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Amoxicillin: In Vitro and Pharmacological Studies

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Amoxicillin is a new semisynthetic penicillin which is active in vitro against gram-positive cocci (except penicillin G-resistant *Staphylococcus aureus*) and most isolates of *Proteus mirabilis* and *Escherichia coli*. Its in vitro activity is quite similar to ampicillin, but it produces higher serum levels after oral administration. The mean peak serum levels of amoxicillin in 11 normal volunteers were 2.30 μg/ml after 125 mg, 3.43 μg/ml after 250 mg, 6.75 μg/ml after 500 mg, and 9.90 μg/ml after 1 g. About 70% of the drug was excreted in the urine during the first 6 hr.

The ability to synthesize derivatives of penicillin G was a major advance in antibiotic therapy. The discovery of ampicillin broadened the spectrum of activity of penicillins to include some gram-negative bacilli. Recently, attempts have been made to improve the absorption of ampicillin by modifying its structure. Amoxicillin (α-amino-*p*-hydroxybenzylpenicillin, BRL-2333) is a new semisynthetic penicillin with antibacterial activity similar to ampicillin (Fig. 1; see references 7 and 10). However, the drug is more effective than ampicillin for the treatment of experimental infections in mice and results in substantially higher serum levels in humans (1, 2, 8). This report describes the results of in vitro susceptibility testing with amoxicillin and the serum levels achieved in normal volunteers after oral administration of this drug.

MATERIALS AND METHODS

Susceptibility tests were performed with 376 clinical isolates of gram-positive cocci and gram-negative bacilli using a micro-dilutor (5). Isolates of *Staphylococcus aureus* were inoculated into tryptose phosphate broth, and isolates of group A beta-hemolytic streptococci and pneumococci were inoculated into tryptose phosphate broth containing 2% human blood (9). After incubation at 37 C for 18 hr, a 0.1-ml sample of this broth containing organisms was added to 9.9 ml of tryptose phosphate broth, and a 50-μliter sample was used as the inoculum for susceptibility testing. The tryptose phosphate broth used for streptococci and pneumococci contained 1.5% human blood. All gram-negative bacilli were inoculated into Mueller-Hinton broth. After incubation at 37 C for 18 hr, a 10^{-5} dilution was made in Mueller-Hinton broth, and a 50-μliter sample was used as the inoculum for susceptibility testing. The total volume in each well was 100 μliters.

The antibiotics studied were amoxicillin (supplied by Beecham-Massengill Pharmaceuticals, Division of Beecham, Inc., Clifton, N.J.), ampicillin (supplied by Bristol Laboratories Syracuse, N.Y.), and cephalothin (supplied by Eli Lilly & Co., Indianapolis, Ind.). The antibiotics were diluted in either tryptose phosphate or Mueller-Hinton broth to a final concentration of 200 μg/ml. Twofold serial dilutions of the antibiotics were made with broth, using 50-μliter samples, and the minimal inhibitory concentration (MIC) was determined after incubation at 37 C for 18 hr. All studies were performed in triplicate.

The organisms used in this study were cultured from specimens obtained from patients between January 1967 and November 1971. A total of 58 isolates of group A beta-hemolytic streptococci, 19 isolates of *Diplococcus pneumoniae*, 49 isolates of *S. aureus*, 50 isolates of *Proteus* spp., 100 isolates of *Escherichia coli*, and 25 isolates each of *Serratia marcescens*, *Pseudomonas aeruginosa*, *Klebsiella* spp. and *Enterobacter* spp. were studied.

Clinical pharmacological studies were conducted in 11 normal volunteers ranging in age from 20 to 36 years old and weighing from 106 to 182 lb. Informed consent was obtained from each subject according to institutional policies. All of the subjects had normal values of serum creatinine, glutamic oxaloacetic and pyruvic transaminases, alkaline phosphatase, and blood-urea nitrogen. There was an interval of at least 2 days between studies in the same individual. Studies were conducted with amoxicillin (supplied by Beecham-Massengill Pharmaceuticals, Division of Beecham, Inc., Clifton, N.J.) and ampicillin (supplied by Bristol Laboratories, Syracuse, N.Y.). The drugs were administered orally after an overnight fast. Blood specimens were obtained prior to administration of the antibiotic, and at 30 min and 1, 2, 3, 4, and 6 hr after administration of the antibiotic. Urine was collected before administration of the antibiotic and during the study period. In the study of the effect of probenecid on the serum levels of amoxicillin, a 0.5-g dose was given 1 hr before amoxicillin and every 6 hr for a total of three doses.

