# NOVEL ß-LACTAMS, THEIR ANTIBACTERIAL AND PHARMACOKINETIC PROPERTIES AND PRE CLINICAL EVALUATION

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# Novel ß-lactams, their antibacterial and pharmacokinetic properties and pre clinical evaluation.

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Since the introduction of the first ß-lactam antibiotics in the early part of the century a myriad of new compounds have been developed. All of these new compounds have been chosen either because of their improved pharmacokinetic properties, antibacterial activity or both.

The aim of this study was firstly, to investigate the in vitro activity of five ß-lactams (representative of new groups of antimicrobials) and the protecting ability of a ß-lactamase inhibitor, tazobactam, on piperacillin, (a ß-lactam susceptible to hydrolysis by ß-lactamase): secondly, to determine the pharmacokinetics and penetration of each drug to various possible sites of infection.

All of the ß-lactams exhibited greater stability to hydrolysis by ß-lactamases than earlier compounds such as ampicillin and cephalorodine. All inhibited a wide range of organisms with the exception of cefixime and aztreonam which had reduced or little activity against gram positive organisms.

The ß-lactamase inhibitor was slightly less active than clavulanic acid, however, was more stable to environmental conditions such as heat and moisture.

All of the ß-lactams penetrated tissues and inflammatory fluids and concentrations for the most part exceeded the minimum inhibitory concentrations of the common pathogens. Tazobactam was found in vivo in a 1:8 ratio when administered with piperacillin.

Key words: ß lactamase inhibitor, ß lactamase stability, broad spectrum, pharmacokinetics, tissue penetration.

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# INTRODUCTION

The ß-lactams, represented by the penicillins and cephalosporins are useful chemotherapeutic agents exhibiting potent antibacterial effects, yet at the same time lacking, for the most part, seriously damaging effects on the host. When discussing the newer novel ß-lactams it is important to consider the evolutionary steps which have led to their development.

## Early observations - naturally occurring ß-lactams

The discovery of penicillin by Fleming in 1928 is so well known, that the events which preceded its discovery are often forgotten. In fact, the conceptual framework that made the discovery of penicillin possible developed over the preceding 50 years.

As early as 1871 Joseph Lister recorded initial observations on the relatively narrow spectrum of activity of naturally produced penicillin, in that 'Penicillium glaucum' cultures inhibited most but not all of the bacterial contaminants derived from air samples. Stimulated by Lister's work on airborne and other routes of bacterial contamination, Tyndall also observed in his experiments the inability of certain bacteria to grow in the presence of the inhibitory mould (1).

The possibility of one organism affecting the

growth of another and the therapeutic possibilities of this antagonism was postulated by Pasteur and Joubert in 1877 (2). Antimicrobial antagonism was demonstrated experimentally by Cornil and Babes in 1885 and they suggested that the phenomenon could be traced to a chemical produced by one organism acting on the other (3). This theory was supported by Garre in 1887 who demonstrated that a substance from a culture of Bacillus fluorescens diffused through solid media and this substance was inhibitory to the growth of Staphylococcus pyogenes (4). Finally, in 1889 Vuillona named this 'Life against life' phenomena as antibiosis (5).

By the end of the nineteenth century it was clear that microbial antagonism could be caused by a specific substance released by one of the interacting organisms. The idea became fact when in 1896 Gosio succeeded in crystallising a phenolic substance from fermentation broths of penicillin mould and a solution of this substance was found to have powerful antiseptic action on Bacillus anthracis (6). The importance of these interactions between mould and bacteria and particularly the effect of penicillin on bacteria was further illustrated by Duchesne in 1897 when he described the antagonism between Escherichia coli and

Penicillium glaucum. He extended his experiments to demonstrate the protective power of nutrient broth which had been used to grow cultures of P. glaucum, for guinea pigs infected with E. coli and Salmonella typhi (7).

The uninterrupted history of the ß-lactams begins with that most famous of laboratory contaminations, when Fleming observed the lysis of Staphylococcus aureus growing on an agar plate, by a contaminating Penicillin (8). To the man who discovered lysozyme it was the lysis of the bacterial strain which was intriguing. However, he was less successful in isolating the lytic agent and probably because he was not interested in chemical purification studies, all future experiments were performed using crude broth cultures. In these studies he demonstrated the effectiveness of penicillin broth on cultures of streptococci, pneumococci and staphylococci. He also demonstrated that penicillin was more toxic to bacteria than animal tissues by directly applying broth to wounds and by injecting mice and rabbits for toxicity. However, he did not extend these experiments to injecting infected animals (8).

Not only Fleming but also other workers experienced chemical problems with penicillin and

therefore it was not until more than ten years later the Oxford group headed by Florey and Chain, made penicillin a practical reality. They began their work in 1939 and within three years had solved many of the practical problems associated with penicillin production and also reported the first clinical use of penicillin (9).

After the first clinical trials it was recognised that larger amounts of penicillin had to be produced so that more patients with serious disease could be treated. It was at this point that co-operation with the US Department of Agriculture was sought so that large scale production of penicillin could be developed. Modifications which were made to the fermentation process included the addition of corn - steep liquor, which improved the penicillin production 10- fold (10), suspending cultures in aerated medium and introducing a screening program to find a strain which would increase the yield of penicillin. From this screening program a strain identified as Penicillium crysogenum (NRRL 1951) was found and the strains used today in the manufacture of penicillin can be traced back to this culture.

It was noted in these early days that different penicillins were produced by different strains,

available nutrients and culture conditions. This fact was highlighted when penicillin was crystallised by both the Americans and the group at Oxford. The American penicillin was found to be predominantly benzylpenicillin (called penicillin G by the Americans and Penicillin 11 by the English) and the English penicillin was 2 - pentenylpenicillin (penicillin F). Six naturally occurring penicillins were known, each differing from one another by their side chains. Of the six penicillins, penicillin G was the most potent.

Chemists were interested in controlling the alteration of the side chain and used the experience gained from previous work, i.e. that by changing the precursor in fermentation, for example by using corn - steep liquor which is rich in phenylacetic acid, high yields of penicillin G could be produced. By using phenoxyacetic acid as precursor Behrens et al formed phenoxymethyl penicillin, better known as penicillin V (11). However, as this compound was less potent than penicillin G there was little interest in the development of penicillin V until its potential as an oral agent was realised, it being more acid stable than penicillin G.

Ultimately this method was found not to be useful because many of the precursors were found to be toxic

to cultures and also the yields of penicillin were very low. No further progress using precursors was made therefore after the introduction of penicillin G and V.

**Semisynthetic antibiotics: six amino penicillinic acid (6 - APA) and its original derivatives**

Stability to ß-lactamase and activity against Gram negative bacteria were the gaps in the spectrum of activity of benzyl penicillin and penicillin V. The prospects for producing a variety of new antibiotics came in 1959 when large amounts of the penicillin nucleus (figure 1) were made available, to which new side chains could be chemically attached. Using this strategy the first compound produced was methicillin, a penicillin exhibiting stability to staphylococcal ß-lactamases, but still lacking any activity against Gram-negative organisms.

The first modified penicillin with activity against Gram-negative organisms was ampicillin (12). This compound also had its drawbacks being unstable to broad spectrum ß-lactamases and lacking activity to certain important pathogens such as Pseudomonas aeruginosa. However, in spite of its shortcomings it was the first antimicrobial described as having broad spectrum activity.

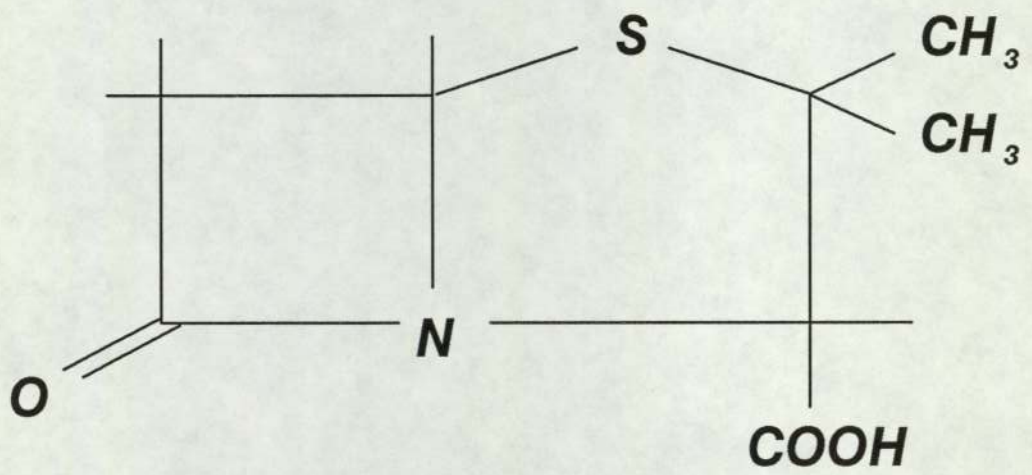


**Figure 1: Penicillin or penem nucleus**

**Cephalosporin or cephem nucleus**

When first isolated the cephalosporins (Figure 2) seemed an unpromising group having lesser activity when compared with the penicillins available. However, they possessed the advantages of being resistant to ß-lactamases and afforded more chances of chemical modification of the nucleus than penicillin.

The first two cephalosporins to be introduced were cephaloridine and cephalothin. Both compounds have stability to the ß-lactamases produced by staphylococci and activity against Gram-negative organisms, with the exception of Ps. aeruginosa and strains such as Enterobacter cloacae which produce ß-lactamases to which these drugs are labile. Another disadvantage is that neither drug can be administered by the oral route.

**Development of the newer ß-lactams**

From the very beginning with the introduction of penicillin, scientists recognised the need to improve the spectrum of activity of antimicrobials. By chemical modification of the natural compounds it was hoped that the biological properties of the compound could be altered without increasing toxicity. These alterations could perhaps modify the antibacterial spectrum giving activity against such organisms as

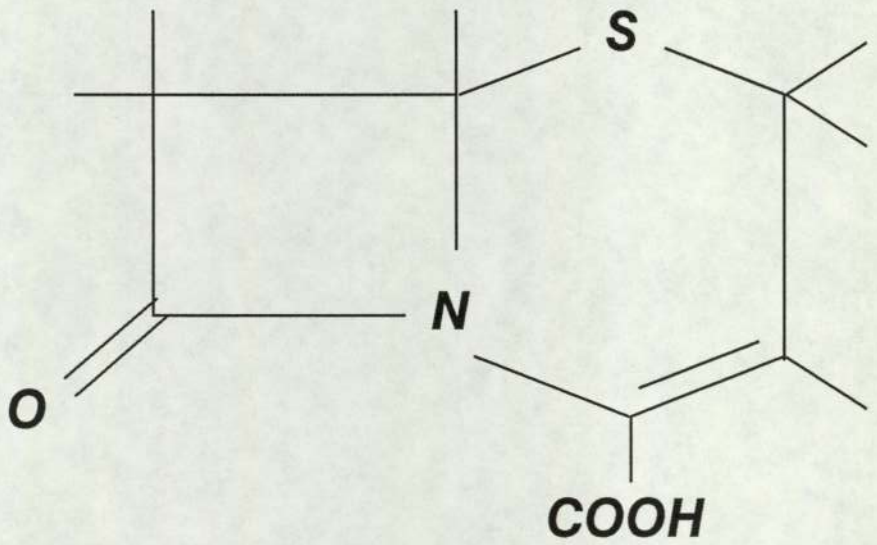


**Figure 2 : cephalosporin or cephem nucleus**

Ps. aeruginosa or high levels of resistance against enzymic hydrolysis or perhaps render the compounds stable to acidity allowing increased oral absorption.

From the first penicillin and cephalosporins have come a succession of antibiotics and the chronological development of the ß-lactams is shown in Figure 3.

## AIM

The object of this ongoing research project was to evaluate some of the newer novel ß-lactams, by comparing their in vitro activity with other compounds currently available (thus confirming the findings of the Pharmaceutical Companies developing the antimicrobial), but more importantly to undertake pre-clinical investigations to determine the pharmacokinetics and penetration of the drugs to various possible sites of infection.

The accurate and precise measurement of antimicrobials requires practical expertise because assay methods have to be developed and tailored to each site under investigation. The confidence in the assay method developed is essential if any conclusions are to be drawn relating concentrations achieved to clinical response. It was therefore, an important part of this research project to develop and evaluate all of the

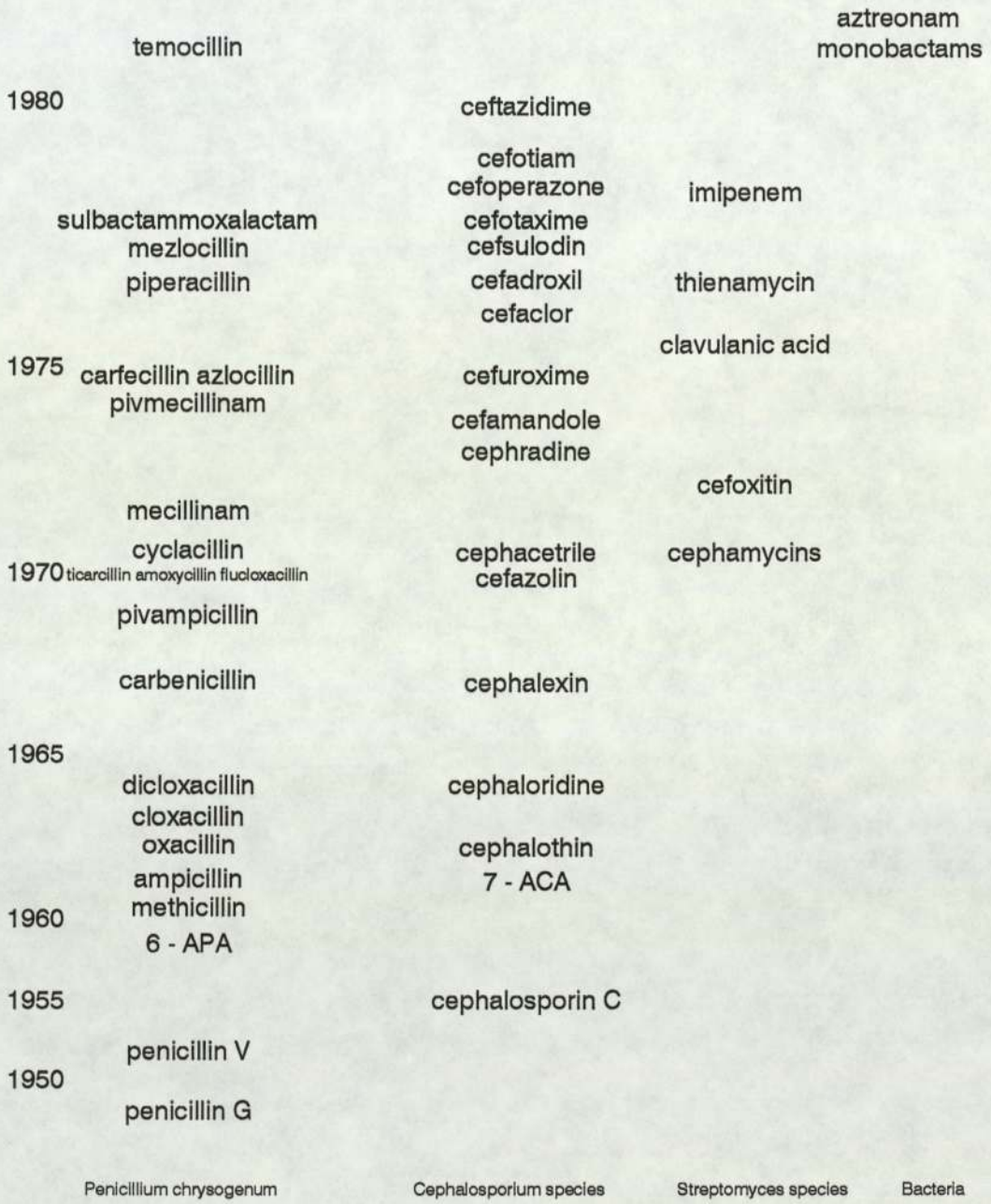


**Figure 3 : Evolution of beta lactams**

assay methods used.

It is hoped ultimately that the data generated by research projects such as this i.e. levels of drug at various possible sites of infection and the susceptibility of organisms associated with infection, can be used to determine breakpoint concentrations (13) which are essential for sensitivity testing in routine laboratories.

## AGENTS STUDIED

### Cefpirome

Cefpirome (HR 810), 3-[2,3-cyclopentento-1-pyridinium) methyl]-7-[2methyoximino-2-(2-aminothiazole-4-yl)-acetamido] ceph-3-em-4 carboxylate (Figure 4), is a new semi-synthetic cephalosporin developed by Hoechst-Roussel for parenteral use. It has some structural similarities to ceftazidime, but preliminary studies (14) suggest that it has enhanced activity against S. aureus and Enterococci compared with other third generation cephalosporins.

### Cefixime

Cefixime (CL 284,635;FK07) (Figure 5) is a new oral cephem antibiotic with in vitro activity similar to cefotaxime, cefmenoxime and ceftizoxime (15). Cefixime is not so readily hydrolysed by plasmid

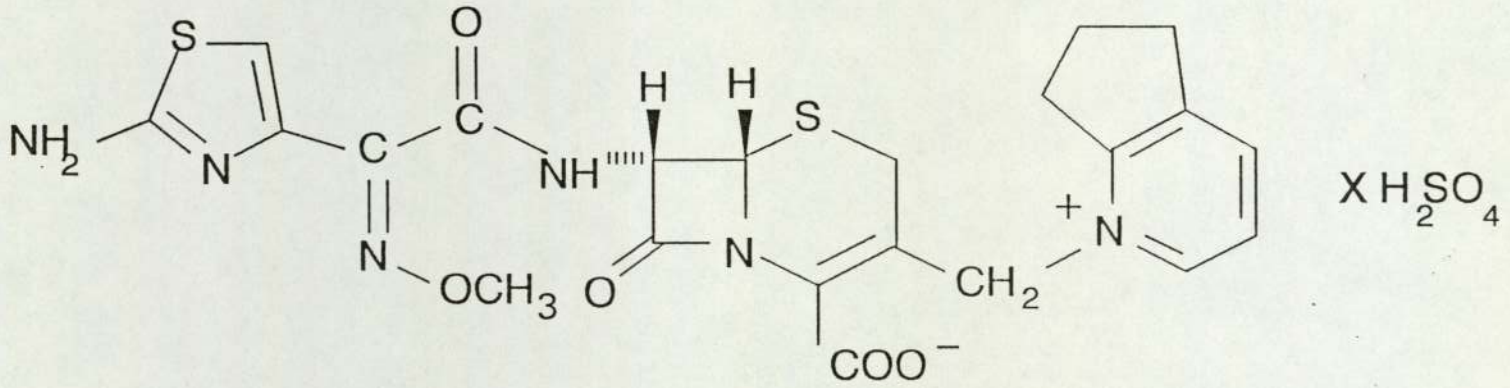


**Figure 4 : Structural formula of cefpirome**

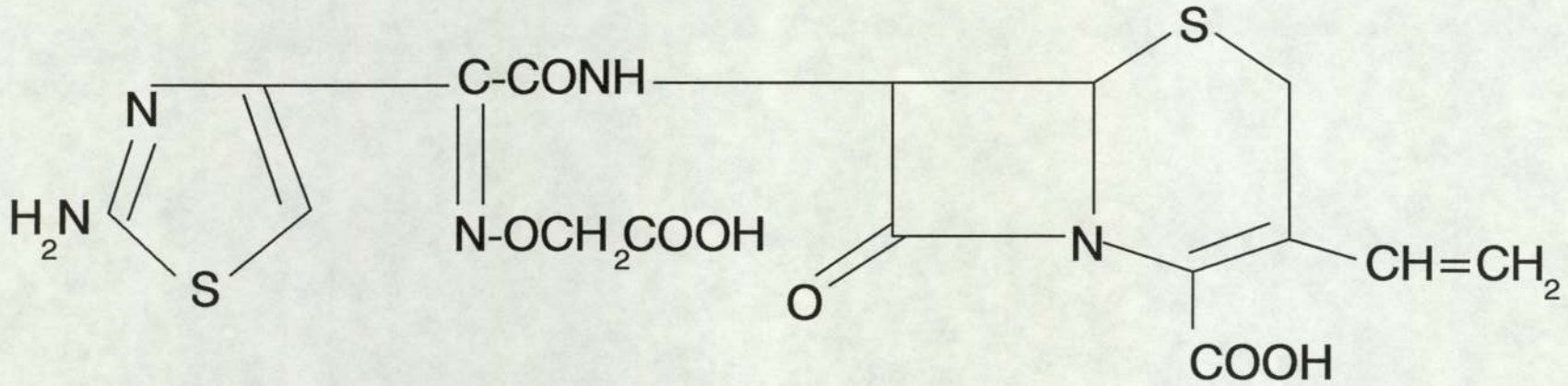


**Figure 5 : Structural formula of cefixime**

mediated ß-lactamases which inactivate currently available oral penicillins and cephalosporins (16). It inhibits a wide variety of organisms including Enterobacteriaceae, Neisseria and Streptococci. Of interest is its activity against respiratory pathogens Haemophilus influenzae and Streptococcus pneumoniae (15, 17). However, poor activity has been reported against S. aureus, enterococci, Ps. aeruginosa and anaerobic bacteria.

**Meropenem**

Meropenem (Figure 6) is a new carbapenem which resembles imipenem in structure and activity (18) and also possesses great stability to ß-lactamase hydrolysis (19). The carbapenems have been characterised as broad spectrum agents with activity similar to that of the first generation cephalosporins against gram positive and that of the third generation cephalosporins against gram negative organisms including Ps. aeruginosa (20). In addition they also have activity against anaerobic organisms (20).

Of considerable importance is meropenems stability to hydrolysis by renal dehydropeptidase 1 (DHP-1) which means that co-administration of the enzyme inhibitor cilastatin is unnecessary (21).

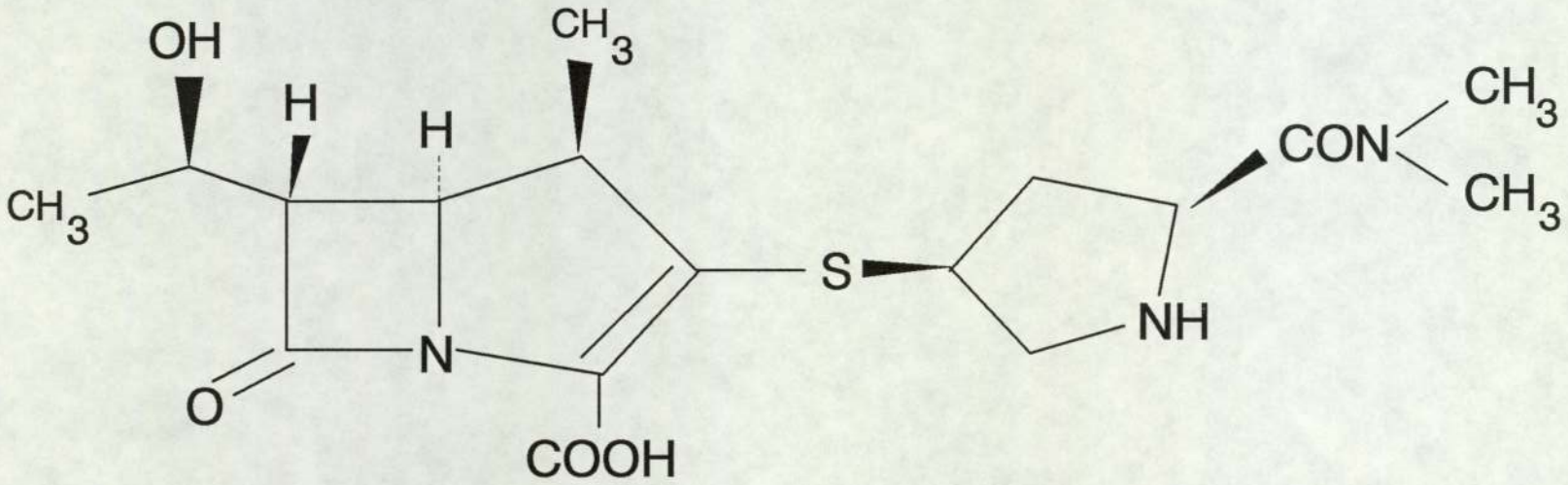


**Figure 6 : Structural formula of meropenem**

**FCE 22101**

In 1975 the first penem was in clinical use (22). Penem is derived from its structural similarity with [pen]em and ceph[em] (23). The first penem had modest activity against staphylococci but was relatively unstable. Other penems which have since been developed including SCH 34343 (24) and CGP 31608 (25).

However, although many penems have been synthesized very few have been fully developed. In these studies we have evaluated the in vitro activity of FCE 22101 (the formula sodium [5R,65,8R]-6-hydroxethyl-2-carbamoyloxymethyl-2-penem-3-carboxylate) (Figure 7).

**Tazobactam**

There have been two approaches to overcoming the increasing problem of resistance attributed to ß-lactamase. Firstly, the development of compounds exhibiting stability to ß-lactamases and secondly the search for ß-lactamase inhibitors. Certain ß-lactams with little intrinsic antibacterial activity have been shown to be potent inhibitors of ß-lactamases (26).

Piperacillin, a broad spectrum penicillin is susceptible to hydrolysis by a range of ß-lactamases, including the plasmid-mediated enzymes found in the Enterobacteriaceae - Richmond and Sykes Group III (27)

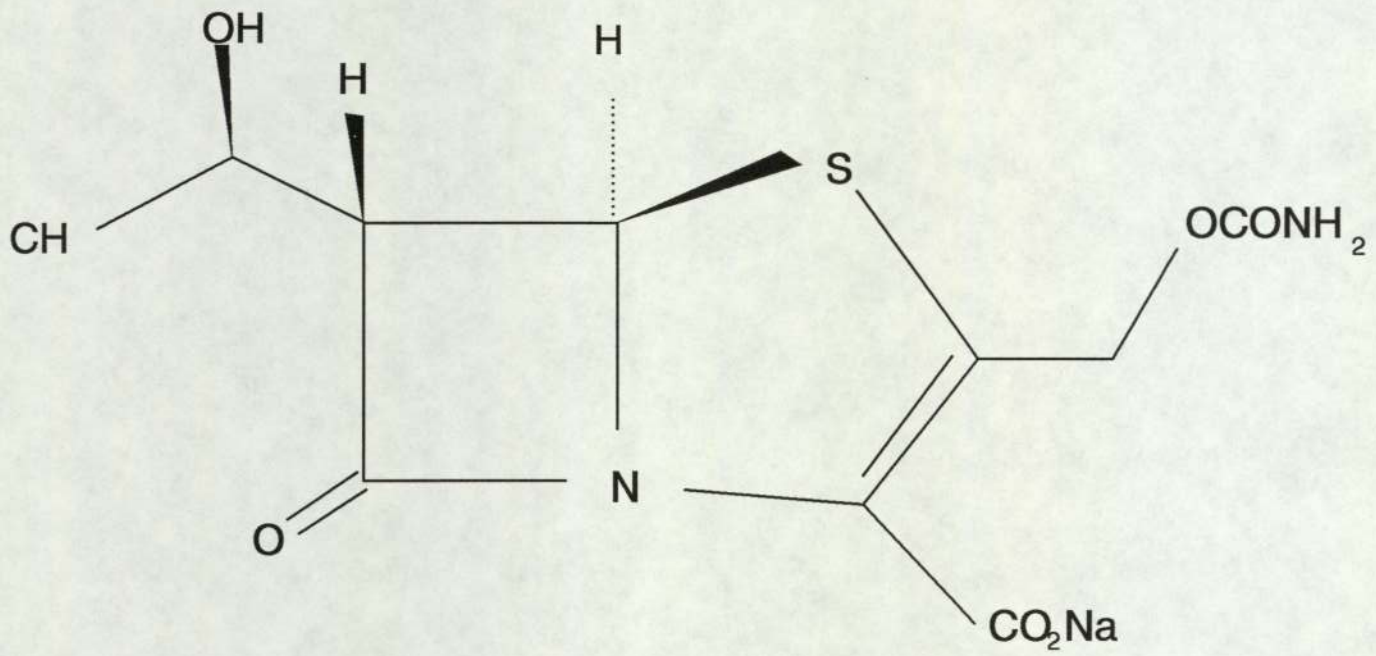


**Figure 7 : Structural formula of FCE 22101**

and the enzymes commonly found in S. aureus and Bacteroides fragilis. Several ß-lactamase inhibitors have been combined with other broad spectrum penicillins such as amoxycillin and ticarcillin with clavulanic acid, ampicillin with sulbactam and pivampicillin with 6 ß-bromopenicillamic acid.

Tazobactam (YTR 830) (Figure 8) which is a penicillamic acid sulphone which has been shown to be an active ß-lactamase inhibitor (28, 29) has been combined with piperacillin in a combination of 1:8. In these studies we have looked at the in vitro activity and also the pharmacokinetics and inflammatory fluid penetration; previous studies indicating that the elimination of tazobactam is slower in the presence of piperacillin (30).

**Aztreonam**

In an attempt to discover novel ß-lactams from natural sources many Pharmaceutical Companies have screened bacterial populations from soil, water and vegetation. Screening of bacteria for the production of ß-lactam antibiotics has resulted in the discovery of a family of structurally similar to monocyclic ß-lactam containing molecules. Although relatively weak antibacterial agents, the naturally occurring monobactams are very stable to hydrolysis by

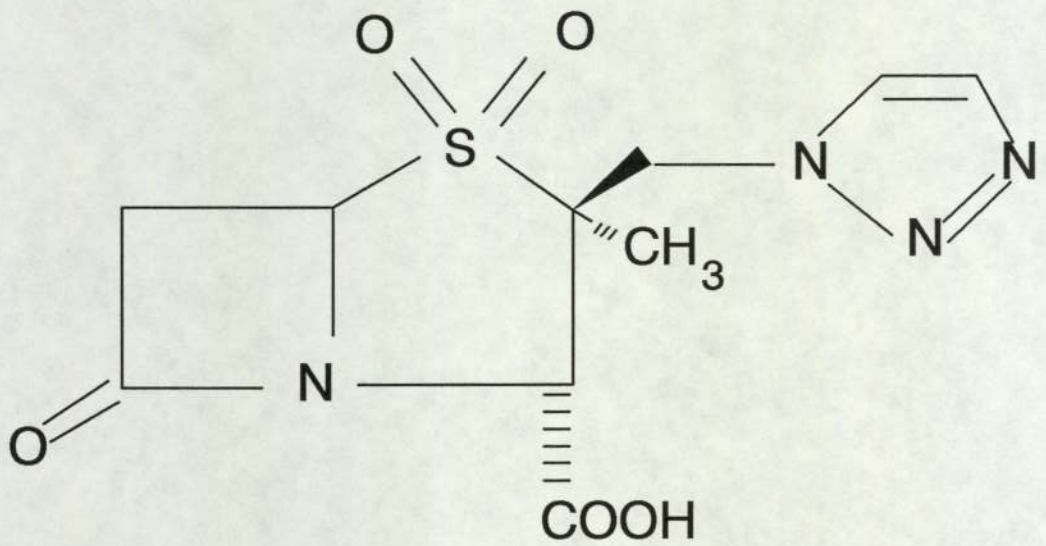


**Figure 8 : Structure of tazobactam**

ß-lactamases.

From one of the first naturally occurring monobactam, SQ 26,180, produced by Chromobacterium violaceum, monobactams have progressed to the chemically synthesized aztreonam (SQ 26,776)(Figure 9). Preliminary unpublished studies suggested that aztreonam has a narrow range of activity covering mainly the Gram negative bacterial species including those with multiple resistance to antibiotics.

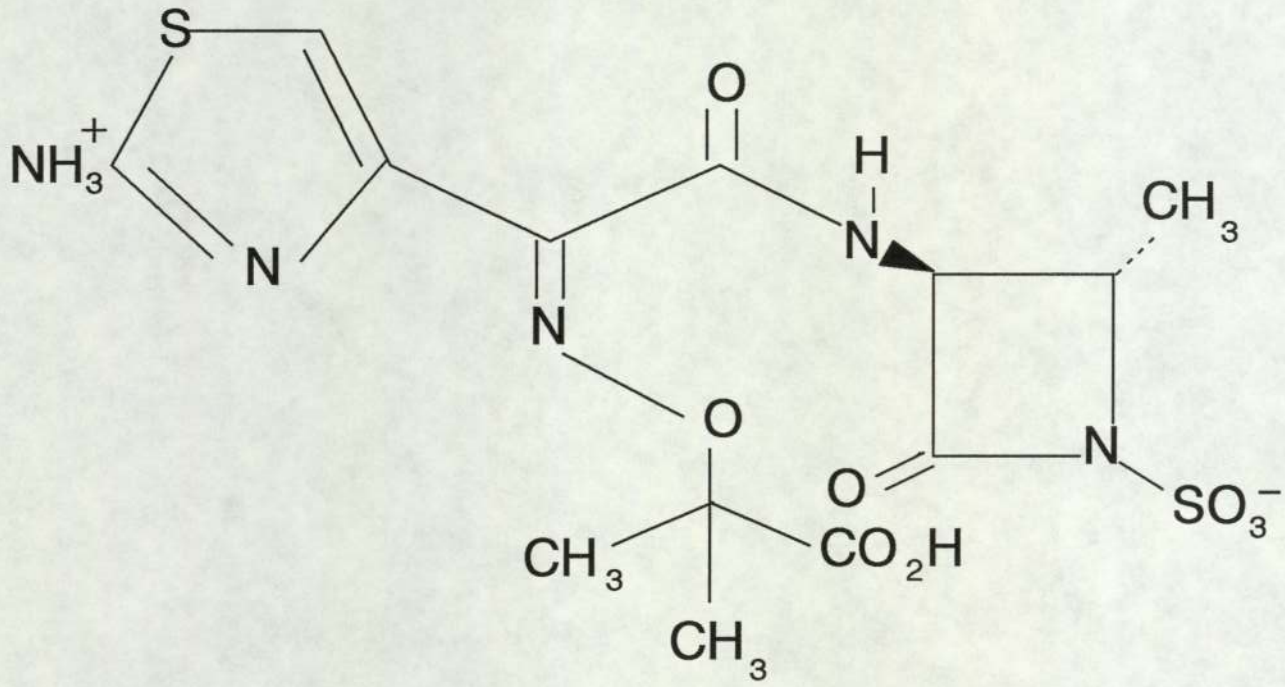


**Figure 9 : Structural formula of aztreonam**

# MATERIALS AND METHODS

## 1. Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration is defined as the smallest amount of antimicrobial necessary to inhibit the growth of an organism. For many years medical microbiologists have used the MIC as the measure of compound's activity against a particular bacterium (31).

Strains examined in these studies were comprised of recent clinical isolates, obtained from the routine Microbiology Department of Dudley Road Hospital. The following identification systems had been used for confirmation. Enterobacteriaceae - Growth characteristics, Gram's reaction, API 20E identification (Biomerieux, Paris, France); Pseudomonas, growth characteristics, Gram reaction, oxidase test; Staphylococci, Gram reaction, catalase, staphaurex (Wellcome, Dartford, England), novobiocin sensitivity; Strep. pneumoniae optochin sensitivity (Unipath, Basingstoke, England); ß-haemolytic Streptococci - bacitracin sensitivity (Unipath); streptex grouping (Wellcome); H influenzae - Gram reaction, growth requirements (X

and V test, Unipath); Neisseria - oxidase, fermentation profile (Southern Group, Hithergreen Hospital, Lewisham, England); anaerobic organisms - biochemical profile (BDH, Poole, Dorset, England).

Strains were maintained until use as follows: Enterobacteriaceae, pseudomonas and staphylococci on nutrient agar slopes and freeze dried cultures; anaerobes and fastidious strains in liquid nitrogen. All strains were checked for purity on appropriate media before use.

Well characterised ß-lactamase producing strains obtained from Glaxo laboratories were also included in the studies.

The antimicrobial agents investigated were from the following sources: Cefpirome from Roussel, Uxbridge, England; tazobactam, piperacillin and cefixime from Lederle Laboratories, Gosport, England; aztreonam from Squibb Institute, Princeton, NJ, USA; meropenem from ICI, Macclesfield, England.

MICs were determined using a routine agar plate dilution method (doubling dilution steps of antimicrobial up and down from 1 mg/l) using Iso-Sensitest agar pH 7.2 (CM 471 Oxoid Ltd,

Basingstoke, England) which was supplemented with 5% horse blood and 20 mg/l Nicotinamide-adenine dinucleotide (NAD) (Sigma Chemicals, Poole, Dorset, UK) to support the growth of fastidious strains. For the anaerobes Wilkins and Chalgren agar (Oxoid CM 619) plus 5% horse blood was used. For the assessment of tazobactam, which has little or no antibacterial activity, its ability to protect piperacillin from ß-lactamase inactivation was determined by comparing MIC's of piperacillin alone and in combination with tazobactam.

Inocula were prepared as follows: Staphylococci, Pseudomonas and Enterobacteriaceae were grown overnight in brain heart infusion broth (Southern Group laboratories) yielding a viable count of about $10^9$ colony - forming units (CFU) per ml. Streptococci were grown in Todd - Hewitt broth (Oxoid CM 189), H. influenzae and neisseria in Levinthal broth, bacteroides (other than fragilis), Clostridium species and anaerobic streptococci in Wilkins and Chalgren broth plus 0.02% 'Tween 80' and Bact. fragilis in Wilkins and Chalgren broth (Oxoid CM 643) plus 0.2% sodium succinate each giving comparable viable counts of about $10^9$ CFU/ml.

The inocula were obtained by transferring 1 µl of a 1:100 dilution of the overnight broth culture (dilution made in sterile distilled water except for fastidious strains where the dilution was made in sterile phosphate buffer saline pH 7.2) to the surface of the antibiotic-containing media and the antibiotic free control, by a Denley multipoint inoculating device (Denley-Tech Ltd., Billingshurst, England). For selected strains undiluted overnight broth culture was used, the final inocula on the plates were therefore approximately $10^4$ and $10^6$ CFU.

The incubation temperature for all strains was 35-37°C aerobic strains in air, fastidious strains in 6% carbon dioxide in air and for the anaerobes an anaerobic cabinet (Don Whitley, Skipton, Yorkshire, England) with an atmosphere of 80% nitrogen, 10% carbon dioxide and 10% hydrogen. The duration of incubation was 18-24 hours with the exception of the anaerobes where incubation was for 48 hours.

The MIC of the antibiotic against all organisms, was defined as that concentration (in mg/l), at which there was a reduction (by counting) to ten or fewer colonies in the original

inoculum. In the case of the higher inoculum ($10^6$ CFU) a faint haze of growth was ignored.

Results are expressed as a range of MIC and also that concentration of antimicrobial which would inhibit fifty per cent of strains ($MIC_{50}$) and that concentration which would inhibit ninety per cent of strains ($MIC_{90}$) stated.

Minimum bactericidal concentration (MBC) is defined as the smallest amount of drug needed to kill an organism. In determining the MBC of an organism and then by direct comparison with the inhibitory concentration, the killing potential of an antimicrobial can be assessed.

All drugs bind to human protein (usually albumin) to a lesser or greater degree and once bound a drug becomes inactive. In vivo, this binding is not static but is always changing due to an equilibrium effect. However, the effect of human serum on an antimicrobial's activity is important to know, particularly if one is dealing with a highly protein-bound drug such as flucloxacillin or ceftriaxone. As the drug will be present at different sites of infection where the amount of protein present may vary, the effect of various concentrations of protein should be

tested. It must also be remembered that antibiotic free human serum alone can have a marked effect on the growth of organisms (32), inhibiting the growth of some bacterial strains or producing a zone of inhibition when performing antibiotic assays. This should also be remembered when selecting antimicrobial strains to ensure that any effect noted is attributed to the antimicrobial alone.

The effect of human serum on the MIC and MBC was studied by a modification of a method of Pearson et al (33). An overnight broth culture of organism (strains selected dependent on antimicrobial) was inoculated into Iso-Sensitest broth containing 0, 20 and 70% human serum (Flow Laboratories, High Wycombe, England) and various concentrations of antimicrobial, to give a final inoculum of $10^5$ orgs/ml. After overnight incubation 100 µl from tubes showing no visible growth was inoculated onto antibiotic free media. After incubation the 99.9% lethality was recorded.

2. **Determination of primary target site of ß-lactams**

It is now well recognised that ß-lactam antibiotics affect sensitive bacteria by inhibiting late stages in the biosynthesis of

their cell wall peptidoglycan. Because peptidoglycan is responsible for maintaining the shape and integrity of cell walls, interference in its synthesis is potentially lethal. The target sites to which ß-lactams bind are identified biochemically as penicillin-binding proteins (PBPs), the cytoplasmic membrane components which form stable acylated derivatives with labelled benzylpenicillin or other ß-lactam antibiotics (34, 35, 36).

The best studied PBP's are those of Esch. coli and therefore this organism is usually used to identify the primary target site of new ß-lactams. Table 1 lists the seven PBPs of Esch. coli and the consequences of inactivation and also the effect on morphology.

3. **Morphological response of E. coli to ß-lactam antibiotics**

The effect of each of some of the antimicrobials upon the morphology of Esch. coli K-12 DCO in broth culture was monitored by differential interference contrast microscopy. Log phase cultures containing approximately $10^7$ cfu/ml were exposed to a concentration of antimicrobial equivalent to 5 times the MIC.

| PBP | Molecular weight | Gene name(s) | Selective inhibitors | Consequences of inactivation |
|---|---|---|---|---|
| 1A | 92,000 | ***pon A mrc A*** | | Rapid cell lysis if |
| 1Bs | ~ 90,000 | ***pon B mrc B*** | Cephaloridine,cefsulodin | both inactivated |
| 2 | 66,000 | ***pbp A mrd A*** | Mecillinam,clavulanic acid imipenem | Spherical,non-growing cells |
| 3 | 60,000 | ***pbp B ftsI*** | Cephalexin,cefuroxime, aztreonam | Filamentous nonseptate cells |
| 4 | 49,000 | ***dacB*** | | Delayed transpeptidation absent |
| 5 | 42,000 | ***dacA*** | | None obvious |
| 6 | 40,000 | ***dacC*** | | None obvious |

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**Table 1 : Properties of Esch.coli PBPs**

At timed intervals control and test broth cultures were sampled onto agar wedges, made from 0.75% agarose (BDH Chemicals, Poole, England) supported on glass slides. After applying a coverslip the slide was examined microscopically. Results were recorded photographically (Photograph 1).

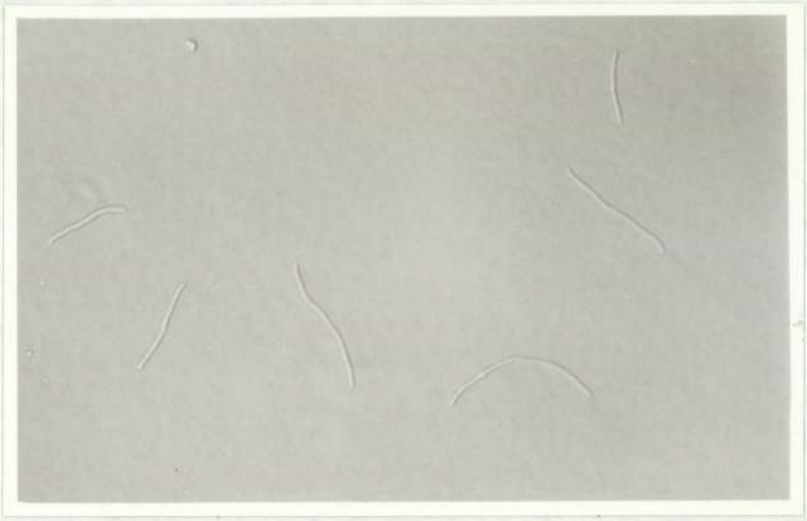
4. **Development of assay methods for the measurement of antimicrobials in body tissues and fluids**

In order for an antimicrobial to be effective its first task is to reach the site of infection and thus the organism causing that infection. When investigating a new drug the pharmacological studies are important because they determine maximum concentrations achieved in the blood and at possible sites of infection such as tissues and body fluids. They also give information about the length of time the drug remains in the body before excretion (usually referred to as the half life of the drug). Urinary excretion indicates the amount of drug excreted by this route and also urine can be screened to determine whether the drug is excreted unchanged or as a metabolite.

Microbiological assays have been used for many years in routine laboratories because they are considered simple, easy to perform, and are

**Photograph 1:**

Morphological response of Esch. coli after exposure to B-lactam antibiotics, filamentation (top), rounding (bottom) compared with a control (middle).



quicker to develop than assays such as high performance liquid chromatography (HPLC). However, there are several practical aspects which have to be considered when developing a new assay. The effect of some of these variables, such as diluent for standards and test, temperature of incubation, stability of antimicrobial, selection of assay organism and adjustment for the presence of contaminating blood have to be considered.

