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Amoxycillin: A new Semi-synthetic Penicillin

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Summary

Amoxycillin (α-amino-*p*-hydroxybenzylpenicillin) is a new semi-synthetic penicillin with a broad spectrum of antibacterial activity similar to that of ampicillin. Penicillin-sensitive strains of staphylococci, streptococci, and pneumococci were sensitive to concentrations of 0·1 μg or less of amoxycillin/ml. Strains of *Haemophilus influenzae* were inhibited by a level of 0·5 μg/ml, and most strains of *Escherichia coli, Proteus mirabilis, Shigella sonnei, Salmonella* species, and *Streptococcus faecalis* were sensitive to a concentration of 5 μg or less of amoxycillin/ml. Penicillinase-producing strains of *Staphylococcus aureus* and strains of *Pseudomonas aeruginosa,* indole-positive *Proteus, Klebsiella,* and *Enterobacter* were insensitive to amoxycillin. The new penicillin was bactericidal in activity, as with other penicillins, and its antibacterial activity was not reduced in the presence of serum. After oral administration to volunteer subjects amoxycillin produced serum concentrations twice as high as those obtained with similar doses of ampicillin, and the penicillin was recovered unchanged in high concentrations in the urine. The absorption of amoxycillin was not greatly influenced by food, and administration of probenecid resulted in increased and more prolonged concentrations of amoxycillin in serum.

Introduction

Amoxycillin (Amoxil; BRL 2333; α-amino-*p*-hydroxybenzylpenicillin) is a new semi-synthetic penicillin synthesized in these laboratories (Long *et al.*, 1971). The compound has a chemical structure related to that of ampicillin (Fig. 1) and its antibacterial spectrum and level of activity are similar to those of ampicillin (Neu and Winshell, 1971a; Sutherland and Rolinson, 1971). However, amoxycillin is better absorbed than ampicillin after oral administration to human subjects and produces serum concentrations considerably higher than those of ampicillin (Neu and Winshell, 1971b; Croydon and Sutherland, 1971). Results are reported here to compare the antibacterial activities and absorption and excretion in man of amoxycillin and ampicillin.

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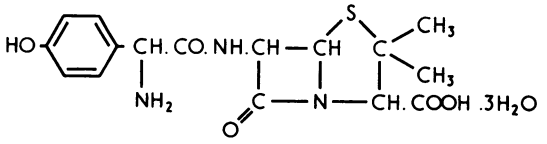
Materials and Methods

Amoxycillin is available as D(-)-α-amino-*p*-hydroxybenzylpenicillin trihydrate (Fig. 1) which, like ampicillin trihydrate, is relatively insoluble in water (0·4% v/w at room temperature),



Mol. wt. 419·46
Amoxycillin, BRL 2333
D(-)-α-amino-p-hydroxybenzylpenicillin trihydrate

FIG. 1—Structure of amoxycillin.

but aqueous solutions may be readily prepared in phosphate buffer, pH 8·0. The penicillin is relatively stable to acid and a 1% solution at 37°C has a half-life in simulated gastric juice (pH 1·5) of 17 hours compared with a half-life of 12 hours for ampicillin. Amoxycillin used in these studies had an assigned potency of 830 μg/mg, and ampicillin trihydrate (Penbritin) a potency of 840 μg/mg, both expressed in terms of anhydrous free acid.

Antibacterial Activity.—Minimum inhibitory concentrations required to inhibit growth of the test organisms for 18 hours at 37°C were measured by serial dilution in agar (Blood Agar Base, Oxoid) or nutrient broth (No. 2, Oxoid). Agar plates were inoculated with one drop (0·003 ml) of an undiluted overnight culture delivered with a multiple inoculating device. For tests in liquid medium the inoculum used was normally one drop (0·03 ml) of an overnight broth culture (about 10^7 cells) in 5 ml of medium.

Binding to Serum Protein.—The extent of binding of amoxycillin and other penicillins to protein of human serum was

measured by ultrafiltration of serum containing known concentrations of the test penicillin through a cellulose acetate (Visking) dialysis membrane (Rolinson and Sutherland, 1965). The amount of penicillin present in the protein-free ultrafiltrate representing unbound antibiotic was measured by microbiological assay with *Bacillus subtilis* A.T.C.C. 6633 as assay organism.

Assay in Serum and Urine.—The concentrations of amoxycillin and ampicillin in serum and urine were measured by standard large plate microbiological assay with *Sarcina lutea* N.C.T.C. 8340 as assay organism (Knudsen, Rolinson, and Stevens, 1961). For the assay of serum specimens standard solutions of amoxycillin or ampicillin were prepared in pooled human serum to give a range of concentrations from 0·01 to 0·5 μg/ml; for urine specimens the standard solutions were prepared in M/20 phosphate buffer, pH 7·0, over the same range of concentrations. The specimens were diluted as required to an estimated concentration midway in the concentration range (0·05–0·1 μg/ml). The plates were incubated overnight at 30°C, the inhibition zone diameters were measured, and the concentrations of the test specimens were derived from the standard line constructed from the standard solutions.

Absorption and Excretion Studies.—The penicillins were administered in gelatin capsules as a single dose of 125, 250, 500, or 1,000 mg to healthy male and female volunteer subjects. Venous blood was taken half an hour, one hour, and two, four, and six hours after administration of the penicillin, and urine was collected over the six-hour period. In fasting studies the subjects received the test penicillin after an overnight fast, and were allowed a standard breakfast after the one-hour specimen was taken. In studies to determine the effect of food on absorption, the penicillin was administered one hour after breakfast.