Antibiotic concentrations were determined by using an agar well method with *Sarcina lutea* NCTC 8340

as the test organism. The organism was inoculated on a slant of antibiotic medium #1 (Difco) and incubated at 37 C for 18 hr. A suspension of the organism was made by washing the slant with 5 ml of normal saline, and additional saline was added so that the suspension had an absorbance of 1.96 at a wavelength of 650 nm. A 0.1-ml sample was added to 65 ml of antibiotic medium #1 (Difco), and the media was poured into plates. Wells (0.75-mm diameter by 0.75-mm deep) were cut into the agar. The wells were filled with 0.1 ml of the specimens, and the plates were incubated at 37 C for 18 hr. Dilutions of the specimens were made in pooled human serum or urine so that zone sizes fell within the standard curve. Zones of inhibition were measured and compared to a standard curve. The standard curve was determined by dissolving amoxicillin standard in pooled human serum or urine to final concentrations of 0.12, 0.25, 0.50, and 1.0 μg/ml and measuring zones of inhibition after incubation at 37 C for 18 hr. All determinations were performed in triplicate.

The standard error of the mean was calculated by the method of Mantel (6). The 95% confidence limits were determined as twice the standard error of the mean.

Chemical Structure Of Amoxicillin Compared To Ampicillin

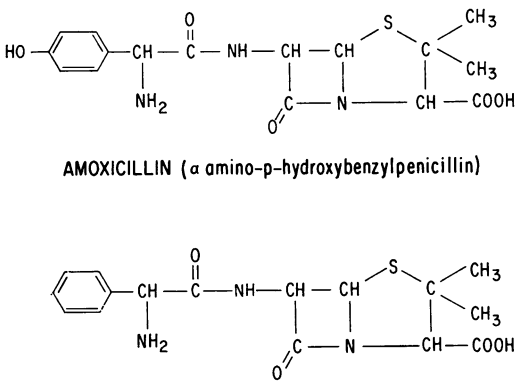

FIG. 1. *Chemical structure of amoxicillin compared o ampicillin.*

RESULTS

The antibacterial activity of amoxicillin is illustrated in Fig. 2. The drug was especially active against group A beta-hemolytic streptococci, pneumococci, and penicillin G-susceptible *S. aureus*. Only 28% of isolates of *S. aureus* resistant to 50 μg of penicillin G per ml were susceptible to amoxicillin at this concentration or less, and none were susceptible to less than 12.5 μg/ml. Seventy-six per cent of *P. mirabilis* isolates were susceptible to 1.56 μg or less of amoxicillin per ml, but 20% were resistant to 12.5 μg/ml or more. Fifty-seven per cent of *E. coli* isolates were susceptible to 6.25 μg/ml or less, but most of the remaining isolates were resistant to 50 μg/ml or more. Only a few of the other gram-negative bacilli were sensitive to amoxicillin.

The activity of amoxicillin was compared to that of ampicillin and cephalothin (Fig. 3). Amoxicillin was slightly more active than ampicil-