Medium:

Specific media are selected for the following attributes: firstly, it needs to be at the pH which is optimum for the activity of the drug, this will ensure maximum sensitivity and secondly, reproducibility with different batches of media. The following media were selected:-

Iso-sensitest agar (Oxoid CM 471) pH 7.2 for the assay of cefpirome and meropenem.

Antibiotic Media No 1 (Oxoid CM 327) pH 6.6 for the assay of cefixime, piperacillin, aztreonam and FCE 22101.

Antibiotic Media No 2 (Oxoid CM 849) for the assay of tazobactam. Tazobactam has very little antibacterial activity and therefore the conventional way of measuring drug concentrations

i.e. measuring zones of inhibition produced when an antimicrobial diffuses from a well or disc into media inoculated with a susceptible bacterial strain, cannot be used. In order for the concentration of inhibitor to be measured, media are seeded with an organism which produces ß-lactamase and a ß-lactam which is susceptible to that ß-lactamase sample containing ß-lactamase inhibitor is then applied to the plate. ß-lactamase inhibitor then diffuses out from the well or disc and comes in contact with the ß-lactam contained in the agar. The susceptible ß-lactam is then protected from hydrolysis by the ß-lactamase inhibitor and can therefore inhibit the growth of the assay organism, thus producing a zone of inhibition. Concentration of inhibitor is thus related to the amount of ß-lactam protected. The ß-lactam added to the culture media was piperacillin at a concentration of 80 mg/l.

All media were prepared following the manufacturer's instructions.

Organism:

When choosing an assay organism not only should sensitivity and specificity be considered but also the availability of the organism to other

scientists. With this in mind organisms from the National Collection of Type Cultures (NCTC) or the American Type Culture Collection (ATCC) should always be considered first before using 'in-house' strains.

The sensitivity of an organism relates to the smallest amount of antimicrobial which can be confidently measured. It is important to remember that often antibiotic-free body fluids and extracts can of themselves have an inhibitory effect on organisms (37). Examples of this are human serum and also sputum. For this reason it is essential that antibiotic free samples be tested to ensure that there is no zone of inhibition.

With regard to sensitivity it must also be remembered that the degree of sensitivity required depends on the expected level at the site to be investigated. Often theoretical levels can be determined so that an assay strain is sought which has the necessary sensitivity. Table 2 shows an example of theoretical levels of a drug in tissue which is constructed knowing serum levels combined with varying percentage penetration.

The specificity of an organism is the confidence that the results obtained are for the

| Time Interval | Serum level mg/l | Predicted level in tissue mg/k assuming theoretical penetration into tissue of | | |
|---|---|---|---|---|
| | | 25% | 50% | 75% |
| 1 | 34 | 8.5 | 17.0 | 25.5 |
| 1.5 | 31 | 7.8 | 15.5 | 23.0 |
| 2 | 20 | 5.0 | 10.0 | 15.0 |
| 3 | 14 | 3.5 | 7.0 | 10.5 |
| 4 | 11.5 | 2.9 | 5.8 | 8.6 |
| 5 | 8 | 2.0 | 4.0 | 6.0 |
| 6 | 6 | 1.5 | 3.0 | 4.5 |
| 7 | 4 | 1.0 | 2.0 | 3.0 |
| 8 | 3 | 0.75 | 1.5 | 2.3 |
| 12 | 1 | 0.25 | 0.5 | 0.75 |

**Table 2 : Theoretical levels in tissue**

Level of antimicrobial at various time intervals after dosing, predicted in tissue assuming penetration equivalent to 25 ,50 and 75% that of simultaneous serum level.

measurement of only one antimicrobial. It is important therefore to ensure that only one antimicrobial is administered to the patient or volunteer, except in the case of tazobactam which is an inhibitor given concomitantly with piperacillin to protect it from inactivation by bacterial ß-lactamases. It should also be determined whether the drug under investigation is metabolised in the body to an antibacterially active form. This is often determined by comparing results obtained by a microbiological assay with those obtained by a chemical method such as HPLC. The following organisms were therefore selected:

Esch. coli

ATCC 25922 for the assay of cefpirome

Providentia stuartii

K166 (DRH strain) for the assay of cefixime

Esch. coli for the assay of meropenem

(NIHJ)

Micrococcus lutea

Z114 (DRH strain) for the assay of FCE

Ps. aeruginosa

NCTC 10701 for the assay of piperacillin

Klebsiella aerogenes

NCTC 1003 for the assay of tazobactam

Esch. coli

(SC 12655 Squibb) for the assay of aztreonam (pharmacokinetic study). This organism was too sensitive for use in the peritoneal study.

Esch. coli

10418 (peritoneal study)

Diluent for standard and test:

The interest in the diluent in which standard material is prepared has recently been increased with the awareness that not only albumin but other components found in serum can have a major effect on the assay of antimicrobials (38). A variation in protein binding depending on animal species has been observed for some ß-lactam antibiotics and Table 3 shows the percentage error in the assayed concentration (compared with the assigned concentration), when calf and horse serum are substituted for human serum for the preparation of standards (37). It is obviously very important to determine the amount of drug bound to serum proteins. A micropartition system, separating free from protein-bound microsolute was therefore employed (Centrifree Micropartition System, Amicon, Mass. USA), to determine the amount of antimicrobial bound at differing drug

| Antimicrobial | % bound to human serum | % error* Horse | Calf |
|---|---|---|---|
| Cephaloridine | 18 | -3 | -10 |
| Cefuroxime | 30 | -2 | -7 |
| Latamoxef | 61 | -8 | -15 |
| Cephazolin | 85 | -36 | -44 |
| Ceftriaxone | 95 | -71 | -74 |
| Aztreonam | 50 | -5 | -42 |

* assayed concentration compared with assigned concentration



**Table 3 : Effect of horse and calf serum standards on the assay of Beta lactams in human serum samples**

Samples prepared in human serum assayed by a microbiological assay method against standard material prepared in either calf or horse serum.

concentrations.

When assaying levels of drug in body fluids other than serum such as mild inflammatory exudates or transudates, it is important to know the expected levels of albumin at the site. In Table 4 the levels of albumin in mild inflammatory exudate are shown.

The measurement of antimicrobials in urine usually presents very few problems as most drugs are concentrated at this site and sample volumes are large. However, a problem may be encountered if the urine sample is not diluted accurately (very important in the meropenem study because the majority of the drug is excreted in the first four hours). It is therefore important that all urine dilutions be made volumetrically to reduce the dilutional error.

The measurement of antimicrobials in bronchial mucosa is of importance when investigating drugs which have a spectrum of activity against the common respiratory pathogens (39). However, there are many technical difficulties eg, size of tissue sample obtained from the patient (often very small samples obtained approximately 7 mg in weight), prevention of loss of moisture from the sample

| | | Plasma | Blister | Blister as % of plasma |
|---|---|---|---|---|
| Albumin g/l | Mean | 50.12 | 38.12 | 76 |
| | Range | 43-54 | 31-52 | |



**Table 4 : Mild inflammatory exudate - blister fluid**

Range of albumin (g/l) measured in inflammatory fluid (produced by Cantharides plasters) compared with those found in plasma.

before assay and selection of diluent for standards. When large quantities of tissue are available experiments are usually performed to determine any possible binding of the antimicrobial to tissue. If there is significant tissue binding, standards must be prepared in tissue homogenate to compensate for this.

When measuring concentrations from this site it is very difficult firstly, to determine the degree to which binding occurs because the acquisition of large quantities of tissue is very difficult and secondly, the availability of a constant supply of antibiotic free tissue to prepare a homogenate (for the preparation of standards) is also unrealistic. For these studies it is therefore usual to spike antibiotic free tissue with antimicrobial and then perform recovery experiments. If an excess of 85% of the drug is recovered from the spiked sample it is assumed that binding is minimal and standards are prepared in phosphate buffered saline.

When measuring levels in large tissue samples such as prostate, as mentioned previously, it is very important that samples be stored in humidifiers before homogenising to prevent loss of

moisture from the tissue. The preparation of the homogenate is usually undertaken by homogenising a fixed weight of tissue and diluent (usually 1:1 volume) for the shortest possible time. A clear supernatant is then obtained by centrifugation. At both of these stages the tissue and diluent should be kept as cold as possible to reduce the risk of drug inactivation by heat.

A very important consideration when assaying tissue is the selection of suitable medium for the preparation of the standard curve. To overcome the possibility of tissue binding the antibiotic standard should be added to antibiotic free tissue before homogenising and then the standard treated in exactly the same way as the test (addition of standard to homogenate supernatant may not reflect the total binding of the whole tissue components). Most workers have obtained either normal antibiotic free tissue samples at operation or at post mortem (within 24 h of death)(40). Instability of the drug during the extraction stage can be determined by dividing a tissue sample containing drug, spiking half with a known concentration of drug and then extracting and assaying both samples. The difference between the

assayed levels of the two samples should be equivalent to the amount of drug added to the one sample if no inactivation has occurred.

Another important consideration is the amount of blood volume remaining in tissue capillaries, the tissue should be rapidly cleaned from superficial blood (blotting or trimming the surface may be necessary to remove the surface blood), however, obviously bloodstained tissue should be discarded. Measuring the blood volume remaining in tissue capillaries has been approached in several ways by different workers. Several methods have been described for measuring haemaglobin for example, a spectrophotometric analysis (40). However, the method which is much favoured because of its simplicity is that of Lowry & Hastings (41). The formula for adjusting for blood is as follows:-

$$X = \frac{\text{Serum concentration of antibiotic X Hb tissue}}{\text{Hb serum}}$$

Tissue concentration:

Supernatant concentration -

X = concentration of antibiotic in tissue

(adjusted value)

Measuring levels of drug in sputum presents problems, in as much that every sputum sample may

differ in consistency i.e. from salivary, mucosalivary, mucoid, purulent, mucopurulent etc. Obtaining antibiotic-free sputum to match each of these descriptions from which standards can be prepared is also very difficult because in reality most patients are already receiving antibiotics. It is therefore generally accepted that standards are prepared in phosphate buffered saline.

Preparation of test before assay

Optimum dilutions for all samples were found by experiment and all buffer solutions used were prepared as per the seventh edition of the Geigy Manual of Scientific Tables.

Serum:

Cefpirome - Samples up to 2 h after IV dose diluted 1:5* Cefixime - samples up to 4 hrs 1:10* and 1:20*, >4 hrs 1:5*

FCE 22101 - No dilution of sample necessary.

Meropenem - No dilution of sample necessary. Standard range selected to accommodate time of sample.

Piperacillin - No dilution of sample.

Tazobactam - Samples 0 - 1 h after an IV dose diluted 1:5*.

Aztreonam - 0-4 h No dilution and 1:5*

>4 h No dilution of sample

* all dilutions made in antibiotic-free pooled human serum (Flow Laboratories).

Peritoneal fluid:

Pre-weighed Whatman 6 mm discs were placed in the peritoneum by the surgeon. After 10 mins discs were removed and then transported to the laboratory as soon as possible. On receipt the discs were weighed to calculate the amount of fluid absorbed by the disc. At this stage test discs were compared with discs which had been soaked in buffer containing 5, 10, 20% human blood. Any disc containing >10% blood staining was discarded.

Inflammatory fluid:

Cefpirome - treated the same as serum.

Cefixime - samples diluted 1:10 in 70% human serum in pH 6.6 phosphate buffer.

FCE - treated the same as serum.

Meropenem - no dilution necessary standard range selected to accommodate time of sample.

Piperacillin samples 0-1 h diluted 1:2 in 70% human serum in pH 7 phosphate buffer. Neat and 1:2 dilution assayed. >1 h samples assayed without dilution.

Tazobactam 0-1 h samples diluted 1:5 in pH 6.6 phosphate buffer. Neat fluid and 1:5 dilution assayed. >1 h samples assayed without dilution.

Aztreonam - No dilution of sample.

| Urine: | Collection time (h) | Optimum urine dilution | |
|---|---|---|---|
| Cefpirome (pH 7.0 phosphate buffer) | 0-4 | 1:500 | |
| | 4-8 | 1:100 | |
| | 8-12 | 1:20 | |
| | 12-24 | 1:5 | |
| Cefixime (pH 6.6 buffer) | 0-2 | 1:10 | 1:100 |
| | 2-4 | 1:50 | 1:500 |
| | 4-8 | 1:50 | 1:500 |
| | 8-12 | 1:10 | 1:100 |
| | 12-24 | 1:5 | 1:50 |
| FCE 22101 (MOPS buffer) | 0-1 | 1:1000 | |
| | 1-2 | 1:100 | |
| | 2-3 | 1:5 | 1:50 |
| | 3-4 | 1:5 | 1:50 |
| | 4-6 | 1:5 | |
| | 6-8 | 1:2 | |
| | 8-12 | 1:2 | |
| Meropenem (pH 7 buffer) | 0-4 | 1:200 | 1:500 |
| | 4-8 | 1:20 | 1:10 |
| | 8-12 | 1:2 | |

| | | | |
|---|---|---|---|
| 12-24 | 1:2 | | |
| Tazobactam | 0-4 | 1:100 | 1:200 |
| (pH 6.6 buffer) | 4-8 | 1:50 | 1:25 |
| 8-12 | 1:2 | | |
| 12-24 | 1:2 | | |
| Piperacillin | 0-4 | 1:100 | 1:200 |
| (pH 6.6 buffer) | 4-8 | 1:100 | 1:50 |
| 8-12 | 1:2 | | |
| 12-24 | 1:2 | | |
| Aztreonam | 0-8 | 1:20 | 1:50 1:100 |
| (pH 6.6 buffer) | 8-12 | 1:20 | 1:50 |
| 12-24 | 1:5 | 1:10 | |

( ) = diluent used for preparing urine dilutions.

Sputum:

Add equal volume of sputum and pH 6.6 phosphate buffer (w/v) and ultrasonicate in a glass tube on ice for 2 mins (Ultrasonics Heat Systems W225 50% duty cycle pulsed).

Bronchial mucosal tissue:

A method for the measurement of concentrations of drug in bronchial mucosa has recently been described by Honeybourne et al (39). The following procedure was therefore employed:-

Tissue taken at biopsy was placed in a known volume of chilled buffer (any obviously blood

stained tissue having been discarded) and then ultrasonicated on ice (Ultrasonics Heat Systems W225) to homogenise the tissue. The concentration in the tissue derived using the following calculation:-

$$\text{assayed conc. (mg/l)} \times \frac{1000}{\text{wt tissue (mg)}} \times \frac{\text{Vol. of buffer (µl)} + \text{wt. of tissue (mg)}}{1000} = \text{concentration in tissue (mg/K)}$$

Prostatic tissue:

Add equal volumes of tissue and chilled pH 7.0 phosphate buffer (weight/volume) and homogenise on ice (Ultraturex TP/18 Janke and Kintel) for approximately 2 mins (this time depends on the resilience of the tissue). Centrifuge at 16,000 RPM 20 mins to obtain a clear supernatant.

Incubation temperatures:

The sensitivity of an assay system can be increased by altering the incubation temperature or by pre diffusion before incubation. None of the antimicrobials required pre diffusion before incubation and the following temperatures were used for the antimicrobials under investigation:-

37°C - cefpirome, cefixime, FCE, piperacillin, tazobactam, aztreonam,

30°C - meropenem.

Sample volume applied to plate:

The amount of sample applied to a plate depends on several factors. Firstly, sensitivity of the assay system, i.e. if the assay organism is not very sensitive to the antimicrobial or if the antimicrobial diffuses poorly then a large sample volume is required to produce a zone of inhibition. However, if an organism is very susceptible then the reverse is true. Secondly, if only small samples are available, for example biopsy samples which may never exceed 10 mg in weight, the weight limits the amount of sample available for assay. Finally, in the assay of fluids by absorption onto blotting paper discs, here the sample size is governed by the way in which the sample is collected.

Obviously where the sample size is large, as is the case with serum and urine, then the amount applied to the plate depends solely on the volume found by experiment to give the most precise and accurate results.

The following sample sizes were used in these studies:-

Cefpirome - urine, serum, blister fluid - 6 mm Whatman discs (Appleton Woods,

Birmingham, England).

- prostate and bronchial mucosal tissue - 5 mm well.

Cefixime - serum, urine, blister fluid, bronchial biopsy, sputum - 6 mm disc.

FCE 22101 - serum - 7 mm well
blister fluid - 5 mm well.

Meropenem - serum, urine, blister fluid, peritoneal fluid - 6mm discs.

Piperacillin - serum, blister fluid, urine - 5 mm well.

Tazobactam - serum, blister fluid, urine - 5 mm well.

Aztreonam - serum, urine, blister fluid, peritoneal fluid - 6 mm discs.

5. **Basic method for the assay of antimicrobials in tissue and body fluids.**

Preparation of media

Media were prepared following the manufacturer's instructions.

Preparation of organism

All strains were stored on nutrient agar slopes at room temperature until required. Before use as

assay indicator organism purity was checked by subculture on to media to obtain single colonies, After purity check, organisms were subcultured into digest or infusion broth and incubated overnight at 37°C.

Preparation of Standard material

The method for preparing meropenem standards is given below. This basic procedure was adapted for each of the antimicrobials investigated.

22.8 mg meropenem ->
(adjusted for potency)
20 mls* distilled water = 1000 mg/l

1000 µl 1000 mg/l solution ->
10 mls distilled water = 100 mg/l

100 µl 1000 mg/l solution ->
10 mls distilled water = 10 mg/l

Using the stock solutions :-

| | | | |
|---|---|---|---|
| 160 µl | 1000 mg/l | - 5 mls* diluent | = 32 mg/l |
| 80 µl | " | " | = 16 mg/l |
| 40 µl | " | " | = 8 mg/l |
| 200 µl | 100 mg/l | - " | = 4 mg/l |
| 100 µl | " | " | = 2 mg/l |
| 50 µl | " | " | = 1 mg/l |
| 250 µl | 10 mg/l | - " | = 0.5 mg/l |
| 125 µl | " | " | = 0.25 mg/l |

Internal controls were prepared from a separate

weighing of powder by an individual other than the one preparing the standards. Internal controls were prepared on every assay occasion to determine the within assay precision and accuracy.

Preparation of internal controls

100 µl 1000 mg/l - 5 mls* diluent = 20 mg/l

150 µl 100 mg/l - " = 3 mg/l

200 µl 10 mg/l - " = 0.4 mg/l

*prepared using volumetric glassware.

Confidence limits of the assay were expressed as coefficients of variation (CV) ie the standard deviation expressed as a percentage of the mean.

The standard ranges and the concentrations chosen for internal controls (IC) were as follows:

Cefpirome:-

| | | |
|---|---|---|
| serum | 32, 16, 8, 4, 2, mg/l | ICs 25 and 3 mg/l |
| blister fluid | " | " |
| urine | " | " |
| bronchial mucosa | " | " |

Cefixime:-

| | | |
|---|---|---|
| serum/ blister/ urine | 0.8, 0.4, 0.2, 0.1, 0.05 | ICs 25 mg/l and 3 mg/l |
| sputum/ mucosal tissue | 0.25, 0.12, 0.06, 0.03 0.015 | ICs 0.2, 0.02. |

FCE 22101:-

serum/ blister/ urine 8, 4, 2, 1, 0.5 mg/l

ICs 6 and 0.75 mg/l

Meropenem:-

serum/ blister fluid/ peritoneal fluid -

0-4 h 32, 16, 8, 4, 2 mg/l

ICs 20 and 3 mg/l

> 4 h 4, 2, 1, 0.5, 0.25 mg/l

ICs 3 and 0.4 mg/l

urines - 32, 16, 8, 4, 2 mg/l ICs 20 and 3 mg/l

Tazobactam:-

serum/ blister fluid/ urine - 8, 4, 2, 1, 0.5,

0.25 mg/l ICs 6 and 0.8 mg/l

Piperacillin:-

serum/ blister fluid/ urine -

64, 32, 16, 8, 4 mg/l. ICs 50 and 6 mg/l

Aztreonam:-

serum 0-4 h - 5, 10, 20, 40, 80 mg/l

ICs 8, 60 mg/l

>4 h - 0.6, 1.25, 2.5, 5, 10, 20,

ICs 1, 15 mg/l

blister fluid 1.25, 2.5, 5, 10, 20,

ICs 2, 15 mg/l

peritoneal fluid 80, 40, 20, 10, 5 mg/l

ICs 60, 8 mg/l

6. **Assay procedure:-**

1. Agar was poured (cooled to 50°C) into assay plate (100 mls into a Nunc disposable plate 25 x 25 cm, 150 mls into a Mast reusable plate 31 x 31 cm)

   For the assay of tazobactam 80 mg/l piperacillin and organism (4% of an overnight broth culture) was added to cooled media before pouring into assay plate.

2. Agar allowed to set. The surface of the agar was then dried after which, plates were refrigerated at +4°C. Plates prepared for the assay of tazobactam were used within 1 hour of preparation.

3. Overnight broth cultures were diluted in distilled water as follows:

   Esch. coli ATCC 25922 1:100

   Prov. stuartii (K166 DRH) 1:1000

   Esch. coli NIHJ 1:500

   S. lutea (Z114 DRH strain) 1:50

   Ps. aeruginosa 10701 1:100

   Esch. coli SC 12655 1:100

4. The surface of agar was flooded with diluted organism, excess water drained from the plate and then the surface of agar re-dried (this was

completed as soon as possible to ensure that the organism does not begin to grow.)

5. Assay plates were stored at +4°C until needed (plates can be stored for up to 8 hrs).
6. Standards, tests and internal controls were applied to the plate in triplicate either by filling wells or by dipping 6 mm discs into the sample and then draining excess fluid onto blotting paper and then placing on to the agar surface. Samples were applied following a random pattern (Table 5, 6)
7. Assay plates were then incubated overnight at the appropriate temperature.
8. Zones were measured using a Leebrook viewer (Leebrook Instruments UK)
9. Bennett's calculation (42) was used to construct a line of best fit from the standard concentrations. From this line the levels in the test and internal controls were determined.

## 7. Protein binding determinations

Protein binding was determined in pooled human serum (Flow Laboratories) using a Centrifree system (Amicon Corporation, Lexington, Mass, USA) of molecular weight exclusion of 50,000. The

| | | | | |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 6 | 7 | 8 | 9 | 10 |
| 11 | 12 | 13 | 14 | 15 |
| 16 | 17 | 18 | 19 | 20 |
| 21 | 22 | 23 | 24 | 25 |
| 26 | 27 | 28 | 29 | 30 |

**Table 5 : Template for application of assay samples.**

| Standard concentration (mg/l) | Random numbers | | |
|---|---|---|---|
| 2 | 7 | 19 | 27 |
| 1 | 4 | 15 | 22 |
| 0.5 | 5 | 12 | 23 |
| 0.25 | 3 | 20 | 29 |
| 0.125 | 1 | 13 | 25 |
| Internal control 1.5 | 6 | 17 | 28 |
| Internal control 0.2 | 2 | 14 | 24 |
| Test 1 | 8 | 11 | 30 |
| Test 2 | 9 | 16 | 26 |
| Test 3 | 10 | 18 | 21 |

**Table 6 : Random pattern.**

Pattern for application of samples to assay plate.

filtrate containing unbound drug, was obtained by centrifugation at 1000 RCF for 5 mins.

Controls of antibiotic prepared in distilled water were also included to ensure that the antimicrobial did not adhere to the surface of the filter.

Filtrates were assayed against standards prepared in buffer at the pH of the medium used for the assay (see previous section on antibiotic assays).

Concentrations assayed:-

Cefpirome - 5, 100 and 200 mg/l

Cefixime - 2, 5 mg/l

FCE 22101 - 5, 200 mg/l

Meropenem - 10, 30

Aztreonam - 25, 50 mm

8. **Validation Studies**

Having obtained a microbiological assay method it is essential to confirm that firstly, there is good correlation with a non-microbiological method (particularly if the drug is metabolised <u>in vivo</u> to microbiological active metabolites) ie. HPLC and secondly that samples obtained from an external source give acceptable results (assayed

concentration should be + 10% of the assigned concentration). An outline of the HPLC methods used for the validation of the microbiological assay methods developed is as follows:-

Cefpirome:-

C18 Nova-Pak-column (Waters Associates, Harrow UK).

25% methanol 1% acetic acid in water - mobile phase flow rate - 1 ml/min

Sample preparation 1:1 serum and acetonitrite centrifuged at 1300 g for 5 mins. Supernatant diluted 1:2 with distilled water for assay.

UV detection at wavelength 270 nm using Uvikon 735 LC (Kontron Instruments, Watford UK)

Cefixime:-

Spherisorb 5 ODS 2 (Jones Chromatography, Glamorgan)

30% methanol 0.5 acetic acid 5mM heptane sulphuric acid in water - mobile phase.

Flow rate 1.5 m/ml

Sample preparation 250 µl serum

75 µl 10% trichloroacetic acid

250 µl acetonitrile

750 µl dichloromethane

Mix for 10 seconds (taking care not to form an emulsion). Centrifuge at 3500 rpm for 10 mins. Clear supernatant injected into column.

UV detection UV wavelength 282 nm

FCE22101:-

C18 μ Bondapak (Waters Associates)

10% methanol 1% phosphoric acid in water.

Flow rate 1.5 ml/min

Sample preparation: 100 μl serum

300 μl methanol

Mix 15 seconds. Spin 10 mins at 3000 rpm. Dilute supernatant 1:2 in buffer before injection.

UV detection at 318 nm.

Aztreonam:-

Radial pak ($C_{18}$ (Waters)

20% methanol 1% acetic acid in water - mobile phase.

Flow rate 3 mls/min

Sample preparation: 1:1 7% perchloric acid.

UV detection 275 nm

9. **Patient Information**

<u>Pharmacokinetic and tissue penetration studies</u>

Healthy male volunteers provided written informed consent for the studies, which had been

approved by the Hospital Ethics Committee. All volunteers underwent a full medical examination one week before the study and were considered normal. These investigations were repeated after the study.

On the night before the study two 0.2% cantharides-impregnated plasters (Adler - Apotheke, Mosel, Germany) (1 x 1 cm) were applied to the volar aspect of the forearm and taped in place. Plasters were removed prior to the administration of the drug. During sampling blisters were kept intact by spraying with Nobecutane (Astra Pharmaceuticals, Kings Langley, England).

Cefpirome:-

Dosage 1 g IV after an overnight fast.

Sample times:

| | |
|---|---|
| Blood - | 0, 5, 20, 35, 50, 60, 90 mins and 2, 3, 4, 5, 6, 7, 8 and 12 h post dose. |
| Blister fluid - | 0, 35, 60 mins and 2, 3, 4 , 5, 6, 7, 8 and 12 h post dose. |
| Urine - | 0-4, 4-8, 8-12, 12-24 h post dose. |

Cefixime:-

400 mg oral dose given with 200 mls of water after an overnight fast.

Sample times:

Blood samples - 0, 0.5, 1, 2, 3, 4, 6, 8, 12 and 25 h post dose.

Blister samples - 0.5, 1, 2, 4, 6, 8, 12 and 25 h post dose

Urine samples - 0-2, 2-4, 4-8, 8-12 and 12-24 h post dose.

FCE 22101:-

1 g dissolved in 20 mls sterile water given intravenously over a 5 min infusion.

Four weeks later the same volunteers were given 1 g as a single oral dose with 200 mls of water.

Sample times:

Blood samples - 0 (end of infusion), 5, 10, 20, 30, 45, 60 and 90 mins and 2, 3, 4, 6, 7 and 8 h post dose.

Blister samples - 0, 15, 30 and 60 mins, 2, 3, 4, 5, 6, 7, and 8 h post dose.

Urine samples - 0-1, 1-2, 2-3, 3-4, 4-6, 6-8, 8-12, 12-24 h post dose.

Meropenem:-

1 g dissolved in 20 mls sterile distilled water,

infused over 5 mins.

Blood samples - 0, 15, 30, 45, 60 and 90 mins, 2, 3, 4, 5, 6, 8 and 12 h post dose.

Blister samples - 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 12 h post dose.

Urine samples - 0-4, 4-8, 8-12, 12-24 post dose.

Aztreonam:-

1 Gram dissolved in 10 mls of sterile water infused over 2 mins.

Sample times:

Blood samples - 15, 30, 45, 60, 90 min, 2, 3, 4, 5, 6, 7, 8, h post dose.

Blister samples - 30 min, 1, 2, 3, 4, 5, 6, 7 and 8 h post dose.

Urine samples - 0-2, 2-4, 4-8, 8-12, 12-24 h post dose.

<u>Bronchial studies</u>

Cefpirome:-

Dosage 1 Gram IV

Sample times:

Blood and bronchial biopsy samples - between 30 min and 7 h post dose.

Cefixime:-

Dosage: Oral dosage over 3 days given as 200 mg

once daily, 200 mg twice daily or 400 mg once daily.

Sample times:

Sputum, blood and bronchial biopsy specimens taken between 1 and 6 h from the last dose of cefixime.

Peritoneal Studies

Cefpirome:-

Dosage 1 Gram IV

Sample times:

Serum and peritoneal samples between 0.5 and 7-6 h post dose.

Meropenem:-

Dosage 1 Gram IV

Sample times:

Serum and peritoneal samples between 0.5 and 5 h.

Aztreonam:-

Dosage 1 Gram IV

Sample times:

Serum and peritoneal samples between 0.42 and 6.28 h.

Penetration in prostatic tissue

Cefpirome:-

Dosage 1 Gram IV

Sample times:

Blood and prostatic tissue samples taken between 1 and 12 h.

10. **Pharmacokinetic analysis**

Analysis in serum was performed with the GPHARM program by assuming a two-compartment model. Pharmacokinetic parameters for the inflammatory fluid were determined by standard graphical methods of individual volunteer data. This included the area under the curve concentration-time curve (AUC), which was calculated by a log-linear trapezoidal rule procedure. The per cent penetration into inflammatory exudate was calculated by comparing the AUC from 0 h to infinity ($AUC_{0-\infty}$) in inflammatory exudate with that in serum.

## RESULTS AND DISCUSSION

### 1. Cefpirome

Microbiological

In Tables 7a, b, c and 8 are shown the results of MIC determination with an inoculum of $10^4$ CFU. The action of cefpirome was compared with that of cefuroxime a second generation cephalosporin and ceftazidime and cefotaxime, third generation cephalosporins.

Against the Enterobacteriaceae cefpirome displayed a high degree of activity which was equal to, or in the case of Citrobacter spp and Enterobacter spp, superior to that of ceftazidime and cefotaxime. Only in the case of Proteus mirabilis was the activity of cefpirome inferior to that of the other extended spectrum cephalosporins. Generally cefpirome was eight to 32-fold more active than cefuroxime.

Cefpirome was approximately four-fold less active than ceftazidime against Ps. aeruginosa, but was four-fold more active than cefotaxime. There tended to be cross susceptibility of Ps. aeruginosa to the cephalosporins i.e. these strains that were more susceptible to cefpirome

| Organism | Antibiotic | $MIC_{50}$ | $MIC_{90}$ | Range |
|---|---|---|---|---|
| Indole positive proteus | cefpirome | ≤0.008 | 0.06 | ≤0.008 - 0.06 |
| (Pr.vulgaris 22 | ceftazidime | 0.015 | 0.06 | 0.015 - 0.25 |
| Pr.rettgeri 2) | cefotaxime | 0.015 | 0.12 | ≤0.008 - 0.25 |
| | cefuroxime | 16 | >128 | 0.5 - >128 |
| Morganella morganii (17) | cefpirome | ≤0.008 | 0.03 | ≤0.008 - 0.03 |
| | ceftazidime | 0.015 | 0.06 | 0.015 - 1 |
| | cefotaxime | 0.015 | 0.25 | ≤0.008 - 0.5 |
| | cefuroxime | 16 | 128 | 2 - 128 |
| Acinetobacter spp. (10) | cefpirome | 1 | 8 | 0.03 - 16 |
| | ceftazidime | 1 | 4 | 0.12 - 8 |
| | cefotaxime | 1 | 16 | 0.06 - 16 |
| | cefuroxime | 4 | 32 | 0.5 - 32 |
| Ps.aeruginosa (46) | cefpirome | 4 | 8 | 1 - 64 |
| | ceftazidime | 1 | 2 | 0.5 - 32 |
| | cefotaxime | 16 | 64 | 2 - 64 |
| | cefuroxime | >128 | >128 | >128 |
| H.influenzae (29) | cefpirome | 0.03 | 0.12 | 0.015 - 1 |
| | ceftazidime | 0.06 | 0.12 | 0.03 - 0.25 |
| | cefotaxime | ≤0.008 | 0.03 | ≤0.008 - 0.25 |
| | cefuroxime | 0.5 | 2 | 0.25 - 4 |
| N.gonorrhoeae (23) | cefpirome | 0.015 | 0.03 | ≤0.008 - 0.03 |
| | ceftazidime | 0.015 | 0.03 | ≤0.008 - 0.06 |
| | cefotaxime | ≤0.008 | ≤0.008 | ≤0.008 - 0.015 |
| | cefuroxime | 0.06 | 0.12 | ≤0.008 - 0.12 |
| Staph.aureus (34) | cefpirome | 0.5 | 2 | 0.5 - 4 |
| | ceftazidime | 8 | 32 | 4 - 64 |
| | cefotaxime | 2 | 8 | 1 - 16 |
| | cefuroxime | 1 | 4 | 0.5 - 16 |

**Table 7A : In vitro activity of cefpirome**

Minimum inhibitory concentrations (MIC's) of cefpirome and three other beta lactams determined. Results expressed as a range of activity and that concentration of antimicrobial which will inhibit 50 and 90% of strains in mg/l.

| Organism | Antibiotic | $MIC_{50}$ | $MIC_{90}$ | Range |
|---|---|---|---|---|
| Esch.coli (50) | cefpirome | 0.03 | 0.5 | ≤0.008 - 16 |
| | ceftazidime | 0.12 | 0.25 | 0.03 - 8 |
| | cefotaxime | 0.06 | 0.25 | ≤0.008 - 32 |
| | cefuroxime | 4 | 16 | 0.06 - 32 |
| Klebsiella spp. (48) | cefpirome | 0.03 | 0.25 | 0.015 - 2 |
| | ceftazidime | 0.12 | 0.25 | 0.03 - 0.5 |
| | cefotaxime | 0.03 | 0.25 | 0.015 - 1 |
| | cefuroxime | 2 | 8 | 1 - 64 |
| Citrobacter spp.(14) | cefpirome | 0.03 | 0.12 | 0.03 - 4 |
| | ceftazidime | 0.12 | 1 | 0.06 - 64 |
| | cefotaxime | 0.06 | 1 | 0.03 - 32 |
| | cefuroxime | 4 | 8 | 2 - >128 |
| Prov.stuartii (20) | cefpirome | 0.12 | 0.25 | 0.03 - 1 |
| | ceftazidime | 0.12 | 0.5 | 0.06 - 1 |
| | cefotaxime | 0.06 | 0.25 | ≤0.008 - 0.5 |
| | cefuroxime | 2 | 16 | 0.25 - 16 |
| Serratia spp. (20) | cefpirome | 0.06 | 0.06 | 0.03 - 0.12 |
| | ceftazidime | 0.12 | 0.12 | 0.03 - 0.25 |
| | cefotaxime | 0.12 | 0.5 | 0.06 - 0.25 |
| | cefuroxime | 64 | 128 | 4 - >128 |
| Enterobacter spp. (22) | cefpirome | 0.03 | 0.12 | 0.03 - 2 |
| | ceftazidime | 0.12 | 0.5 | 0.06 - 16 |
| | cefotaxime | 0.12 | 0.5 | ≤0.008 - 32 |
| | cefuroxime | 4 | 16 | 1 - >128 |
| Pr.mirabilis (50) | cefpirome | 0.03 | 0.25 | ≤0.008 - 0.25 |
| | ceftazidime | 0.015 | 0.06 | 0.015 - 0.06 |
| | cefotaxime | ≤0.008 | 0.03 | ≤0.008 - 0.12 |
| | cefuroxime | 0.5 | 2 | 0.25 - 8 |

**Table 7B : In vitro activity of cefpirome**
(continued)

| Organism | Antibiotic | $MIC_{50}$ | $MIC_{90}$ | Range |
|---|---|---|---|---|
| Ent.faecalis (10) | cefpirome | 4 | 16 | 1 - 16 |
| | ceftazidime | 128 | >128 | 16 - >128 |
| | cefotaxime | 16 | 128 | 0.25 - >128 |
| | cefuroxime | 16 | 128 | 2 - >128 |
| Str.pneumoniae (18) | cefpirome | 0.015 | 0.015 | ≤0.008 - 0.12 |
| | ceftazidime | 0.12 | 0.12 | 0.06 - 1 |
| | cefotaxime | ≤0.008 | 0.015 | ≤0.008 - 0.12 |
| | cefuroxime | 0.015 | 0.015 | ≤0.008 - 0.25 |
| Bact.fragilis (30) | cefpirome | 32 | 64 | 8 - >128 |
| | ceftazidime | 16 | 32 | 4 - >128 |
| | cefotaxime | 8 | 8 | 1 - 128 |
| | cefuroxime | 8 | 128 | 1 - >128 |

**Table 7C : In vitro activity of cefpirome**

(continued)

| Organism | Number of strains | Mode MIC | Range |
|---|---|---|---|
| Fusobacterium | 4 | 2 | 2-32 |
| Bacteroides spp* | 13 | 64 | 0.12->128 |
| Bact.ureolyticus | 3 | 0.03 | 0.03-0.06 |
| Cl.perfringens | 4 | 0.5 | 0.25-1 |
| Cl.difficle | 10 | 8 | 8-16 |
| Shigella sonnei | 5 | 0.03 | 0.03 |
| N.meningitidis | 5 | ≤ 0.008 | ≤ 0.008-0.05 |
| Salmonella spp | 5 | 0.06 | 0.03-0.12 |
| Group A streptococci | 5 | 0.015 | ≤ 0.008-0.015 |
| Group B streptococci | 5 | 0.06 | 0.03-0.06 |

*Includes three strains each of Bact.distasonis,Bact.ovatus,Bact.uniformis and four strains of Bact.thetaiotaomicron

## Table 8 : Susceptibility of miscellaneous strains to cefpirome.

Activity expressed as a range or mode MIC in mg/l.

were more susceptible to cefotaxime and ceftazidime.

Table 9 shows the susceptibilities of known ß-lactamase producing strains to cefpirome. It appears the broad spectrum chromosomal K1 enzyme-Richmond & Sykes, Group IV (27) would probably hydrolyse cefpirome as there was reduced susceptibility and a marked inoculum effect. This was also observed in the case of cefotaxime (MICs 1 and 4 mg/l at $10^4$ and $10^6$ CFU inocula) but not for ceftazidime (MIC 0.5 and 0.5 mg/l, respectively). Generally an increase in inoculum had little effect on cefpirome.

Cefpirome showed high activity against H. influenzae (including nine ß-lactamase producing strains) in common with the other cephalosporins tested. One strain with an MIC of 1 mg/l to cefpirome did not produce ß-lactamase and this strain was presumed to have a permeability barrier or altered PBPs.

Cefpirome was also highly active against Neisseria including six ß-lactamase-producing strains of Neisseria gonorrhoeae.

Results for staphylococci are biased by the inclusion of eight methicillin resistant strains

| Strain | ß-lactamase group* | MIC $10^4$ | MIC $10^6$ |
|---|---|---|---|
| Esch.coli 1541E | 1 | 0.06 | 0.06 |
| Ent.cloacae 1051E(P99) | 1 | 0.06 | 0.06 |
| Ps.aeruginosa 1563E | 1 | 4 | 8 |
| Esch.coli 1193E(TEM-1) | 111 | 0.06 | 0.06 |
| Esch.coli 1725E(TEM-2) | 111 | 0.25 | 0.25 |
| K.aerogenes 1082E | 1V | 2 | 16 |
| Esch.coli 2138E(OXA-1) | V | 0.25 | 0.5 |
| Ps.aeruginosa 1559E(PSE-4) | Dalgleish | 2 | 2 |

* Richmond & Sykes (1973) ( 27 )

**Table 9 : MIC of cefpirome for known ß-lactamase producing strains**

| Organism No. | Type | Agar MIC | Broth MIC | Broth MBC | Broth + 20% HS MIC | Broth + 20% HS MBC | Broth + 70% HS MIC | Broth + 70% HS MBC |
|---|---|---|---|---|---|---|---|---|
| G261 | Ps.aeruginosa | 2 | 4 | 8 | 16 | 16 | 8 | 32 |
| G270 | " | 4 | 8 | 8 | 8 | 16 | 8 | 8 |
| F236 | S.aureus (meth S) | 0.5 | 0.5 | 1 | 1 | 1 | 0.5 | 1 |
| F201 | S.aureus (meth R) | 4 | 8 | 16 | 8 | 16 | 16 | 16 |
| P35 | E.faecalis | 4 | 16 | 64 | 16 | 32 | 16 | 32 |
| P16 | " | 2 | 8 | 32 | 8 | 64 | 4 | 64 |
| I191 | Esch.coli | 0.03 | 0.12 | 0.25 | 0.06 | 0.06 | 0.03 | 0.06 |
| I190 | " | 0.03 | 0.06 | 0.06 | 0.03 | 0.03 | 0.03 | 0.06 |
| H112 | Klebsiella spp. | 0.06 | 0.12 | 0.12 | 0.12 | 0.12 | 0.06 | 0.12 |
| H142 | " | 0.03 | 0.12 | 0.12 | 0.06 | 0.06 | 0.06 | 0.06 |
| J192 | P.mirabilis | 0.008 | 0.06 | 0.25 | 0.06 | 0.12 | 0.25 | 0.5 |
| J191 | " | 0.03 | 0.5 | 0.5 | 0.5 | 1 | 0.5 | 0.5 |

## Table 10 : Effect of serum on the activity of cefpirome

Minimum inhibitory (MIC) and minimum cidal concentrations (MBC) determined in Iso Sensitest broth containing 0 , 20 and 70% human serum (HS) at an inoculum of $10^5$ orgs/ml. Results expressed in mg/l.

to which the cephalosporins tested display reduced activity. MICs of cefpirome for methicillin susceptible strains was 0.5 - 1 mg/l and for methicillin resistant strains 1 - 4 mg/l.

With the exception of Enterococcus faecalis, cefpirome was highly active against ß-haemolytic streptococci. However, cefpirome was the only cephalosporin tested which had any useful activity (MIC range 1-16 mg/l).

Against anaerobes cefpirome showed poor activity against Bact. fragilis and only good activity Bacteroides ureolyticus and Clostridium perfringens.

The MICs and MBCs (Table 10) of cefpirome for the two strains of Esch. coli, Klebsiella spp, Ps. aeruginosa and S. aureus were the same or within one dilutional step. In the case of Pr. mirabilis and E. faecalis the MBC was four-fold or greater than the MIC.

The effect of 20 and 70% serum on the MIC and MBC was minimal (that is the value did not alter by more than 2-fold in the presence of either concentration of serum) Table 10. The human serum protein binding of cefpirome was 18% at 5 mg/l/l, 21% or 100 mg/l and 12% at 200 mg/l. The

morphological response observed at 0.06 mg/l was filamentation.

Assay validation

In Table 11 the results of a cross validation study are shown for ten coded samples (supplied by Roussel Laboratories) and the results are shown graphically in Figure 10.

The confidence limits of the assay for the two internal controls are:-

| Assigned conc mg/l | Mean assayed conc | SD | CV |
|---|---|---|---|
| 25 | 25.306 | 2.2414 | 8.86 |
| 3 | 2.922 | 0.2078 | 7.11 |

Volunteer Study

The mean concentrations of cefpirome (six volunteers) obtained in serum and inflammatory fluid after 1 Gram intravenous dose are shown in Figure 11, the derived pharmacokinetic parameters in Table 12 and urinary excretion in Table 13.

Serum concentration of cefpirome declined sharply over the first hour from a mean of 97.4 mg/l at 5 min to 33.7 mg/l at 1 h. This implies a rapid distribution phase of the drug with a mean half-life of 0.3 h. The volume of distribution of cefpirome at steady state was 21.3 L (range 17.3 -

| Code | Assigned concentration mg/l | Microbiological mg/l | HPLC mg/l |
|---|---|---|---|
| A | 24 | 24.98 | 24.5 |
| B | 5.1 | 4.96 | 5.1 |
| C | 54 | 56.85 | 56.5 |
| D | 2.16 | 2.18 | 0.9 |
| E | 45 | 46.25 | 49.7 |
| F | 15 | 15 | 16.2 |
| G | 8.4 | 8.09 | 8.4 |
| H | 60 | 59 | 61.3 |
| L | 12 | 13.25 | 12.4 |
| M | 30 | 28.26 | 33 |
| Correlation coefficient | | 0.998 | 0.998 |
| Slope | | 1.0133 | 1.0585 |
| y intercept | | -0.0231 | -0.2613 |
| p | | <0.001 | <0.001 |

## Table 11 : Cefpirome crossvalidation

Human serum spiked with concentrations of cefpirome (ten samples).
Samples assayed by HPLC and the microbiological assay method.

