CHAPTER I

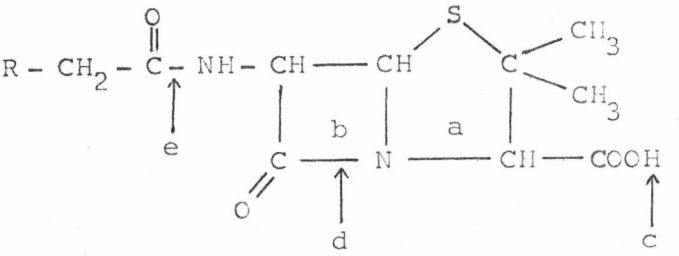
INTRODUCTION

Background and rationale

Penicillin was first discovered from Penicillium natatum by Sir Alexander Fleming in 1929, but it was not until the early 1940s that Florey, Chain and their colleagues demonstrated its therapeutic effectiveness in man and introduced into clinical medicine, since the era of antibiotic chemotherapy had begun (1,2). The early penicillin was a mixture of several penicillin compounds, designated as, F, G, K, O and X. Penicillin G or benzylpenicillin was found to be the most satisfactory and widely used for therapeutic purposes (1,2,3). Although, it has had enormous impact on the morbidity or mortality of many bacterial infections, benzyl penicillin has three notable deficiencies : it is not well absorbed from the gastrointestinal tract because of the destruction by gastric acid, it has a narrow spectrum with poor activity against the enterobacteriaceae and it is susceptible to destruction by bacterial enzymes, the penicillinase or β-lactamase (3,4). In order to overcome the acid instability of benzyl penicillin, the phenoxymethylpenicillin (Penicillin V) was synthesized. Penicillin V is not destroyed in the stomach and therefore can be administered orally (1). When given orally, it is absorbed sufficiently well to acheive therapeutic blood levels. However, only 30-40 percent of the oral dose was found in urine (5,6)

indicating that the oral absorption of phenoxymethylpenicillin is not complete even though it is superior to that of benzyl penicillin. The antibacterial spectrum of phenoxymethylpenicillin is still narrow to those gram positive species only and it remains entirely vulnerable to penicillinase action (7).

Benzyl penicillin and phenoxymethylpenicillin are natural penicillins. They are produced biosynthetically from Penicillium chrysogenum by fermentation. Benzyl penicillin or phenoxymethylpenicillin is formed when the precursor, phenylacetic acid or phenoxyacetic acid is added to the culture medium (1). Deacylation of benzyl penicillin is brought about by amidase enzymes of bacterial origin which splitts off the side chain leaving the 'penicillin nucleus' or 6-aminopenicillanic acid (6-APA). The 6-APA nucleus itself consists of a thiazolidine ring fused to a β-lactam ring (Figure 1). This structure is converted into bacteriologically inert penicilloic acid by enzyme β-lactamase, which splits open the β -lactam ring (Figure 1). The new or semisynthetic penicillins are derived by grafting different side chains onto 6-APA nucleus so confering wide different pharmacological and antibacterial properties. Also, the deficiencies of the earlier penicillins could be improved (2,4)



R = C_6H_5- benzyl penicillin

R = C_6H_5O- phenoxymethyl-penicillin

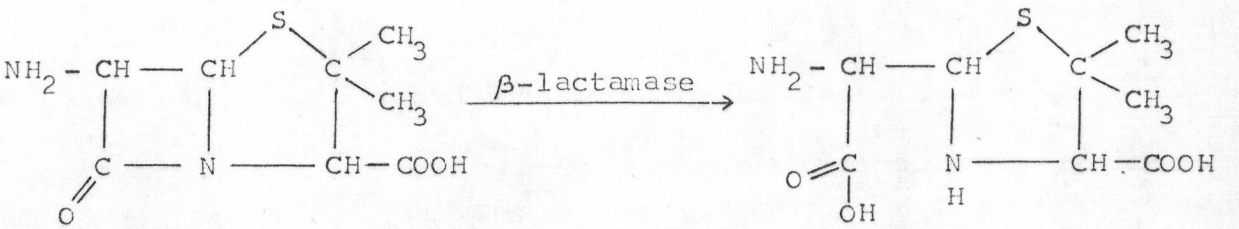
a thiazolidine ring

b β-lactam ring

c site of salt/ester formation

d site of action of penicillinase (β- lactamase)

e site of action of amidase



6-Aminopenicillanic acid (6-APA)