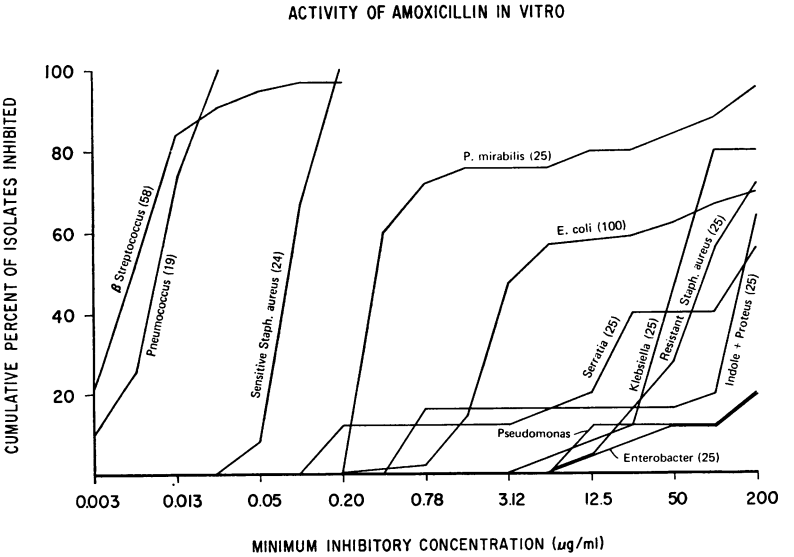

FIG. 2. *Activity of amoxicillin in vitro against clinical isolates of gram-positive cocci and gram-negative bacilli. The number in parentheses refers to the number of isolates tested.*

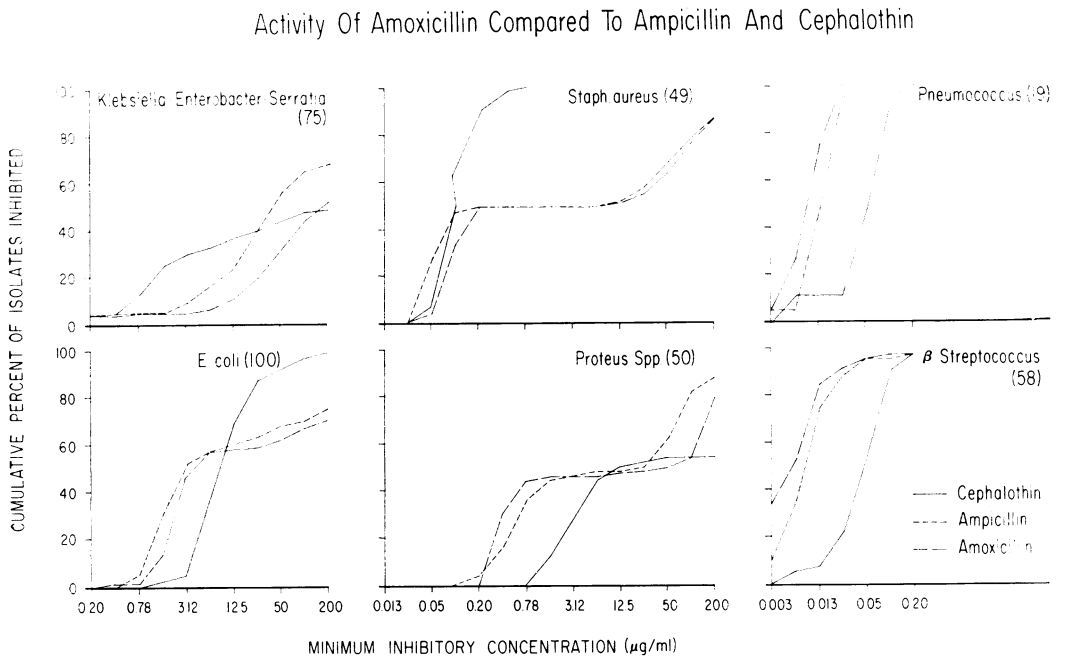

FIG. 3. *Comparison of the in vitro activity of amoxicillin, ampicillin, and cephalothin.*

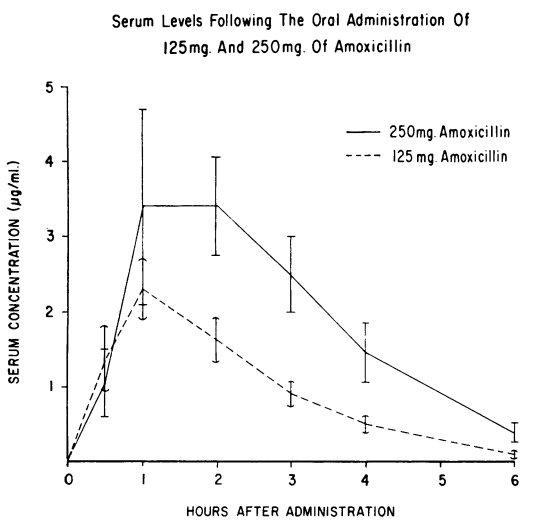

FIG. 4. *Mean serum levels following the oral administration of 125-mg and 250-mg doses of amoxicillin to 11 normal volunteers. The bars indicate the 95% confidence limits.*