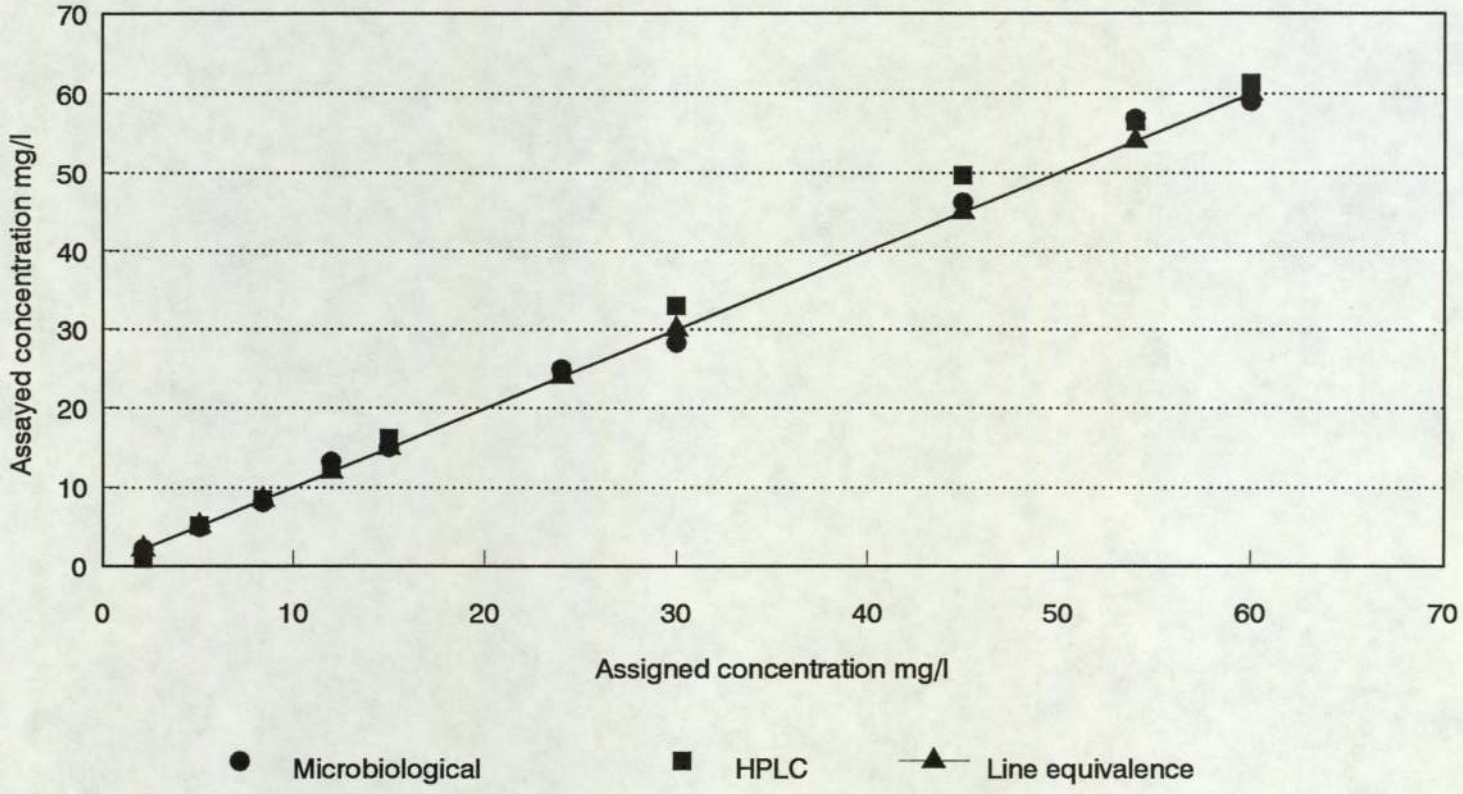



**Figure 10 : Validation of cefpirome microbiological assay**

Antibiotic free serum spiked with cefpirome , samples then assayed by HPLC and the microbiological assay. Results obtained by both methods (assayed concentration) plotted against the assigned concentration (mg/l).

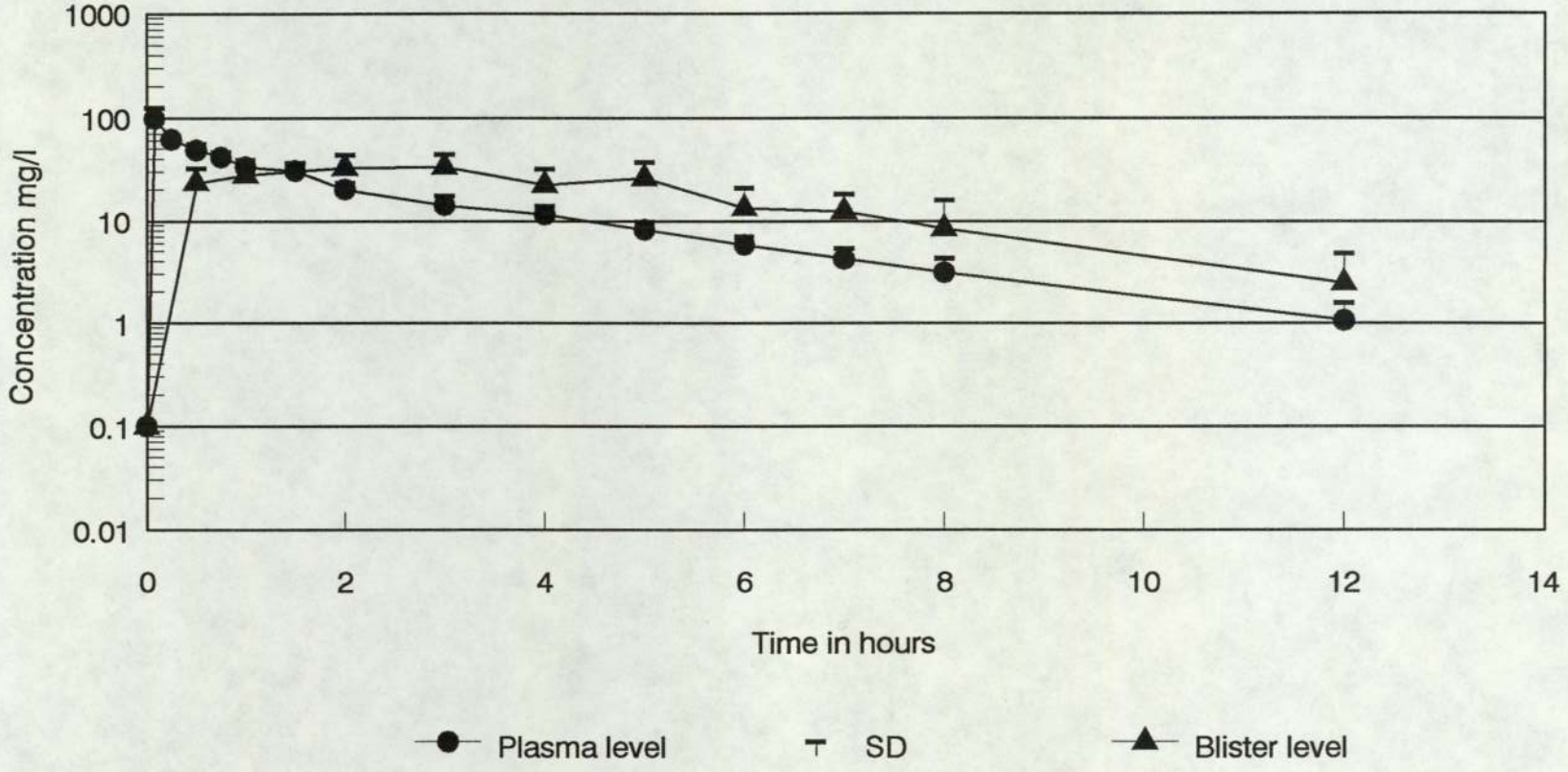



**Figure 11 : Mean plasma and inflammatory fluid concentrations following a single 1 g intravenous infusion of cefpirome.**

Data obtained from six volunteers. Concentration (mg/l) plotted against time (timed from the end of the infusion).

| Parameter in indicated fluid | | Mean ± SD | (range) |
|---|---|---|---|
| Serum | | | |
| $C_{max}$ | (mg/l) | 97.4 ± 28.5 | (70 - 146.5) |
| $T_{max}$ | (h) | 0.08 | |
| T ½ ß | (h) | 2.3 ± 0.3 | (2 - 2.6) |
| T ½ α | (h) | 0.3 ± 0.1 | (0.2 - 0.5) |
| $AUC_{0-\infty}$ | (mg/l.h) | 156.3 ± 27.8 | (117.3 - 196.2) |
| V dss | (l) | 21.3 ± 3.1 | (17.3 - 25.6) |
| Blister fluid | | | |
| $C_{max}$ | (mg/l) | 39.2 ± 7.9 | (31.2 - 49.1) |
| $T_{max}$ | (h) | 1.9 ± 1.0 | (0.6 - 3) |
| T ½ ß | (h) | 2.5 ± 0.7 | (1.7 - 3.8) |
| $AUC_{0-\infty}$ | (mg/l.h) | 200 ± 80.5 | (93.5 - 306.7) |
| Serum clearance (ml/min) | | 109.5 ± 20.2 | (85 - 142.2) |
| Renal clearance (ml/min) | | 82.1 ± 19.5 | (53.6 - 113) |
| % penetration into blister fluid | * | 123.6 ± 31.3 | (79.7 - 156.3) |
| % 24 h urinary recovery of administered dose | | 75.5 ± 6.9 | (63.1 - 81.6) |

T max Time that concentration C max was reached in serum or blister fluid

T ½ ß Terminal elimination half-life in serum or blister fluid

T ½ α Half-life during distribution phase

AUC 0 - ∞ Area under serum (or blister fluid) concentration time curve from zero to infinity

V dss Volume of distribution at steady state

* AUC blister fluid x 100/AUC serum

**Table 12 : Pharmacokinetic parameters of cefpirome following a single 1g intravenous dose**

| Time | Vol 1 | Vol 2 | Vol 3 | Vol 4 | Vol 5 | Vol 6 |
|---|---|---|---|---|---|---|
| 0 - 4 | 631.6 | 607.7 | 677.1 | 509.4 | 564.8 | 414 |
| 4 - 8 | 123.1 | 96.6 | 84.6 | 154.1 | 164.25 | 145.5 |
| 8 - 12 | 35 | 30.5 | 24.7 | 45.4 | 67.98 | 44.7 |
| 12 - 24 | 17.1 | 8.9 | 8.5 | 24.6 | 19.37 | 26.4 |
| Total mg | 806.8 | 743.7 | 794.8 | 733.5 | 816.4 | 630.6 |
| % excreted | 80.7 | 74.4 | 79.5 | 73.4 | 81.6 | 63.1 |



**Table 13 : Urinary excretion of cefpirome**

Urinary excretion following a single
1 gram intravenous dose of cefpirome.

25.6). The serum elimination half-life was 2.3 h (range 2.0 - 2.6).

There was excellent penetration of cefpirome into inflammatory fluid: the mean peak inflammatory fluid concentration ($C_{max}$) of 39.2 mg/l (range 31.2 - 49.1) was obtained by a mean time of 1.9 h (range 0.6 - 3) post dose. The mean per cent penetration of the drug into inflammatory fluid as calculated from individual ratios of $AUC_{0-oo}$ blister and $AUC_{0-oo}$ serum was 123.6% (range 79.7 - 156.3). Cefpirome concentrations in inflammatory fluid were two to three times greater than serum as from 1 to 2 h after administration. The mean elimination half-life of cefpirome from inflammatory fluid was 2-5 h (similar to the serum half-life). At 12 h post dose the mean inflammatory fluid concentration was 2.5 mg/l and the mean serum concentration was 1.1 mg/l. The serum clearance of cefpirome was 109.5 ml/min (SD$\pm$ 20.2), with a renal clearance of 82.1 ml/min (SD$\pm$ 19.5), urinary excretion was rapid with recovery of 56.7% of administered dose by 4 h and the 24 h urinary recovery was 75.5% of the administered dose.

Intraperitoneal penetration

Thirty-five sets of serum and peritoneal fluid samples in that one set from each of 17 patients and two sets from each of nine patients, were obtained 0.5 to 7.6 h post dose. The results are shown graphically in Figure 12.

The serum elimination half-life of cefpirome was 2.1 h. The intraperitoneal levels peaked in the first 2 h following injection, achieving a mean concentration of 44.4 mg/l (SD 9.2) during 0-2 h. The elimination half-life of cefpirome in peritoneal fluid was 2 h. The mean intraperitoneal concentration of cefpirome exceeded 10 mg/l at 6 h post dose. The mean per cent penetration of cefpirome into peritoneal fluid (mean of the individual peritoneal levels expressed as a percentage of the corresponding serum concentration) was 99.4% (SD 28.6) over the first 2 h and 97.7% (SD 34.3) over the 8 h of the study period.

Penetration into prostatic tissue

Data from twenty three patients undergoing prostatectomy were studied. Individual results are shown in Figure 13. The line of best fit is drawn through these points. Serum, tissue and

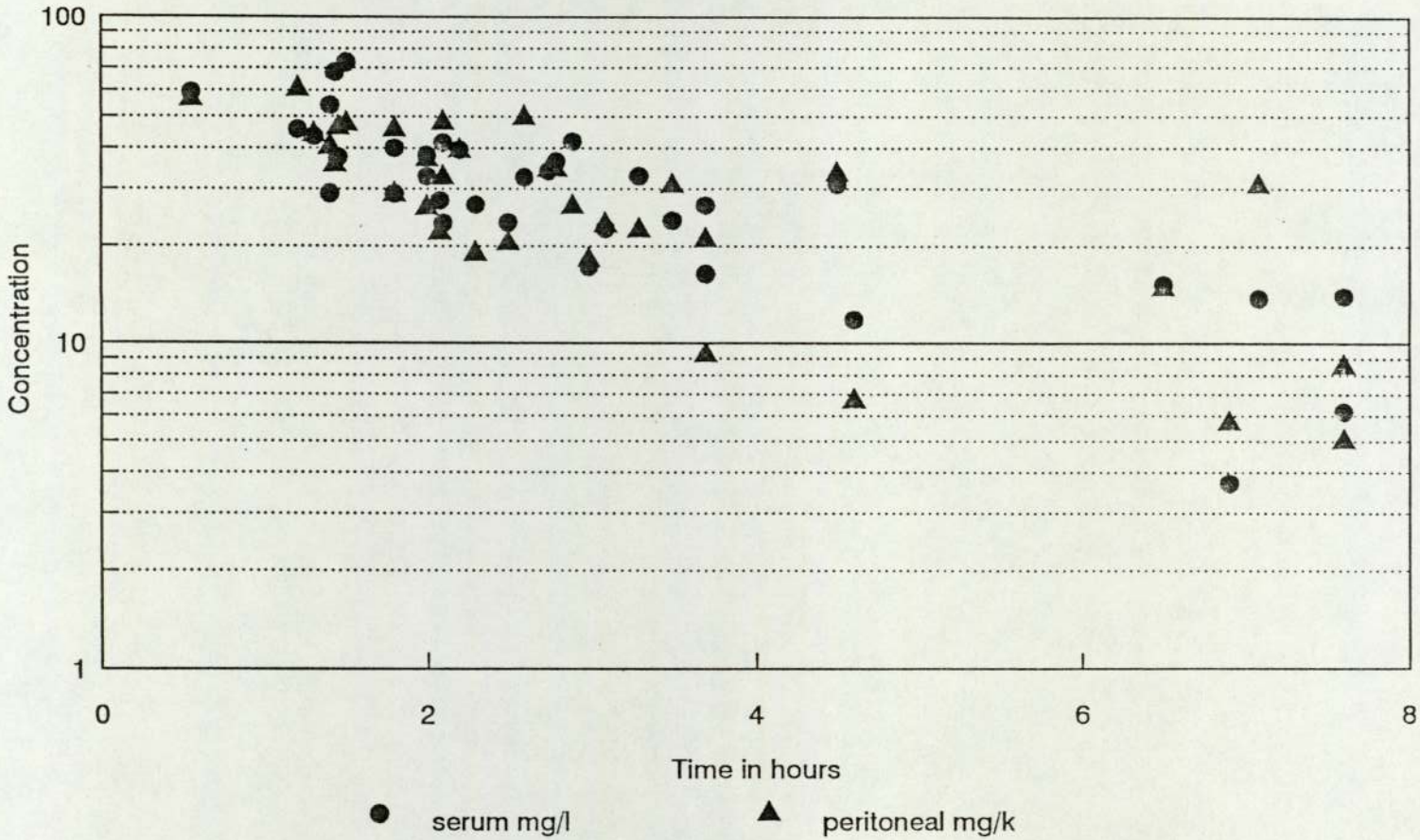



**Figure 12 : Concentrations of cefpirome in peritoneal fluid**

Levels of cefpirome in serum and simultaneous peritoneal fluid following a single 1 g intravenous dose of cefpirome plotted against time (timed from the end of the infusion).

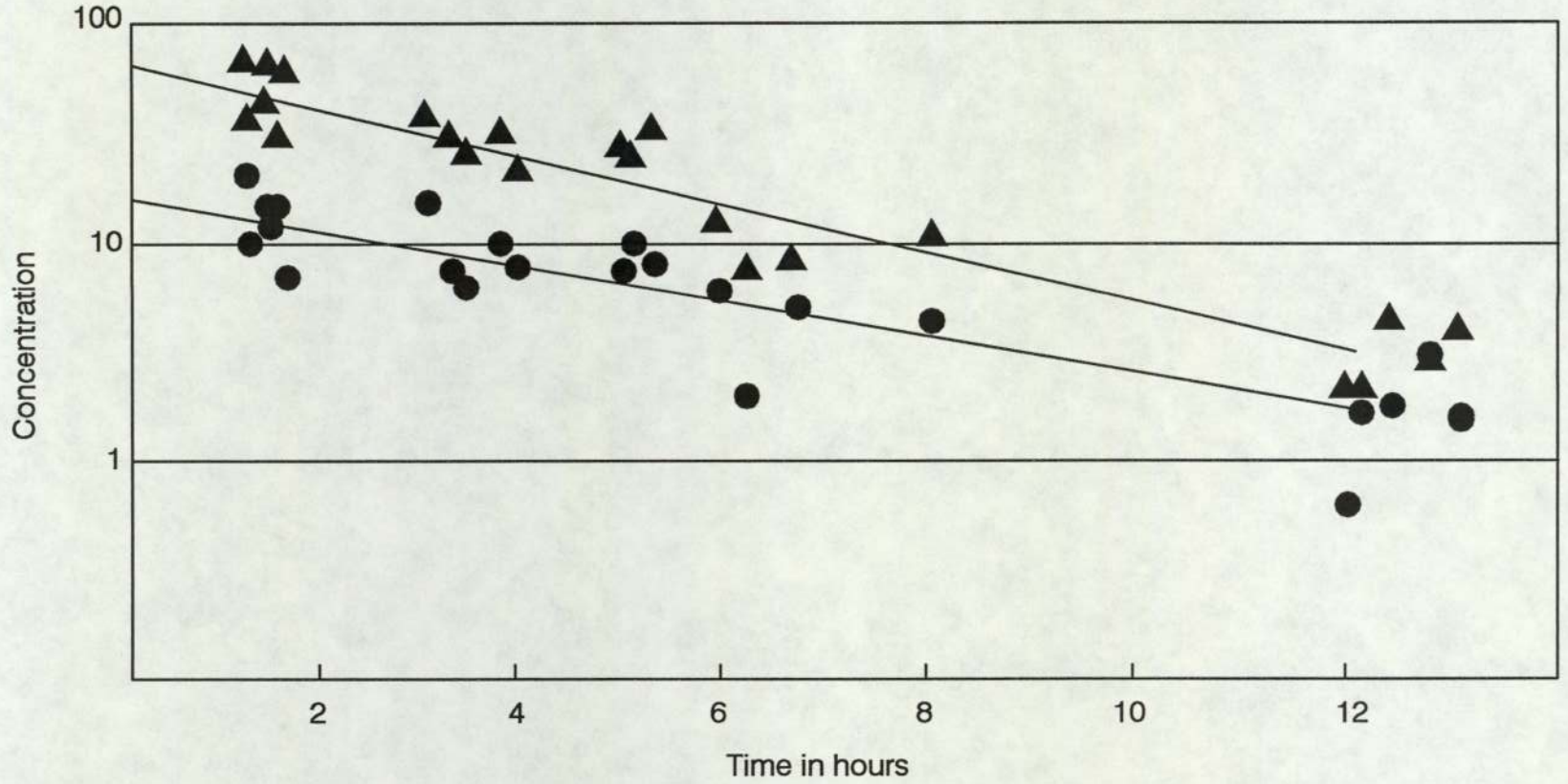



**Figure 13 : Serum and prostatic levels of cefpirome**

Levels in patients undergoing transurethral resection following a single 1 g intravenous dose of cefpirome. Concentration in serum and tissue plotted against time (timed after the end of the infusion).

percentage penetration for 1-2, 3-4, 5-8, and 12-13 h are shown in Figure 14.

The half-life in serum as measured graphically was three hours and the half-life in prostatic tissue was 3.3 h. The percentage penetration at one to two hours was 28.5% (SD 13.2) and 31.6% (SD 7) at 3-4 h.

Penetration into bronchial mucosa

Thirty seven patients were studied and Figure 15 shows the concentrations measured in serum and bronchial mucosa plotted against time since administration of drug. Mean serum and mucosal concentrations and percentage penetration at a mean time of 3.2 h were 34.48 mg/l, 19.28 mg/K and 56.28%,respectively.

Bronchial mucosal concentrations were below the lower limit of sensitivity of the assay in five cases. However, in three of these patients biopsy weights and serum concentrations were both low.

## Discussion

The in vitro study confirms the work of others that cefpirome is a very potent extended spectrum cephalosporin and results agree closely to that of other workers (14). However, Seibert et al (43)

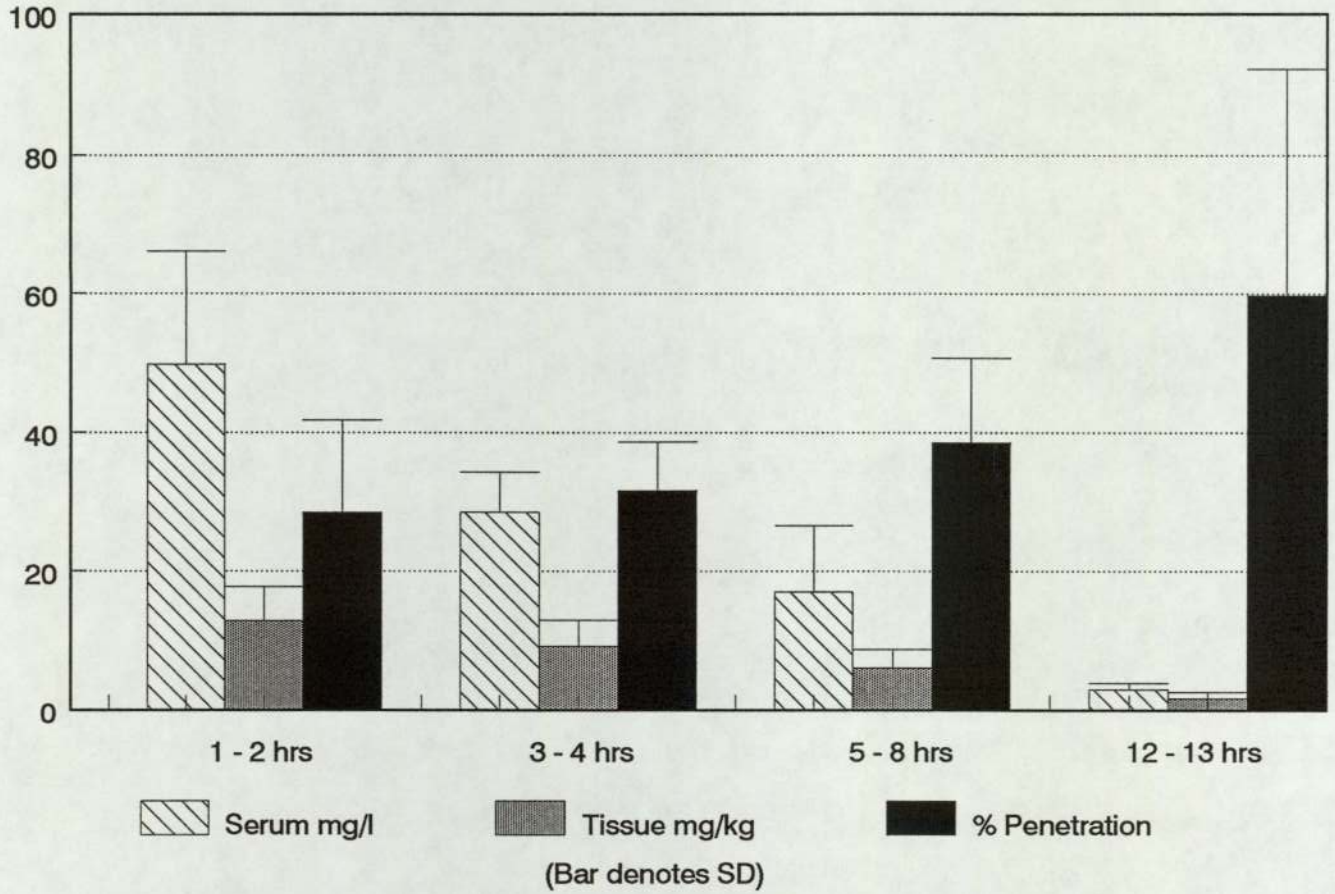



**Figure 14 : Levels of cefpirome in serum and prostatic tissue**

Levels in prostatic tissue and simultaneous serum samples following a single 1 g intravenous dose of cefpirome. Mean concentration plotted at four time bands.

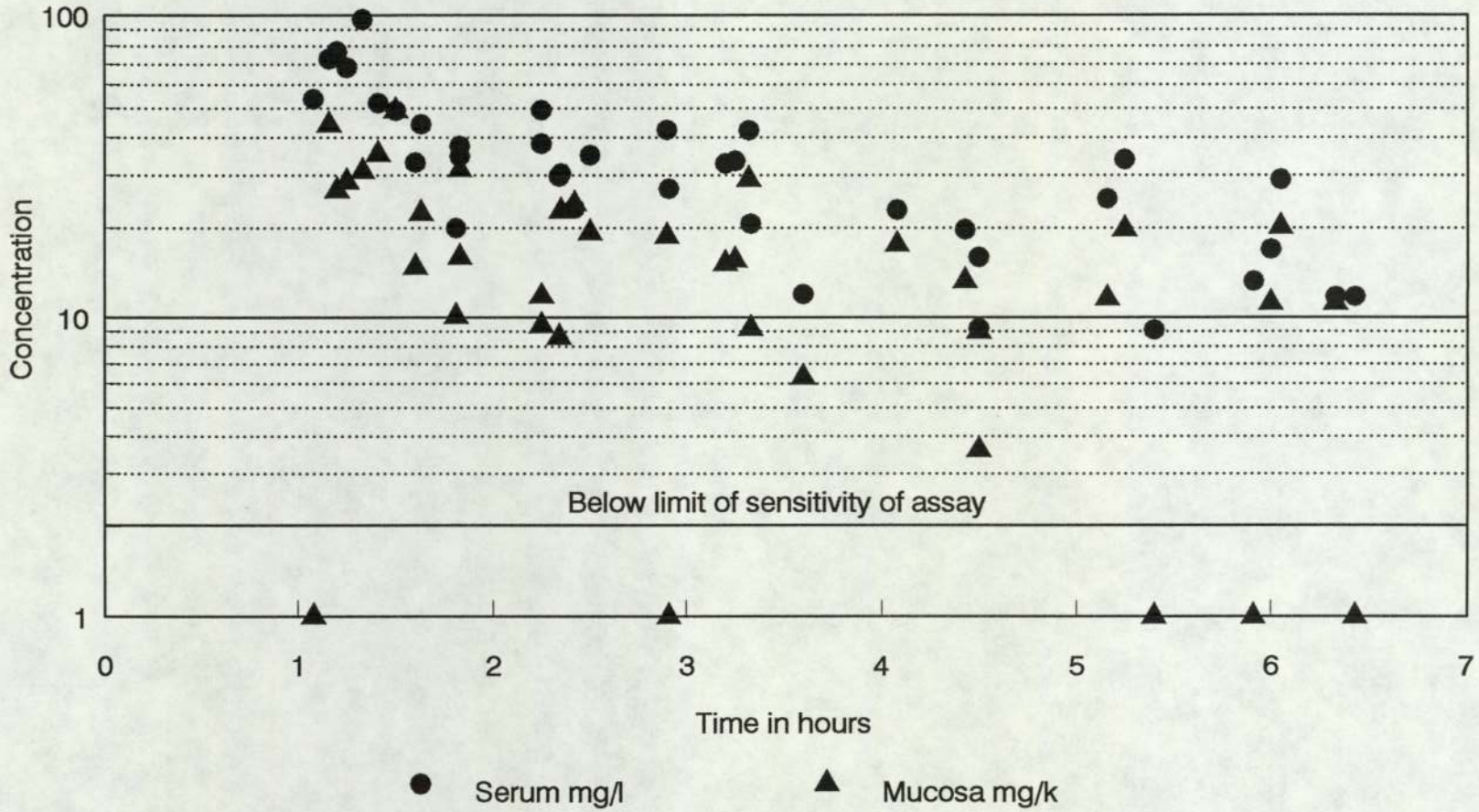



**Figure 15 : Penetration of cefpirome into bronchial mucosa**

Levels of cefpirome in bronchial mucosa and simultaneous serum samples following a single 1 g intravenous dose. Concentration plotted against time (timed from the end of the infusion).

using similar inocula, found cefpirome to be about twice as active against Gram-negative bacilli and Gram- positive cocci (43). Although there is agreement with Seibert et al (43) that cefpirome had a marked ß-lactamase stability, this study would suggest that the compound is hydrolysed by the chromosomally coded K1 ß-lactamase whereas the previous workers did not find significant hydrolysis. Cefpiromes poor activity against Bact. fragilis may also be partly due to hydrolysis by ß-lactamase.

It is suggested by the results of the differential interference microscopy i.e. filamentation that the primary target site for cefpirome is PBP 3. This study also agrees with Machka and Braveney (14) in that cefpirome is a bactericidal compound, except perhaps clinically against E. faecalis. In the Machka and Braveney study (14) a sample volume of only 10 µl was taken to determine lethality, whereas in this study a 100 µl volume was used to determine a 99.9% lethality. Differences may therefore be attributed to differences in methodology.

Following a single 1 g intravenous dose, cefpirome was rapidly distributed (T1/2 = 0.3 h)

with an apparent volume of distribution of 21.3 litres. Cefpirome penetrated rapidly into inflammatory fluid producing a high mean peak concentration of 39.2 mg/l. Availability of the drug in inflammatory fluid was high (123.6%) reflecting a high degree of penetration into inflammatory fluid.

Cefpirome appears to be eliminated predominantly by the kidneys, the rate of renal elimination being similar to the glomerular filtration rate. The fate of 25% administered dose not recovered in urine within 24 h is unknown. Kiesel and Seeger (44) have shown that only a small amount of the drug is recovered in the bile of animals.

Similar studies on the pharmacokinetics and tissue penetration following a single intravenous dose of ceftazidime, a drug to which cefpirome is chemically related, have been reported by Armstrong et al (45). It is interesting to note that the volume of distribution of cefpirome is 1.5 to 2 times greater than ceftazidime and this is reflected in its excellent tissue penetration (123%) compared to ceftazidime (89%). The mean peak inflammatory fluid concentration of cefpirome

was comparable to that obtained with 1G of ceftazidime (45 mg/l) and cefpirome persisted longer in inflammatory fluid with an elimination half-life of 2.5 h compared with 1.8 h for ceftazidime. Thus the pharmacokinetics and tissue penetration of cefpirome are superior or comparable to ceftazidime.

In the intraperitoneal study the serum concentrations were similar to those obtained in the healthy volunteer pharmacokinetic study. Cefpirome penetrated well into peritoneal fluid. MIC 90s for Enterobacteriaceae and S. aureus are between 0.03 and 0.5 mg/l. These inhibitory concentrations are exceeded for a considerable length of time. The intraperitoneal concentration of cefpirome exceeded the $MIC_{90}$ of Ps. aeruginosa (8 mg/l) for over 6 h and the $MIC_{90}$ of E. faecalis (16 mg/l) for over 4 h. Activity against E. faecalis is important in view of the emerging importance of this low-grade pathogen in abdominal surgery. Intraperitoneal concentrations of cefpirome achieved in this study would not be adequate to treat infections involving Bact. fragilis ($MIC_{90}$, 64 mg/l).

The measurement of antimicrobials in prostatic

tissue is of clinical interest because acute prostatitis may be difficult to treat possibly due to the poor diffusion of most antibiotics into the prostate gland. Men who are not cleared of an acute infection may develop chronic prostatitis which requires longer courses of antibiotics or even surgery (46). Thus it has been suggested that all cases of urinary tract infections in men should be treated with an agent that penetrates the prostate to prevent this condition (47). Also pre-operative prophylaxis for prostatectomy may well prevent post operative wound or urinary infection. It is therefore of interest that tissue and serum levels of cefpirome exceeded the $MIC_{90}$ of the majority of urinary pathogens (<1 mg/l).

Established methods for predicting clinical efficacy of antimicrobials are based on comparative in vitro activities in relation to serum or plasma concentrations. In the respiratory tract levels in sputum have also been measured. However, there are many technical problems such as a difficulty with sampling, sputum pooling, contamination with blood and the presence of ß-lactamase which have resulted in

wide variation in sample concentrations. More recently bronchial mucosa has been used to represent the site of infection in exacerbations of chronic bronchitis and possibly bronchiectasis (39, 48). There is also evidence that bronchial mucosa concentration are better predictors of efficacy than serum levels, particularly if a drug concentrates in tissue as is the case for newer agents, where very low serum concentrations are found with simultaneous high tissue concentrations (49). In this study we have shown that cefpirome penetrates well into bronchial mucosa and that $MIC_{90}$ for the common respiratory pathogens are exceeded for up to 6h post dose.

These studies have shown that cefpirome has a broad spectrum of activity, that it penetrates well into body fluids and tissues and that the $MIC_{90}$ of the majority of pathogens is exceeded for up to 8 h. However cefpirome should be combined with a suitable antianaerobic agent if a mixed infection including anaerobes is suspected. These data suggest that twice daily dosage with 1 G of cefpirome should be sufficient for prophylaxis and treatment of infection.

2. Cefixime

Microbiological

In Tables 14a,b the in vitro activity of cefixime compared with other cephalosporins for 352 clinical isolates and amoxycillin are shown. In Table 15 $MIC_{90}$s are given for miscellaneous groups of organisms.

Cefixime exhibited good activity against the common Enterobacteriaceae (Esch. coli, Klebsiella, Pr. mirabilis, Salmonella) with $MIC_{90}$s being less than equivalent to 1 mg/l. Providencia spp were also highly susceptible to cefixime ($MIC_{90}$ 0.12 mg/l). However, Acinetobacter spp and Enterobacter spp were more resistant with $MIC_{90}$s of 16 and >128 mg/l respectively. Ps. aeruginosa were uniformly resistant to cefixime. N. gonorrhoeae and meningitidis were very susceptible to cefixime ($MIC_{90}$ 0.008 mg/l) including three ß-lactamase producing strains of N. gonorrhoeae. Both ß-lactamase and non ß-lactamase producing strains of H. influenzae were susceptible to cefixime have $MIC_{90}$s of 0.06 mg/l, cefixime being considerably more active than amoxycillin. Against the streptococci cefixime was similar in activity to amoxycillin against Strep. pneumoniae

| Organism (No. of strains) | Agent | $MIC_{50}$ (mg/l) | $MIC_{90}$ (mg/l) | Range (mg/l) |
|---|---|---|---|---|
| Esch.coli (66) | cefixime | 0.12 | 1 | 0.015 - 32 |
| | cefotaxime | 0.03 | 0.12 | 0.008 - 1 |
| | cefuroxime | 2 | 8 | 0.12 - 64 |
| | cephalexin | 4 | 16 | 1 - >128 |
| | amoxycillin | 32 | >128 | 0.5 - >128 |
| Pr.mirabilis (50) | cefixime | 0.015 | 0.015 | 0.008 - 0.12 |
| | cefotaxime | 0.015 | 0.03 | 0.008 - 1 |
| | cefuroxime | 0.5 | 4 | 4 - >128 |
| | cephalexin | 8 | 16 | 4 - >128 |
| | amoxycillin | 0.5 | >128 | 0.25 - >128 |
| Klebsiella spp.(58) | cefixime | 0.03 | 0.12 | 0.004 - 1 |
| | cefotaxime | 0.03 | 0.12 | 0.008 - 2 |
| | cefuroxime | 2 | 16 | 0.25 - >128 |
| | cephalexin | 4 | 8 | 2 - >128 |
| | amoxycillin | >128 | >128 | 4 - >128 |
| Enterobacter spp.(51) | cefixime | 1 | >128 | 0.03 - >128 |
| | cefotaxime | 0.12 | 16 | 0.03 - 64 |
| | cefuroxime | 16 | >128 | 1 - >128 |
| | cephalexin | 64 | >128 | 4 - >128 |
| | amoxycillin | 128 | >128 | 2 - >128 |

**Table 14A : In vitro activity of cefixime**

Activity of cefixime compared with that of other beta lactam antibiotics against 352 isolates. Results given as a range of activity and that concentration which was found to inhibit 50 (MIC 50) and 90% (MIC 90) of strains in mg/l.

| Organism (No. of strains) | Agent | $MIC_{50}$ (mg/l) | $MIC_{90}$ (mg/l) | Range (mg/l) |
|---|---|---|---|---|
| H.influenzae (32) | cefixime | 0.03 | 0.06 | 0.015 - 0.5 |
| | cefotaxime | 0.015 | 0.06 | 0.008 - 0.12 |
| | cefuroxime | 0.5 | 1 | 0.5- 2 |
| | cephalexin | 16 | 32 | 4 - 128 |
| | amoxycillin | 2 | 16 | 0.25 - 32 |
| N.gonorrhoeae (41) | cefixime | 0.004 | 0.008 | 0.002 - 0.12 |
| | cefotaxime | 0.004 | 0.008 | 0.004 - 0.12 |
| | cephalexin | 0.5 | 2 | 0.06 - 8 |
| | amoxycillin | 0.03 | 0.12 | 0.03 - 128 |
| | penicillin | 0.015 | 0.06 | 0.008 - 128 |
| St.aureus (34) | cefixime | 16 | >128 | 2 - >128 |
| | cefotaxime | 2 | 64 | 1 - >128 |
| | cefuroxime | 1 | >128 | 0.25 - >128 |
| | cephalexin | 4 | >128 | 1 - >128 |
| | amoxycillin | 1 | 64 | 0.12 - >128 |
| Str.pneumoniae (20) | cefixime | 0.12 | 0.12 | 0.03 - 0.12 |
| | cefotaxime | 0.015 | 0.015 | 0.008 - 0.015 |
| | cefuroxime | 1 | 1 | 0.5 - 2 |
| | cephalexin | 0.03 | 0.12 | 0.015 - 0.25 |
| | amoxycillin | 0.015 | 0.03 | 0.015 - 0.03 |

**Table 14B : In vitro activity of cefixime**

(continued)

| Organism (No. of strains) | $MIC_{90}$ (mg/l) |
|---|---|
| Prov.species (20) | 0.12 |
| Acinetobacter species (15) | 16 |
| Salmonella species (12) | 0.25 |
| Ps.aeruginosa (42) | >128 |
| N.meningitidis (7) | 0.008 |
| Gp A streptococci (10) | 0.06 |
| Gp B streptococci (10) | 0.25 |
| E.faecalis (10) | >128 |
| Clostridia species (17) | 4 |
| Bacteroides species (15) | >128 |

**Table 15 : Activity of cefixime against miscellaneous groups of organisms**

($MIC_{90}$ 0.12 mg/l) and $MIC_{90}$ for Group A and B streptococci were 0.06 and 0.25 mg/l respectively. E. faecalis were uniformly resistant to cefixime. Cefixime was less active against S. aureus with a wide range of MICs (20->128 mg/l) and an $MIC_{90}$ of >128 mg/l. Anaerobic organisms were relatively resistant to cefixime with $MIC_{90}$s in excess of 4 mg/l.

Assay Validation

In Table 16 the results of the microbiological assay for human serum samples spiked with varying concentrations of cefixime are given and these data are shown graphically in Figure 16. The lower limit of sensitivity of the assay was 0.015 mg/l and the coefficient of variation for the two internal controls 7.4%.

In order to validate the microbiological plate assay samples from volunteer 1 and volunteer 4 (samples collected on two separate trial days) were assayed by HPLC, the results of which are shown in Figure 17 and Table 17. From the results of both validation studies it would appear that the microbiological assay gives acceptable results. The protein binding of cefixime in human serum was 65 and 70% for 2 and 5 mg/l respectively

| Assigned concentration mg/l | Assayed concentration mg/l |
|---|---|
| 10 | 9.72 |
| 6 | 5.44 |
| 8 | 7.24 |
| 3 | 3.54 |
| 2.5 | 2.61 |
| 1.5 | 1.43 |
| 0.8 | 0.87 |
| 0.6 | 0.71 |
| 5 | 4.45 |
| 0.5 | 0.52 |
| 2 | 2.21 |
| 1.9 | 2.07 |
| 0.9 | 0.85 |
| 1.7 | 1.69 |
| 4.5 | 3.91 |
| 1 | 0.92 |
| Correlation coefficient | 0.995 |
| Slope | 0.9191 |
| y intercept | 0.1447 |
| p | <0.001 |

**Table 16 : Validation of the microbiological assay for the measurement of cefixime**

Human serum spiked with cefixime and then assayed by the microbiological assay. Results compared with the assigned concentration.

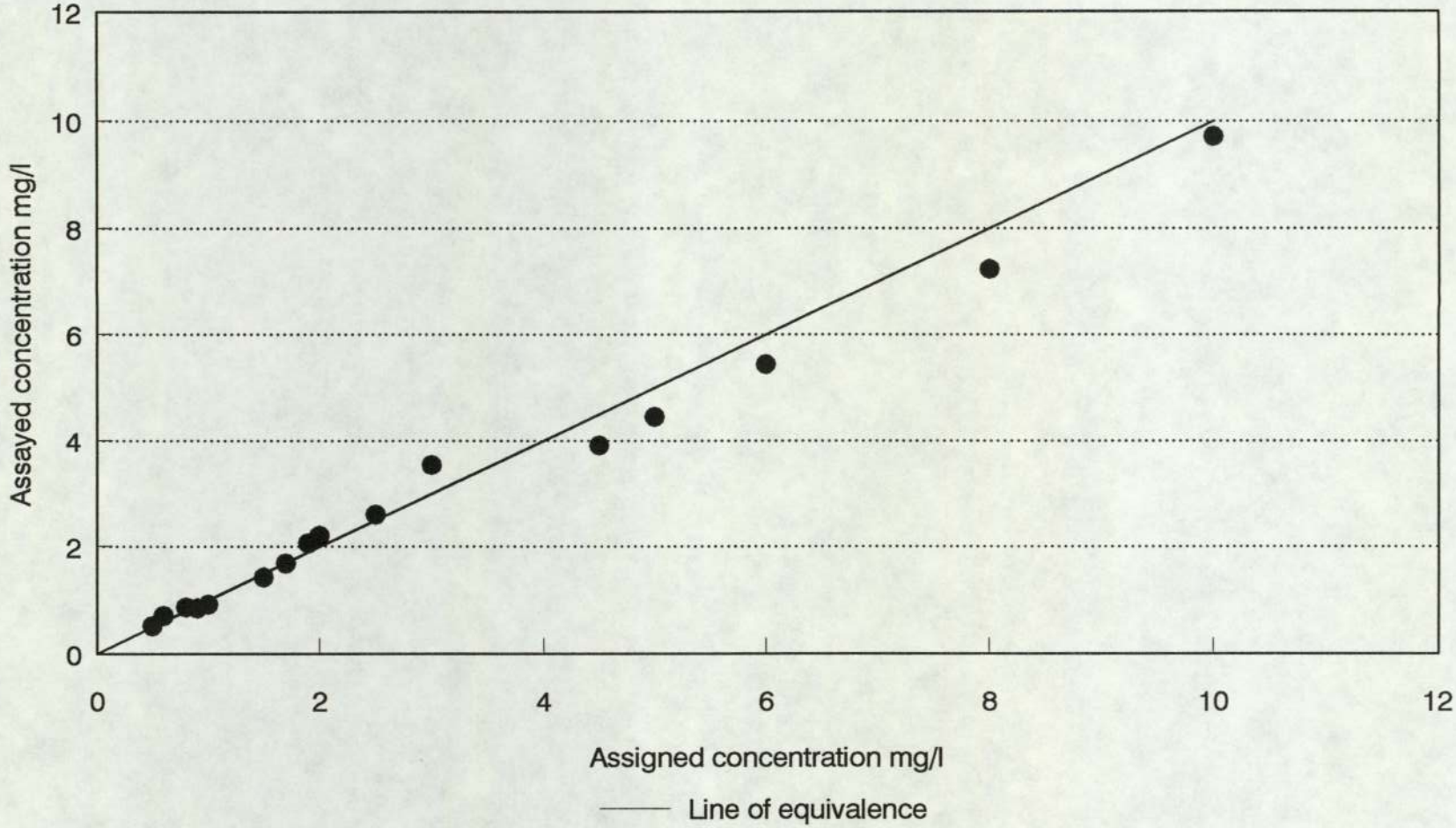



**Figure 16 : Cefixime assay validation**

Samples spiked with concentrations of cefixime.
Samples assayed by the microbiological assay method.
Assigned concentration plotted against assayed concentration.