Penicilloic acid

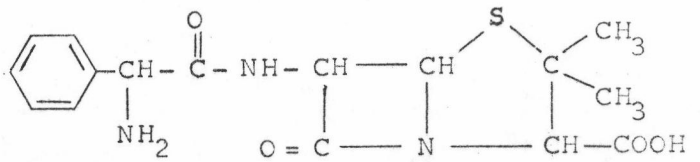
Figure 1 Structural formulae of benzyl penicillin, phenoxymethylpenicillin, 6-aminopenicillanic acid (6-APA) and penicilloic acid (3).

In 1961 ampicillin, the first semisynthetic penicillin, was synthesized from 6-APA nucleus and introduced into clinical uses (1,7). Unlike benzyl penicillin, it possesses a broader antibacterial spectrum. It is active against a wide range of gram positive and gram negative microorganisms. This broad spectrum activity and along with a low toxicity soon made it a very widespread used antibiotic, particularly for respiratory, urinary and mixed infections (8). Although, the broad spectrum

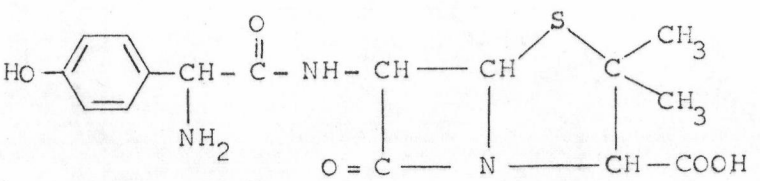
activity of ampicillin improved on that of benzylpenicillin and phenoxymethylpenicillin, its oral absorption is rather incomplete. Earlier studies showed that when ampicillin was given orally, about 24-45 percent of the dose was absorbed and the urinary recovery was only about 30-50 percent of the dose (10-14). Sjovall, J. (15) also presented data showing limited oral absorption of ampicillin upon an increased dose. Thus, poor absorption can, only to a certain degree, be compensated by increasing the doses. In serious infections, the drug must be administered parenterally in order to achieve maximum effectiveness. Interestingly, it is beleived that unabsorbed drug probably causes rather high incidence of diarrhoea by interaction with the normal intestinal flora (16). However, ampicillin remains susceptible to the destruction by β-lactamase produced by many resistant strains of bacteria including Escherichia coli and penicillin-resistant Staphylococcus aureus (1,7).

It was natural therefore to continue the search for other penicillins that improve oral absorption characteristic and resist to β-lactamase enzymes. There has been great interest in mechanisms by which greater oral absorption of ampicillin was achieved and thereby concentrations of drug in serum and tissues are increased. Greater absorption also should decrease the amount of drug in the intestine and decrease the gastrointestinal side effects due to the presence of unabsorbed drug. Several different methods to acheive maximum absorption of ampicillin have been tried. One approach was the modification of the structure to improve oral absorption, without essentially altering

the antibacterial activity of the parent compound. For example, by substituting the hydroxyl group at p-position of α-aminobenzylpenicillin resulted in a new compound named, amoxycillin (Figure 2) which was better absorbed than ampicillin itself (17-19).