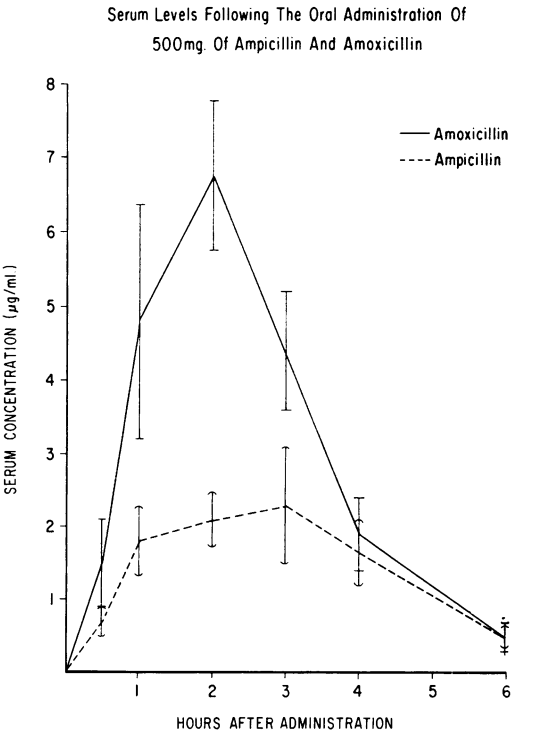

FIG. 5. *Mean serum levels following the oral administration of 500 mg of amoxicillin and 500 mg of ampicillin.*

lin and considerably more active than cephalothin against group A beta-hemolytic streptococci and pneumococci. Cephalothin was the most active antibiotic against *S. aureus*, and inhibited all penicillin G-susceptible and -resistant isolates at a concentration of 0.78 μg/ml or less. Amoxicillin

and ampicillin were similar in activity against *Proteus* spp. and more active than cephalothin. Ampicillin was slightly more active than amoxicillin against *E. coli* and considerably more active against members of the *Klebsiella-Enterobacter-Serratia* group.

Serum levels obtained after oral administration of 125 mg and 250 mg of amoxicillin are shown in Fig. 4. The mean peak serum level after the 125-mg dose was achieved at 1 hr and was 2.30 μg/ml. A mean serum level of 0.47 μg/ml was present at 4 hr. The mean peak serum level after the 250-mg dose occurred between 1 and 2 hr and was 3.43 μg/ml. A mean serum level of 1.45 μg/ml was present at 4 hr.

A comparison was made between serum levels achieved after oral administration of 500 mg of amoxicillin and 500 mg of ampicillin (Fig. 5). With amoxicillin, the mean peak serum level occurred at 2 hr and was 6.75 μg/ml. With ampicillin, the mean peak serum level occurred at 3 hr and was 2.28 μg/ml. Between 1 and 3 hr, the differences in the serum levels were statistically significant.

Mean serum levels obtained after the oral administration of 1 g of amoxicillin with and without probenecid are shown in Fig. 6. The mean peak serum level obtained when the drug was given alone was 9.90 μg/ml, and a mean serum level of 1.11 μg/ml was still present at 6 hr. When amoxicillin was given with probenecid, the mean peak serum level obtained was 16.04 μg/ml. The