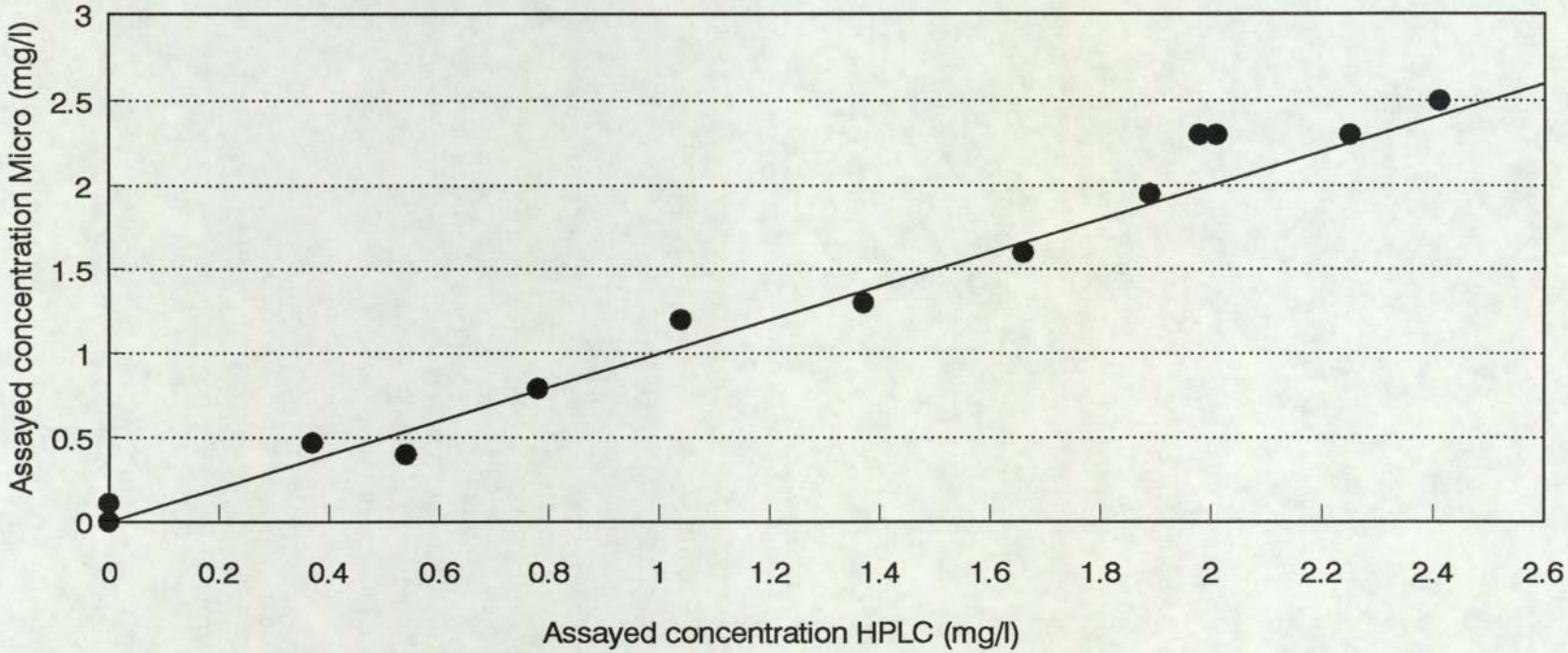



**Figure 17 : Assay of cefixime in volunteer samples using two methods of assay.**

13 serum samples containing varying concentrations of cefixime assayed by HPLC and the microbiological assay methods. Data points plotted for each sample showing result obtained by both methods.

| Vol No. | Time (h) | HPLC (mg/l) | Micro (mg/l) |
|---|---|---|---|
| 1 | Pre | NDL | NDL |
| | 0.5 | NDL | 0.11 |
| | 1 | 0.78 | 0.79 |
| | 1.5 | 1.37 | 1.30 |
| | 2 | 1.89 | 1.95 |
| | 3 | 2.41 | 2.5 |
| | 4 | 2.25 | 2.3 |
| | 12 | 0.37 | 0.47 |
| | 26 | NDL | NDL |
| 4 | Pre | NDL | NDL |
| | 1 | 0.54 | 0.4 |
| | 1.5 | 1.04 | 1.2 |
| | 3 | 2.01 | 2.3 |
| | 6 | 1.98 | 2.3 |
| | 8 | 1.66 | 1.6 |

| | |
|---|---|
| Correlation coefficient | 0.993 |
| slope | 1.0524 |
| y intercept | 0.0044 |
| p | <0.001 |

**Table 17 : Cefixime samples assayed by HPLC and plate assay**

Pharmacokinetic Study

The mean serum and inflammatory fluid levels are shown in Figure 18, the derived pharmacokinetic parameters in Table 18 and the urinary excretion in Table 19.

Absorption of cefixime was rather slow following oral administration with a mean T max of 3.7 h (range 3-4 h). The maximum concentrations achieved in serum (Cmax) were between 2.5 and 3.95 mg/l for five of the volunteers, however one of the volunteers achieved a level of 6.2 mg/l at 4 h, 12 h after the dosing the level had fallen to 0.9 mg/l.

The elimination half-life was relatively long for a cephalosporin with a mean of 3.8 h (range 3.4 - 4.2 h).

Penetration into inflammatory fluid was slow (mean 6.7 h) with a mean peak concentration of 3.2 mg/l (range 1.6 - 4.4 mg/l). The percentage penetration gave a mean value of 132.6 (SD 36.2%).

Urinary recovery over 24 h accounted for 19.9% of the total oral dose and ranged from 12.4 - 27.1%. The cefixime concentration in the 12 - 24 h samples of all the volunteers exceeded 4.9 mg/l.

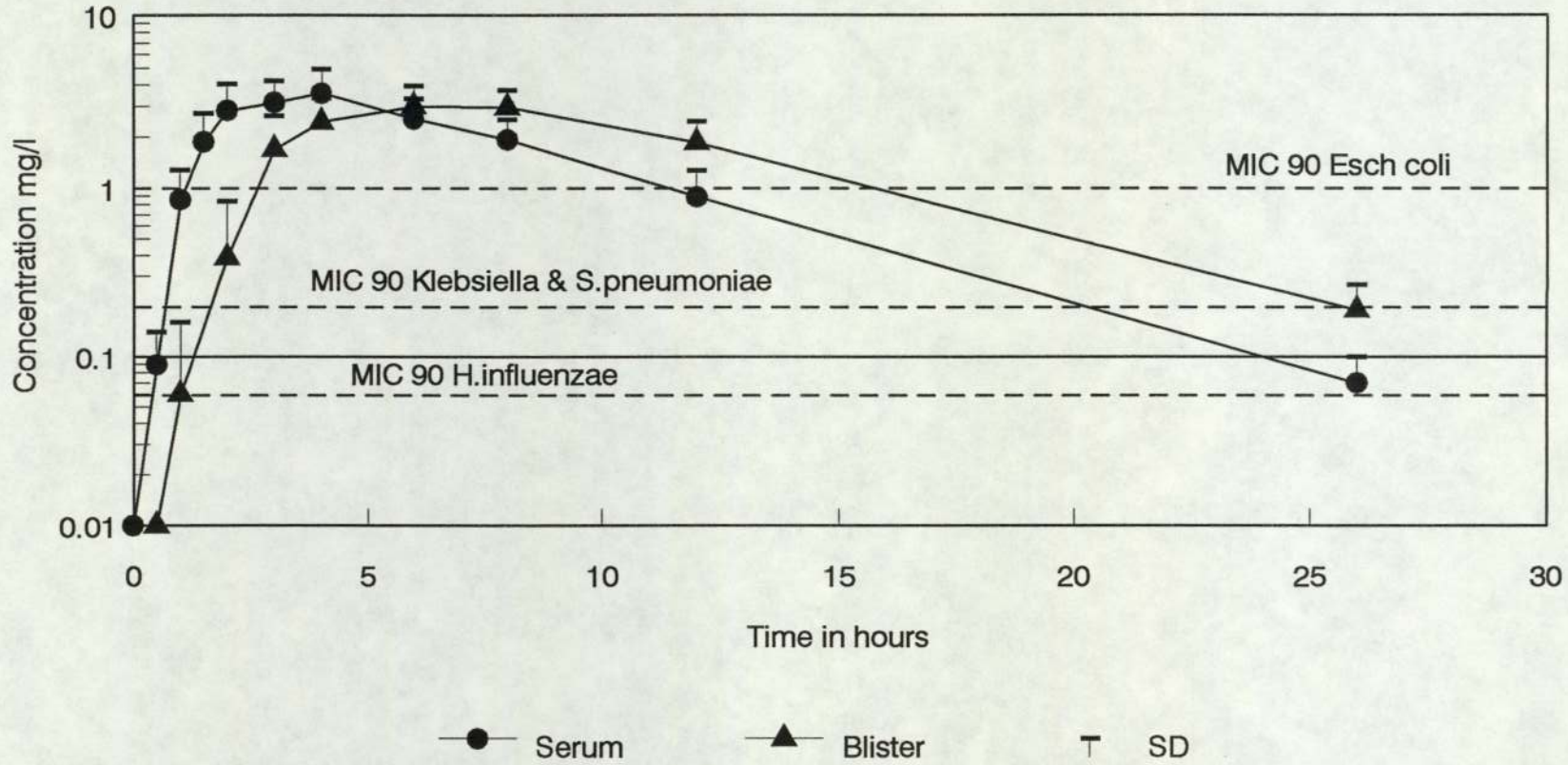



**Figure 18 : Mean serum and inflammatory fluid concentrations following a single 400 mg oral dose of cefixime.**

Data obtained from six volunteers. The concentration found to inhibit 90% (MIC 90) of Esch.coli , Klebsiella , S.pneumoniae and H.influenzae also shown.

| | Mean value ± SD | Range |
|---|---|---|
| Serum : | | |
| $T_{max}$ (h) | 3.7 ± 0.5 | 3.0 - 4.0 |
| Cmax (mg/l) | 3.7 ± 1.3 | 2.5 - 6.2 |
| T 1/2 (h) | 3.8 ± 0.3 | 3.4 - 4.2 |
| AUC 0 - ∞ (mg.h/l) | 30.4 ± 11.0 | 19.7 - 48.1 |
| Inflammatory fluid : | | |
| $T_{max}$ (h) | 6.7 ± 1.0 | 6.0 - 8.0 |
| Cmax (mg/l) | 3.2 ± 1.0 | 1.6 - 4.4 |
| T 1/2 (h) | 4.1 ± 0.4 | 3.5 - 4.5 |
| AUC 0 - ∞ (mg.h/l) | 36.5 ± 0.4 | 18.7 - 45.2 |

**Table 18 : Pharmacokinetic parameters of cefixime in serum and tissue fliud following a single 400 mg oral dose**

Data derived from six volunteers.

| Time | Vol 1 | Vol 2 | Vol 3 | Vol 4 | Vol 5 | Vol 6 |
|---|---|---|---|---|---|---|
| 0-2 hours. | 3.22 | 4.20 | 9.40 | 3.88 | 2.70 | 6.36 |
| 2-4 hours | 13.95 | 20.25 | 29.60 | 9.14 | 12.39 | 13.16 |
| 4-8 hours | 19.95 | 35.00 | 44.0 | 38.64 | 43.48 | 66.96 |
| 8-12 hours | 8.40 | 10.53 | 10.11 | 11.22 | 7.32 | 10.14 |
| 12-24 hours | 4.14 | 7.44 | 15.37 | 5.31 | 6.05 | 5.61 |
| Total mg. | 49.66 | 77.42 | 108.48 | 68.19 | 71.94 | 102.23 |
| % excreted. | 12.42 | 19.36 | 27.12 | 17.04 | 17.98 | 25.55 |

**Table 19 : Cefixime Urinary Excretion**

Urinary excretion following a single
400 mg oral dose of cefixime.

Concentrations of cefixime in sputum and bronchial mucosa.

The groups into which the patients were allocated for the study is shown in Table 20. Each of these groups were divided into three on the basis of a randomly allocated drug regime.

Results obtained are shown graphically in Figures 19 a, b and c, and mean serum, sputum and mucosa concentration for the six groups in Table 21. In Figures 19 a, b and c undetectable levels have been given an arbitrary value of 0.01 mg/l so that these are visible in a log scale.

In all three groups there were sputum samples in which no cefixime could be detected (lower limit of sensitivity of the assay 0.015 mg/l). They comprised of six of the 200 mg o.d. group, 5 of the 200 mg b.d. group and 2 of the 400 mg o.d. group. In contrast cefixime levels could be measured in all but one bronchial biopsy samples. The mean percentage penetration into bronchial mucosa was 39%, 39.6% and 36% for the 200 mg o.d., 200 mg b.d. and 400 mg o.d. groups respectively.

It should be noted that the mean serum concentration in the 400 mg o.d. group was higher in those undergoing bronchial biopsy. However,

| Group | No. patients | Sample | Dose (on each of three days) |
|---|---|---|---|
| A | 9* | sputum | 200 mg o.d. |
| B | 10 | bronchial biopsy | 200 mg o.d. |
| C | 9* | sputum | 200 mg b.d. |
| D | 10 | bronchial biopsy | 200 mg b.d. |
| E | 10 | sputum | 400 mg o.d. |
| F | 10 | bronchial biopsy | 400 mg o.d. |

(* = one patient withdrawn : group A due to a severe chest infection superimposed on carcinoma of the lung ; group C due to loss of sample during processing)

**Table 20 : Dosage regime for the study on the penetration of cefixime into the respiratory tract.**

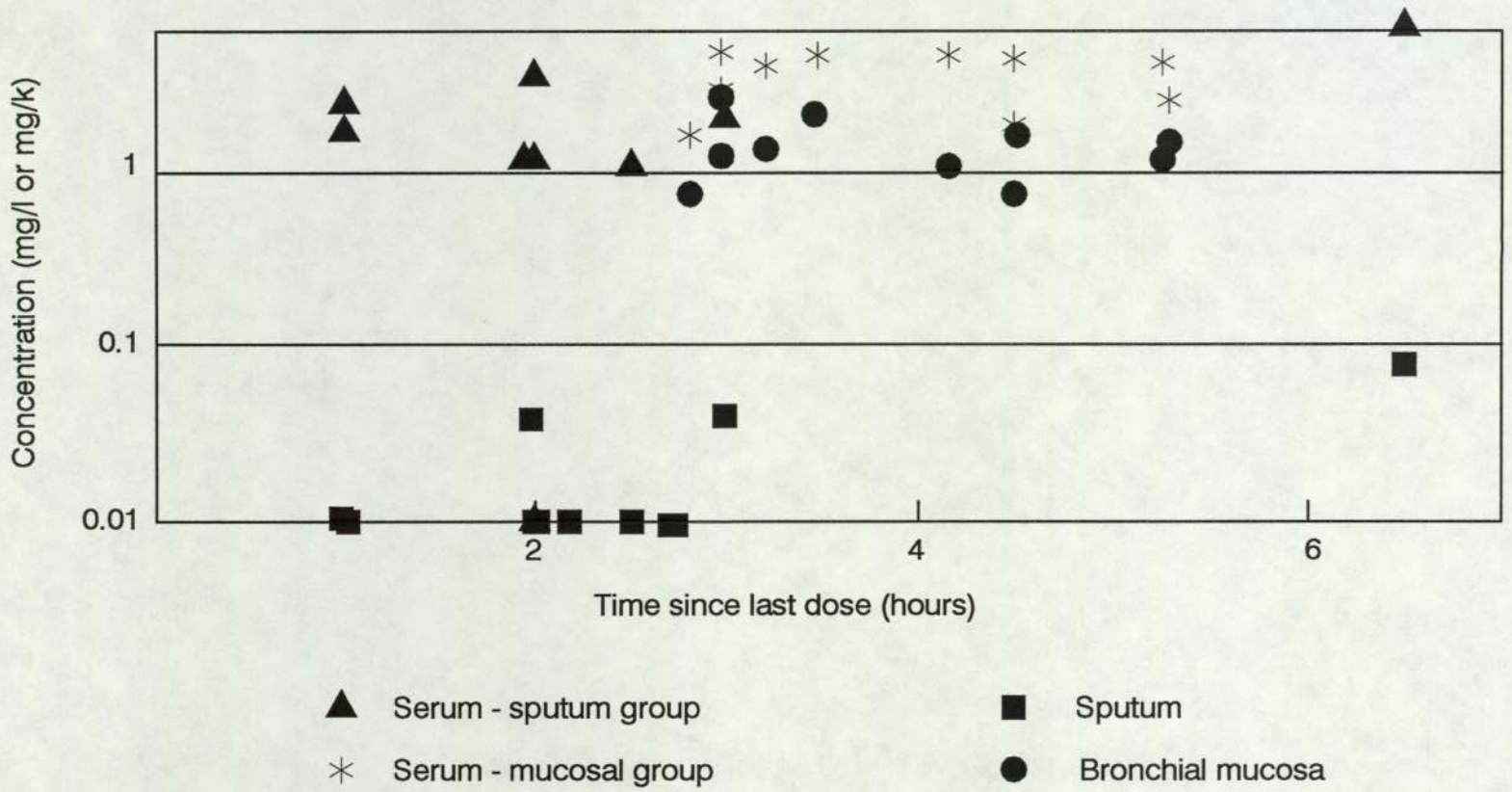



**Figure 19A : Serum , bronchial mucosal and sputum levels of cefixime (200 mg oral dose)**

Lower limit of detection of the assay 0.01 mg/l.

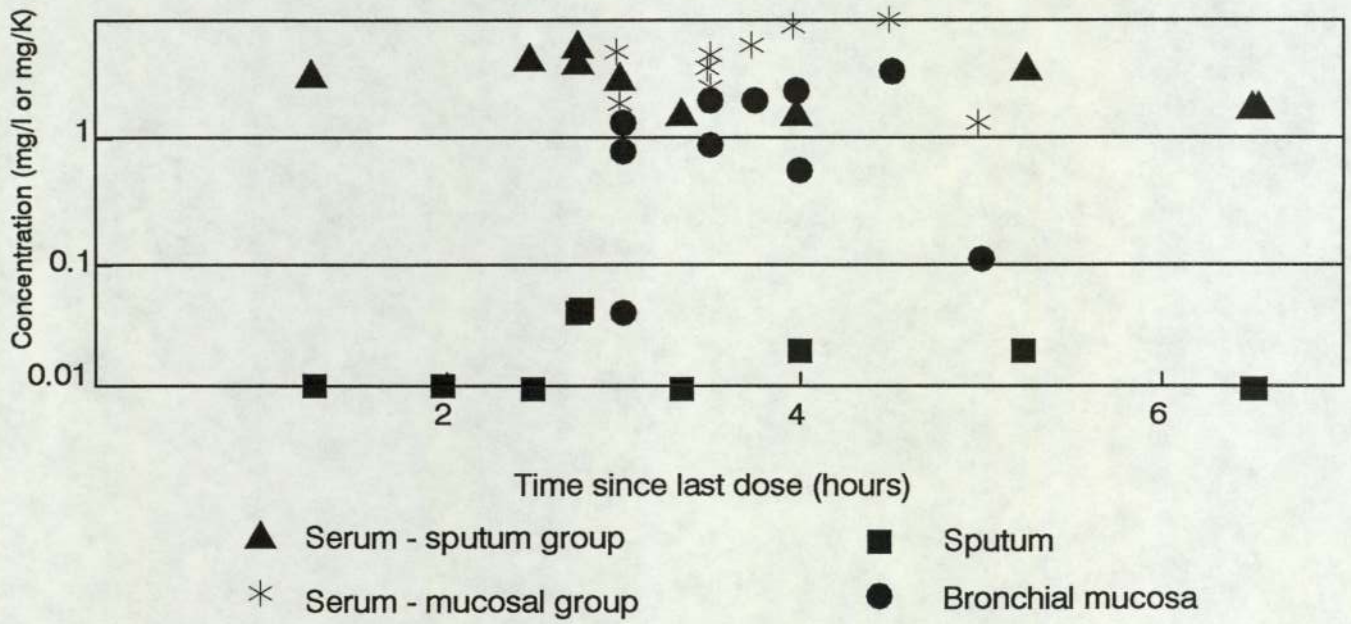



**Figure 19 B : Serum , bronchial mucosal and sputum levels of cefixime (200 mg oral b.d.dose)**

Lower limit of detection of the assay 0.01 mg/l

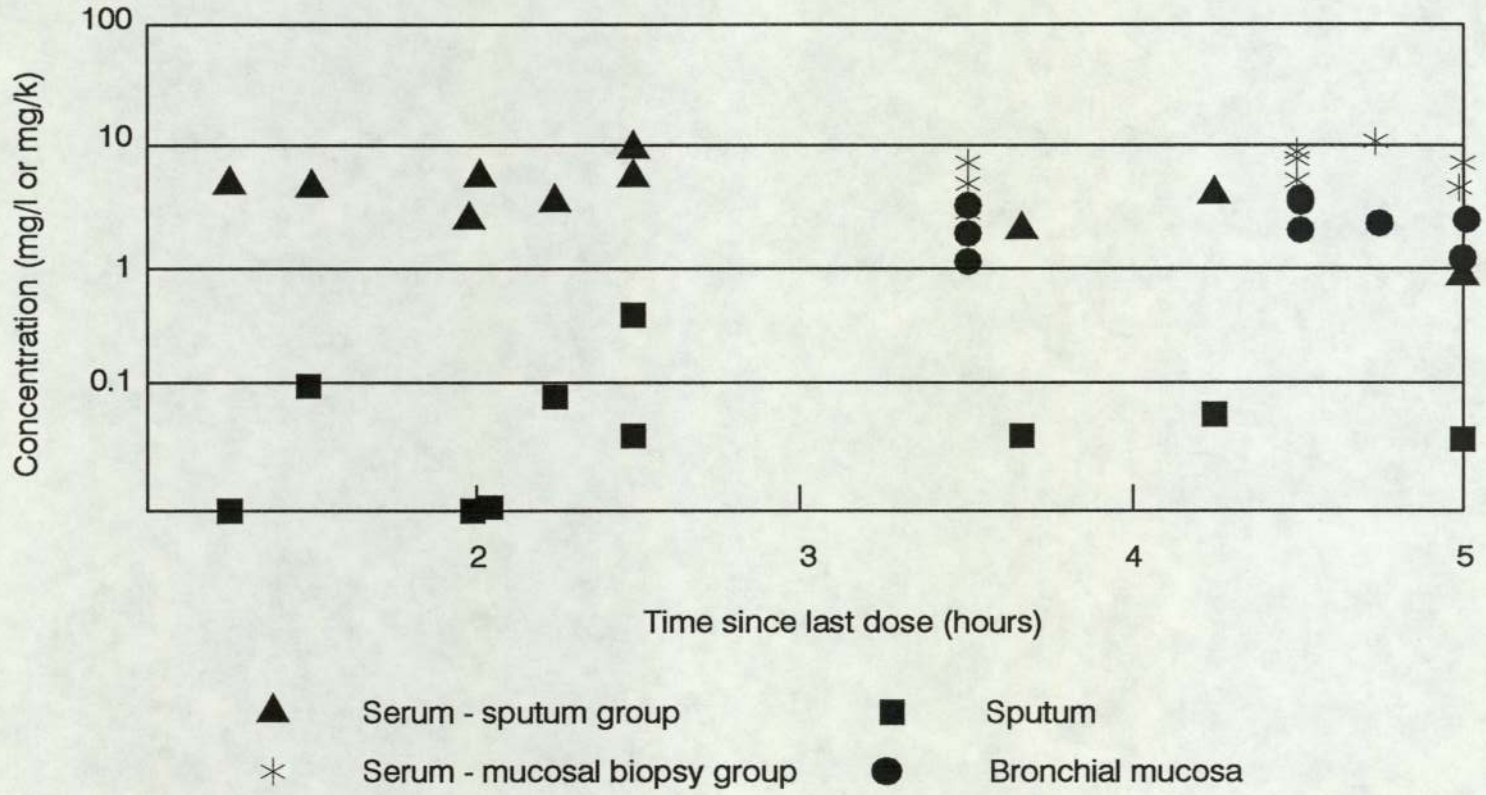



**Figure 19C : Serum , bronchial mucosa and sputum levels for cefixime 400 mg daily.**

Lower limit of sensitivity of the assay 0.01 mg/l

| | Dosage regimen | | | | | |
|---|---|---|---|---|---|---|
| | 200 mg o.d. | | 200 mg b.d. | | 400 mg o.d. | |
| | sputum study | biopsy study | sputum study | biopsy study | sputum study | biopsy study |
| Mean concentration mg/l | 2.3 | 3.9 | 3.2 | 4.6 | 4.2 | 6.6 |
| Mean sputum or mucosal concentration mg/l | 0.02 | 1.5 | 0.02 | 1.6 | 0.07 | 2.4 |
| Mean time of last dose (hours) | 2.5 | 3.9 | 3.5 | 3.8 | 2.7 | 4.3 |

(Undetectable concentrations have been excluded when calculating means)

**Table 21 : Study on the penetration of cefixime into the respiratory tract.**

there were no differences in serum concentrations in the other two dosage groups.

**Discussion**

The pharmacokinetic parameters of cefixime and its in vitro activity are markedly different from those of currently available oral cephalosporins including cefuroxime axetil (50). There is general agreement between the pharmacokinetic data obtained in this study and that reported previously (17). However, the mean serum elimination half-life was slightly shorter being 3.1 h compared with 3.8 h. In this study 19.9% of the administered dose was recovered and other studies have confirmed the rate of recovery around 20% or less (17). The urinary recovery of cefixime is therefore much less than that of other oral agents such as cephalexin, cephradine and cephadroxil where the recovery is in excess of 80% (51, 52).

The serum elimination half-life of cefixime is significantly longer than other oral cephalosporins (cephradine and cephalexin <1 h, (51). There are two possible explanations for this, one, that the absorption from the intestine

may be longer and slower than other cephalosporins and secondly, as there are appreciable amounts of drug in bile (53) then enterohepatic recycling may occur.

Penetration into inflammatory fluid was fairly slow (6.7 h), however, levels achieved were only slightly lower than those found in serum.

With regard to the penetration of cefixime into the respiratory tract in all three groups studied the bronchial mucosa concentrations were far higher than the sputum concentrations. Cefixime was undetectable in thirteen patients in spite of the fact that levels were found in simultaneous mucosa and serum samples. This highlights the problems of interpretation of sputum concentrations. As mentioned previously sputum pooling, contamination with saliva and the variable origin of sputum in the bronchial tree, all help to explain the consistent results obtained. However, it is encouraging that cefixime reaches 35 to 40% of serum concentrations in bronchial mucosa which should lead to concentrations above the $MIC_{90}$ for most of the common respiratory pathogens.

Although serum and urine concentrations are

lower than for other cephalosporins the in vitro study confirms the findings of previous workers (15, 17) and as can be seen in Figure 18 the $MIC_{90}$ of most Enterobacteriaceae, H. influenzae, Neisseria spp and Strep. pneumoniae are readily exceeded. Bacteria not susceptible to cefixime include, Staphylococci, Enterococci, Ps. aeruginosa and anaerobes.

Because of the long serum half-life, high in vitro activity and good penetration into tissue, a twice daily dose for respiratory and systemic infections and a once daily dose for urinary tract infections (most of the common urinary pathogens with the exception of Staphylococcus saprophyticus) should allow adequate therapy.

3. **Meropenem**

In vitro activity

In Table 22a b, c the in vitro activity of meropenem is compared with other ß-lactams including imipenem another carbopenem. Against the Enterobacteria meropenem was the most active of the antimicrobials tested and against Ps. aeruginosa equal in activity to imipenem. Of the four antimicrobials investigated, piperacillin was

| Organism (No.) | Drug | $MIC_{50}$ | $MIC_{90}$ | Range |
|---|---|---|---|---|
| Esch.coli (50) | Meropenem | 0.008 | 0.015 | 0.008 - 0.03 |
| | Cefotaxime | 0.03 | 0.12 | 0.008 - 1 |
| | Piperacillin | 2 | 128 | 0.12 - >128 |
| | Imipenem | 0.06 | 0.12 | 0.015 - 0.5 |
| Klebsiella spp.(50) | Meropenem | 0.015 | 0.03 | ≤0.002 - 0.06 |
| | Cefotaxime | 0.03 | 0.12 | 0.008 - 2 |
| | Piperacillin | 4 | >128 | 0.25 - >128 |
| | Imipenem | 0.12 | 0.12 | 0.03 - 0.5 |
| P.mirabilis (46) | Meropenem | 0.06 | 0.06 | 0.015 - 0.06 |
| | Cefotaxime | 0.015 | 0.03 | 0.008 - 1 |
| | Piperacillin | 0.25 | 16 | 0.12 - >128 |
| | Imipenem | 1 | 2 | 0.12 - 4 |
| P.vulgaris (19) | Meropenem | 0.03 | 0.06 | 0.015 - 0.12 |
| | Cefotaxime | 0.015 | 0.25 | 0.015 - 0.25 |
| | Piperacillin | 0.5 | 1 | 0.12 - 4 |
| | Imipenem | 2 | 4 | 0.12 - 4 |
| M.morganni (15) | Meropenem | 0.03 | 0.06 | 0.015 - 0.06 |
| | Cefotaxime | 0.015 | 4 | 0.008 - 4 |
| | Piperacillin | 0.25 | 32 | 0.12 - 128 |
| | Imipenem | 1 | 2 | 0.5 - 2 |
| Enterobacter spp.(40) | Meropenem | 0.015 | 0.12 | 0.008 - 0.25 |
| | Cefotaxime | 0.12 | 16 | 0.03 - 64 |
| | Piperacillin | 4 | 32 | 0.5 - >128 |
| | Imipenem | 0.25 | 1 | 0.06 - 2 |
| Citrobacter spp.(19) | Meropenem | 0.015 | 0.06 | 0.008 - 0.06 |
| | Cefotaxime | 0.06 | 1 | 0.03 - 32 |
| | Piperacillin | 2 | 32 | 0.25 - >128 |
| | Imipenem | 0.12 | 1 | 0.12 - 4 |
| Serratia spp.(25) | Meropenem | 0.015 | 0.06 | 0.008 - 0.5 |
| | Cefotaxime | 0.5 | 16 | 0.06 - 128 |
| | Piperacillin | 2 | >128 | 0.5 - >128 |
| | Imipenem | 0.5 | 2 | 0.12 - 8 |

**Table 22A : In vitro activity of meropenem compared with other beta lactam antibiotics.**

Minimum inhibitory concentrations (MIC) determined. Results expressed as a range of activity or as a concentration which will inhibit 50 or 90% of isolates in mg/l.

| Organism (No.) | Drug | $MIC_{50}$ | $MIC_{90}$ | Range |
|---|---|---|---|---|
| Prov.stuartii (22) | Meropenem | 0.06 | 0.12 | 0.015 - 0.12 |
| | Cefotaxime | 0.06 | 0.5 | 0.008 - 0.5 |
| | Piperacillin | 4 | 128 | 0.5 - >128 |
| | Imipenem | 1 | 4 | 0.06 - 4 |
| Acinetobacter spp.(19) | Meropenem | 0.25 | 1 | 0.12 - 1 |
| | Cefotaxime | 16 | 32 | 2 - 32 |
| | Piperacillin | 32 | 64 | 4 - .128 |
| | Imipenem | 0.25 | 2 | 0.12 - 4 |
| Ps.aeruginosa (39) | Meropenem | 0.5 | 2 | 0.12 - 4 |
| | Cefotaxime | 16 | 128 | 1 - >128 |
| | Piperacillin | 4 | 64 | 1 - 128 |
| | Imipenem | 2 | 4 | 0.5 - 8 |
| S.aureus (39) | Meropenem | 0.25 | 1 | 0.06 - 2 |
| | Cefotaxime | 1 | 32 | 0.25 - 64 |
| | Piperacillin | 1 | 32 | 0.25 - 128 |
| | Imipenem | 0.03 | 0.5 | 0.015 - 2 |
| S.epidermidis (35) | Meropenem | 0.5 | 1 | 0.06 - 2 |
| | Cefotaxime | 4 | 8 | 0.25 - 32 |
| | Piperacillin | 2 | 8 | 0.12 - >128 |
| | Imipenem | 0.06 | 1 | 0.015 - 8 |
| S.saprophyticus (40) | Meropenem | 0.12 | 0.25 | 0.06 - 0.25 |
| | Cefotaxime | 4 | 4 | 0.5 - 64 |
| | Piperacillin | 1 | 2 | 0.25 - 8 |
| | Imipenem | 0.06 | 0.06 | 0.008 - 0.25 |
| Gp A Streptococci (15) | Meropenem | 0.008 | 0.008 | 0.008 |
| | Cefotaxime | 0.015 | 0.015 | 0.015 |
| | Piperacillin | 0.06 | 0.06 | 0.06 |
| | Imipenem | 0.004 | 0.008 | 0.004 - 0.008 |
| Gp BStreptococci (13) | Meropenem | 0.03 | 0.06 | 0.03 - 0.06 |
| | Cefotaxime | 0.03 | 0.06 | 0.03 - 0.06 |
| | Piperacillin | 0.25 | 0.25 | 0.12 - 0.25 |
| | Imipenem | 0.015 | 0.015 | 0.015 |

**Table 22B : In vitro activity of meropenem compared with other beta lactam antibiotics.**

(continued)

| Organism (No.) | Drug | $MIC_{50}$ | $MIC_{90}$ | Range |
|---|---|---|---|---|
| E.faecalis (24) | Meropenem | 2 | 4 | 2 - 4 |
| | Cefotaxime | 8 | 64 | 1 - 128 |
| | Piperacillin | 2 | 4 | 1 - 4 |
| | Imipenem | 0.5 | 1 | 0.5 - 1 |
| S.pneumoniae (38) | Meropenem | 0.008 | 0.06 | 0.004 - 0.5 |
| | Cefotaxime | 0.015 | 0.25 | 0.015 - 1 |
| | Piperacillin | 0.03 | 0.25 | 0.015 - 2 |
| | Imipenem | 0.004 | 0.03 | 0.004 - 0.25 |
| H.influenzae (29) | Meropenem | 0.12 | 0.25 | 0.06 - 0.25 |
| | Cefotaxime | 0.03 | 0.03 | 0.015 - 0.06 |
| | Piperacillin | 0.12 | 64 | 0.015 - >128 |
| | Imipenem | 1 | 4 | 0.12 - 8 |
| M.catarrhalis (27) | Meropenem | 0.008 | 0.015 | 0.004 - 0.03 |
| | Cefotaxime | 0.25 | 1 | 0.008 - 1 |
| | Piperacillin | 0.25 | 0.5 | 0.008 - 2 |
| | Imipenem | 0.015 | 0.12 | 0.008 - 0.12 |
| N.gonorrhoeae (22) | Meropenem | 0.015 | 0.015 | 0.004 - 0.03 |
| | Cefotaxime | 0.015 | 0.015 | 0.004 - 0.03 |
| | Piperacillin | 0.03 | 0.5 | 0.008 - 64 |
| | Imipenem | 0.03 | 0.12 | 0.015 - 0.12 |
| N.meningitidis (9) | Meropenem | 0.008 | 0.008 | 0.008 |
| | Cefotaxime | 0.004 | 0.008 | 0.004 - 0.008 |
| | Piperacillin | 0.015 | 0.06 | 0.008 - 0.06 |
| | Imipenem | 0.03 | 0.12 | 0.03 - 0.12 |

**Table 22C : In vitro activity of meropenem compared with other beta lactam antibiotics.**

(continued)

the least active. Meropenem displayed a high degree of stability to plasmid and chromosomally mediated ß-lactamase as shown in Table 23.

Against Gram-positive organisms meropenem was slightly less or equal in activity to imipenem. However, in common with other ß-lactams the activity of meropenem was reduced against Staphylococci known to be resistant to methicillin (mode MIC to meropenem 2 mg/l). Of interest is the activity of meropenem against E. faecalis an organism which is resistant to third generation cephalosporins. Against the fastidious organism meropenem displayed a high degree of activity and showed greater activity than imipenem against H. influenzae. Unlike piperacillin meropenem was stable to the ß-lactamase producing strains of H. influenzae and N. gonorrhoeae.

Table 24 summarises the activity of meropenem against miscellaneous organisms including anaerobes. Meropenem was highly active against the anaerobic strains tested with a mode MIC less than or equivalent to 0.12 mg/l. The morphological response to meropenem was to cause rounding of cells.

| Strain | | | | MIC $10^4$ |
|---|---|---|---|---|
| E.cloacae | 1194E | Gp 1 | Tem 2 | 0.25 |
| E.cloacae | 1051E | Gp 1 | P99+ | 0.015 |
| K.pneumoniae | 1976E | Gp 3 | SHV1 | 0.03 |
| K.aerogenes | 1082E | Gp 4 | K1+ | 0.03 |
| Esch.coli | 1725E | Gp 3 | Tem 2 | 0.03 |
| Esch.coli | 1894E | Gp 5 | Oxa 3 | 0.015 |
| Esch.coli | 1573E | Gp 5 | OxA 2 | 0.015 |
| Esch.coli | 2138E | Gp 5 | Oxa 1 | 0.008 |
| Esch.coli | CIBA-82 | Gp 3 | Tem 1 | 0.008 |
| Ps.aeruginosa | Plas R151 | | PSE 2 | 1 |
| Ps.aeruginosa | Plas RM149 | | PSE 3 | 1 |

**Table 23 : Activity of meropenem against known beta lactamase producing strains.**

| Organism (No.tested) | Mode MIC | Range |
|---|---|---|
| Gp C Streptococci (6) | 0.015 | 0.015 |
| P.rettgeri (5) | 0.03 | 0.015 - 0.03 |
| Anaerobic Streptococci (27) | 0.12 | 0.004 - 0.5 |
| Clost.difficle (4) | 0.008 | 0.008 - 2 |
| Clost.perfringens (6) | 0.008 | 0.008 - 0.015 |
| B.fragilis (42) | 0.12 | 0.06 - 1 |

**Table 24 : In vitro activity of meropenem against miscellaneous organisms.**

Minimum inhibitory concentration (MIC) expressed as a range of activity or as mode MIC in mg/l.

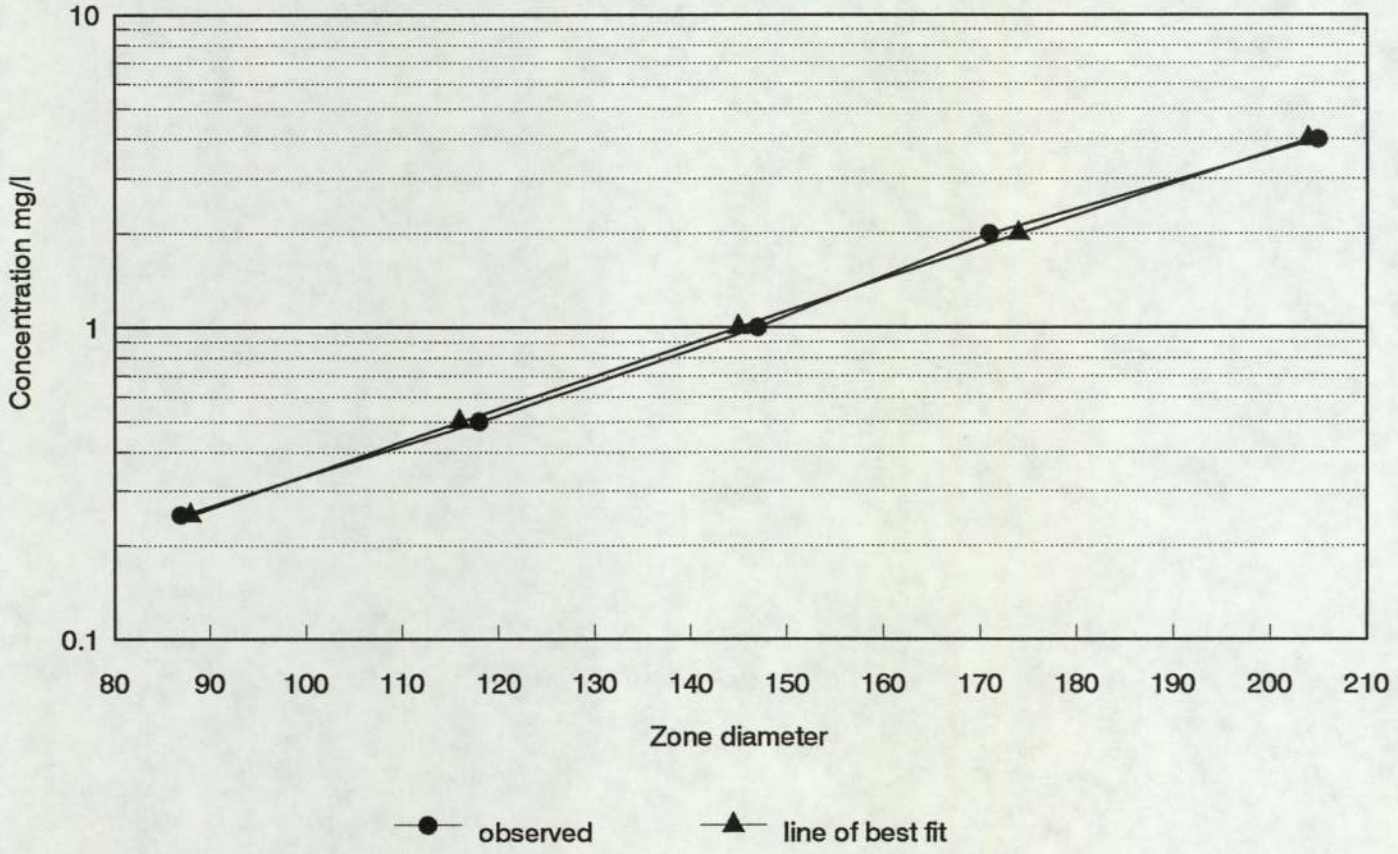



**Figure 20 : Microbiological assay of meropenem.**

Standard material prepared in human serum over a concentration range of 0.25 to 4 mg/l. Zone diameters (arbritrary units) plotted against concentration in mg/l.