Ampicillin or α-aminobenzylpenicillin



Amoxycillin

Figure 2 Structure of ampicillin and amoxycillin

Eshelman, F.N. (20) summarized the reports of the pharmacokinetics of amoxycillin compared with ampicillin. He showed that the mean peak serum concentrations after oral administration of amoxycillin 500 mg were 1.2 to 2.6 times higher than those obtained after administration of ampicillin 500 mg. The mean percentage of urinary recovery was as high as 62 percent compared to 42 percent after ampicillin administration. In Verbist's studies (21) the absorption of ampicillin and amoxycillin were compared using the following three parameters: the mean peak serum levels, the area under the curve and the percentage of recovery in urine. He reported that the absorption of amoxycillin was 2.1 to 2.9 times higher than that of ampicillin, when the corresponding 500 mg dose was given. An explanation for the difference in the absorption of ampicillin and amoxycillin was

given by Brogden, et al.(22). Both ampicillin and amoxycillin are practically water-soluble as free acid and both have a low lipid solubility compared with other penicillins. Acid stability, a critical factor for oral absorption was not significantly different, for ampicillin the half-life of 1% solution at pH 1.5, 37°C was 12 hours compared to the half-life of 17 hours for amoxycillin (23-25). The plausible explanation is that the p-hydroxyl group of amoxycillin enable it to be more readily accepted than ampicillin by the natural transport mechanisms in the intestinal mucosa for aminoacids and short peptide chains. It is interesting that m-hydroxy ampicillin is less well absorbed than ampicillin itself (26).

In another sophisticated approach to improve oral absorption of ampicillin, special interest has been paid on the esterification of the ampicillin molecule, at a position that does not alter the stability of the agent, and yet allows for rapid hydrolysis to ampicillin. The hydrolysis is greatly enhanced by non-specific esterase enzymes present in the intestinal mucosa and serum (27). This method appears to be most successful to improve oral absorption of ampicillin. The esters are called prodrugs or pro-ampicillin. They are virtually microbiologically inactive, but following absorption, they are rapidly hydrolysed to liberate ampicillin, the active compound. By this sort of approach, it is possibly necessary to send much larger quantities of the prodrug of ampicillin across the gut mucosa than is the case with unchanged compound. Some prodrugs of ampicillin are :

Hetacillin

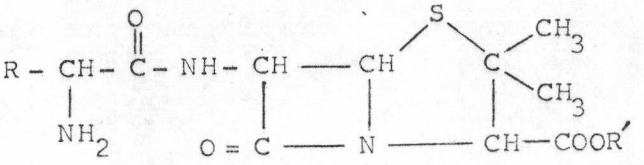
Metampicillin

Pivampicillin

Talampicillin

Bacampicillin

Bacampicillin, pivampicillin and talampicillin are called thiazolidine esters, they differ from ampicillin by the attachment of an ester group to the carboxyl group at C_3 of the thiazolidine ring. The attachment thereafter forming a less polar compound than ampicillin itself. It is thought that the improved absorption of these thiazolidine esters is due to high lipid solubility conferred by the ester groups (27-32).



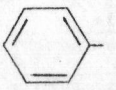
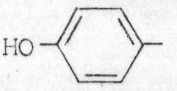
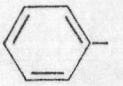
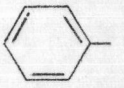
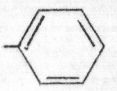
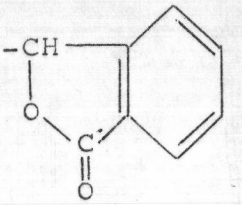
	R	R′
Ampicillin		H
Amoxycillin		H
Bacampicillin		$-CH(CH_3)OCOOC_2H_5$
Pivampicillin		$-CH_2OCOC(CH_3)_3$
Talampicillin		

Figure 3 Structures of some ampicillin derivatives compared with ampicillin (4).