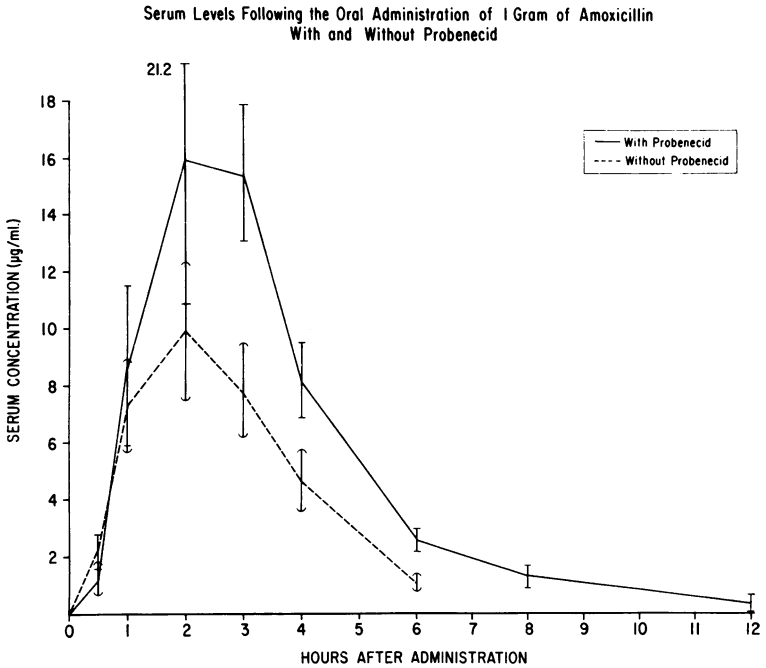

FIG. 6. *Mean serum levels following the oral administration of 1 g of amoxicillin with and without probenecid.*

TABLE 1. *Urinary excretion during 6 hr after administration of amoxicillin*

Drug	Dose (mg)	Mean urinary excretion		Mean urinary concn (μg/ml)
		mg	%	
Amoxicillin	125	98 (67–121)[a]	78	482 (137–950)
	250	175 (125–250)	70	843 (376–1,359)
	500	349 (126–495)	70	1579 (590–2,200)
	1,000	727 (300–1,000)	73	3313 (880–6,400)
Ampicillin	500	321 (231–443)	64	1041 (610–1,650)

[a] Numbers in parentheses indicate range of values.

mean serum levels at 8 and 12 hr were 1.30 μg/ml and 0.30 μg/ml, respectively.

The peak serum level in different individuals occurred from 30 min to 4 hr after drug administration. Considering the highest serum level achieved in each subject with the different doses of amoxicillin, the means were 2.40 μg/ml with 125 mg, 4.69 μg/ml with 250 mg, 7.34 μg/ml with 500 mg, 10.74 μg/ml with 1 g, 17.41 μg/ml with 1 g of amoxicillin plus probenecid, and 2.68 μg/ml with 500 mg of ampicillin.

The urinary excretion of the antibiotics was determined during the first 6 hr of each study (Table 1). The urinary concentrations varied considerably depending on the amount of urine excreted. The mean proportion of amoxicillin excreted in the urine with each dose varied between 70 and 78%.

DISCUSSION

Amoxicillin is a new semisynthetic penicillin with an antibacterial spectrum similar to ampicillin. The results of our in vitro susceptibility studies are similar to those of other investigators (7, 10). However, only 60% of our isolates of *E. coli* were inhibited by 12.5 μg or less of amoxicillin per ml compared to 80% of isolates in other studies. Most of our patients are exposed to multiple antibiotics which may account for this difference. Interestingly, 40% of our isolates of nonpigmented *S. marcescens* were inhibited by 25 μg or less of this drug per ml compared, to less than 10% of isolates in other series.

Serum levels in this study were lower than those obtained by Croyden and Sutherland, although a similar assay method was used (2). Our results agreed more closely with those of Neu et al. (8), but we obtained lower serum levels with ampicillin. Our serum levels of ampicillin were similar to those of Klein et al. (3) in their earlier studies of this drug. All of the studies of amoxicillin have demonstrated that this drug produces substantially higher serum levels than the same dose of ampicillin.

Recent studies have suggested that a single oral dose of 3.5 g of ampicillin plus probenecid is effective for the treatment of gonorrhea in males (4). The mean highest serum level of ampicillin achieved with this regimen was 35.7 μg/ml. By substituting 1 g of amoxicillin, we achieved a mean highest serum level of 17.41 μg/ml with a mean serum level of 0.30 μg/ml present at 12 hr. It seems probable that equally good clinical results could be achieved with lower doses of amoxicillin.

The major advantage of amoxicillin over ampicillin is that it produces substantially higher serum levels after oral administration. Clinical trials are in progress to determine its effectiveness for the treatment of infections.

ACKNOWLEDGMENTS

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