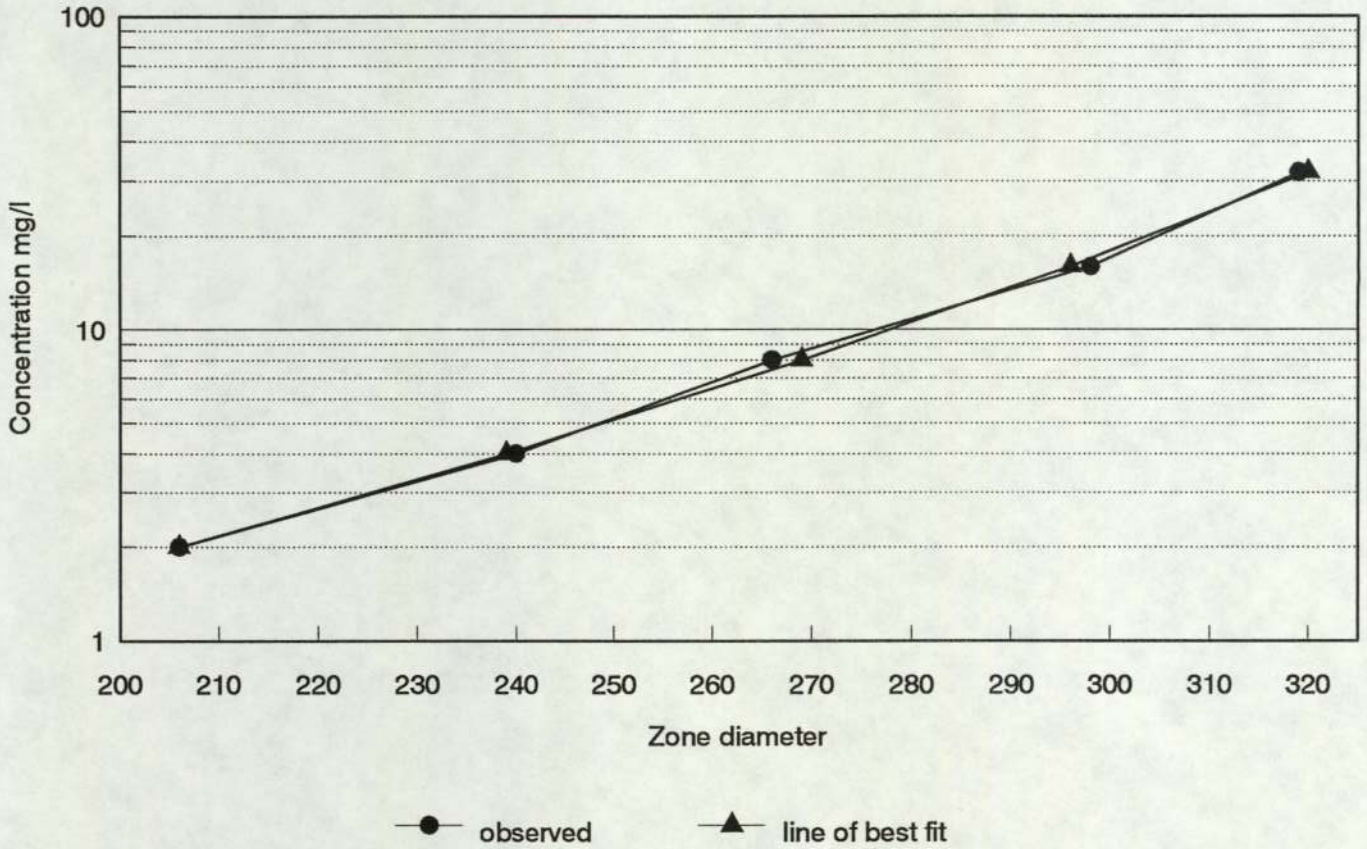



**Figure 21 : Microbiological assay of meropenem**

Standard material prepared in human serum over a concentration range of 2 to 32 mg/l. Zone diameters (arbritrary units) plotted against concentration in mg/l.

Assay validation

In Figures 20 and 21 the observed lines and lines of best fit are given for the low range of standards (0.25 - 4 mg/l) and the high range of standards (2 - 32 mg/l). After developing an assay, the method was validated by assaying samples supplied by ICI Laboratories. In Figure 22 the results are shown.

Because of the instability associated with imipenem (another carbapenem) it was considered that the stability of meropenem in urine be determined (during four hour urine collection times there could be a considerable loss in activity). Antibiotic free urine was therefore spiked with 8 mg/l of meropenem and then stored under the same conditions as those which would be used for the volunteers i.e. 'cooler bag' at approximately 4°C in the dark. There was no statistical difference between the mean concentration of meropenem before and after storage with a p value of 0.319.

Validation of the assay method used for measuring levels of meropenem in peritoneal fluid was also undertaken. In Table 25 are shown the theoretical levels of meropenem assuming 77%

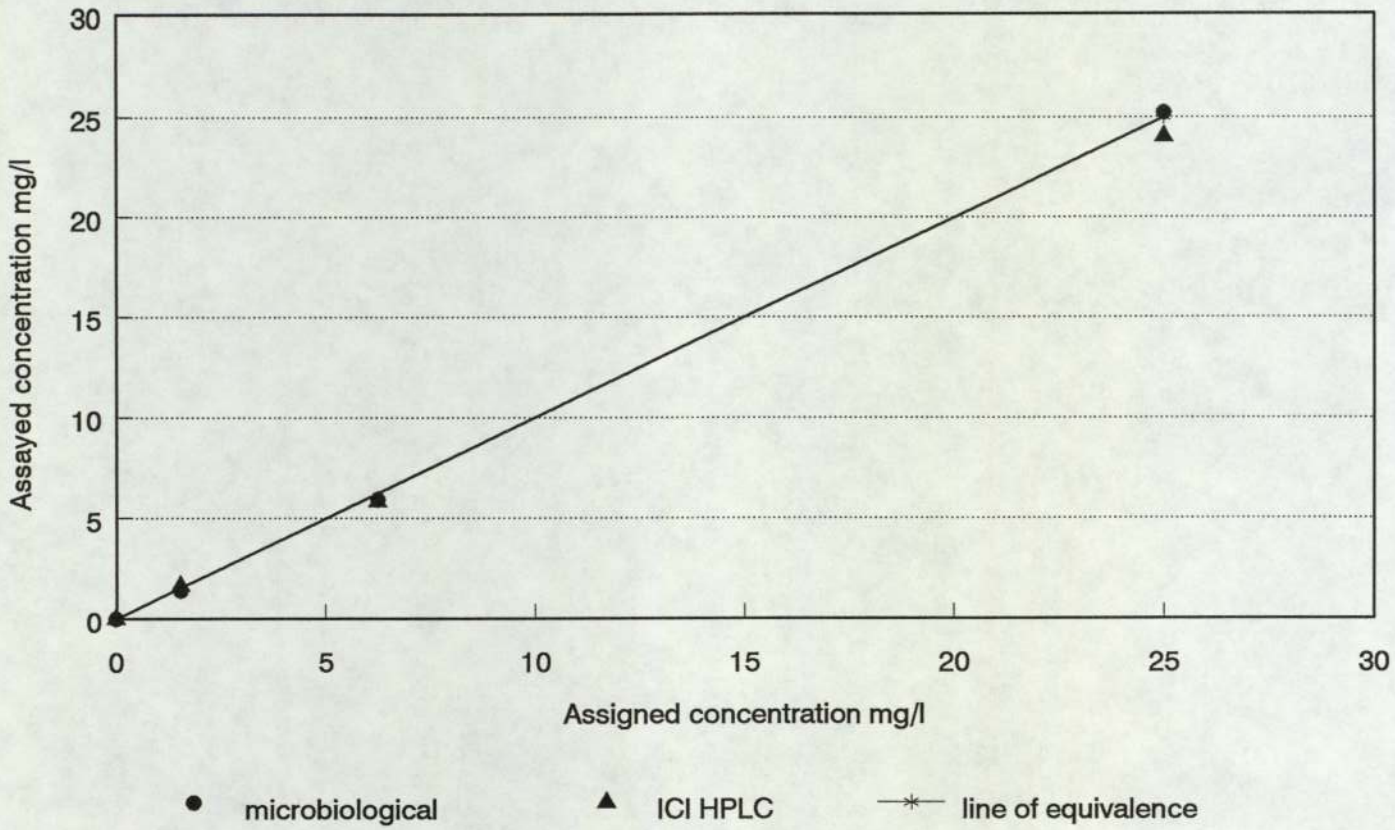



**Figure 22 : Validation of meropenem microbiological assay.**

Samples containing meropenem supplied by ICI Laboratories. Samples assayed by the microbiological assay (DRH) and by HPLC (ICI). Results obtained by both methods plotted along with a line of equivalence.

| Time (h) | Plasma level (mg/l) | Peritoneal level (mg/l) |
|---|---|---|
| 0.5 | 55 | 38.5 |
| 1.0 | 28 | 19.6 |
| 2.0 | 12 | 8.4 |
| 3.0 | 5.2 | 3.6 |
| 4.0 | 3.0 | 2.1 |
| 5.0 | 1.2 | 0.8 |
| 6.0 | 0.7 | 0.5 |
| 7.0 | 0.4 | 0.35 |

**Table 25 : Theoretical levels of meropenem in peritoneal fluid**

Assuming that the penetration of meropenem is similar to that of imipenem (ie.77 % of simultaneous plasma levels) , the concentration one could expect for meropenem were calculated.

penetration (peritoneal level expressed as a percentage of the plasma level) which would be a similar penetration to imipenem. Samples are collected by placing 6 mm blotting paper discs in the peritoneum to absorb peritoneal fluid and the level in peritoneal fluid calculated using the formula shown in Table 26. Four concentrations of meropenem 40, 20, 10 and 5 mg/l were assayed using varying volumes pipetted into 6 mm blotting paper discs i.e. 10, 15, 20 µl (chosen because these have been the volumes recorded in previous studies). Results of assigned concentration versus assayed concentration are shown in Figure 23.

Pharmacokinetics and penetration into inflammatory fluid study

Table 27 shows the results for the internal controls for the assay of meropenem in plasma. In Figure 24 the concentration of meropenem in plasma and inflammatory fluid are shown graphically and Tables 28 and 29 the pharmacokinetics are shown in more detail.

The mean concentration of meropenem at the first sample time (0.25h post dose) was 55.6 mg/l (range 43.7 - 66.5 mg/l) and thereafter there was

$$\frac{AC \quad X \quad VS}{VPF} = CL$$

where :

CI = corrected level mg/l

AC = assayed concentration mg/l

VS = volume of standard pipetted onto disc

VPF = volume of peritoneal fluid absorbed by disc



**Table 26 : Calculation of level of antimicrobial in peritoneal fluid**

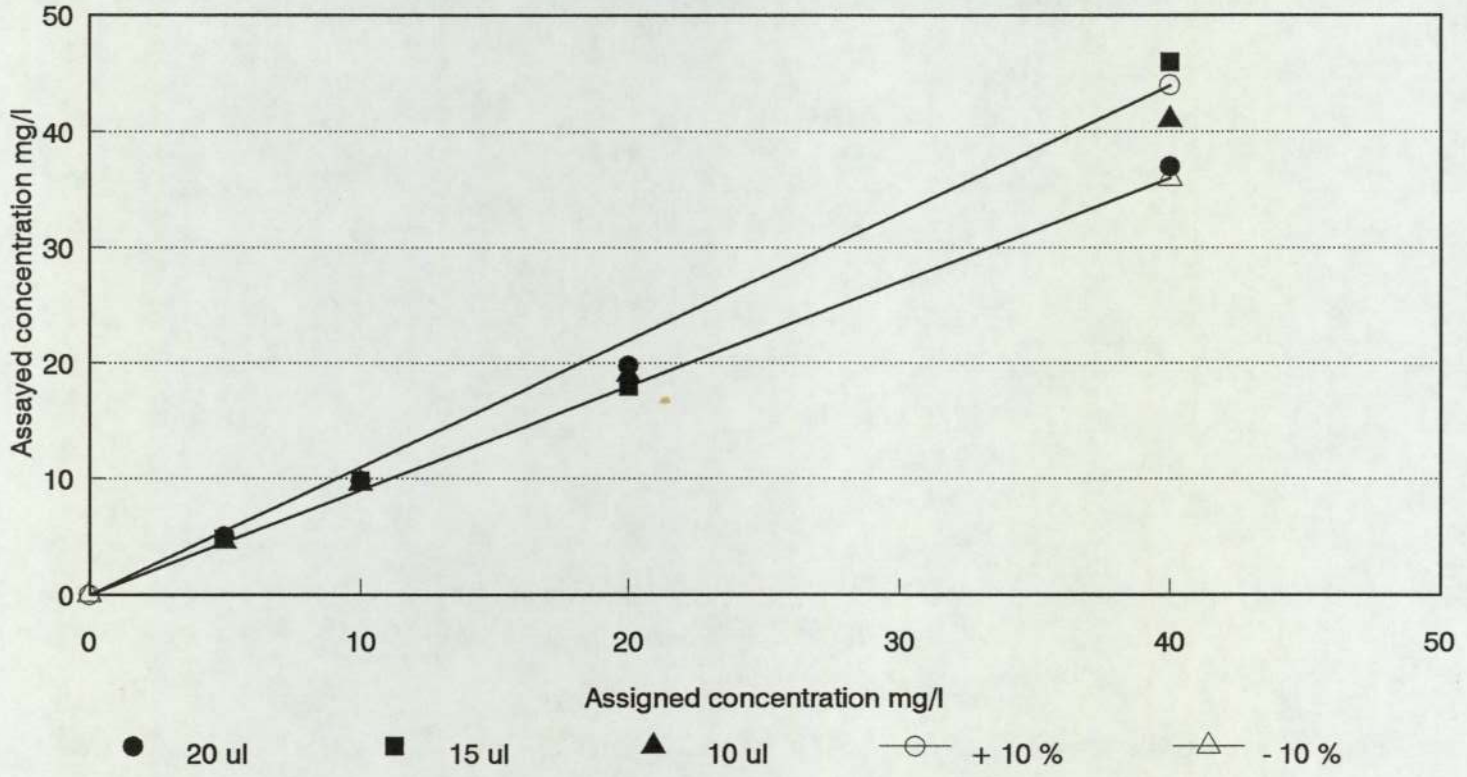



**Figure 23 : Peritoneal penetration of meropenem - validation of assay**

Standard material prepared in pH 7 phosphate buffer containing 20% human serum. Concentrations of meropenem equivalent to 5 , 10 , 20 and 40 mg/l prepared in the same medium. 10 , 15 and 20 ul of each of the concentrations pipetted onto 6 mm discs and then the disc assayed. Assayed concentration plotted versus assigned concentration (mg/l).

| | Assigned concentration mg/l | | |
|---|---|---|---|
| | 0.4 | 3.0 | 20 |
| Total | 11 | 30 | 24 |
| Mean | 0.41 | 3.07 | 20.33 |
| SD | 0.026 | 0.21 | 1.21 |
| CV | 6.3 | 6.8 | 5.95 |



**Table 27 : Confidence limits of the microbiological assay for measuring concentrations of meropenem in clinical samples.**

Assayed results of internal controls prepared for the volunteer study. Mean , standard deviation and coefficient of variation calculated for each concentration.

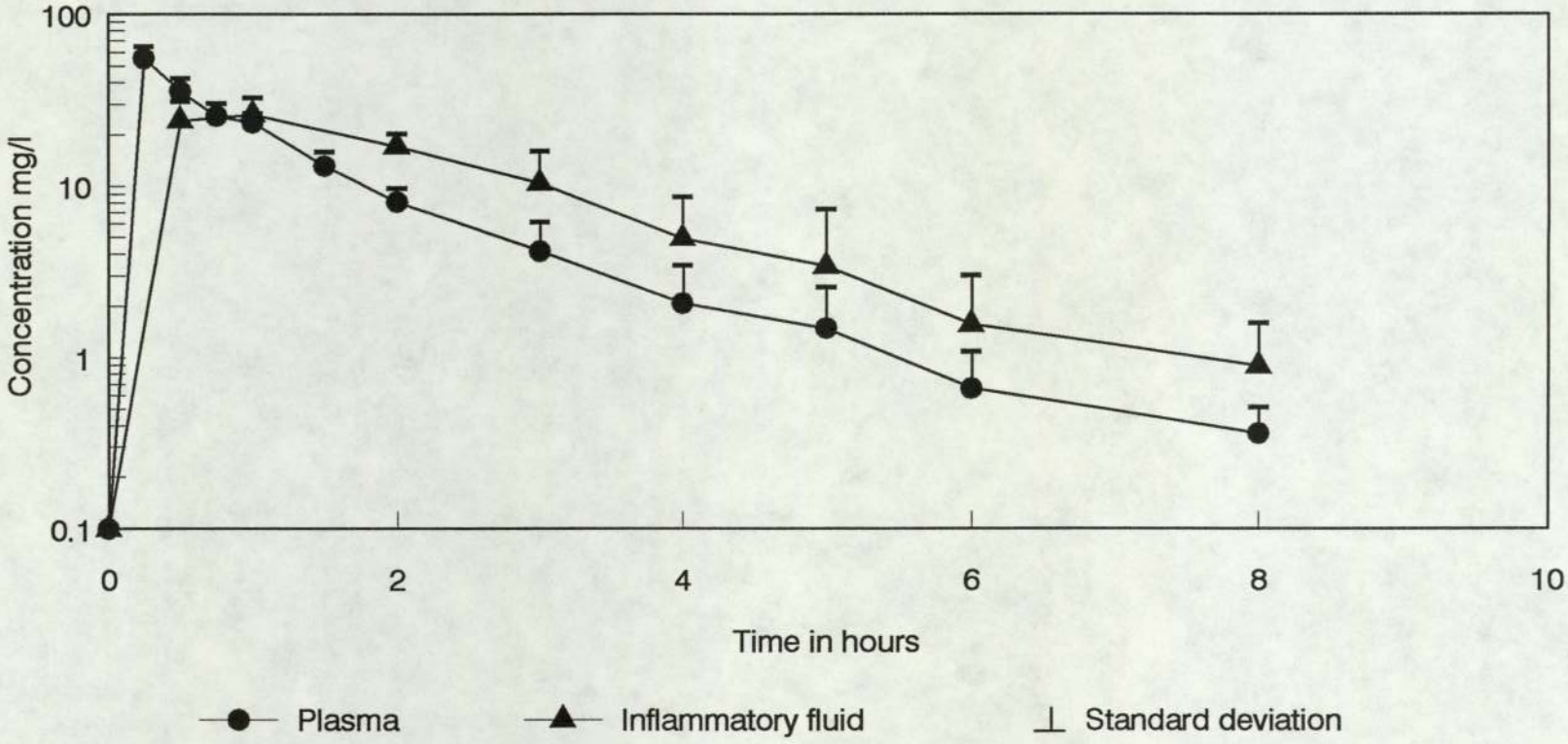



**Figure 24 : Plasma and inflammatory fluid levels of meropenem**

Data derived from volunteers following a single 1 g intravenous dose of meropenem. Concentration (mg/l) plotted against time (timed from the end of the infusion).

| Plasma : | | |
|---|---|---|
| C max (mg/l) (@ 0.25 h) | 55.6 | range 43.7 - 66.7 |
| T/2 b (h) | 1.1 | (0.2) |
| Clearance total (ml/min) | 253 | (51.5) |
| Clearance renal (ml/min) | 181 | (46.5) |
| AUC (mg/l h) | 66.9 | (13.7) |
| Vd ss (l) | 20.6 | (5.9) |

( ) = Standard deviation

**Table 28 : Pharmacokinetics of meropenem**

Data derived from six volunteers following a single 1 g intravenous dose of meropenem.

| | | |
|---|---|---|
| Inflammatory fluid : | | |
| | | (SD) |
| C max (mg/l) | 28.3 | (5.0) |
| T max (h) | 0.75 | (0.3) |
| T/2 b (h) | 1.1 | (0.4) |
| AUC (mg/l h) | 73.4 | (16.1) |
| Penetration (%) | 110.7 | (15.6) |
| Urinary recovery 65.4 (% in 24 h) | | (8.8) |

(SD) = standard deviation

## Table 29 : Pharmacokinetics of meropenem

Data derived from volunteers following a single 1 g dose of meropenem.

a log linear decline in plasma concentration with time to a mean of 0.7 mg/l at 6 h. At 12 h all levels were below the lower limit of sensitivity of the assay. The mean plasma elimination half-life of meropenem was 1.1 h and as the standard deviation was small it would appear that there is high reproducibility between volunteers. After examination of individual data it would appear that distribution of meropenem was essentially complete at 1h post dose when the mean plasma level was 23.6 mg/l.

Meropenem penetrated rapidly into inflammatory fluid with a peak concentration of 28.3 mg/l at approximately 0.75 h. Levels in inflammatory fluid paralleled those found in plasma and the mean elimination half-life of 1.1 h was the same as that found in plasma. The mean percentage penetration of meropenem into inflammatory fluid was 110.7% (range 88 - 130.4%) and in all volunteers the levels in inflammatory fluid exceeded those found in plasma by 1 h. Levels in inflammatory fluid at 6 h were approximately double those found in plasma (range 0.14 - 2.13 mg/l). The mean volume of distribution of meropenem at steady state was 20.6 l.

| Assigned concentration mg/l | Total | Mean | SD | CV |
|---|---|---|---|---|
| 20 | 6 | 20.94 | 1.35 | 6.4 |
| 3 | 8 | 3.12 | 0.21 | 6.7 |

**Table 30 : Confidence limits for meropenem assay**

Phosphate buffer containing known concentrations of meropenem (20 and 3 mg/l) assayed by the microbiological assay.
Mean , standard deviation and coefficient of variation calculated for both concentrations.

| Volunteer | Start | End | % difference | Trial % urine recovery | Trial date |
|---|---|---|---|---|---|
| 1 | 101.3 | 99.4 | - 2 | 57.8 | 18/10/89 |
| 2 | 82.7 | 87 | + 5 | 52.3 | 25/10/89 |
| 3 | 114.5 | 90.7 | - 21 | 43 | 18/10/89 |
| 4 | 85.2 | 73.3 | - 14 | 62.2 | 25/10/89 |
| 5 | 90.5 | 81.8 | - 10 | 46.9 | 25/10/89 |



**Table 31 : Stability of meropenem in urine.**

Pre antibiotic free urine from volunteers 1 - 5 spiked with meropenem. These samples assayed before and after placing in the `cooler bag' with the volunteer urine collections.
NB. spiked urines left in the `cooler baf' for the duration of the study.

Tables 30 and 31 show the results for the internal controls used for the assay of meropenem in urine and the stability of meropenem in pre-trial urine from each of the volunteers (spiked with meropenem). There was no statistical difference between the assayed concentration before and after storage (p value 0.152).

In Table 32 the urinary excretion for each of the volunteers is shown. The mean recovery of meropenem from the urine in 24 h was 65.4% (range 52 - 73%) of the administered dose; 62.3% of the drug was excreted in the first 4 h. The mean total clearance of meropenem from the body was 253 ml/min (range 208 - 329 ml/min) and the mean renal clearance was 182 ml/min (range 131 - 241 ml/min).

Peritoneal Study

Twenty four patients undergoing elective gastrointestinal surgery entered the study and five of the patients had two samples taken making a total of 29 time points. In Figure 25 peritoneal levels and simultaneous plasma concentrations are shown graphically.

Plasma levels are similar to those found in the pharmacokinetic study on healthy volunteers. Meropenem penetrated rapidly into the peritoneal

| | mg excreted | | | | | | |
|---|---|---|---|---|---|---|---|
| Time in hours | Vol 1 | Vol 2 | Vol 3 | Vol 4 | Vol 5 | Vol 6 | Mean |
| 0 - 4 | 605.85 | 707.7 | 699.55 | 681 | 498 | 550.71 | 623.80 |
| 4 - 8 | 39.47 | 20.95 | 27.13 | 27.11 | 20.03 | 32.5 | 27.87 |
| 8 - 12 | 5.21 | 2.1 | 3.58 | 2.38 | 0.67 | 0.15 | 2.35 |
| 12 - 24 | 1.19 | 0.71 | 0.50 | 3.65 | 1.7 | 0.96 | 1.45 |
| % excreted in 24 h | 65 | 73 | 73 | 71 | 52 | 58 | 65.33 |



**Table 32 : Urinary excretion of meropenem**

Urinary excretion following a single 1 g intravenous dose.

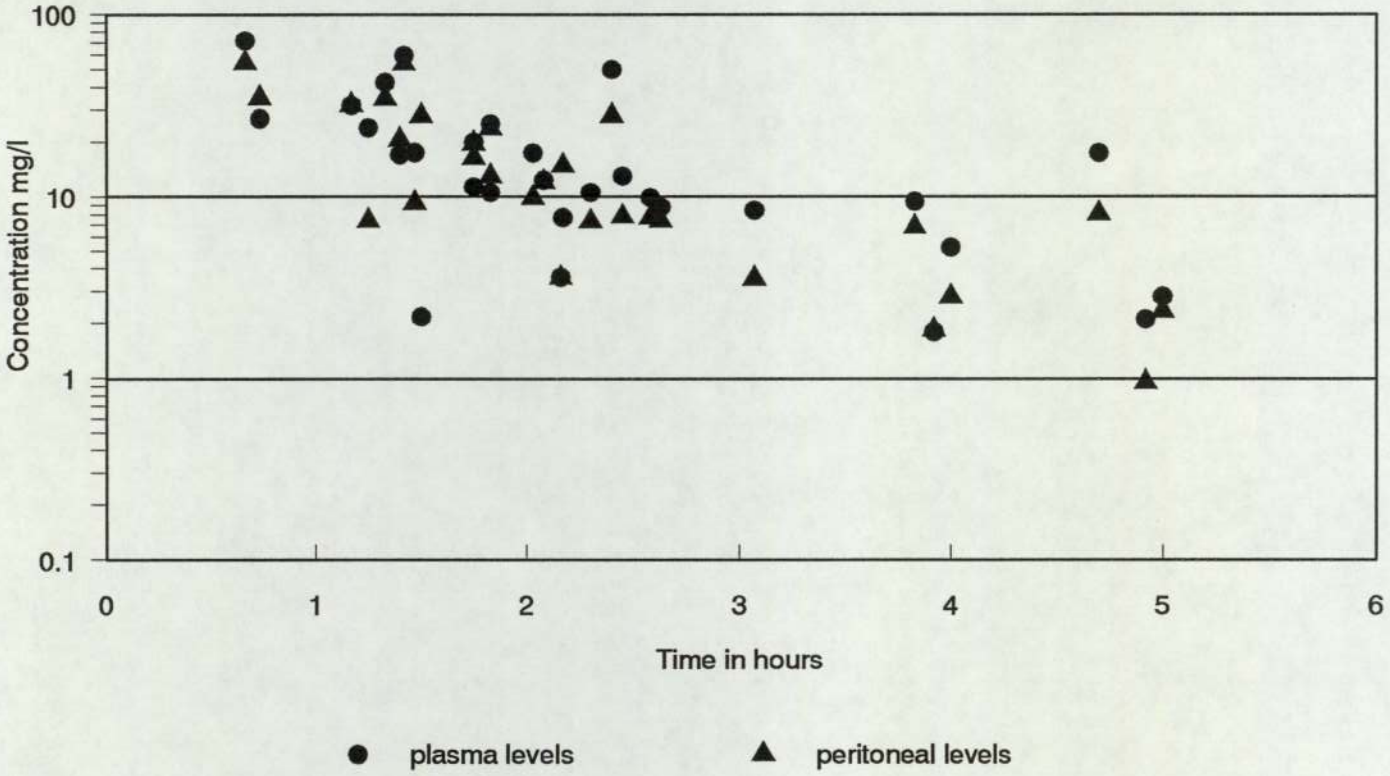



**Figure 25 : Intraperitoneal penetration of meropenem**

Peritoneal fluid and simultaneous plasma levels measured in 24 patients (5 patients at two time intervals) using a microbiological assay method. Concentration (mg/l) plotted against time.

fluid and percentage penetration (peritoneal fluid concentration expressed as a percentage of the plasma concentration) was approximately 90% over the first 4 h (range 31 - 194%). As with the plasma concentration there was a log linear decline in peritoneal fluid level with time.

**Discussion**

Meropenem like imipenem has a broad spectrum of activity and the results compare with those found by other workers (54, 55). Meropenem appears to be unaffected by common plasmid-mediated and class 1 ß-lactamases and also appears to be stable to the ß-lactamases produced by Bact. fragilis.

Meropenem displayed good activity against Staphylococci and although MIC values for methicillin resistant Staphylococci are exceeded in plasma, like imipenem the use of meropenem against these strains is controversial (56, 57, 58). It may be prudent therefore, to use meropenem with caution for infections attributed to these strains.

The pharmacokinetics of meropenem are broadly in agreement with those already published (21). However, one exception is the urinary recovery. Bax et al found a mean urinary recovery of 79% in

comparison with this study with a mean of 65.4%. The reason for this slight difference in recovery could not be attributed to instability of meropenem in urine (Table 31). However, the more likely explanation is a reflection of the dilutional inaccuracies in the 0 to 4 h urine when most of the drug is eliminated.

There appear to be two major differences in the pharmacokinetics of meropenem and imipenem, firstly, higher urinary recovery of meropenem. In similar studies (59) even after co-administration of cilastatin, a DHP-1 inhibitor, recovery of meropenem was greater (imipenem urinary recovery with and without cilastatin, 14.7 and 55.6%, respectively). The second difference is the more efficient penetration of meropenem into tissue. When percentage penetrations are compared, even with co-administration of the inhibitor, the penetration of imipenem into inflammatory fluid is somewhat less than meropenem (without inhibitor 67.8, with inhibitor 73.2%). Similarly, in the peritoneal study a peritoneal penetration of 90% for meropenem compared with 77% for imipenem (60). Obviously crossover studies should be performed to support these observations. However, the

suggestion that meropenem penetrates tissues more efficiently is further supported by the finding that the volume of distribution of meropenem (20.6 l) is somewhat higher than for other ß-lactams, for example, imipenem 16.7 l (61) and cefixime 13.6 l (62).

The rapidity of penetration of meropenem into both inflammatory exudate and peritoneal fluid is noteworthy and it would be expected that in the inflamed peritoneum levels would be higher than those observed in this study were the peritoneum non-inflamed.

Meropenem was found to have a high level of activity against the majority of Enterobacteriaceae, methicillin sensitive Staphylococci and Bact. fragilis, with $MIC_{90}$s of less than or equivalent to 0.25 mg/l. These levels are exceeded in plasma, inflammatory fluid and peritoneal fluid for at least 6 - 8 h. However, for Ps. aeruginosa and E. faecalis these levels are only achieved for 4 - 6 h.

These data suggest that a twice daily dosing of 1 g of meropenem might be sufficient to treat most pathogens and would also be suitable for the prophylaxis and treatment of intra-abdominal

infections.

4. **FCE 22101**

In vitro activity

In Table 33a, b, c, d the results for 429 isolates are summarised at an inoculum of $10^4$CFU. Overall FCE 22101 was not the most active ß-lactam tested, but it still displayed a high degree of activity and in most cases activity was comparable to imipenem, ceftriaxone, ceftazidime and moxalactam. Against Pr. mirabilis, the indole positive Proteus, and Prov. stuartii FCE 22101 was equal in activity to imipenem, however, less active than either ceftriaxone or moxalactam. Against Ps. aeruginosa FCE 22101 could be considered to have no useful activity.

In Table 34 the activity of all the agents tested against well characterised ß-lactamase producing strains is shown. Cefuroxime was not active against Group I and IV strains and ceftriaxone was not active against Gp I. The other agents were active against all strains and increasing the inoculum from $10^4$ to $10^6$ CFU did not affect activity suggesting that there was minimal ß-lactamase hydrolysis occurring.

| Organism(No.tested) | Antibiotic | MIC mg/l Range | 50% | 90% |
|---|---|---|---|---|
| Ps.aeruginosa (48) | FCE 22101 | 0.5 - >128 | 128 | >128 |
| | Ceftriaxone | 1 - 64 | 4 | 32 |
| | Moxalactam | 0.06 - 64 | 8 | 32 |
| | Imipenem | 0.25 - 8 | 1 | 4 |
| | Cefuroxime | 8 - >128 | >128 | >128 |
| | Ceftazidime | 0.06 - 4 | 1 | 32 |
| St.aureus (30) | FCE 22101 | 0.06 - 2 | 0.12 | 0.5 |
| (including 10 methicillin | Ceftriaxone | 2 - >128 | 16 | 32 |
| resistant isolates) | Moxalactam | 4 - 64 | 16 | 64 |
| | Imipenem | 0.06 - 1 | 0.06 | 0.12 |
| | Cefuroxime | 0.5 - >128 | 4 | 32 |
| | Ceftazidime | 8 - 64 | 16 | 64 |
| St.pneumoniae (17) | FCE 22101 | 0.015 - 0.25 | 0.03 | 0.25 |
| | Ceftriaxone | ≤0.008 - 0.06 | ≤0.008 | 0.06 |
| | Moxalactam | 1 - 8 | 1 | 8 |
| | Imipenem | ≤0.008 - 0.06 | ≤0.008 | 0.06 |
| | Cefuroxime | ≤0.008 - 0.5 | 0.015 | 0.25 |
| | Ceftazidime | 0.06 - 2 | 0.12 | 2 |
| H.influenzae (34) | FCE 22101 | 0.25 - 1 | 1 | 1 |
| (including 11 ß lactamase | Ceftriaxone | ≤0.008 - 0.06 | ≤0.008 | 0.6 |
| positive isolates) | Moxalactam | 0.03 - 2 | 0.06 | 2 |
| | Imipenem | 4 - 16 | 8 | 16 |
| | Cefuroxime | 0.5 - 16 | 1 | 8 |
| | Ceftazidime | 0.03 - 1 | 0.12 | 1 |

**Table 33A : In vitro activity of FCE 22101 compared with other ß lactams**

Minimum inhibitory concentrations (MIC) determined for FCE 22101 and comparator antibiotics against clinical isolates.Results expressed as a range of activity and also as the concentration which will inhibit 50% (MIC 50) and 90% (MIC 90) of strains.

| Organism(No.tested) | Antibiotic | MIC mg/l Range | 50% | 90% |
|---|---|---|---|---|
| Indole +ve Proteus (46) | FCE 22101 | 1 - 4 | 2 | 4 |
| (including 25 P.vulgaris | Ceftriaxone | <0.015 - 1 | 0.015 | 0.25 |
| 18 M.morgannni | Moxalactam | 0.06 - 16 | 0.12 | 4 |
| 3 P.rettgeri) | Imipenem | 0.25 - 16 | 2 | 4 |
| | Cefuroxime | 1 - >128 | 32 | >128 |
| | Ceftazidime | 0.03 - 2 | 0.03 | 0.25 |
| Serratia species (17) | FCE 22101 | 1 - 8 | 2 | 4 |
| (including 15 S.marcescens | Ceftriaxone | <0.015 - 1 | 0.12 | 0.25 |
| 2 S.liquefaciens) | Moxalactam | 0.06 - 4 | 0.25 | 0.25 |
| | Imipenem | 0.12 - 4 | 0.25 | 4 |
| | Cefuroxime | 4 - >128 | >64 | >128 |
| | Ceftazidime | 0.5 - 8 | 0.12 | 0.25 |
| Prov.stuartii (18) | FCE 22101 | 0.5 - 2 | 1 | 2 |
| | Ceftriaxone | 0.015 - 0.12 | 0.06 | 0.12 |
| | Moxalactam | 0.03 - 0.25 | 0.06 | 0.12 |
| | Imipenem | 0.12 - 2 | 1 | 2 |
| | Cefuroxime | 0.25 - 64 | 1 | 16 |
| | Ceftazidime | 0.06 - 1 | 0.12 | 0.5 |
| Acinetobacter (8) | FCE 22101 | 0.12 - 2 | 1 | 2 |
| | Ceftriaxone | 0.5 - 16 | 8 | 16 |
| | Moxalactam | 2 - 32 | 32 | 32 |
| | Imipenem | 0.06 - 0.25 | 0.12 | 0.25 |
| | Cefuroxime | 1 - 32 | 32 | 32 |
| | Ceftazidime | 1 - 16 | 4 | 16 |

**Table 33B : In vitro activity of FCE 22101 compared with other ß lactams**
(continued)

| Organism(No.tested) | Antibiotic | MIC mg/l Range | 50% | 90% |
|---|---|---|---|---|
| Esch.coli (50) | FCE 22101 | 0.25 - 1 | 0.5 | 0.5 |
| | Ceftriaxone | <0.015 - 8 | 0.03 | 0.25 |
| | Moxalactam | 0.03 - 16 | 0.06 | 0.25 |
| | Imipenem | 0.06 - 1 | 0.12 | 0.5 |
| | Cefuroxime | 0.25 - 32 | 4 | 8 |
| | Ceftazidime | 0.03 - >128 | 0.12 | 4 |
| Klebsiella species (50) | FCE 22101 | 0.5 - 1 | 0.5 | 1 |
| | Ceftriaxone | 0.015 - 4 | 0.06 | 0.5 |
| | Moxalactam | 0.03 - 1 | 0.06 | 0.12 |
| | Imipenem | 0.12 - 1 | 0.25 | 0.25 |
| | Cefuroxime | 0.5 - 128 | 2 | 16 |
| | Ceftazidime | <0.015 - 4 | 0.06 | 0.25 |
| Enterobacter species (10) | FCE 22101 | 0.5 - 8 | 2 | 4 |
| (including 4 E.aerogenes | Ceftriaxone | 0.12 - 16 | 0.12 | 2 |
| & 6 E.cloacae) | Moxalactam | 0.06 - 4 | 0.12 | 1 |
| | Imipenem | 0.12 - 1 | 0.25 | 0.5 |
| | Cefuroxime | 4 - 128 | 8 | 16 |
| | Ceftazidime | 0.12 - 8 | 0.25 | 0.5 |
| Pr.mirabilis (50) | FCE 22101 | 1 - 4 | 1 | 2 |
| | Ceftriaxone | <0.015 - 0.12 | <0.015 | <0.015 |
| | Moxalactam | 0.06 - 0.12 | 0.06 | 0.06 |
| | Imipenem | 0.12 - 8 | 2 | 4 |
| | Cefuroxime | 1 - >128 | 0.5 | 4 |
| | Ceftazidime | 0.03 - 0.12 | 0.03 | 0.06 |

**Table 33C : In vitro activity of FCE 22101 compared with other beta lactams.**

Activity of assessed at $10^4$CFU. Results expressed as a range of activity and that concentration which will inhibit 50 and 90 % of strains (mg/l).

| Organism(No.tested) | Antibiotic | MIC mg/l Range | 50% | 90% |
|---|---|---|---|---|
| N.gonorrhoeae (23) | FCE 22101 | ≤0.008 - 0.5 | 0.06 | 0.25 |
| (including 11 ß lactamase | Ceftriaxone | ≤0.008 | ≤0.008 | ≤0.008 |
| positive isolates) | Moxalactam | ≤0.008 - 0.12 | 0.03 | 0.06 |
| | Imipenem | ≤0.008 - 2 | 0.06 | 0.25 |
| | Cefuroxime | ≤0.008 - 0.12 | 0.015 | 0.12 |
| | Ceftazidime | ≤0.008 - 0.06 | ≤0.008 | 0.06 |
| Bacteroides spps.(29) | FCE 22101 | 0.015 - 1 | 0.03 | 0.5 |
| | Ceftriaxone | 0.5 - >128 | 8 | >128 |
| | Moxalactam | 0.5 - >128 | 4 | 64 |
| | Imipenem | 0.06 - 8 | 0.5 | 1 |
| | Cefuroxime | 1 - >128 | 8 | 64 |
| | Ceftazidime | 2 - >128 | 8 | >128 |

**Table 33D : In vitro activity of FCE 22101 compared with other ß lactams**
(continued)

| | | | MIC (mg/l) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | FCE 22101 | | Ceftriaxone | | Moxalactam | | Imipenem | | Cefuroxime | | Ceftazidime | |
| Organism | ß lactamase type | Group | $10^4$ | $10^6$ | $10^4$ | $10^6$ | $10^4$ | $10^6$ | $10^4$ | $10^6$ | $10^4$ | $10^6$ | $10^4$ | $10^6$ |
| E.cloacae | P99+ | 1 | 2 | 2 | 16 | 32 | 4 | 4 | 0.25 | 0.25 | >128 | >128 | 8 | 16 |
| Esch.coli | D31 | 1 | 1 | 1 | 0.25 | 0.25 | 0.25 | 0.25 | 0.12 | 0.25 | 32 | 64 | 2 | 4 |
| Esch.coli | TEM-1 | 111 | 0.5 | 0.5 | 0.06 | 0.06 | 0.06 | 0.06 | 0.5 | 0.5 | 4 | 4 | 0.25 | 0.25 |
| Esch.coli | TEM-2 | 111 | 0.5 | 1 | 0.06 | 0.06 | 0.12 | 0.12 | 0.25 | 0.5 | 2 | 4 | 0.12 | 0.25 |
| K.pneumoniae | SHV-1 | 111 | 0.5 | 0.5 | 0.03 | 0.03 | 0.06 | 0.06 | 0.25 | 0.25 | 4 | 4 | 0.12 | 0.12 |
| K.aerogenes | K1 + | 1V | 0.5 | 1 | 4 | 16 | 0.06 | 0.06 | 0.25 | 1 | 128 | >128 | 0.12 | 0.12 |
| E.cloacae | Broad spectrum | 1V | 2 | 2 | 2 | 2 | 0.12 | 0.25 | 0.5 | 0.5 | >128 | >128 | 8 | 16 |
| Esch.coli | OXA -1 | V | 0.5 | 1 | 0.06 | 0.06 | 0.25 | 0.25 | 0.25 | 0.5 | 8 | 8 | 0.12 | 0.25 |
| Esch.coli | OXA -3 | V | 0.5 | 0.5 | 0.03 | 0.03 | 0.06 | 0.06 | 0.25 | 0.5 | 2 | 4 | 0.12 | 0.25 |

**Table 34 : Activity of FCE 22101 and other agents against characterised beta lactamase producing strains.**

Activity tested at two inocula and results expressed as an MIC (mg/l)

Against S. aureus the activity of FCE 22101 was similar to that of imipenem. Mode MICs for methicillin resistant Staphylococci were similar (0.25 mg/l) to those for methicillin sensitive strains (0.12 mg/l). However, as with imipenem, FCE 22101 should be used with caution against these strains (see section on meropenem).

Except for moxalactam all compounds tested were highly active against Strep. pneumoniae. One strain having a reduced susceptibility to penicillin (MIC 0.12 mg/l) was susceptible to 0.25 mg/l FCE 22101, 0.03 mg/l imipenem and 2 mg/l ceftazidime. FCE 22101 displayed good activity against other streptococci including E. faecalis (Table 35).

All strains of H. influenzae including ß-lactamase producing strains (11 strains) were susceptible to FCE 22101. Also included in the strains tested were five strains with reduced MICs to ampicillin (MIC 0.5 mg/l). The exact mechanism of resistance was not known however, it could not be attributed to ß-lactamase production as all strains when tested with nitrocefin were ß-lactamase negative. These strains were all susceptible to 0.5 - 1 mg/l FCE 22101, whereas

| Organism (No. tested) | MIC range (mg/l) |
|---|---|
| Gp A streptococci (5) | 0.06 - 0.12 |
| Gp B streptococci | 0.25 |
| Ent.faecalis (10) | 1 - 4 |



## Table 35 : Activity of FCE 22101 against streptococci

Minimum inhibitory concentrations of FCE 22101 determined at an inoculum of $10^4$ organisms / ml against clinical isolates. Results expressed as a range of activity in mg/l.

they showed a decrease in susceptibility to moxalactam (MIC 0.5 - 2 mg/l), ceftriaxone (MIC 0.04 - 0.06 mg/l), ceftazidime (MIC 0.5 - 1 mg/l) and cefuroxime (MIC 1 - 16 mg/l). Imipenem, however, was active against these strains.

Ceftriaxone was the most active agent against the strains of N. gonorrhoeae tested. FCE 22101 was as active as imipenem and cefuroxime. The fourteen ß-lactamase producing strains included for testing were equally as susceptible as the ß-lactamase negative strains.

Of the 29 Bacteroides tested, 25 were Bact. fragilis, 2 were Bacteroides thetaotamicron and one strain each of Bacteroides ovatus and Bacteroides vulgatus. All strains were highly susceptible to FCE 22101. One strain of B. thetaiotaomicron known to be resistant to cefoxitin with an MIC of 32 mg/l was resistant to all agents (MIC>128 mg/l) except for imipenem and FCE 22101 having MICs of 8 and 0.5 mg/l respectively.