The oral absorption of ampicillin compared with its esters have been reported by several investigators. Clayton, J.P. (28) reported that the mean peak serum level attained after administration of talampicillin 370 mg, equivalent to 250 mg ampicillin was 6.2 mcg/ml and the urinary recovery of ampicillin was 83 percent compared to the mean peak serum level of 2.6 mcg/ml and 54 percent urinary recovery obtained after ingestion of ampicillin. Likewise, following a single oral dose of pivampicillin HCl equivalent to ampicillin 500 mg, the average peak serum levels ranged 8-10 mcg/ml were reported, compared to 2-6 mcg/ml attained after ampicillin administration. On the other hand, about 55-75 percent of the drug was recovered in urine within 6 hours after pivampicillin administration, whereas only about 30-40 percent of oral ampicillin was recovered in the same period of time (21,31). For bacampicillin, earlier reports showed that the mean peak serum concentrations of ampicillin obtained after bacampicillin administration were 2-3 times higher and the peak appeared earlier than those obtained after the administration of ampicillin. The AUC, an indication of the extent of bioavailability of drug, was 1.5 times greater for bacampicillin than for ampicillin. The urinary recovery of ampicillin after bacampicillin administration was over 70 percent (33-38).

While the superiority of absorption of the ester prodrugs over ampicillin is undoubted, the relative positions of the esters has yet to be resolved. The most relevant study was that of Sjovall, et al. (35) who investigated the oral absorption of amoxycillin, bacampicillin and pivampicillin in equimolar doses

in 11 healthy volunteers. Bacampicillin was most rapidly absorbed, yeilding the highest peak serum concentrations. The mean of the individual peak concentrations were 8.3 mcg/ml, 7.1 mcg/ml, 7.7 mcg/ml and 3.7 mcg/ml for bacampicillin, pivampicillin, amoxycillin and ampicillin respectively. Urinary recovery for amoxycillin and bacampicillin were both over 70 percent, while it was 65 percent for pivampicillin. None of these differences were however statistically significant, due to the wide scatters of the individual values. The results were confirmed by the study of Ehrnebo, et al. (39) who showed that the bioavailability of bacampicillin and pivampicillin in 5 healthy male subjects were not significantly different. From their unpublished data, Sjovall and Magni (4) could find no significant differences in a crossover study in 10 healthy volunteers between talampicillin and bacampicillin, in terms of peak concentrations, the area under the curves and urinary recoveries of ampicillin.

Unfortunately, the earlier studies evidenced that talampicillin and pivampicillin produced higher incidence of upper gastrointestinal side effects than bacampicillin (16,40). Bacampicillin was very well tolerated with a low incidence of diarrhoea (16). There were clinical evidences that bacampicillin administered only twice daily could be therapeutically effective against numerous gram positive and gram negative bacterial infections (41-44), due to the higher serum concentrations of ampicillin attained after bacampicillin administration, the corresponding tissue concentrations were higher and the drug stayed longer than those attained after oral administration of ampicillin (45). However, in serious infections, the dose of bacampicillin as

high as 3200 mg has been recommended and well tolerated by many patients. The trend towards an increased frequency of adverse reactions with increasing doses of bacampicillin would have little clinical significance. The adverse reactions are generally of mild or moderate types (46).

In this study, the attention is concentrated on bacampicillin since it appears preferable among the present ampicillin prodrugs due to its fewer side effects and well tolerant.

As mentioned before that it is not possible to increase the blood concentrations of ampicillin greatly by increasing the doses because of a non-linearity between doses and peak concentrations and doses and the area under the curves, but this non-linearity is not as pronounced with increased doses of the ampicillin esters (15,32,38). The studies of the effect of food on the absorption of ampicillin and ampicillin esters were reported by many investigators. They agreed that prior or concurrent ingestion of food reduced the peak concentrations produced by oral administration of ampicillin (20,47,48), but no significant effect was observed on the absorption of ampicillin esters (29,34,46,47). Some studies even noted an increase in the absorption of ampicillin esters with food (37,49).

An extensive study was therefore carried out to investigate the pharmacokinetics of ampicillin after bacampicillin administration in Thai-healthy volunteers. Also, the study of dose-drug concentration relationship and the effect of food on oral absorption of bacampicillin were performed.

Objectives :

1. To compare the concentrations of ampicillin obtained from both serum and urine after single oral administration of two different doses of ampicillin with those obtained after single oral administration of bacampicillin.

2. To calculate and compare the pharmacokinetic parameters of ampicillin obtained after a single oral dose of ampicillin with those obtained after single oral administration of bacampicillin.