In Table 36 the effect of serum on the activity of FCE 22101 is shown. There was very little difference between MICs and MBCs in the presence or absence of serum. For two strains however, in

| Organism | 0 % human serum | | 20 % human serum | | 70 % human serum | |
|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC |
| Esch.coli | 1 | 4 | 1 | 2 | 1 | 4 |
| Esch.coli | 1 | 2 | 1 | 2 | 1 | 2 |
| Kleb.pneumoniae | 1 | 2 | 1 | 2 | 1 | 2 |
| Kleb.pneumoniae | 1 | 2 | 1 | 4 | 1 | 2 |
| Pr.mirabilis | 2 | > 16 | 4 | > 16 | 4 | > 16 |
| Pr.mirabilis | 4 | 8 | 4 | > 16 | 4 | 4 |
| St.aureus | 0.06 | 2 | 0.12 | 1 | 0.25 | 1 |
| St.aureus (methicillin resistant) | 0.5 | 2 | 0.5 | 2 | 0.5 | 2 |
| Ent.faecalis | 2 | 8 | 4 | 4 | 4 | 4 |
| Ent.faecalis | 4 | 16 | 4 | 8 | 4 | 8 |

**Table 36 : Effect of human serum on the activity of FCE 22101**

Minimum inhibitory (MIC) and cidal concentrations (MBC) determined against ten isolates at an inoculum of $10^5$ organisms/ ml in Iso Sensitest broth containing 0 , 20 and 70% pooled human serum

the presence and absence of serum a marked difference in MIC and MBC was noted. One strain of methicillin sensitive S. aureus when grown in the absence of serum there was a 32-fold difference and for one strain of Pr. mirabilis, when there was a >8-fold difference.

Assay validation

In the developmental stage the stability of FCE 22101 in plasma and urine had been questioned and in the handling information supplied by the drug company it was recommended that samples be stabilised immediately in buffer. This procedure is often difficult to carry out when volunteer studies are undertaken and therefore it was not only considered necessary to validate the assay but also to determine the stability of FCE 22101 in plasma and urine stored at +4°C.

In Figure 26 the results for 23 patients assayed by HPLC (Southmead Hospital) and the microbiological assay (Dudley Road Hospital). It was felt that the good correlation (0.99) obtained justified the use of the assay.

In Table 37 the results for a plasma sample stored at +4°C over a seven hour period and assayed at hourly intervals are shown. There was

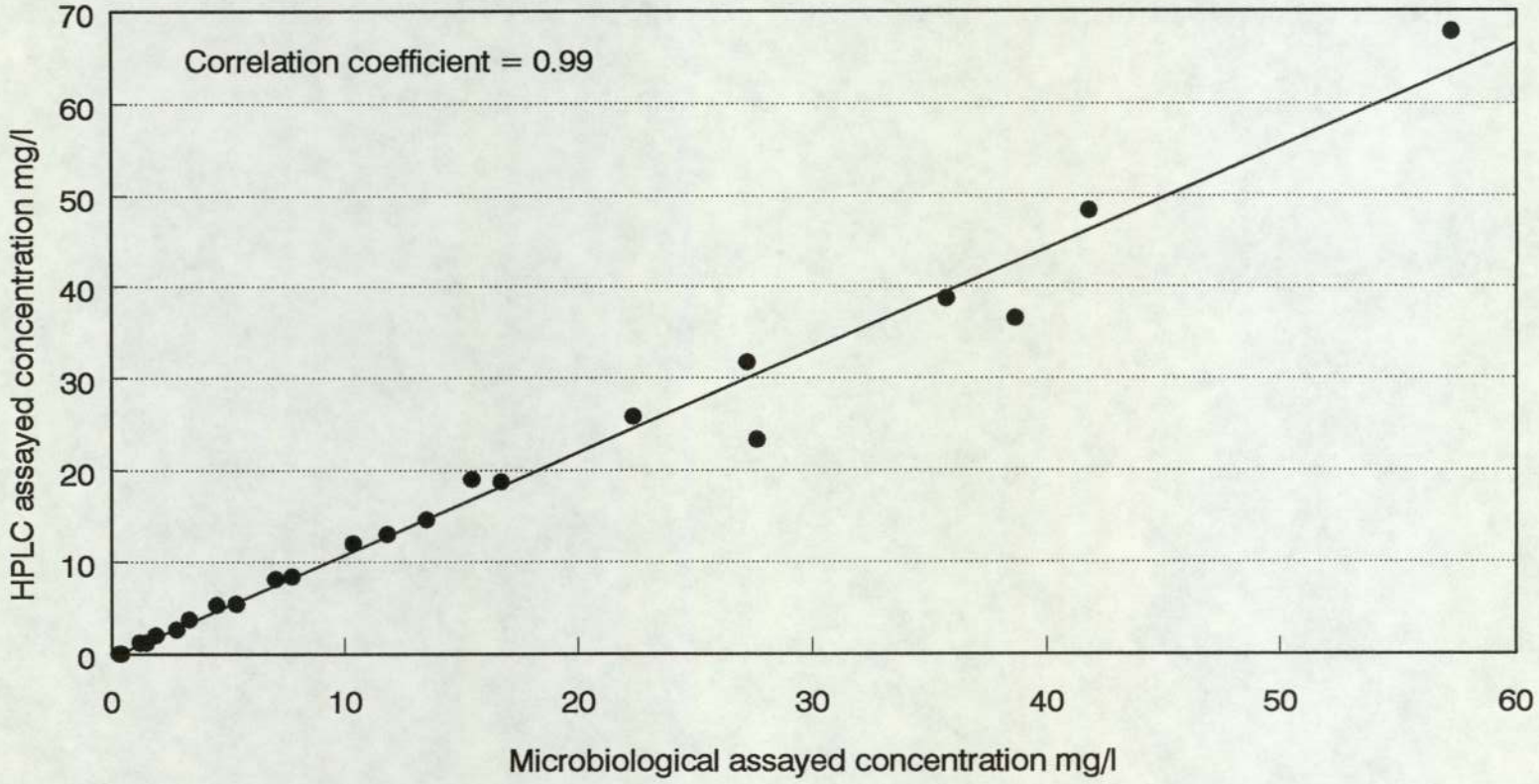



**Figure 26 : FCE 22101 external validation**

Serum samples assayed by the microbiological assay method (DRH) and by HPLC (Southmead Hospital). Values obtained for the microbiological assay plotted against the levels obtained by HPLC.

| Assigned concentration time 0 | Time interval after storage at 4°C | Assayed concentration mg/l |
|---|---|---|
| 6.35 | 1 h | 6.05 |
| | 2 h | 7.1 |
| | 4 h | 6.5 |
| | 4 h | 4.85 |
| | 5 h | 5.4 |
| | 6 h | 6.45 |
| | 7 h | 5.85 |

p = 0.299 (assigned concentration compared with 7 assayed concentrations)

**Table 37 : Determination of the stability of FCE 22101 in plasma**

Human plasma containing 6.35 mg/l FCE 22101 at time zero assayed at hourly intervals for 7 hours.

no statistical difference between the assigned concentration (to) and the assayed concentrations of the seven hour period with a p value of 0.299. Similarly it was found that greater than 90% of drug was recovered from urine samples spiked with 50 and 200 mg/l FCE 22101 stored for 4 h at +4°C. It was therefore felt unnecessary to stabilise the samples immediately with MOPs buffer.

In Figures 27 and 28 the results are shown graphically when samples from two volunteers, assayed by HPLC and the microbiological assay method, are compared. In both the samples from the IC study and the oral study there was no statistical difference in results obtained ($p > 0.5$ and $0.5 - 0.1$, respectively).

The mean serum protein binding of FCE 22101 in human serum was 40.6% and varied little with the concentration studied; from 40% at 5 mg/l to 35.5% for 200 mg/l.

Pharmacokinetic study

Mean plasma and inflammatory fluid levels are shown in Figures 29 and 30 and the derived pharmacokinetic data in Table 38. Because of an adverse event analysis is given for five volunteers only (slightly raised serum creatinine

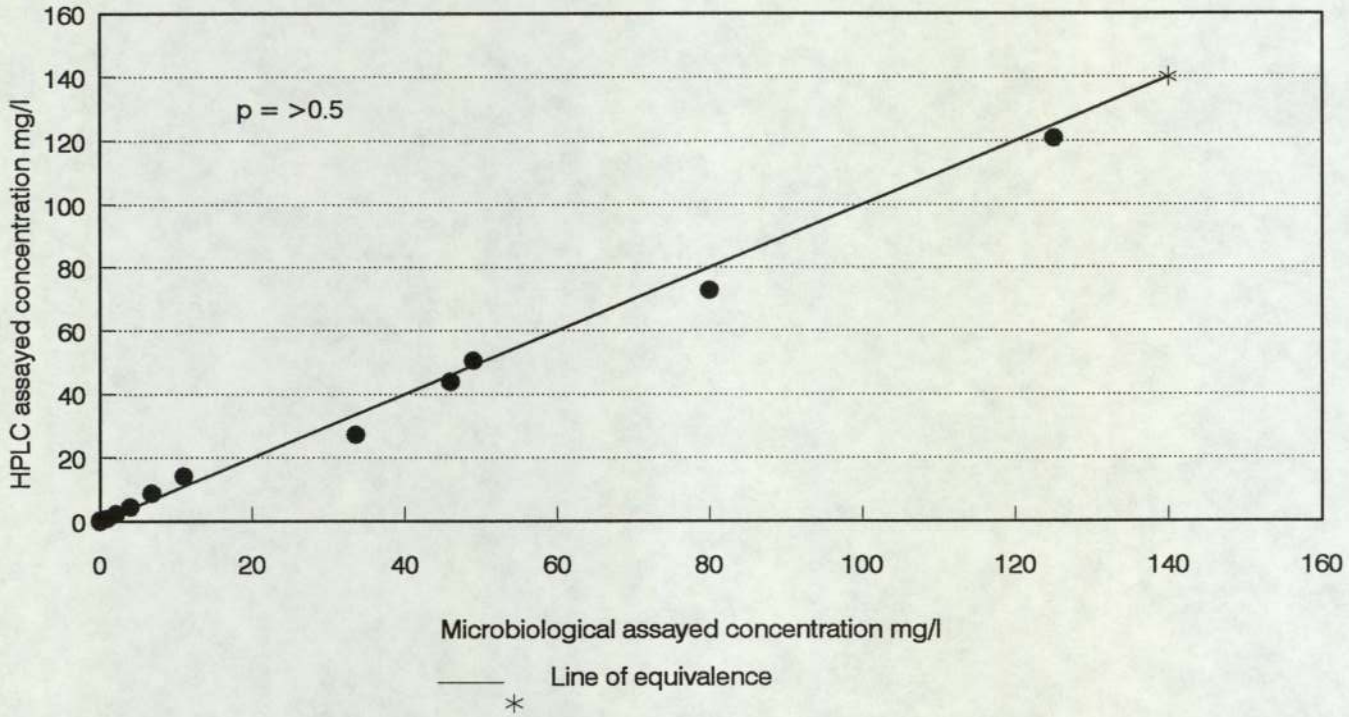



**Figure 27 : Comparison of HPLC and microbiological assay methods for the assay of FCE 22101**

Eleven serum samples assayed by both methods. Data points plotted from results obtained by both methods.

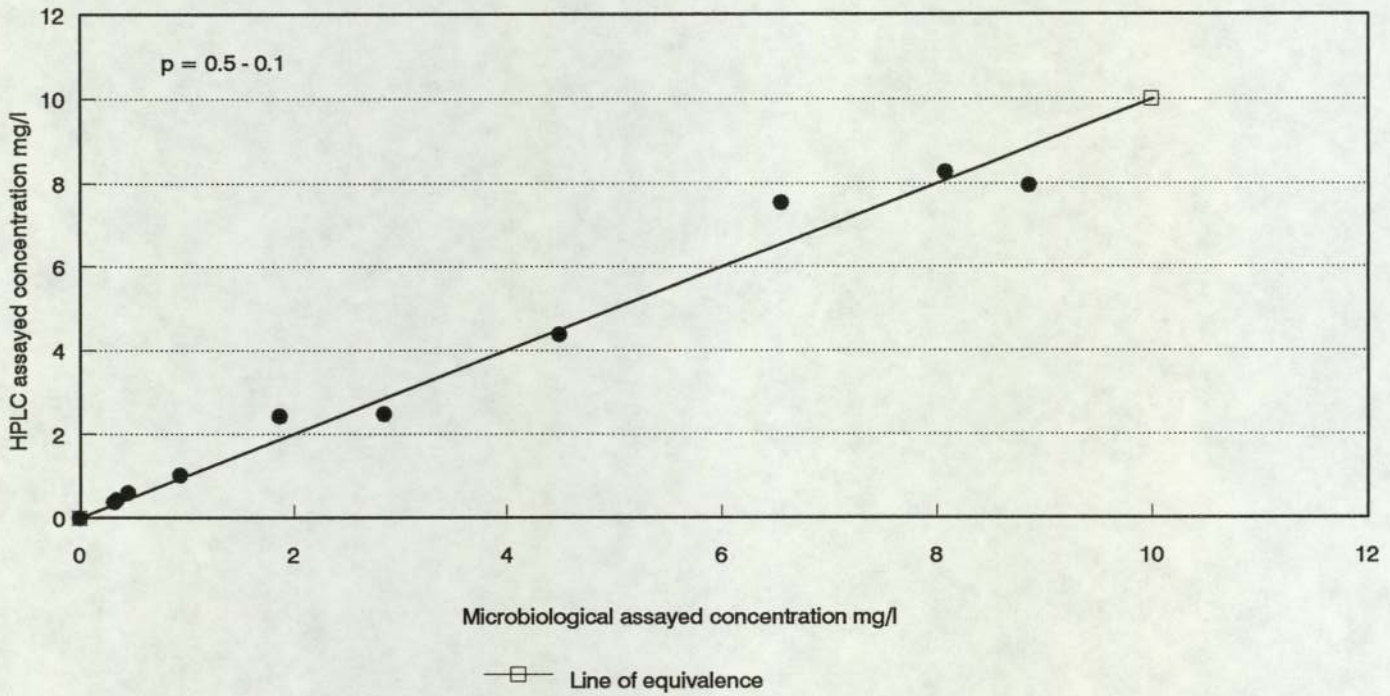



**Figure 28 : Comparison of HPLC and microbiological methods for the assay of FCE 22101**

Ten samples obtained after oral administration of drug assayed by both methods. Data points plotted from results obtained.

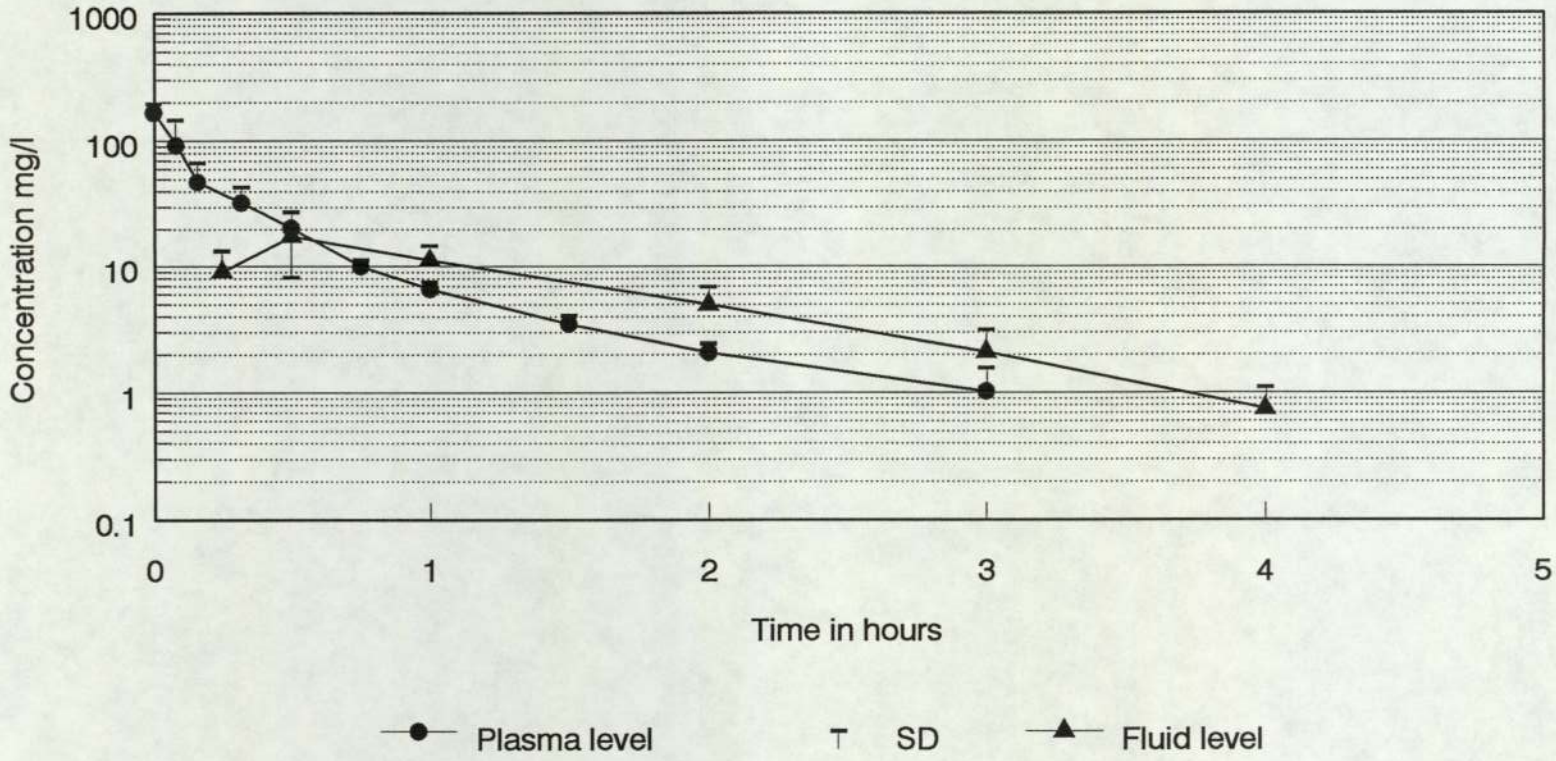



**Figure 29 : Mean plasma and inflammatory fluid concentrations of FCE 22101 following a single 1 g intravenous dose.**

Data obtained for six volunteers. Concentration in mg/l plotted against time (timed from the end of the infusion).

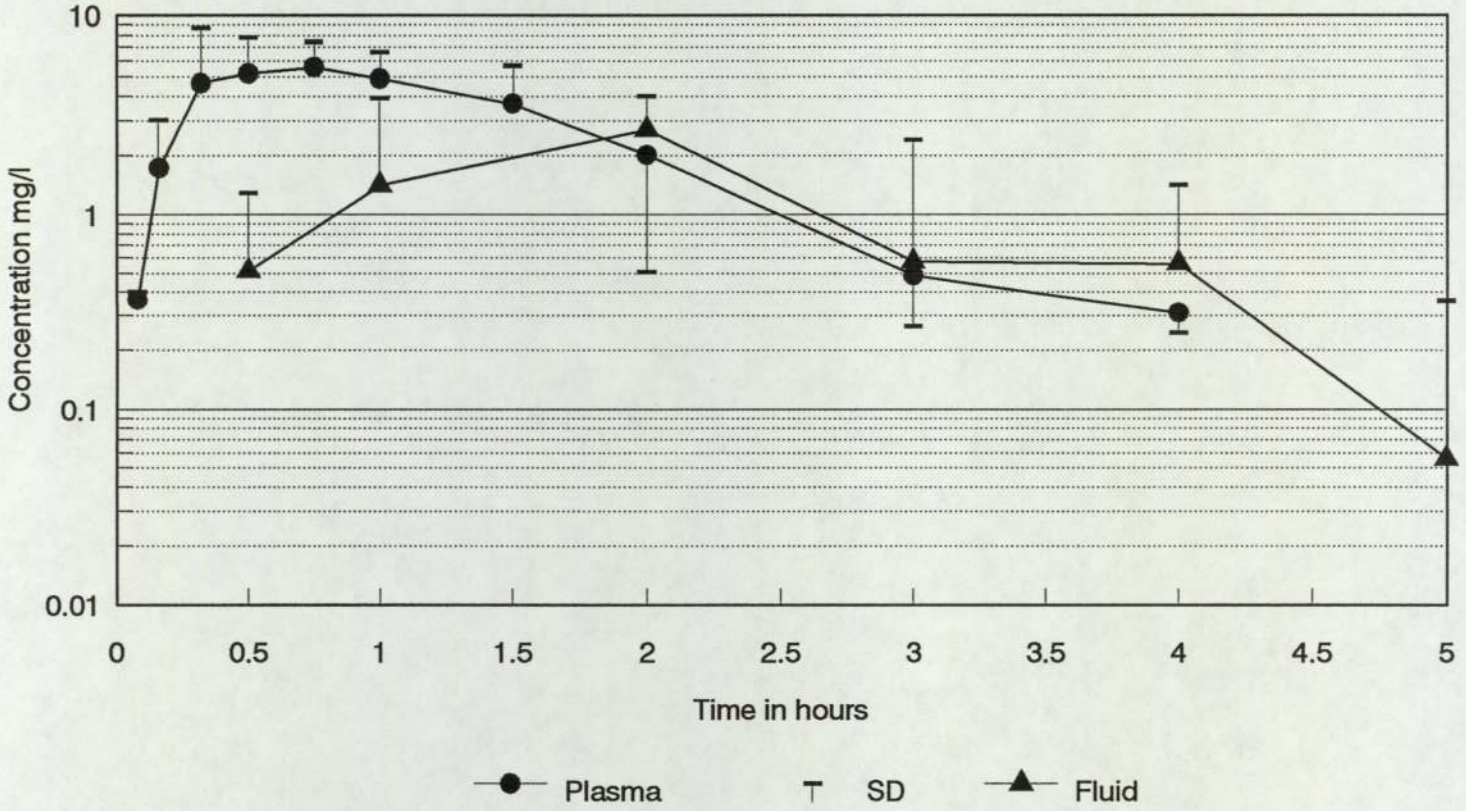



**Figure 30 : Mean plasma and inflammatory fluid levels following a single 809.2 mg oral dose of FCE 22101**

Data obtained from 6 volunteers. Concentration in plasma and inflammatory fluid (mg/l) plotted against time after administered dose.

| Parameters in indicated fluid | IV Mean ± SD | IV (Range) | Oral Mean ± SD | Oral (Range) |
|---|---|---|---|---|
| Plasma : | | | | |
| $C_{max}$ (mg/l) | 167 ± 29.2 | (125 - 177) | 6.9 ± 2.0 | (4.6 - 8.9) |
| $T_{max}$ (h) | | | 0.8 ± 0.5 | (0.3 - 1.5) |
| $T_{½}$ (h) | 0.8 ± 0.3 | (0.6 - 1.3) | 0.6 ± 0.2 | (0.4 - 0.8) |
| $AUC_{0-\infty}$ (mgh/l) | 41.5 ± 4.3 | (33.2 - 44.7) | 9.5 ± 2.5 | (6.7 - 13.2) |
| $Vd_{ss}$ (l) | 16.6 ± 5.5 | (11.0 - 25.5) | | |
| Inflammatory fluid : | | | | |
| $C_{max}$ (mg/l) | 17.8 ± 9.4 | (0.2 - 36.1) | 2.9 ± 1.4 | (1.5 - 4.9) |
| $T_{max}$ (h) | 0.6 ± 0.2 | (0.5 - 1.0) | 1.8 ± 0.5 | (1.0 - 2.0) |
| $T_{½}$ (h) | 0.6 ± 0.2 | (0.5 - 1.0) | 0.9 ± 0.2 | (0.6 - 1.1) |
| $AUC_{0-\infty}$ (mgh/l) | 25.3 ± 5.1 | (20.2 - 33.6) | 8.2 ± 2.7 | (4.6 - 12.0) |
| % penetration | 60.9 ± 9.8 | (49.2 - 76.7) | 86.1 ± 15.3 | (68 - 108.3) |
| % excreted | 30.9 ± 8.7 | (22.8 - 47.9) | 11.0 ± 5.8 | (7.0 - 20.5) |
| Plasma clearance (ml/min) | 406.2 ± 48.9 | (373.2 - 502.5) | | |
| Renal clearance (ml/min) | 124.0 ± 32.4 | (85.1 - 180.6) | | |
| Bioavailability of oral drug (%) | | | 28.9 ± 8.5 | (20.2 - 38.0) |

**Table 38 : Pharmacokinetics of FCE 22101**

Pharmacokinetics of FCE 22101 compared in the same volunteers following a single 1g oral dose and 1g given intravenously.

in Volunteer 4).

When FCE 22101 is given intravenously a peak concentration of 167 mg/l is obtained at the end of the infusion. Thereafter drug levels fall rapidly with levels being below the lower limit of sensitivity of the assay after 4 h. After oral administration a mean peak concentration of 6.9 mg/l (range 4.6 - 8.9 mg/l) occurred at a mean time of 0.8 h. After 1.5 h plasma levels were comparable for both routes of administration. The mean elimination of half life for IC and oral routes were 0.8 and 0.6 h, respectively.

FCE 22101 penetrated the inflammatory fluid rapidly after intravenous administration with a mean peak concentration of 17.8 mg/l (range 10.2 - 36.1 mg/l) at a mean time of 0.6 h. Penetration was slightly slower after oral administration with a $C_{max}$ of 2.9 mg/l and $T_{max}$ of 1.8 h. Levels in inflammatory fluid exceeded those in plasma for both routes of administration. After oral dosing the drug could be detected in inflammatory fluid for up to 5 h, whereas in plasma, levels could only be measured for up to 4 h. The elimination half life from inflammatory fluid was 0.6 and 0.9 h for intravenous and oral administration,

respectively.

The percentage penetration into inflammatory fluid was 60.9% after intravenous administration and 86.1% after oral dosing. The volume of distribution following intravenous administration was 16.6 h (range 11.0 - 25.5 h).

In Tables 39 and 40 the urinary excretion for both routes administration are shown. Urinary recovery was greater following intravenous administration (30.9%) compared with oral administration (11.0%). The total and renal clearances of FCE 22101 following IV administration were 406.2 ml/min (range 373.2 - 502.5 ml/min) and 124.9 ml/min (range 85.1 - 180.6 ml/min), respectively.

The bioavailability of the oral preparation was estimated by comparing the $AUC_{0-oo}$ (after correction for the amount of active compound in the dose) and the mean found to be 28.9% (range 20.2 - 38%).

**Discussion**

Although in vitro antibacterial activity of FCE 22101 against the Enterobacteriaceae is somewhat less than some of the other ß-lactams tested, its narrow range of activity suggests that it is more predictable in its degree of activity.

| Time | Vol 1 | Vol 2 | Vol 3 | Vol 4 | Vol 5 | Vol 6 |
|---|---|---|---|---|---|---|
| 0 - 1 | 222.5 | 174.9 | 244.4 | 391.5 | 192 | 239.8 |
| 1 - 2 | 28.5 | 98.0 | 30.6 | 59.2 | 21.8 | 29.7 |
| 2 - 3 | 13.4 | 2.97 | 13.2 | 14.6 | 9.6 | 8.1 |
| 3 - 4 | 5.1 | 1.94 | 5.6 | 7.2 | 2.4 | 14.0 |
| 4 - 6 | 3.2 | 1.80 | 3.0 | 4.4 | 1.6 | 4.1 |
| 6 - 8 | 0.54 | 0.60 | 0.84 | 1.20 | 0.47 | 1.30 |
| 8 - 12 | 0.46 | 0.22 | 0.27 | 0.56 | 0.12 | 1.0 |
| 12 - 24 | NDL | NDL | NDL | NDL | NDL | NDL |
| Total mg excreted | 273.7 | 280.43 | 297.91 | 478.66 | 227.99 | 298.0 |
| % of dose excreted | 27.4 | 28.0 | 29.8 | 47.9 | 22.8 | 29.8 |

Mean % of dose excreted = 30.95

## Table 39 : Urinary excretion of FCE 22101

Urinary excretion following a single
1 g intravenous infusion of FCE 22101.

| Time | Vol 1 | Vol 2 | Vol 3 | Vol 5 | Vol 6 |
|---|---|---|---|---|---|
| 0 - 1 | 109.7 | 14.7 | 17.0 | 24.9 | 55.6 |
| 1 - 2 | 45.9 | 24.9 | 35.7 | 20.8 | 29.5 |
| 2 - 3 | 5.9 | 12.9 | 8.6 | 7.5 | 8.8 |
| 3 - 4 | 2.0 | 2.7 | 2.3 | 1.8 | 3.7 |
| 4 - 6 | 1.9 | 1.6 | 0.6 | 0.8 | 1.5 |
| 6 - 8 | 0.3 | 0.7 | 0.4 | 0.2 | 0.5 |
| 8 - 12 | NDL | NDL | NDL | 0.2 | NDL |
| 12 - 24 | NDL | NDL | NDL | NDL | NDL |
| Total mg excreted | 165.7 | 57.5 | 64.6 | 56.2 | 99.6 |
| % of dose excreted | 20.5 | 7.1 | 8.0 | 6.9 | 12.3 |

Mean % of dose excreted = 9.1

## Table 40 : Urinary excretion of FCE 22101

Excretion following a single 809 mg oral dose of FCE 22101.

FCE 22101 appears to be resistant to a variety of ß-lactamases including those produced by H. influenzae, N. gonorrhoeae and Bacteroides. It is also noteworthy that FCE 22101 displayed good activity against the strain of cefoxitin-resistant B. thetaiotaomicron. FCE 22101 has similar activity to imipenem against S. aureus. Although both compounds have good in vitro activity against methicillin resistant strains of Staphylococci it must be remembered that clinical failures have been recorded when imipenem has been used against these strains. In addition, FCE 22101 was highly active against Strep. pneumoniae and E. faecalis.

The pharmacokinetic study suggests that FCE 22101 penetrates rapidly into inflammatory fluid following both oral and intravenous administration and that levels in plasma and inflammatory fluid exceed the $MIC_{90}$ for the majority of Enterobacteriaceae, Staphylococci and Bacteroides for 2 and 3 h, respectively.

These data suggest that because of the short elimination half life the drug would have to be administered at least 4 or maybe 6 times a day to maintain good levels.

5. **Tazobactam**

In vitro activity

A total of 116 bacterial isolates were examined to see the effect of varying concentration of clavulanic acid and tazobactam on the activity of piperacillin. Strains included ß-lactamase and non-ß-lactamase producing strains. All strains producing extracellular ß-lactamase were tested at $10^4$ and $10^6$ CFU.

In Table 41a, b, c, d, e and f the results can be seen for all isolates and in Figure 31 results are shown graphically for two strains of Esch. coli one ß-lactamase producing I147 and one ß-lactamase negative strain I252. Overall against Gram negative rods clavulanic acid appeared the most potent ß-lactamase inhibitor. In most cases a concentration of 1 mg/l only being required to bring the ß-lactamase producing Enterobacteriaceae into the sensitive range, whereas a concentration of 5 mg/l tazobactam was necessary to achieve the same results. Against ß-lactamase negative strains neither compound had a marked effect on activity i.e. 'synergy' not demonstrated. However, for 10 strains a concentration of 10 mg/l clavulanic acid alone was inhibitory.

| Organism | | P | 0.5 C * | 1 C * | 5 C * | 10 C * | 0.5 T * | 1 T * | 5 T * | 10 T * |
|---|---|---|---|---|---|---|---|---|---|---|
| Proteus | | | | | | | | | | |
| J191 | | .25 | .25 | .25 | .25 | .12 | .25 | .25 | .25 | .25 |
| J201 | | .12 | .12 | .12 | .06 | .03 | .06 | .12 | .12 | .06 |
| J253 | @ | 16 | .5 | .25 | .25 | .25 | .25 | .25 | .12 | .12 |
| J258 | @ | 16 | .5 | .25 | .25 | .5 | .25 | .25 | .03 | .12 |
| J256 | | .12 | .12 | .12 | .25 | .5 | .03 | .03 | .25 | .03 |
| J 51 | | .25 | .25 | .25 | .25 | .12 | .25 | .25 | .06 | .12 |
| J170 | @ | 16 | .5 | .25 | .5 | .25 | 2 | .25 | .5 | .06 |
| J205 | | .5 | .5 | .5 | .5 | .25 | .5 | .5 | .5 | .5 |
| J206 | | .5 | .5 | .5 | .5 | .25 | .5 | .5 | .5 | .5 |
| J203 | | .5 | .5 | .5 | .5 | .25 | .5 | .5 | .5 | .5 |
| J126 | | .5 | .5 | .5 | .5 | .25 | .5 | .5 | .5 | .5 |
| K235 | @ | 16 | 16 | 16 | 32 | 8 | 8 | 8 | 2 | 4 |
| K301 | @ | 32 | 32 | 32 | 64 | 64 | 8 | 16 | 4 | 4 |
| K355 | @ | 32 | 32 | 32 | 64 | 64 | 8 | 16 | 4 | 4 |
| K297 | @ | 32 | 8 | 8 | 16 | 8 | 8 | 16 | 2 | 4 |
| K233 | @ | 16 | 16 | 32 | 64 | 64 | 16 | 16 | 16 | 16 |
| K243 | | 4 | 8 | 4 | 4 | 2 | 8 | 4 | 4 | 4 |
| K335 | @ | 32 | 8 | 4 | 4 | 1 | 8 | 8 | 4 | 4 |
| K336 | | 256 | 8 | 4 | 4 | 4 | 128 | 128 | 4 | 4 |
| K376 | @ | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 |
| K385 | | 4 | 4 | 4 | 32 | 32 | 2 | 2 | 2 | 2 |
| K426 | | 1 | 1 | .5 | 1 | .5 | .5 | 1 | .5 | .5 |
| K170 | | 2 | 2 | 2 | 2 | 8 | 2 | 2 | 2 | 1 |
| K 35 | | 1 | 1 | 1 | 2 | 4 | 1 | 1 | 1 | 1 |
| K260 | | 4 | 8 | 8 | 16 | 8 | 4 | 4 | 2 | 2 |
| K270 | @ | 256 | 8 | 4 | 8 | 16 | 128 | 128 | 2 | 2 |
| K259 | | 2 | 2 | 2 | 4 | 8 | 2 | 2 | 2 | 2 |
| K258 | | .5 | .5 | .5 | .5 | .5 | .5 | .5 | .5 | .5 |
| K353 | @ | 128 | 16 | 16 | 64 | 64 | 128 | 128 | 16 | 16 |

## Table 41A : Activity of piperacillin alone and in combination with clavulanic acid or tazobactam.

P = MIC of piperacillin alone (mg/l) C = clavulanic acid T = tazobactam
@ = beta lactamase producing strain
* Results (expressed as piperacillin MIC) when piperacillin combined with fixed concentrations of 0.5 , 1 , 5 or 10 mg/l of either clavulanic acid or tazobactam.

| Organism | P | 0.5 C | 1 C | 5 C | 10 C | 0.5 T | 1 T | 5 T | 10 T |
|---|---|---|---|---|---|---|---|---|---|
| Esch.coli | | | | | | | | | |
| I252 | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 2 | 2 |
| I147 @ | >256 | 16 | 2 | 2 | 1 | 256 | >256 | 1 | 2 |
| I153 @ | >256 | 16 | 4 | 2 | - | 256 | 256 | 2 | 2 |
| I146 | 2 | 2 | 1 | 1 | .25 | 1 | 1 | 1 | 1 |
| I145 | 8 | 2 | 1 | .5 | .25 | 1 | .5 | .5 | .5 |
| I143 | 8 | 4 | 2 | 1 | .5 | 4 | 4 | 2 | 1 |
| I139 @ | 32 | 8 | 4 | 2 | 1 | 4 | 4 | 2 | 2 |
| I247 | 4 | 4 | 4 | 2 | 2 | 4 | 4 | 2 | 4 |
| I117 | 1 | 1 | 1 | .5 | - | 1 | 1 | 1 | 1 |
| I118 | 4 | 4 | 4 | 2 | 1 | 4 | 4 | 2 | 2 |
| Klebsiella | | | | | | | | | |
| H34 @ | 256 | 8 | 4 | 2 | 1 | 128 | 128 | 1 | 1 |
| H130@ | 32 | 2 | 1 | 1 | .5 | 8 | 8 | 1 | 1 |
| H132@ | >256 | 128 | 32 | 16 | 8 | >256 | >256 | 128 | 2 |
| H200 | 2 | 2 | 1 | 1 | .5 | 2 | 2 | 1 | 2 |
| H154@ | 256 | 16 | 8 | 8 | 1 | 128 | 128 | 2 | 2 |
| H114 | 2 | 1 | 1 | 1 | .5 | 1 | 1 | 1 | 1 |
| H115 | 2 | 1 | 1 | .5 | .015 | 1 | 1 | 1 | 1 |
| H116@ | 256 | 16 | 8 | 2 | 1 | 128 | 64 | 1 | 1 |
| H117 | 4 | 2 | 2 | 1 | .5 | 2 | 2 | 1 | 2 |
| H118 | 4 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 |
| Proteus | | | | | | | | | |
| J195 @ | 64 | 4 | 2 | 1 | .5 | 2 | 2 | 1 | 1 |
| J98 @ | 8 | 1 | 1 | 1 | .5 | 1 | 1 | 1 | 1 |
| J176 | .25 | .25 | .25 | .25 | .12 | .25 | .25 | .12 | .12 |
| J174 @ | 32 | .5 | .5 | .25 | .12 | .5 | .25 | .12 | .12 |
| J151 @ | 64 | 1 | 1 | .5 | .25 | 16 | 8 | .5 | .5 |
| J241 @ | 4 | .5 | .5 | .5 | .5 | .5 | .5 | .5 | .5 |
| J240 | 2 | .5 | .5 | .25 | .25 | .25 | .5 | .5 | .5 |
| J239 | 2 | .5 | .5 | .25 | .25 | .25 | .5 | .5 | .5 |

**Table 41B : Activity of piperacillin alone and in combination with clavulanic acid or tazobactam**

| Organism | P | 0.5 C | 1 C | 5 C | 10 C | 0.5 T | 1 T | 5 T | 10 T |
|---|---|---|---|---|---|---|---|---|---|
| Misc GNR | | | | | | | | | |
| K284 | 2 | 2 | 2 | 2 | 8 | 2 | 2 | 2 | 2 |
| K316 @ | 256 | 64 | 64 | 32 | 32 | 128 | 128 | 64 | 32 |
| P.aeruginosa | | | | | | | | | |
| G 92 | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 32 | 32 |
| G328 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| G329 @ | 64 | 2 | 2 | 2 | 2 | 8 | 8 | 2 | 4 |
| G182 | 4 | 2 | 2 | 4 | 4 | 4 | 4 | 2 | 4 |
| G183 | 32 | 64 | 32 | 32 | 32 | 32 | 32 | 16 | 8 |
| G239 @ | 32 | 4 | 4 | 2 | 2 | 32 | 32 | 8 | 4 |
| G244 | 32 | 32 | 16 | 8 | 4 | 16 | 32 | 32 | 16 |
| G245 @ | 64 | 4 | 4 | 4 | 4 | 16 | 16 | 4 | 4 |
| G246 @ | 64 | 8 | 4 | 2 | 4 | 32 | 32 | 16 | 8 |
| G247 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| S.aureus | | | | | | | | | |
| F318 @ | 32 | 8 | 2 | 2 | 2 | .5 | .5 | .5 | .015 |
| | 256 | 128 | 64 | 64 | 64 | 64 | 64 | 64 | 64 |
| F253 | 1 | 1 | 1 | .5 | .25 | 1 | 1 | 1 | .5 |
| | 1 | 1 | 1 | .5 | .5 | 1 | 1 | 1 | 1 |
| F284 | 1 | 1 | .5 | .25 | | 1 | 1 | .5 | .5 |
| | 2 | 1 | 1 | .5 | .12 | 1 | 1 | .5 | .5 |
| F275 | 1 | 1 | 1 | .5 | .12 | 1 | 1 | 1 | .5 |
| | 2 | 1 | 1 | 1 | .5 | 1 | 1 | 1 | 1 |
| F274 | 1 | 1 | .5 | .5 | .12 | 1 | 1 | .5 | .5 |
| | 2 | 1 | 1 | 1 | .25 | 1 | 1 | 1 | .5 |
| F273 | 1 | 1 | .5 | .5 | .12 | 1 | 1 | .5 | .5 |
| | 1 | 1 | 1 | 1 | .25 | 1 | 1 | 1 | 1 |
| F272 | 1 | 1 | 1 | .5 | .12 | 1 | 1 | 1 | .5 |
| | 1 | 1 | 1 | 1 | .5 | 1 | 1 | 1 | .5 |
| F271 | 1 | 1 | 1 | .5 | .06 | 1 | 1 | 1 | .5 |
| | 1 | 1 | 1 | .5 | .25 | 1 | 1 | 1 | 1 |

**Table 41C : Activity of piperacillin alone and in combination with clavulanic acid or tazobactam.**

(continued)

| Organism | | P | 0.5 C | 1 C | 5 C | 10 C | 0.5 T | 1 T | 5 T | 10 T |
|---|---|---|---|---|---|---|---|---|---|---|
| S.aureus | | | | | | | | | | |
| F282 | | 1 | 1 | .5 | .25 | | .5 | .5 | .5 | .5 |
| | | 1 | 1 | 1 | .5 | .12 | 1 | 1 | 1 | 1 |
| F256 | | 1 | 1 | .5 | .25 | | .5 | 1 | .5 | .5 |
| | | 1 | 1 | .5 | .5 | .03 | .5 | 1 | .5 | .5 |
| E748 | @ | 8 | 1 | .5 | .12 | | 1 | 1 | .5 | .25 |
| | | >256 | 2 | 2 | .12 | | 8 | 4 | 1 | .5 |
| E745 | @ | 2 | 1 | 1 | .25 | | 1 | 1 | .5 | .5 |
| | | 64 | 4 | 2 | .5 | | 4 | 8 | 2 | 1 |
| E687 | @ | 64 | 8 | 8 | 8 | 8 | 8 | 8 | 4 | 2 |
| | | >256 | 64 | 64 | 32 | 16 | 128 | 128 | 16 | 8 |
| G600 | | .5 | .5 | .5 | .12 | | .5 | .5 | .5 | .25 |
| | | .5 | .5 | .5 | .12 | | .5 | .5 | .5 | .25 |
| E466 | @ | 4 | 1 | .5 | .25 | | 1 | 1 | .5 | .015 |
| | | 128 | 2 | 1 | .25 | | 2 | 2 | 1 | .5 |
| G630 | @ | 2 | .5 | .5 | .12 | | 1 | .5 | .25 | .25 |
| | | 64 | 1 | .5 | .12 | | 2 | 1 | .5 | .5 |
| H.influenzae | | | | | | | | | | |
| A233 | @ | | | NO GROWTH | | | | | | |
| | | 32 | .12 | .06 | .03 | | .12 | .06 | .03 | .015 |
| A229 | @ | 1 | .06 | .06 | .03 | | .06 | .06 | .03 | .03 |
| | | 8 | .12 | .06 | .03 | | .12 | .06 | .03 | .03 |
| A228 | @ | 8 | .25 | .12 | .12 | | .25 | .12 | .12 | .12 |
| | | 64 | .25 | .12 | .12 | | .25 | .12 | .12 | .12 |
| A241 | @ | 8 | .06 | .03 | .008 | | .06 | .03 | .03 | .008 |
| | | 32 | .06 | .03 | .015 | | .12 | .06 | .06 | .015 |
| A159 | @ | 8 | .03 | .015 | .008 | | .03 | .015 | .008 | .008 |
| | | 64 | .03 | .015 | .008 | | .03 | .015 | .008 | .008 |
| A235 | @ | 1 | .03 | .015 | .008 | | .015 | .008 | .004 | .004 |
| | | 64 | .03 | .015 | .008 | | .03 | .015 | .008 | .008 |

**Table 41D : Activity of piperacillin alone and in combination with clavulanic acid or tazobactam.**

(continued)

| Organism | P | 0.5 C | I C | 5 C | 10 C | 0.5 T | 1 T | 5 T | 10 T |
|---|---|---|---|---|---|---|---|---|---|
| H.influenzae | | | | | | | | | |
| A178 | 64 | .06 | .03 | .015 | .015 | .03 | .03 | .015 | .008 |
| | 128 | .25 | .06 | .03 | .015 | .12 | .03 | .015 | .008 |
| A231 | 8 | .06 | .06 | .06 | .03 | .06 | .06 | .06 | .03 |
| | 128 | .12 | .12 | .06 | .03 | .12 | .12 | .06 | .03 |
| A246 | .015 | .015 | .004 | .004 | .004 | .008 | .004 | .004 | .008 |
| | .015 | .015 | .004 | .004 | .004 | .008 | .004 | .004 | .008 |
| A288 | .015 | .015 | .008 | .008 | .004 | .015 | .008 | .008 | .008 |
| | .03 | .03 | .008 | .008 | .008 | .015 | .008 | .008 | .008 |
| N.gonorrhoeae | | | | | | | | | |
| E314 | .25 | .06 | .03 | | | .03 | | | |
| | .5 | .06 | .03 | | | .03 | | | |
| E 61 | 4 | .015 | .004 | | | .015 | | | |
| | 8 | .015 | .004 | | | .015 | | | |
| E 65 | 1 | .03 | .008 | .004 | | .008 | .008 | | |
| | 32 | .06 | .015 | .008 | | .015 | .015 | | |
| E190 | .25 | .06 | .015 | | | | | | |
| | 1 | .06 | .015 | | | | | | |
| E188 | 1 | .03 | .015 | | | | | | |
| | 16 | .06 | .015 | | | | | | |
| E 29 | 2 | .06 | .015 | | | .015 | | | |
| | 16 | .12 | .015 | | | .015 | | | |
| E108 | .001 | | | | | | | | |
| | .002 | .002 | .002 | | | | | | |
| E323 | .015 | .004 | .002 | | | | | | |
| | .015 | .004 | .002 | | | .002 | | | |
| E227 | .25 | .25 | .25 | | | .25 | .25 | | |
| | .5 | .25 | .25 | | | .25 | .25 | | |
| E213 | .25 | | | | | | | | |
| | .25 | .002 | | | | | | | |

**Table 41E : Activity of piperacillin alone and in combination with clavulanic acid or tazobactam.**
(continued)

| Organism | P | 0.5 C | 1 C | 5 C | 10 C | 0.5 T | 1 T | 5 T | 10 T |
|---|---|---|---|---|---|---|---|---|---|
| Bacteroides | | | | | | | | | |
| B187 @ | 16 | 16 | 16 | 16 | 0.25 | 16 | 16 | 16 | 2 |
| | 128 | 128 | 128 | 64 | 2 | 128 | 128 | 128 | 64 |
| B264 @ | 128 | 128 | 128 | 128 | 64 | 128 | 128 | 128 | 256 |
| | >256 | >256 | >256 | >256 | >256 | >256 | >256 | >256 | >256 |
| B311 | 8 | 1 | 1 | 0.12 | | 1 | 0.5 | 0.25 | |
| | 8 | 1 | 1 | 0.25 | | 1 | 0.5 | 0.5 | |
| B316 @ | 128 | 128 | 128 | 128 | 64 | 128 | 128 | 128 | 256 |
| | >256 | >256 | >256 | >256 | >256 | >256 | >256 | >256 | >256 |
| B55 @ | 8 | 1 | | | | 0.5 | 0.5 | 0.12 | |
| | 128 | 16 | 0.12 | 0.12 | | 16 | 16 | 16 | |
| B71 @ | 128 | 1 | 0.12 | 0.12 | | 8 | 8 | 0.5 | |
| | 256 | 16 | 4 | 4 | 2 | 64 | 64 | 2 | 0.25 |
| B315 @ | 64 | 1 | 0.12 | 0.12 | | 4 | 4 | | |
| | 128 | 2 | 4 | 4 | 2 | 8 | 8 | 0.5 | 0.12 |
| B314 | 8 | 1 | 0.12 | 0.12 | | 1 | 1 | 0.25 | |
| | 8 | 1 | 0.12 | 0.12 | | 1 | 1 | 0.5 | |
| B312 @ | 128 | 1 | 0.12 | 0.12 | | 4 | 4 | 0.12 | |
| | 256 | 16 | 8 | 8 | 2 | 32 | 32 | 2 | 2 |
| B317 @ | 16 | 0.5 | .008 | .008 | | 0.25 | 0.25 | 0.12 | |
| | 64 | 4 | 2 | 2 | 2 | 8 | 8 | 2 | 2 |

**Table 41F : Activity of piperacillin alone and in combination with clavulanic acid or tazobactam.**

(continued)

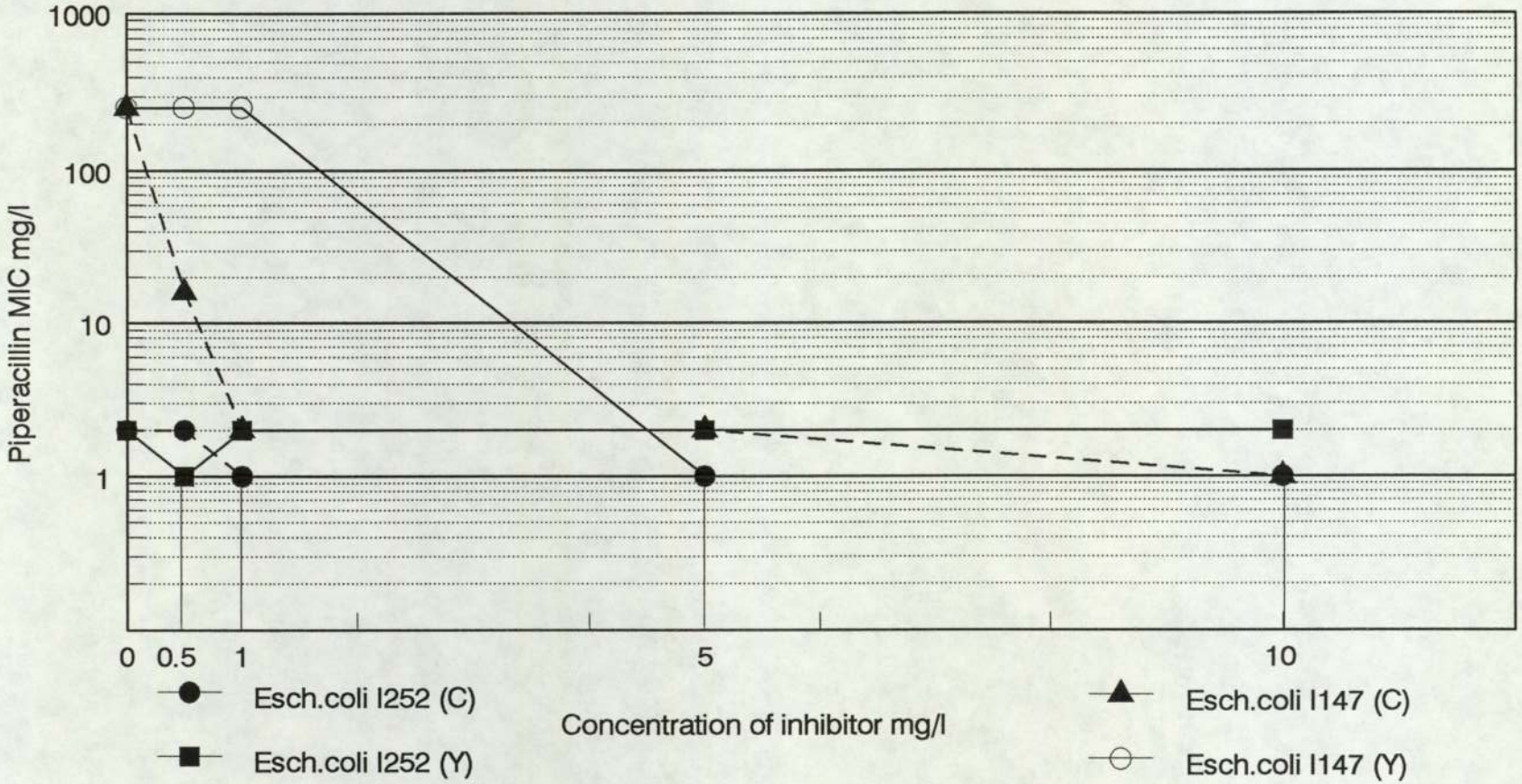



**Figure 31 : Effect of clavulanic acid and tazobactam on the activity of piperacillin**

The inhibitory effect of clavulanic acid (C) and tazobactam (Y) determined using two strains of Esch.coli (I 252 - non beta lactamase producing strain , I147 - beta lactamase producing strain).

Against Staphylococci tazobactam was equal in activity when compared with clavulanic acid. For both compounds against a methicillin-resistant strain of S. aureus (E687) MICs as expected were only reduced slightly (presumably two mechanisms of resistance) and the addition of either compound did not reduce the MIC into what could be considered the sensitive range.

Against Bact. fragilis both clavulanic acid and tazobactam had a marked effect on the activity of piperacillin except for two strains whose MICs were not reduced by the addition of either inhibitor (exact mechanism of resistance not known). Both inhibitors at high concentration ($\geq$5 mg/l) alone also had an inhibitory effect on some strains of Bacteroides.

The activity of tazobactam against H. influenzae and N. gonorrhoeae was equal to that of clavulanic acid, reducing the MICs dramatically from more than 8 mg/l to <0.25 mg/l with the addition of 0.5 mg/l of the inhibitor. Against N. gonorrhoeae both inhibitors displayed a marked inhibitory effect in the absence of piperacillin.

Assay Validation

There are problems peculiar to the assay of

inhibitors. In order for the activity of the inhibitor to be determined it is necessary to add an indicator antimicrobial to the culture media. As the indicator organism also has to be incorporated there is a fine balance between the concentration of antimicrobial and the size of inoculum to obtain the degree of sensitivity required. It is therefore necessary to titrate the amount of indicator antimicrobial to be added. It was found (by visual comparison of growth) that a final concentration of 100 mg/l piperacillin in the culture medium gave acceptable results. For optimum results plates have to be prepared fresh and used within one hour.

Another problem associated with the assay of inhibitors is the confidence that only one compound is being measured by the assay system. It is therefore necessary to include both compounds in the internal controls to ensure that an overestimation of results is not seen.

The confidence limits for the assay are as follows:

| Assigned concentration mg/l | | Mean | SD | CV |
|---|---|---|---|---|
| Piperacillin | 50 | 49.52 | 2.88 | 5.82 |
| | 6 | 5.73 | 0.213 | 3.72 |
| Tazobactam | 6 | 5.94 | 0.367 | 6.19 |
| | 0.8 | 0.79 | 0.069 | 8.76 |

Unfortunately an external validation of the assay was not possible because the Drug Company was unable to transport the samples from North America. However, in Figures 32 and 33 representative graphs for the study are shown from which the confidence limit data are derived.

Pharmacokinetics

The pharmacokinetics for piperacillin when co-administered with tazobactam are shown in Table 42 and Figure 34. Mean plasma concentrations at 0.5 h were 223.7 mg/l falling to 9.2 mg/l at 4 h. concentrations of piperacillin at any one time point were similar with the exception of those obtained for volunteer 6 in whom consistently lower levels were obtained. These differences could perhaps be attributed to his being taller

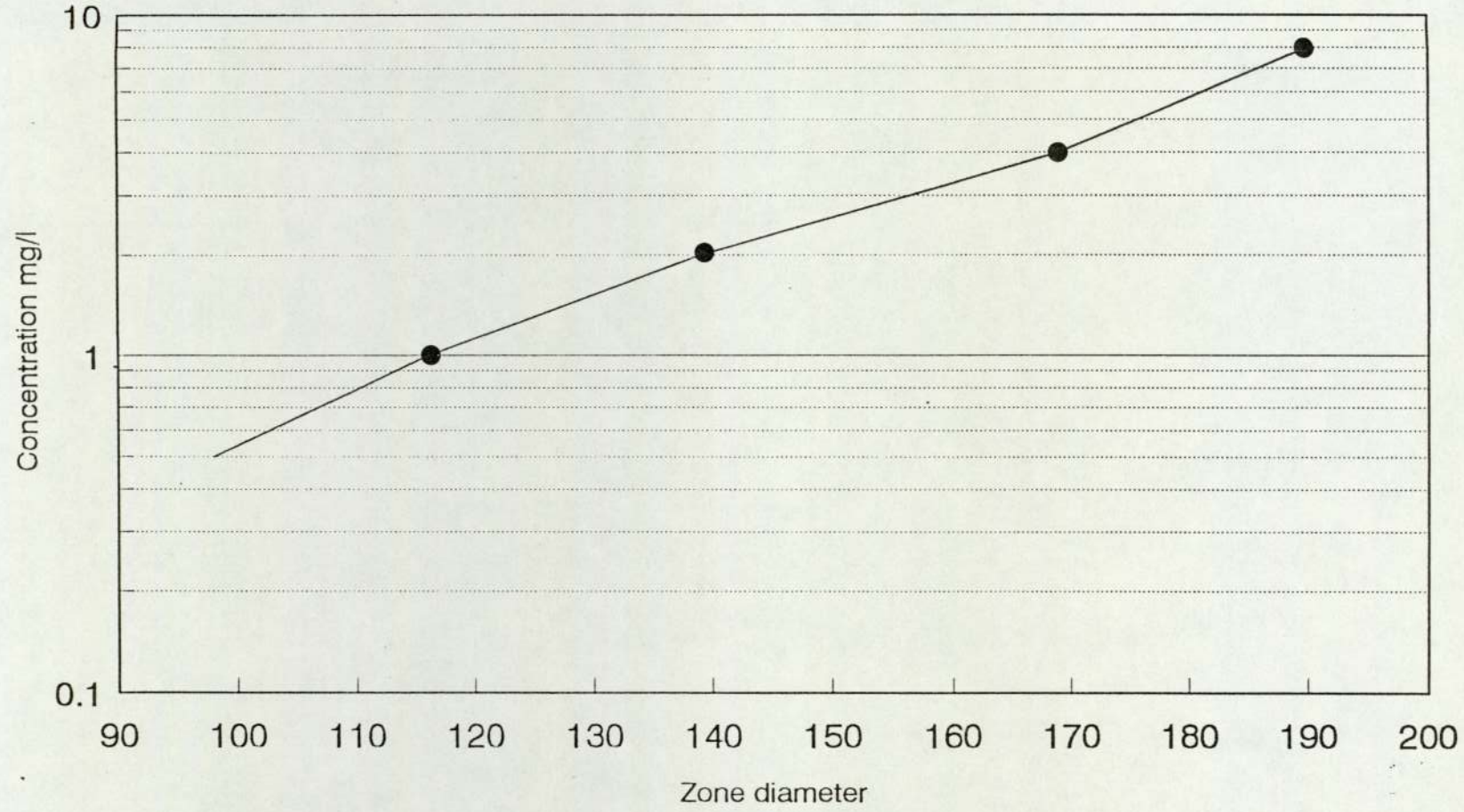



**Figure 32 : Assay of tazobactam in clinical samples**

Representative graph obtained during volunteer study. X axis zone diameter of standard (arbritrary units) , Y axis concentration of standard (mg/l).

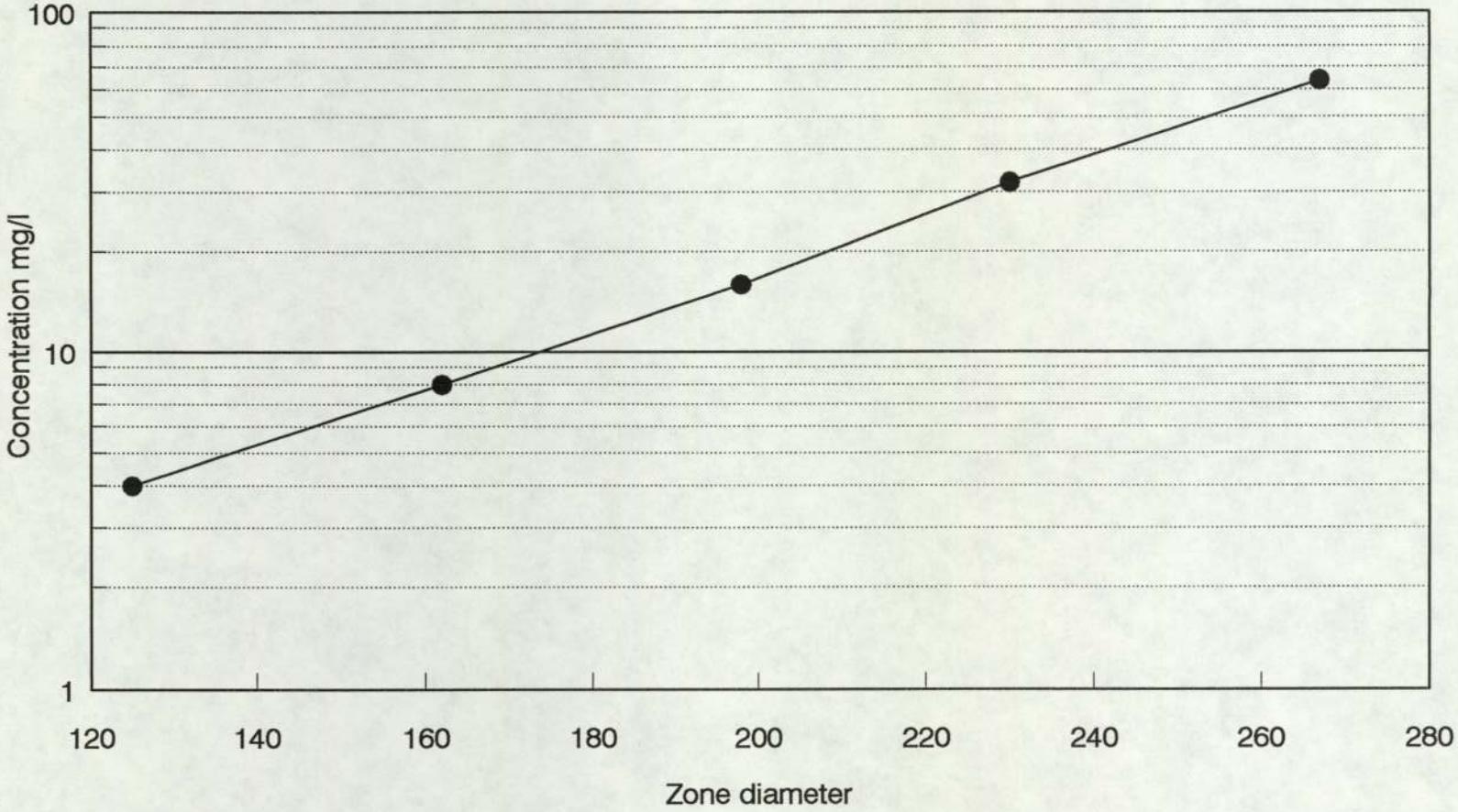



**Figure 33 : Piperacillin assay development**

Assay of piperacillin standards prepared in human serum using Oxoid Antibiotic medium Number 1 and indicator organism Pseudomonas aeruginosa NCTC 10701. Zone diameters (arbritrary units) for each of the standard concentrations plotted.

| | Mean | S D | Range |
|---|---|---|---|
| Plasma | | | |
| C 0.5 mg/l | 223.7 | 49.7 | 136-290.4 |
| C 4 mg/l | 9.4 | 3.2 | 5.4-13.6 |
| t/2 h | 1.0 | 0.15 | 0.82-1.2 |
| $AUC_{0-\infty}$ | 485 | 82.1 | 370-592 |
| Total Clearance ml/min | 145 | 22.7 | 107-166 |
| Renal clearance mg/min | 73.1 | 6.5 | 69.0-86.2 |
| Inflammatory Fluid | | | |
| $C_{max}$ | 77.2 | 32.6 | 42.8-126 |
| $t_{max}$ | 2.1 | 1.1 | 0.5-3.0 |
| $AUC_{0-\infty}$ | 237 | 20.9 | 207-257 |
| % penetration | 49.6 | 5.9 | 41.8-55.9 |
| Urine | | | |
| % excretion 24 h | 49.8 | 4.7 | 42.6-55.5 |

**Table 42 : Pharmacokinetics of piperacillin combined with tazobactam.**

Mean plasma and inflammatory fluid concentrations of piperacillin (1 g IV) after coadministration with tazobactam (0.5 g).

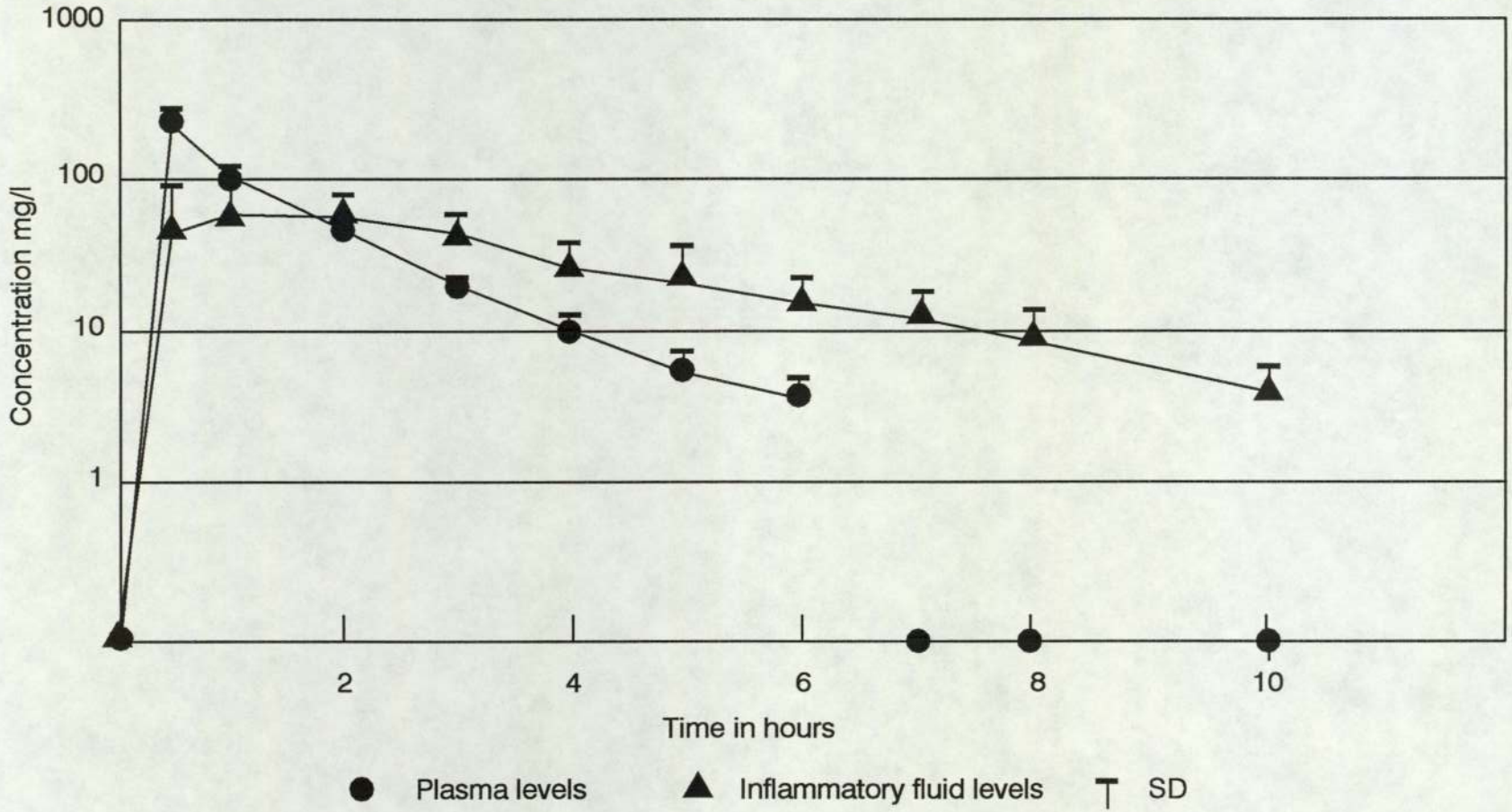



**Figure 34 : Mean plasma and inflammatory fluid levels of piperacillin**

Data otained from six volunteers following a single 4 g infusion of piperacillin combined with 500 mg of tazobactam. Concentration (mg/l) plotted against time (timed from the end of the infusion).

and heavier than the other volunteers. The mean plasma elimination half life of piperacillin was 1 h, however, volunteer 6 had the shortest half life of 0.82 h. The mean total renal clearance of piperacillin were 145 and 73.1 mg/min, respectively, and 49.8% of the drug was recovered in urine in 24 h.

Inflammatory fluid was penetrated rapidly by piperacillin with a mean peak concentration of 77.2 mg/l at 2.1 h. There was considerable variation between the volunteers and the most rapid and extensive penetration was seen in volunteer 6 (126 mg/l at 0.5 h after the end of the infusion). The mean percentage penetration into inflammatory fluid was 49.8%.

The pharmacokinetics of tazobactam was influenced by the presence of piperacillin as can be seen in Table 43 and Figures 35, 36 and 37. Although the mean plasma concentrations at 0.5 h after administration were not markedly different, there was a rise in tazobactam concentration when co-administered with piperacillin (24.3 mg/l alone, 27.2 mg/l in combination). However, there was a significant difference between concentrations of tazobactam at 4 h with levels of

| | | Mean | S D | Range | |
|---|---|---|---|---|---|
| Plasma | | | | | |
| c 0.5 | A mg/l | 24.3 | 8.0 | 13.7-37.1 | |
| | B mg/l | 27.2 | 6.2 | 16.8-36.3 | p=0.6 |
| c.4 | A mg/l | 0.6 | 0.12 | 0.44-0.74 | |
| | B mg/l | 1.2 | 0.25 | 0.9-1.5 | p=0.0003 |
| t/2 | A h | 1.13 | 0.21 | 0.94-1.5 | |
| | B h | 1.11 | 0.31 | 0.90-1.67 | p=0.5 |
| $AUC_{0-\infty}$ | A mg/l.h | 42.4 | 15.8 | 23.0-66.9 | |
| | B mg/l.h | 49.0 | 12.4 | 34.5-68.1 | p=0.07 |
| Total clearance | A ml/min | 203.5 | 74.0 | 123.1-296 | |
| | B ml/min | 134.2 | 39.7 | 97.6-21.0 | p=0.035 |
| Renal | A ml/min | 130.3 | 51.3 | 66.7-206.7 | |
| | B ml/min | 75.7 | 19.9 | 60.1-115 | p=0.02 |
| Vol, Dist. (B) | A l | 17.8 | 9.8 | 6.6-34.5 | |
| | B l | 11.6 | 2.1 | 9.7-15.0 | p=0.11 |
| Blister | | | | | |
| $C_{max}$ | A mg/l | 6.4 | 2.2 | 4.7-10.7 | |
| | B mg/l | 11.3 | 8.2 | 5.8-27.1 | |
| $T_{max}$ | A h | 0.94 | 0.7 | 0.5-2.0 | p=0.05 |
| | B h | 1.83 | 1.3 | 0.5-3.0 | |
| t/2 | A h | 0.94 | 0.14 | 0.66-1.0 | p=0.05 |
| | B h | 1.3 | 0.4 | 0.76-1.8 | |
| $AUC_{0-\infty}$ | A mg/l.h | 18.0 | 1.29 | 16.5-19.7 | p=0.002 |
| | B mg/l.h | 30.8 | 4.9 | 24.6-37.8 | |
| penetration | A % | 46.7 | 15.0 | 28.3-71.3 | p=0.03 |
| | B % | 67.7 | 25.9 | 43.9-108.6 | |
| Urine | | | | | |
| 24h excretion | A % | 63.7 | 7.9 | 54.2-75.3 | |
| | B % | 56.8 | 2.7 | 42.1-61.4 | p=0.05 |

## Table 43 : Pharmacokinetics of tazobactam alone and in combination with piperacillin

Pharmacokinetics of tazobactam (500 mg) alone (A) and in combination (B) with piperacillin (4 g).

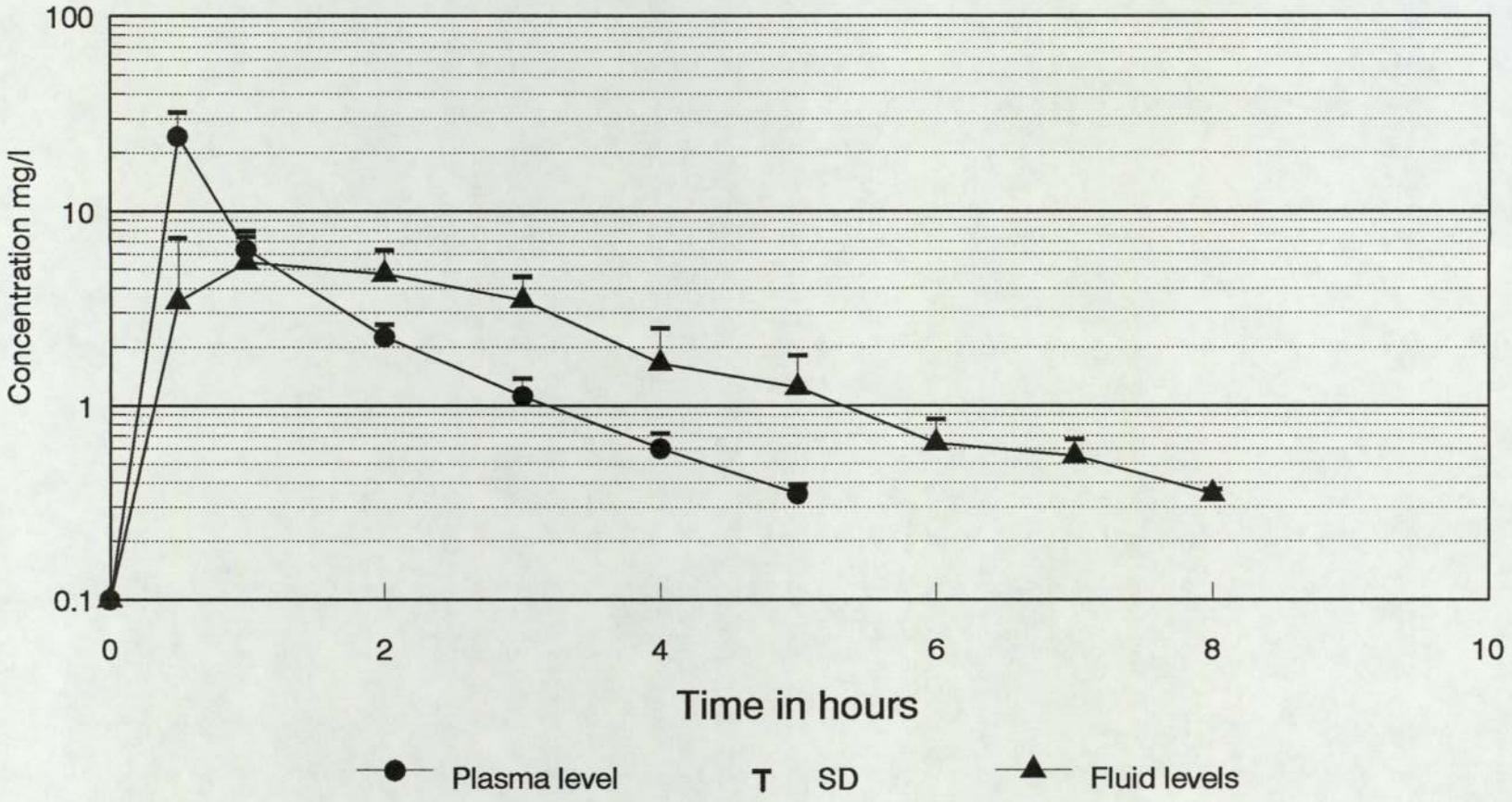



**Figure 35 : Mean plasma and inflammatory fluid concentrations of tazobactam**

Mean plasma and inflammatory fluid concentrations (six volunteers) following a single 500 mg intavenous dose of tazobactam.

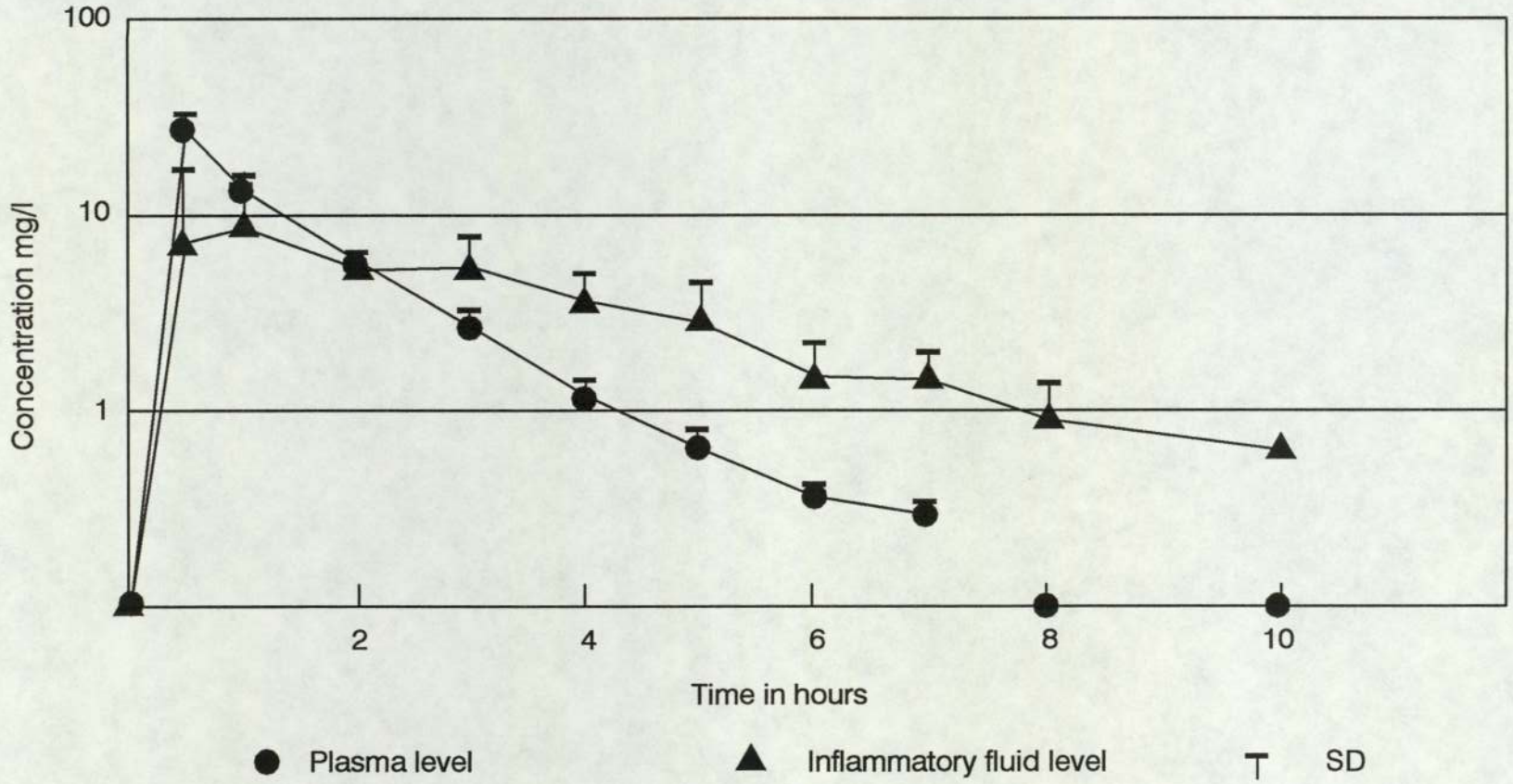



**Figure 36 : Concentrations of tazobactam following a single intravenous infusion of 500 mg tazobactam combined with 4 g of piperacillin.**

Data obtained from six healthy volunteers. Concentration in plasma and inflammatory fluid plotted against time (timed from the end of the infusion).

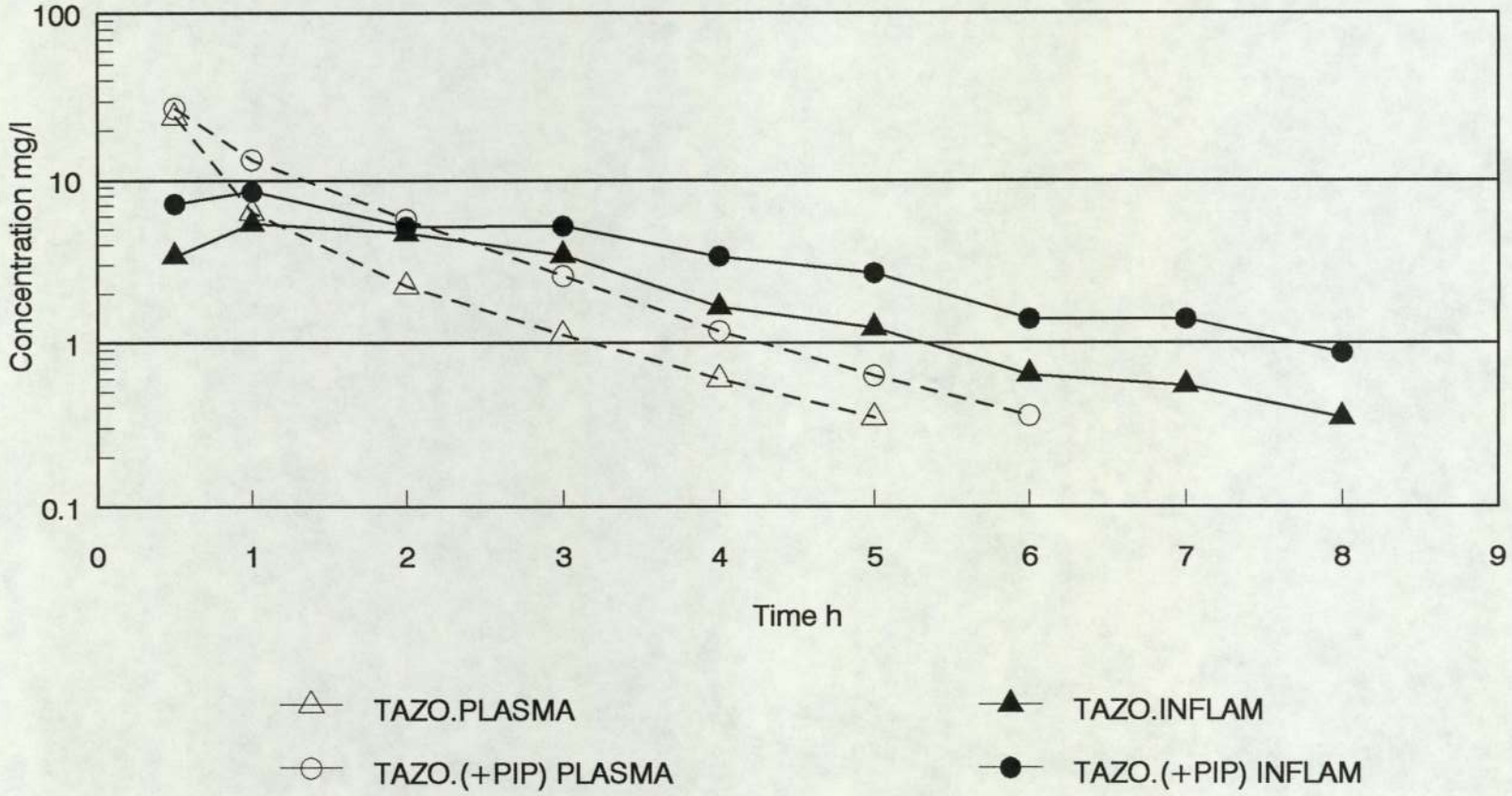



**Figure 37 : Plasma and inflammatory fluid concentrations of tazobactam alone and combined with pipercaillin**

Mean data obtained from volunteers following a 500 mg dose of tazobactam and tazobactam combined with 4 g of piperacillin. Concentration (mg/l) plotted against time (timed from the end of the infusion).

1.2 mg/l in combination and 0.6 mg/l when the drug was given alone ($p = 0.0003$). The mean elimination half life was the same in both studies (1.1 h).

There were significant differences in the total ($p = 0.035$) and renal ($p = 0.02$) clearances of tazobactam, both being more rapid when tazobactam was given alone. The urinary recovery of tazobactam over 24 h was greater when the drug was given alone, 63.7%, compared with 56.8% when co-administered with piperacillin (Table 44). Tazobactam penetrated the inflammatory fluid rapidly with a peak concentration being achieved at 1.6 - 1.8 h post administration. Results being similar for both studies. There was however, a significant increase in the maximum concentration attained when piperacillin was co-administered ($C_{max}$ alone 6.4 mg/l, 11.3 mg/l in combination - $p = 0.05$). There was also a longer elimination half life from the inflammatory fluid when piperacillin was co-administered than when tazobactam was given alone (means 1.3 h and 0.94 h respectively).

The mean percentage penetration of tazobactam into inflammatory fluid was also greater after

| Time (hours) | Volunteer 1 comb. Pip | Tazo | Tazo | Volunteer 2 comb. Pip | Tazo | Tazo | Volunteer 3 comb. Pip | Tazo | Tazo | Volunteer 4 comb. Pip | Tazo | Tazo | Volunteer 5 comb. Pip | Tazo | Tazo | Volunteer 6 comb. Pip | Tazo | Tazo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 - 4 | 1779 | 255 | 283 | 202 | 283 | 319 | 1936 | 261 | 363 | 1693 | 260 | 338 | 1925 | 274 | 261 | 1409 | 218 | 257 |
| 4 - 8 | 143 | 15 | 9 | 113 | 10.5 | 6 | 168 | 15.2 | 10 | 149 | 19.6 | 10.2 | 131 | 15.8 | 8.7 | 282 | 55.4 | 38.9 |
| 8 - 12 | 9.8 | 0.65 | 0.4 | 15 | 1.2 | 0.5 | 18.8 | 1.2 | 3.5 | 17.1 | - | 1.9 | 14.2 | 1.9 | 1.2 | 8.7 | 1 | 1.6 |
| 12 - 24 | 8.4 | 0.17 | - | 73 | 127 | - | 57 | - | - | 12.9 | 0.91 | - | 49 | 0.4 | - | 4.7 | 0.68 | 0.5 |
| Total mg | 1940 | 271 | 292 | 2221 | 307 | 326 | 2128 | 277 | 377 | 1872 | 281 | 350 | 2075 | 292 | 2708 | 1705 | 275 | 298 |
| % excreted | 48.5 | 54.1 | 58.5 | 55.5 | 61.4 | 65.1 | 53.2 | 55.4 | 75.3 | 46.8 | 56.2 | 70 | 51.9 | 58.4 | 54.2 | 42.6 | 55 | 59.6 |

**Table 44 : Urinary excretion of tazobactam combined alone and combined with tazobactam**

Urinary excretion following administration of 500 mg tazobactam alone compared with 500 mg tazobactam and 4 g piperacillin.

co-administration of piperacillin (46.7% alone, 67.7% in combination), however, there was considerable individual variation.

In Table 45 the ratio of piperacillin to tazobactam in plasma and inflammatory fluid is shown. In plasma a ratio of 8:1 is maintained over a 6 h period and no significant difference in ratio was observed at 0.5 and 6 h ($p = 0.4$). The increase in ratio with time observed in inflammatory fluid was however, not statistically significant ($p = >0.5$) and is probably attributed to the occasionally large ratios. The mean ratio of piperacillin : tazobactam in inflammatory fluid was 8.1:1 (SD 5.1).

**Discussion**

Tazobactam is similar to clavulanic acid in its ability to protect piperacillin to hydrolysis by ß-lactamase. It would appear from the in vitro study that a ratio of piperacillin : tazobactam of 4 or 8:1 would reduce the piperacillin $MIC_{90}$ of the majority of ß-lactamase producing strains of Enterobacteriaceae, S. aureus (but not methicillin resistant strains) and Bact. fragilis to 32 mg/l or less. Exceptions include certain strains possessing chromosomal cephalosporinases (63).

| Time (h) | Plasma (SD) | Inflammatory exudate (SD) |
|---|---|---|
| 0.5 | 8.2 (0.14) | 5.1 (0.7) |
| 1 | 7.5 (0.60) | 6.1 (0.64) |
| 2 | 7.6 (1.2) | 11.9 (9.4) |
| 3 | 7.0 (0.3) | 9.5 (7.6) |
| 4 | 7.9 (1.7) | 7.7 (2.8) |
| 5 | 9.2 (4.5) | 7.5 (3.2) |
| 6 | 10.0 (4.5) | 9.1 (1.7) |

**Table 45 : Ratio of piperacillin : tazobactam in plasma and inflammatory exudate.**

Data derived in volunteers following a single 4 g dose of piperacillin coadministered with 500 mg tazobactam.

The pharmacokinetics of piperacillin broadly agree with those previously described (64). However, we noted lower urinary recovery of 49.6% in 24 h compared with 79.8% in 24 h. In preliminary studies Lederle Laboratories also found recovery rates of 49% when piperacillin was given alone and 46% when co-administered with tazobactam (O. Kuye: personal communication).

The major finding of this study is the influence that piperacillin had in the pharmcokinetics of tazobactam. Plasma and inflammatory fluid concentration of tazobactam were higher when co-administered with piperacillin and also $AUC_{0-oo}$ were also greater. Plasma elimination half lives were however, not different in the two studies. The plasma and renal clearances of tazobactam were reduced in the presence of piperacillin and this may well be explained by the inhibition of the tubular secretion of tazobactam (if this occurs) by piperacillin. Piperacillin, when given alone, has a renal clearance of 213 ml/min (64) which argues strongly that piperacillin undergoes active renal secretion. It was also interesting to note how the urinary recovery of tazobactam was lower

following co-administration, again suggesting that a reduction of urinary elimination is the major reason for the pharmacokinetic difference.