3. To compare some pharmacokinetic parameters calculated from serum data with those obtained from urinary excretion data.

4. To study the dose-drug concentration relationship of ampicillin and bacampicillin.

5. To verify whether food affected the oral absorption of bacampicillin.

Materials and Methods

Subjects

Inclusion criteria : Fourteen healthy adult volunteers, seven males and seven females are included in this study. They are Thai, aged 20-45 years.

Exclusion criteria : History of cephalosporin or penicillin hypersensitivity.
Known hepatic or renal diseases

Diseases of the gastrointestinal tract.

Other antibiotics treatment.

Ethical consideration : All participants are fully informed about the nature and the possible hazards of this study as there are hypersensitivity reactions and gastrointestinal disturbances. They also give their consent to take part.

Drug dosage : Bacampicillin tablet contains bacampicillin hydrochloride 400 mg

Ampicillin capsule contains Ampicillin B.P. 250 mg

Ampicillin capsule contains Ampicillin B.P. 500 mg

Drug administration : The drugs were given orally in a single dose.

Experimental design : The study was designed in a randomized crossover fashion with an interval of one week between the treatments.

Sampling : Blood samples were drawn from the antecubital vein immediately before the administration of the drug and at different time intervals after drug intake for a period of up to 8 hours. The blood samples were allowed to clot, collected for serum samples and kept in deep freeze condition ($-60^{\circ}C$) until analysed.

Urine samples were collected immediately before drug intake and at different time intervals for 8 hours after drug administration. The volume of each urine sample was recorded and an aliquot portion was kept in a test tube and was deep frozen until analysed. All samples were analysed within one week after collection.

Sample analyses(35,51): The ampicillin concentrations in serum and urine samples were determined microbiologically by the agar diffusion method using Sarcina lutea ATCC 9341 as the test organism and Antibiotic media No. 1 as substrate. The standard solutions for serum samples were prepared using Sterile Human Plasma as diluent and those for urine samples Sorensen's phosphate buffer, pH 7.0 was used. The samples were diluted to the appropriated concentrations with the same diluent used in preparing the standard solutions.

Pharmacokinetic analyses : Pharmacokinetic evaluations were performed by assuming that a first-order one-compartment open model was valid for this drug. For each subject the following parameters were calculated: absorption rate constant (Ka), overall elimination rate constant (Ke), bio-

logical half-life ($t_{\frac{1}{2}}$), area under the serum concentration-time curve (AUC), urinary excretion rate constant (k_e), apparent volume of distribution (Vd) and total clearance (Cl_T).

Statistical analyses : Serum concentrations and pharmacokinetic parameters were compared statistically using Student's t test and Analysis of variance.

Significance of the study :

1. The pharmacokinetic study of any drug can serve as a part of the preclinical study. Clinical uses of a new drug are usually based upon this type of study. It also enable us to deduce the intrinsic properties of a drug and suggest the most suitable way of handling it.

2. This study should provide several meaningful information about the pharmacokinetics of a new drug, bacampicillin in Thai healthy adult volunteers. The results will be compared with previously reported studies which were performed in other countries. The effects of races and tribes on the pharmacokinetics of this drug could thus be notified.

3. From the pharmacokinetic information obtained, we can justify whether the new drug, bacampicillin really has substantial advantages over ampicillin.

4. This study will also provide information about the

effect of food on the bioavailability of bacampicillin, which therefore suggest the suitable way of the drug administration for maximum effectiveness.

5. The study of dose-drug concentration relationship of the two progressive doses of ampicillin and bacampicillin will manifest whether the bioavailability of ampicillin following ampicillin and bacampicillin oral administration increase in proportional with the increased doses.

6. If the pharmacokinetic parameters calculated from urinary excretion data agrees very well with those calculated from serum data, so it may be possible to follow the time course of drug concentrations by means of urine collection, whenever the pharmacokinetic information of this drug is needed. The urine collection is sometimes chosen in preference to drawing blood periodically since it is a convenient, safe and non-invasive method.