The pharmacokinetic study has shown that the ratio as administered (8:1) is maintained over a 6 h period in both plasma and inflammatory fluid and this has been found in in vitro studies to reduce the MICs of piperacillin to ß-lactamase producing strains. It would appear from both of these studies that tazobactam merits clinical trial in systemic infections in its role of protecting piperacillin against hydrolysis by ß-lactamase producing bacterial pathogens.

6. **Aztreonam**

In vitro activity

The results for 394 recent clinical isolates are shown in Tables 46a, b and 47. Overall aztreonam was the most active of the compounds tested and was approximately twice as active as moxalactam. Of particular note is the high degree of activity against indole positive Proteus, Prov. stuartii and Serratia marcescens. Two strains of Klebsiella species were more resistant to aztreonam having MICs of 8 and 16 mg/l. These

strains were however, more susceptible to cefoxitin (MICs 1 and 4 mg/l) and moxalactam (MIC 0.12 and 0.06 mg/l). From preliminary studies it would appear that aztreonam is hydrolysed by the ß-lactamase produced by these strains. However, more detailed experiments are required to identify the exact mechanism of resistance.

Aztreonam appeared to be equal in activity to piperacillin against the strains of Pseudomonas tested. Two strains resistant to carbenicillin and piperacillin (MIC >128 mg/l) were susceptible to aztreonam having MICs of 2 mg/l. Generally aztreonam was twice as active as moxalactam. H. influenzae and N. gonorrhoeae were highly susceptible to aztreonam having MICs 90 $\leq$0.25 mg/l. Aztreonam was also equally active against the ß-lactamase producing and non ß-lactamase producing strains. However, the activity of aztreonam was reduced against two strains of non ß-lactamase producing H. influenzae with raised MICs to ampicillin (1 and 2 mg/l). MICs for these strains to aztreonam were 1 mg/l.

Aztreonam displayed only modest activity against Bact. fragilis and all strains of S. aureus and Streptococci tested were resistant.

| Strain (no. tested) | Antibiotic | Range MIC mg/l | MIC 50% | MIC 90% |
|---|---|---|---|---|
| Esch.coli (50) | Aztreonam | 0.03 - 2 | 0.06 | 0.25 |
| | Moxalactam | 0.03 - 2 | 0.06 | 1 |
| | Cefoxitin | 2 - 32 | 4 | 16 |
| | Piperacillin | 0.25 - 128 | 4 | 64 |
| Klebsiella species (50) | Aztreonam | ≤0.15 - 16 | 0.06 | 0.12 |
| | Moxalactam | 0.06 - 0.5 | 0.12 | 0.25 |
| | Cefoxitin | 1 - 16 | 2 | 4 |
| | Piperacillin | 1 - >128 | 8 | >128 |
| Enterobacter species (20) | Aztreonam | 0.03 - 64 | 0.06 | 0.5 |
| | Moxalactam | 0.06 - 32 | 0.06 | 0.25 |
| | Cefoxitin | 2 - >128 | >128 | >128 |
| | Piperacillin | 0.5 - 128 | 2 | 32 |
| Pr.mirabilis (30) | Aztreonam | ≤0.015 - 0.12 | ≤0.015 | 0.03 |
| | Moxalactam | 0.06 - 0.5 | 0.06 | 0.12 |
| | Cefoxitin | 2 - >128 | 2 | 16 |
| | Piperacillin | 0.25 - >128 | 0.5 | 64 |
| Indole +ve Proteus (20) | Aztreonam | ≤0.015 - 0.03 | ≤0.015 | ≤0.015 |
| | Moxalactam | 0.06 - 0.25 | 0.12 | 0.25 |
| | Cefoxitin | 2 - 16 | 4 | 8 |
| | Piperacillin | 0.12 - 16 | 0.5 | 2 |
| Citrobacter species (15) | Aztreonam | 0.03 - 2 | 0.06 | 0.25 |
| | Moxalactam | 0.06 - 0.5 | 0.06 | 0.5 |
| | Cefoxitin | 2 - 128 | 2 | 64 |
| | Piperacillin | 4 - 64 | 8 | 32 |

## Table 46A : In vitro activity of aztreonam

Activity of aztreonam compared with that of other beta lactam antibiotics at an inoculum of $10^4$CFU. Results expressed as a range of activity and also that concentration which was found to inhibit 50 (MIC 50) and 90% (MIC 90) of strains in mg/l.

| Strain (no. tested) | Antibiotic | Range MIC mg/l | MIC 50% | MIC 90% |
|---|---|---|---|---|
| Ps.aeruginosa (50) | Aztreonam | 2 - 32 | 4 | 8 |
| (including 2 Tem-1 | Moxalactam | 4 - 128 | 8 | 16 |
| strains) | Cefoxitin | 32 - >128 | >128 | >128 |
| | Piperacillin | 2 - 128 | 4 | 32 |
| | Carbenicillin | 16 - >128 | 32 | >128 |
| H.influenzae (50) | Aztreonam | ≤0.015 - 1 | 0.03 | 0.12 |
| (including 11 ß lactamase | Moxalactam | ≤0.015 - 0.5 | 0.03 | 0.12 |
| + ve strains) | Cefoxitin | 1 - 4 | 2 | 2 |
| | Piperacillin | ≤0.015 - 2 | 0.03 | 0.5 |
| | Ampicillin | 0.06 - 4 | 0.06 | 2 |
| Bact.fragilis (10) | Aztreonam | 16 - >128 | 32 | >128 |
| | Moxalactam | 0.5 - 16 | 1 | 8 |
| | Cefoxitin | 4 | 4 | 4 |
| | Piperacillin | 2 - >128 | 4 | 128 |
| | Penicillin | 8 - >128 | 16 | >128 |
| N.gonorrhoeae (330 | Aztreonam | ≤0.015 - 1 | 0.06 | 0.25 |
| (including 2 ß lactamase | Moxalactam | ≤0.015 - 0.25 | 0.03 | 0.06 |
| + ve strains) | Cefoxitin | 0.06 - 0.5 | 0.25 | 0.5 |
| | Piperacillin | ≤0.015 - 4 | ≤0.015 | 0.06 |
| | Penicillin | ≤0.015 - 64 | 0.06 | 0.12 |
| Staph.aureus (20) | Aztreonam | >128 | >128 | >128 |
| | Moxalactam | 2 - 16 | 4 | 8 |
| | Cefoxitin | 1 - 8 | 4 | 8 |
| | Piperacillin | 0.5 - 64 | 2 | 64 |
| | Penicillin | 0.03 - 32 | 0.25 | 32 |

**Table 46B : In vitro activity of aztreonam**
(continued)

| | | MIC (mg/l) | |
|---|---|---|---|
| Strain (no.tested) | Antibiotic | Range | Geometric mean |
| S.marcescens (12) | Aztreonam | <0.015 - 0.12 | 0.07 |
| | Moxalactam | 0.25 - 0.5 | 0.3 |
| | Cefoxitin | 4 - 16 | 9.0 |
| | Piperacillin | 1 - 16 | 1.5 |
| P.stuartii (9) | Aztreonam | ≤0.015 - 0.06 | 0.03 |
| | Moxalactam | 0.06 | 0.03 |
| | Cefoxitin | 1 - 8 | 3.4 |
| | Piperacillin | 64 - 128 | 87 |
| Group A strep.(6) | Aztreonam | 8 - 16 | 12.7 |
| | Moxalactam | 0.5 - 1 | 0.9 |
| | Cefoxitin | 0.25 - 0.5 | 0.45 |
| | Piperacillin | 0.03 - 0.06 | 0.03 |
| | Penicillin | 0.008 - 0.015 | 0.009 |
| E.faecalis (7) | Aztreonam | >128 | >128 |
| | Moxalactam | >128 | >128 |
| | Cefoxitin | >128 | >128 |
| | Piperacillin | 2 - 4 | 3.3 |
| | Penicillin | 2 - 4 | 2.6 |
| S.pneumoniae (9) | Aztreonam | >128 | >128 |
| | Moxalactam | 2 | 2 |
| | Cefoxitin | 1 - 2 | 1.26 |
| | Piperacillin | 0.015 - 0.03 | 0.018 |
| | Penicillin | 0.03 | 0.03 |

**Table 47 : Susceptibility of miscellaneous and clinical isolates to aztreonam and other beta lactam antibiotics.**

Organisms tested at an inoculum of $10^4$ CFU. Results expressed as a range of activity and geometric mean in mg/l.

The activity of aztreonam against known ß-lactamase producing strains is shown in Table 48. Aztreonam was active against strains containing the TEM-1, OXA-1 and SHV-1 enzymes. The two strains possessing chromosomal cephalosporinases and also the 'broad spectrum' enzyme (K1+) were relatively resistant to aztreonam (moxalactam and cefoxitin were active against these strains).

The effect of serum on the activity of aztreonam is shown in Table 49. For the two strains of Esch. coli, Pr. mirabilis and Klebsiella species the addition of serum had little effect on activity. For Pseudomonas an increase in serum (75%) increased the MBC four fold.

The morphological response to aztreonam was the production of filaments.

Assay Validation

In Figure 38 a comparison of results when patient samples were assayed by both HPLC and the microbiological assay are shown graphically. There was no statistical difference between results ($p = 0.803$). In Table 50 the confidence limits of the assay are shown for the pharmacokinetics and an external validation was

| | Aztreonam | | Moxalactam | | Piperacillin | | Cefoxitin | | Gentamicin | |
|---|---|---|---|---|---|---|---|---|---|---|
| inoculum | $10^4$ | $10^6$ | $10^4$ | $10^6$ | $10^4$ | $10^6$ | $10^4$ | $10^6$ | $10^4$ | $10^6$ |
| E. coli TEM-1+* | 0.06 | 0.12 | 0.12 | 0.12 | 64 | 128 | 4 | 4 | 0.12 | 0.25 |
| TEM-1- | 0.06 | 0.25 | 0.5 | 0.5 | 2 | 4 | 2 | 8 | 0.06 | 0.5 |
| E. cloacae P99+ | NG | 8 | NG | 4 | NG | 64 | NG | 64 | NG | 0.25 |
| P99- | 0.03 | 0.06 | 0.06 | 0.12 | 1 | 1 | 4 | 4 | 0.12 | 0.25 |
| E. coli D31 | 8 | 8 | 1 | 1 | 16 | 16 | 64 | 64 | 0.25 | 0.25 |
| E. coli Oxa-1+ | 0.12 | 0.12 | 0.12 | 0.12 | 32 | 32 | 8 | 8 | 0.12 | 0.25 |
| Klebsiella $K_1$+ | 32 | 128 | 0.06 | 0.12 | >128 | >128 | 2 | 2 | 0.25 | 0.25 |
| $K_1$- | 0.03 | 0.06 | 0.06 | 0.12 | 1 | 2 | 2 | 2 | 0.25 | 0.25 |
| K. pneumoniae SHV-1 | 0.25 | 0.25 | 4 | 8 | 64 | >128 | 64 | 64 | 0.25 | 0.25 |

* Isogenic strains containing (+) or not (-) the ß-lactamase enzyme

NG = no growth

**Table 48 : Activity of aztreonam**

The MIC (mg/l) of five antibiotics against known ß-lactamase producing bacteria at inocula $10^4$ and $10^6$ cfu

| Organism | Broth | | Broth + 25% serum | | Broth + 50% serum | | Broth + 70% serum | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| P.mirabilis | 0.06 | 0.25 | 0.06 | 0.06 | 0.06 | 0.25 | 0.06 | 0.12 |
| P.mirabilis | 0.015 | 0.06 | 0.015 | 0.06 | 0.015 | 0.03 | 0.03 | 0.12 |
| Ps.aeruginosa | 4 | 16 | 4 | 16 | 4 | 32 | 4 | 128 |
| Ps.aeruginosa | 8 | 64 | 4 | 64 | 4 | 128 | 4 | 128 |
| Klebsiella species | 0.25 | 0.25 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | 0.25 |
| Klebsiella species | 0.12 | 0.12 | 0.12 | 0.12 | 0.06 | 0.12 | 0.06 | 0.25 |
| Esch.coli | 0.25 | 0.25 | 0.03 | 0.03 | 0.06 | 0.06 | 0.06 | 0.25 |
| Esch.coli | 0.12 | 0.12 | 0.03 | 0.03 | 0.06 | 0.06 | 0.12 | 0.12 |



**Table 49 : The effect of serum on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aztreonam.**

MIC's and MBC's to aztreonam determined for eight strains in Iso Sensitest broth containing 0 , 25 , 50 or 70% human serum at an inoculum of $10^5$ organisms/ml. results expressed in mg/l.

not possible because of the difficulty in transporting samples from Princetown in the USA.

Having developed a microbiological assay it was felt important to determine the stability of aztreonam in plasma and urine when stored under different conditions. This information could then be used for collecting samples in the pharmacokinetic study.

The results of the stability studies are tabulated in Tables 51 and 52. In plasma samples it would appear that in the 100 mg/l and 15 mg/l spiked samples aztreonam was relatively stable for up to 6 h even at 37°C. However, after this period aztreonam was less stable at room temperature and 37°C. Similarly, in urine the same trend was observed. From this study it was confirmed that aztreonam was stable in urine at +4°C for the time intervals selected.

In Table 53 the results to determine the lower limit of sensitivity for the microbiological assay using 6 mm discs and varying microlitre volumes of standard are shown. From previous studies the average volume of peritoneal fluid absorbed by the discs placed in the peritoneum is 20 - 25 µl. From these results it can be seen

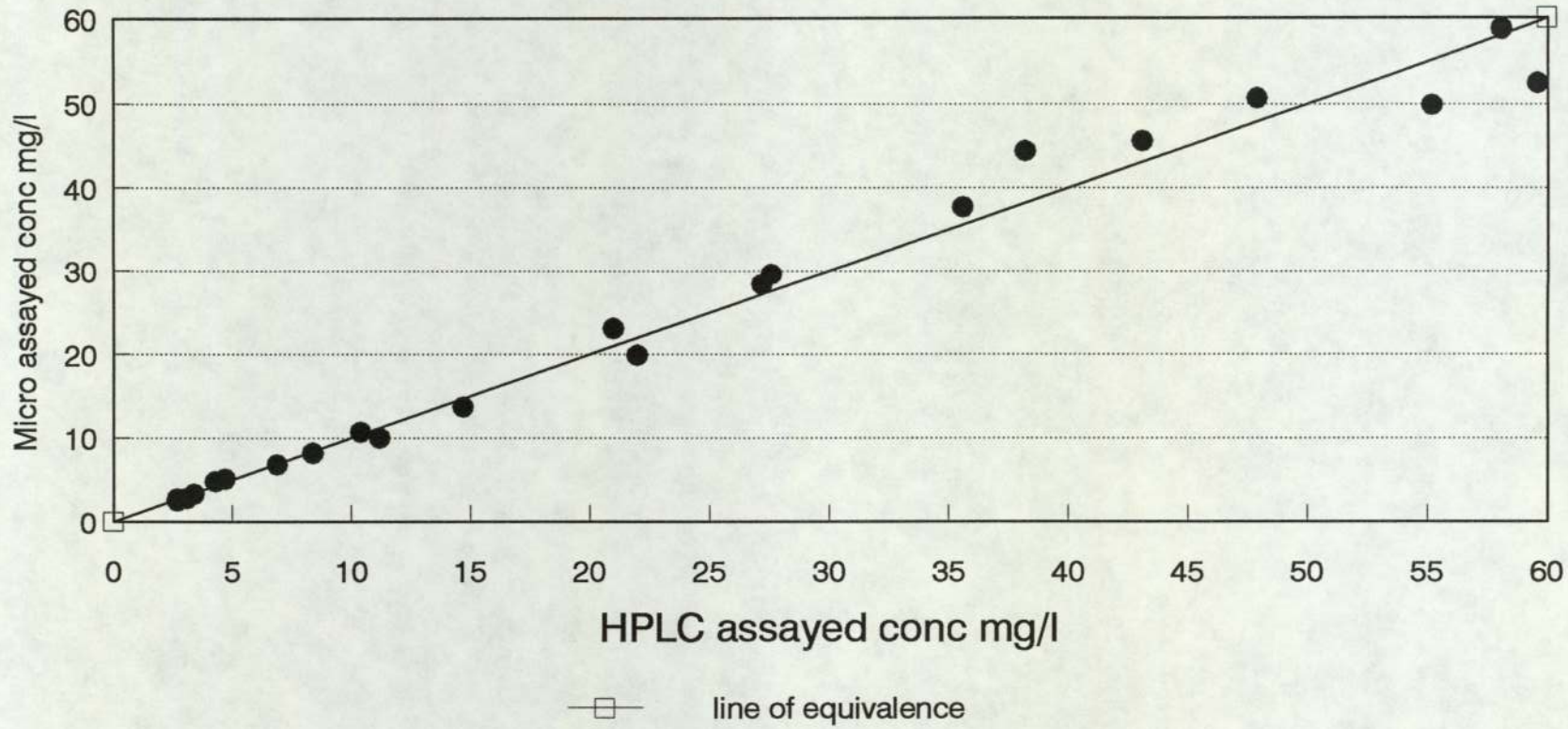



**Figure 38 : Comparison of HPLC and microbiological assay methods used for the assay of aztreonam**

Human serum spiked with concentrations of aztreonam assayed by HPLC and microbiological assay methods. Results obtained by both methods plotted and a line of equivalence constructed between the observed points.

| Assigned concentration (mg/l) | Mean assayed conc (mg/l) | SD | CV |
|---|---|---|---|
| 60 | 60.39 | 4.75 | 7.91 |
| 15 | 14.75 | 0.859 | 5,82 |
| 8 | 8,07 | 0.494 | 6.12 |
| 2 | 2.05 | 0.138 | 6.73 |
| 1 | 1.025 | 0.067 | 6.54 |

**Table 50 : Validation of the aztreonam microbiological assay.**

Antibiotic free human serum spiked with aztreonam
and then assayed by the microbiological assay method.
Mean assayed concentration , standard deviation and coefficient
of variation calculated for each concentration.

| | 100 mg/l | | | | | 15 mg/l | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | -40 | -20 | +4 | RT | 37 | -40 | -20 | +4 | RT | 37 |
| 0 | | | 98.5 | | | | | 15.4 | | |
| 1 | 81 | 103 | 86 | 101 | 92 | 14.4 | 12.3 | 14.2 | 11.8 | 13.7 |
| 2 | 102 | 89 | 112 | 92 | 87 | 13.5 | 13.7 | 13.9 | 13.1 | 14.1 |
| 4 | 104 | 99 | 93 | 94 | 109 | 13.9 | 13.0 | 16.1 | 13.9 | 14.3 |
| 6 | 109 | 109 | 94 | 104 | 98 | 17.4 | 15.6 | 16.3 | 14.4 | 14.9 |
| 24 | 89 | 81 | 91 | 80 | 57 | 12.8 | 14.9 | 13.6 | 11.2 | 7.6 |
| 48 | 83 | 80 | 90 | 90 | 45 | 12.4 | 11.8 | 11.0 | 9.6 | 3.2 |

RT = room temperature

## Table 51 : Stability of aztreonam in plasma

Antibiotic free plasma spiked with two concentrations of aztreonam. Samples stored at -40 , -20 , 4 , room temperature and 37 'C for a 48 h period. Samples assayed at various times during this period. Levels measured at each time interval compred with time zero.

| | 100 mg/l | | | | | 15 mg/l | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | -40 | -20 | +4 | RT | 37 | -40 | -20 | +4 | RT | 37 |
| 0 | | | 91 | | | | | 13.6 | | |
| 1 | 113 | 90 | 91 | 119 | 116 | 11.9 | 13.8 | 14.5 | 17.9 | 15.7 |
| 2 | 140 | 100 | 104 | 119 | 104 | 15.7 | 13.8 | 15.2 | 12.9 | 12.7 |
| 4 | 81 | 93 | 123 | 123 | 142 | 18.1 | 19.3 | 16.9 | 19.3 | 18.7 |
| 6 | 124 | 122 | 97 | 122 | 104 | 15.1 | 15.9 | 14.0 | 16.1 | 16.5 |
| 24 | 84 | 99 | 98 | 63 | 70 | 12.1 | 10.6 | 10.8 | 9.8 | 11.1 |
| 48 | 84 | 75 | 94 | 80 | 68 | 13.3 | 11.3 | 16.4 | 5.9 | 6.0 |

RT = room temperature

**Table 52 : Stability of aztreonam in urine.**

Antibiotic free urine spiked with aztreonam and then stored at various temperatues for a 48 h period. Samples assayed at various time intervals and results compared with time zero.

that if only 15 µl is absorbed a level of 2.5 mg/l can still be detected.

In protein binding experiments no difference was observed between concentration with 50% binding at a concentration of 25 mg/l and 47.2% at 50 mg/l.

Pharmacokinetic Study

The mean levels of aztreonam in plasma are given in Table 54 and shown graphically in Figure 39. The derived pharmacokinetic data are given in Table 55.

The mean plasma level of aztreonam showed a rapid initial distribution phase, which was essentially complete at 1 h, followed by a steady decline. The mean plasma level 30 mins after the dose was 50 mg/l falling to 2.5 mg/l at 8 h. The terminal plasma half-life was 1.93 h.

Aztreonam penetrated the inflammatory fluid rapidly with a mean $C_{max}$ of 25.4 mg/l (SD 5.9) at a mean $T_{max}$ of 1.8 h (SD 0.83). At 1 h the level in inflammatory fluid was approximately one and a half that in plasma. By 8 h the level in inflammatory fluid had fallen to 5 mg/l.

In Table 56 the urinary recovery of aztreonam is tabulated. A mean of 73.7% of the administered

| | Average zone diameter | | | |
|---|---|---|---|---|
| Assigned conc (mg/l) | 25 µl | 20 µl | 15 µl | 10 µl |
| 160 | 264 | 254 | 251 | 238 |
| 80 | 238 | 244 | 237 | 218 |
| 40 | 221 | 211 | 205 | 196 |
| 20 | 193 | 194 | 186 | 165 |
| 10 | 166 | 168 | 155 | 141 |
| 5 | 136 | 131 | 123 | 101 |
| 2.5 | 102 | 106 | 86 | - |

**Table 53 : Measurement of aztreonam in peritoneal fluid.**

Determination of the lower limit of sensitivity of the assay.

Samples containing varying concentrations of aztreonam pipetted onto 6 mm blotting paper discs at various volumes from 10 to 25 ul. Samples assayed and zone diameters obtained recorded.

| Time (h) after IV injection | Mean aztreonam conc. mg/l | |
|---|---|---|
| | plasma | inflammatory fluid |
| 0.25 | 72.5 ± 12.6 | ND |
| 0.5 | 53.5 ± 4.9 | 16.2 ± 8.9 |
| 0.75 | 47.5 ± 6.1 | ND |
| 1.0 | 42.1 ± 6.1 | 22.7 ± 8.4 |
| 1.5 | 30.1 ± 2.9 | ND |
| 2.0 | 24.1 ± 3.5 | 21.8 ± 3.8 |
| 3.0 | 16.0 ± 2.6 | 21.7 ± 4.1 |
| 4.0 | 11.0 ± 1.7 | 16.1 ± 2.9 |
| 5.0 | 7.6 ± 0.9 | 11.8 ± 1.6 |
| 6.0 | 5.3 ± 0.7 | 10.0 ± 1.9 |
| 7.0 | 3.9 ± 0.9 | 6.72 ± 1.5 |
| 8.0 | 2.6 ± 0.7 | 4.9 ± 1.0 |

ND = not determined

**Table 54 : Concentrations of aztreonam in plasma and inflammatory fluid.**

Mean plasma and inflammatory fluid levels obtained for 6 healthy volunteers following a single 1g intravenous dose of aztreonam.

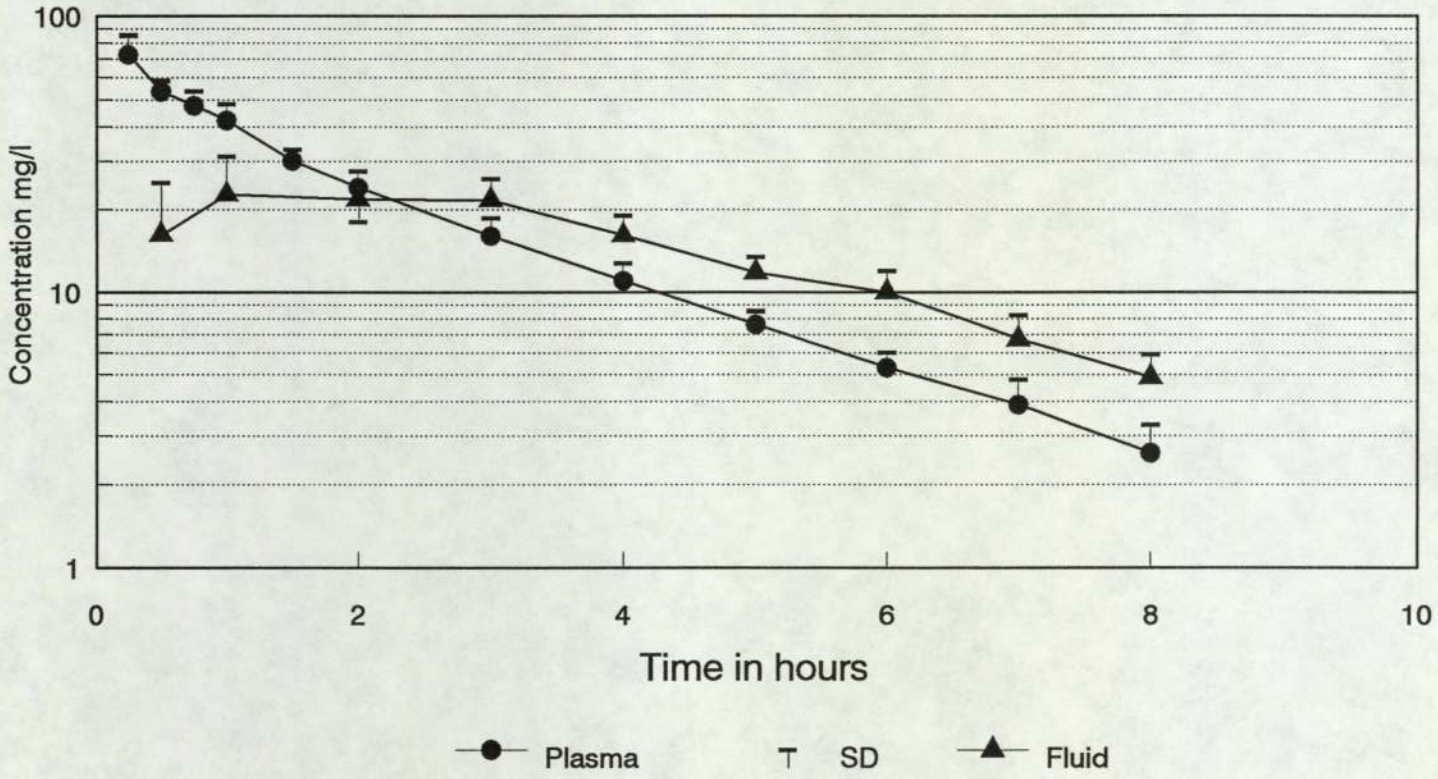



**Figure 39 : Mean plasma and inflammatory fluid concentrations of aztreonam**

Mean levels from 6 healthy volunteers following a single 1g intravenous dose of aztreonam. Concentration in mg/l plotted versus time in hours (timed from the end of the infusion).

| Plasma | |
|---|---|
| C max (mg/l) | 72.5 ± 12.6 |
| (at time of first sampling) | |
| T ½ α (h) | 0.361 ± 0.028 |
| T ½ ß (h) | 1.93 ± 0.14 |
| AUC o - ∞ (mg/l.h) | 189.3 ± 16.6 |
| Vdss | 17.2 ± 3.1 |
| | |
| Inflammatory fluid | |
| C max (mg/l) | 25.4 ± 5.9 |
| T max (h) | 1.8 ± 0.83 |
| T ½ ß (h) | 2.14 ± 0.14 |
| | |
| Plasma clearance (ml/min) | 89.0 ± 7.1 |
| Renal clearance (ml/min) | 65.9 ± 10.5 |
| % recovery in urine in 24h | 73.7 ± 6.41 |

## Table 55 : Pharmacokinetics of aztreonam

Pharmacokinetic parameters derived from data obtained for 6 healthy volunteers following a single 1g intravenous dose of aztreonam.

drug was recovered in urine by 24 h. Urine samples were examined for the presence of metabolites and using the method described none were found.

Intraperitoneal penetration study

In Figure 40 levels of aztreonam in plasma and simultaneous peritoneal fluid for thirty four patients undergoing elective gastrointestinal surgery are shown. Three patients had two sets of samples taken at different time intervals making a total of 40 time points.

Plasma levels greater than 10 mg/l were found for up to 4.5 h after administration of aztreonam. Levels in plasma were similar to those found in the pharmacokinetic study and the plasma half-life (taken from observations after 90 min) was found to be 1.65 h which is similar to that found in the volunteer study (1.93 h).

The half-life of aztreonam in peritoneal fluid was 1.5 h and mean levels of more than 10 mg/l were found for up to 5 h post dose. The mean percentage penetration of aztreonam into peritoneal fluid was 100.2%.

| Time | Vol 1 | Vol 2 | Vol 3 | Vol 4 | Vol 5 | Vol 6 |
|---|---|---|---|---|---|---|
| 0 - 2 | 429.4 | 492.8 | 416.5 | 416 | 431.3 | 503 |
| 2 - 4 | 131 | 146.6 | 180.9 | 126.9 | 210.5 | 147.2 |
| 4 - 8 | 102.9 | 79.2 | 121.4 | 91.8 | 103.8 | 135.8 |
| 8 - 12 | 9.4 | 12.2 | 18.8 | 15.8 | 28.7 | 30.8 |
| 12 - 24 | 4.8 | 4.1 | 4.7 | 6.2 | 5.8 | 6.7 |
| Total excreted | 677.5 | 734.9 | 742.3 | 656.7 | 780.1 | 823.5 |
| % excreted | 68 | 74 | 74 | 66 | 78 | 83 |

**Table 56 : Urinary excretion of aztreonam (mg) following a single 1g IV dose**

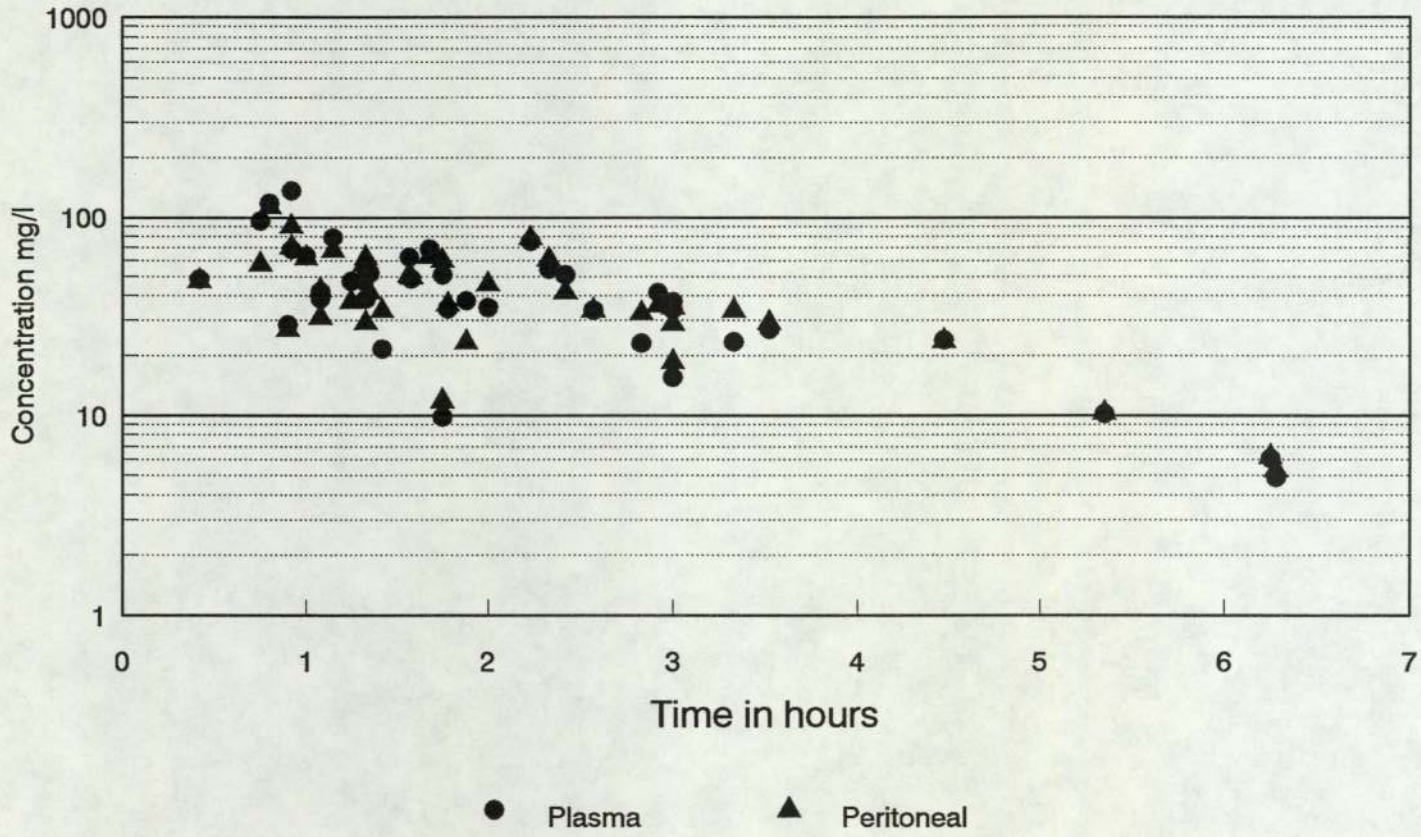



**Figure 40 : Intraperitoneal penetration of aztreonam**

Levels in peritoneal and simultaneous plasma samples in 34 patients following a single 1g intravenous dose of aztreonam. Concentration in mg/l plotted against time in hours (timed from the end of the infusion)

**Discussion**

The antibacterial spectrum of aztreonam contrasts with other ß-lactams in that it displays high activity against the Enterobacteriaceae yet has little or no activity against aerobic Gram positive cocci or Bacteroides species. It also has only modest activity against Ps. aeruginosa.

From the in vitro study it would appear that aztreonam is resistant to hydrolysis by the TEM-1, OXA and SHV-1 plasma mediated ß-lactamases and this is important as they account for over 90% of the ß-lactamases found in Gram negative organisms (65). It would appear however, that aztreonam is hydrolysed to some extent by certain chromosomally-mediated cephalosporinases and broad spectrum ß-lactamases - Richmond and Sykes, 1973 Groups I and IV respectively (66) have also described the instability of aztreonam to Group I enzymes but not to Group IV.

Aztreonam would appear to be bactericidal, there being little difference between MIC and MBC, and serum did not exert much influence on the activity of aztreonam (this can be expected from a drug which is only modestly protein bound).

The results of the pharmacokinetic study are

similar to those previously described (67) with the exception of the terminal half-life in this study being 1.93 h and in the previous study 1.66 h. The method of calculating the half-life may account for these differences.

Aztreonam appears to be eliminated predominantly by the kidneys. The fate of 25% of the administered dose not recovered is unknown. However, it is possible that the drug is eliminated by the biliary route. No metabolites were found by HPLC in the limited search undertaken in urine samples. However, as the search was not exhaustive, metabolism and consequent renal elimination might occur undetected by this method.

Aztreonam is very active against the majority of Enterobacteriaceae having $MIC_{90}$s of less than or equivalent to 0.5 mg/l. By extrapolation of the data from the pharmacokinetic study levels exceeding 0.5 mg/l will be attained for about 12 h in both plasma and blister fluid (similar results for the peritoneal fluid).

It would appear from these data that a twice daily dosing of 1G of aztreonam would be adequate to treat most infections by susceptible pathogens.

However, in the treatment of Ps. aeruginosa having an $MIC_{90}$ of 8 mg/l more frequent dosing may be required. For prophylaxis in gastrointestinal surgery a single 1 G dose would seem sufficient. However, in the case of an established infection a three or four times daily dosing may be necessary and in mixed infections the addition of other agents to cover anaerobes, and Gram positive cocci may be prudent.

# CONCLUSIONS

## Cefpirome

Cefpirome is a very potent extended spectrum parenteral cephalosporin with marked stability to hydrolysis by ß-lactamase. However, this study suggests that the compound is hydrolysed by the chromosomally-coded K1 ß-lactamase. Cefpirome shows poor activity against Bact. fragilis. Differential interference microscopy suggests that the primary target site of cefpirome in PBP3 (filamentation observed). Cefpirome is a bactericidal compound whose activity is not significantly altered by the presence of human serum or an increase in inoculum.

Following intravenous administration cefpirome penetrated rapidly into mild inflammatory exudate, peritoneal fluid and bronchial mucosa. Levels at all sites exceeded the $MIC_{90}$ for the majority of pathogens (with the exception of anaerobes) for up to a 6 h period. Cefpirome appears to be eliminated predominantly by the kidney.

Cefpirome has a wide spectrum of activity, including activity against some strains of E. faecalis and penetrates well into tissues and fluids. However, its poor activity against Bact. fragilis means that it

has to be combined with another agent if the presence of anaerobes is suspected. Data from patients treated with cefpirome are currently being evaluated and it will be interesting to see if the side effects, observed in some patients, have been elucidated.

Cefixime

These in vitro and pharmacokinetic studies have shown that cefixime has markedly different properties compared with other oral agents currently available such as cefuroxime axetil. Cefixime is active against the common respiratory and urinary tract pathogens with the exception of Staphylococci, Enterococci, Ps. aeruginosa and anaerobes.

The serum elimination half-life of cefixime is significantly longer than other oral cephalosporins (cefixime approximately 3.5 h, cephradine <1 h). In these studies only 19.9% of the administered dose was recovered in urine (appreciable amounts have been found in bile) which is much less than other oral agents (cephradine in excess of 80%). Penetration into inflammatory fluid was slow. However, levels obtained were only slightly lower than those found in serum. Levels in bronchial mucosa were higher than those found in sputum (approximately 35-40% of serum concentrations).

The major difference between cefixime and the other currently available oral ß-lactam antibiotics is its long elimination half life. Most oral ß-lactams need to be administered twice or three times daily. To have an antimicrobial such as cefixime with a similar spectrum of activity but which only has to be given once a day is obviously an advantage. Because of its anti Gram-negative activity it can obviously be used for the treatment of uncomplicated urinary tract infections. However, perhaps its most useful contribution is in the treatment of upper and lower respiratory tract infections, pharyngitis, tonsillitis and childhood acute otitis media.

Meropenem

Meropenem like imipenem has a broad spectrum of activity and appears unaffected by common plasmid-mediated, class 1 ß-lactamases and the ß-lactamase produced by Bact. fragilis. Meropenem displayed good activity in vitro against Staphylococci including methicillin resistant strains. However, treatment failures have been encountered when imipenem has been administered and therefore it would seem prudent to use meropenem with caution for infections attributed to these strains.

Meropenem penetrates well into inflammatory and

peritoneal fluid (approximately 90% compared with serum) levels in plasma, inflammatory fluid and peritoneal fluid exceed the $MIC_{90}$ of most pathogens for a 6 to 8 h period. However, for Ps. aeruginosa and E. faecalis levels are only exceeded for a 4 to 6 h period. Of considerable importance is meropenem's stability to hydrolysis by renal dehydropeptidase 1 (DHP-1) which means that co-administration of the enzyme inhibitor cilastatin is unnecessary. Approximately 65% of the administered dose is excreted in urine which is higher than that found for imipenem.

Meropenem is a very promising carbapenem, combining the spectrum of activity and stability to ß-lactamases seen with imipenem, yet, lacking the instability problems (instability to human dehydropeptidases, heat and moisture) associated with imipenem. This is obviously a major advantage not only clinically but also when undertaking in vitro experiments.

FCE 22101

The in vitro activity of FCE 22101 is somewhat less than that of other ß-lactams. However, because of its narrow range of activity it makes it more predictable in its degree of activity. FCE 22101 is stable to a variety of ß-lactamases and unlike many

other ß-lactams displayed good activity against anaerobes and E. faecalis. Although displaying good activity against methicillin resistant strains, clinical failures have been recorded when imipenem has been used against these strains.

FCE 22101 penetrates rapidly into inflammatory fluid following both oral and intravenous administration. However, these data suggest that because of the short elimination half life (approximately 0.8 h), the drug would have to be administered at least 4 or maybe 6 times a day to maintain good levels. Although FCE 22101 is an interesting drug it is difficult to envisage a clinical situation where it would be chosen in preference to a drug which does not have to be administered so frequently. This may be the reason for the delay in introducing it for general use.

Tazobactam

Tazobactam is similar to clavulanic acid in its ability to protect piperacillin to hydrolysis by ß-lactamase. It would appear from in vitro data that a ratio of piperacillin: tazobactam of 4 or 8:1 would reduce the MIC of the majority of ß-lactamase producing strains to 32 mg/l or less. Exceptions are those strains possessing chromosomal ß-lacatmases.

These studies have shown that piperacillin has a major influence on the pharmacokinetics of tazobactam. Plasma and inflammatory fluid concentrations of tazobactam were higher when co-administered with piperacillin and also $AUC_{0-00}$ were also greater. These studies have also shown that the 8:1 ratio as administered is maintained over a 6 h period in plasma and inflammatory fluid.

The advantage of protecting a ß-lactam antibiotic with a ß-lactamase inhibitor such as tazobactam is obvious (commonest mechanism of resistance to ß-lactam antibiotics is the TEM enzyme). However, another very important role which combinations such as tazobactam and piperacillin can play, are in those clinical situations where mixed infections are encountered, for example abdominal sepsis. Often in these situations several antimicrobials, often including an aminoglycoside are given. Toxicity associated with antimicrobials such as aminoglycosides, can therefore be avoided if a combination such as tazobactam and piperacillin are administered.

Aztreonam

The antibacterial activity of aztreonam is somewhat different than other ß-lactams in that it displays high activity against the Enterobacteriaceae

yet little or no activity against Gram positive cocci or Bacteroides species. It also has only modest activity against Ps. aeruginosa. Aztreonam is resistant to hydrolysis by the plasmid mediated ß-lactamases. However, aztreonam would appear to be hydrolysed to some extent by Type I chromosomally mediated ß-lactamases. Aztreonam would appear to be bactericidal and its activity is unaffected by the presence of human serum or an increase in inoculum.

Aztreonam penetrates well into inflammatory and peritoneal fluid. Levels in plasma, inflammatory fluid and peritioneal fluid exceed the $MIC_{90}$ of the majority of Enterobacteriaceae ($\leq$0.5 mg/l) for about a 12 h period. In the case of mixed infections the addition of other agents to lower anaerobes and Gram positive cocci may be advisable.

The spectrum of activity of aztreonam is somewhat narrower than that of the third generation cephalosporins, this, may be an advantage because aztreonam's lack of activity against Gram positive bacteria and anaerobes may reduce the potential gastro-intestinal disturbance associated with broad spectrum antimicrobials. This plus its stability to a wide variety of ß-lactamases makes aztreonam a useful antimicrobial. However, because of its narrow spectrum

of activity its use should be restricted to infections due to susceptible aerobic, Gram negative organisms.

In summary, the 5 ß-lactams which have been studied in this thesis display a range of useful properties as antimicrobials which suggest they have a useful part in the treatment of infectious diseases. No single ß-lactam yet has a combination of full spectrum of activity, low toxicity and pharmacokinetic/delivery properties and it means that further development of ß-lactams will continue.

## Publications resulting from this thesis

Stone JW, Linong G, Andrews JM and Wise R. Cefixime, in-vitro activity, pharmacokinetics and tissue penetration. J Antimicrob Chemother 23:221-228, 1989.

Wise R, Ashby JP and Andrews JM. The antibacterial activity of meropenem in combination with gentamicin or vancomycin. J Antimicrob Chemother 24 (Suppl A):233-238, 1989.

Saxby MF, Arkell DG, Andrews JM and Wise R. Penetration of cefpirome into prostatic tissue. J Antimicrob Chemother 25:488-490, 1990. (C)

Wise R, Logan MN, Cooper M, Ashby JP and Andrews JM. Meropenem pharmacokinetics and penetration into an inflammatory exudate. Antimicrob Agent Chemother 34:1515-1517, 1990.

Wise R, Logan MN, Cooper M and Andrews JM. Pharmacokinetics and tissue penetration of tazobactam administered alone and with piperacillin. Antimicrob Agent Chemother 35:1081-84, 1991.

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* Translations