Results

ANTIBACTERIAL ACTIVITY

The antibacterial spectrum of amoxycillin against Gram-positive and Gram-negative bacteria is shown in Table I. It can be seen that the compound was active at low concentrations against Gram-positive cocci, except penicillin-resistant staphylococci, and against Gram-positive aerobic and anaerobic bacilli. Penicillinase-producing strains of *Staphylococcus aureus* were resistant to amoxycillin as a result of β-lactamase activity. Gram-negative bacteria sensitive to amoxycillin included gonococci, meningococci, *Haemophilus influenzae*, *Bordetella pertussis*, *Pasteurella septica*, *Escherichia coli*, *Proteus mirabilis*, and *Salmonella* and *Shigella* species. Strains of *Pseudomonas aeruginosa*, indole-positive *Proteus* species, *Klebsiella* and *Enterobacter* species, *Serratia marcescens*, and *Bacteroides fragilis* were relatively insensitive.

TABLE I—*Antibacterial Spectrum of Amoxycillin*

Organism	Minimum Inhibitory Concentration (μg/ml)*
Staphylococcus aureus N.C.T.C. 6571	0·1
Staph. aureus†	250
Streptococcus pyogenes	0·01
Str. viridans	0·01
Str. pneumoniae	0·02
Str. faecalis	0·5
Bacillus anthracis	0·25
B. subtilis A.T.C.C. 6633	0·25
Corynebacterium diphtheriae	0·02
Erysipelothrix rhusiopathiae	0·02
Listeria monocytogenes N.C.T.C. 5348	0·1
Sarcina lutea N.C.T.C. 8340	0·005
Clostridium tetani	0·05
Cl. welchii	0·05
Bacteroides fragilis	25
Brucella abortus N.C.T.C. 8226	0·25
B. melitensis N.C.T.C. 8223	0·1
B. suis N.C.T.C. 5061	0·25
Bordetella pertussis	0·5
Haemophilus influenzae	0·25
Neisseria gonorrhoeae	0·02
N. meningitidis	0·02
Pasteurella septica N.C.T.C. 948	0·5
Escherichia coli N.C.T.C. 10418	5·0
Salmonella typhi	1·25
Shigella sonnei	2·5
Klebsiella aerogenes	250
Proteus mirabilis	2·5
P. morganii	250
P. rettgeri	50
P. vulgaris	250
Pseudomonas aeruginosa	>500
Serratia marcescens	100
Vibrio cholerae N.C.T.C. 8021	5·0

*Serial dilution in agar. †Penicillinase-producing strain.

The activities of amoxycillin and ampicillin against Gram-positive cocci, *H. influenzae*, and gonococci are compared in Table II and against Gram-negative bacilli in Table III. Amoxycillin was as active as ampicillin against staphylococci and pneumococci and was slightly more active than the latter against β-haemolytic streptococci and enterococci. Strains of *H. influenzae* were inhibited by concentrations of both penicillins, of 0·1–0·5 μg/ml, amoxycillin being slightly less active than ampicillin. Both penicillins were somewhat less active than benzylpenicillin against sensitive strains of gonococcus, but showed slightly greater activity than the latter against penicillin-insensitive strains. Amoxycillin generally showed activity similar to that of ampicillin against Gram-negative bacilli and most strains of *E. coli*, *P. mirabilis*, *Sh. sonnei*, and *Salmonella* species were sensitive to 5 μg or less of amoxycillin/ml. Ampicillin-resistant strains of Gram-negative bacilli were found to be resistant also to amoxycillin, indicating complete cross-resistance between these two penicillins.

TABLE II—*Activity of Amoxycillin and Ampicillin against Gram-positive Cocci, H. influenzae, and Gonococci*

Organism	No. of Strains	Penicillin	M.I.C.* (μg/ml) and No. of Strains							
			1·0	0·5	0·25	0·12	0·05	0·02	0·01	0·005
Staph. aureus	29	Amoxycillin			6	20	3			
		Ampicillin			2	21	6			
β-haemolytic streptococci	28	Amoxycillin						3	25	
		Ampicillin						15	11	2
Str. pneumoniae	18	Amoxycillin					6	6	6	
		Ampicillin					7	6	4	1
Str. faecalis	53	Amoxycillin	11	39	3					
		Ampicillin	29	24						
H. influenzae	98	Amoxycillin	8	29	41	20				
		Ampicillin	5	21	46	20	6			
N. gonorrhoeae	13	Amoxycillin			5	1	3	3	1	
		Ampicillin			3	4		2	4	
		Benzylpenicillin	1	3		1		2	3	3

*Serial dilution in agar.

TABLE III—*Activity of Amoxycillin and Ampicillin against Gram-negative Bacilli*

Organism	No. of Strains	Penicillin	M.I.C.* (μg/ml) and No. of Strains							
			>100	100	50	25	12·5	5·0	2·5	1·25 or less
E. coli	206	Amoxycillin	23	1	1	2	46	115	13	5
		Ampicillin	19	5	1	1	26	113	37	4
P. mirabilis	90	Amoxycillin	13					11	28	38
		Ampicillin	13					1	26	50
Sh. sonnei	26	Amoxycillin	5	1	1		4	11	4	
		Ampicillin	2	4				11	8	1
Salm. species	20	Amoxycillin	2						8	10
		Ampicillin	2						7	11
Klebsiella-Enterobacter	29	Amoxycillin	25	2	1				1	
		Ampicillin	21	3	2	1	1		1	
S. marcescens	18	Amoxycillin	7	6	3	1		1		
		Ampicillin	5	10		2		1		

*Serial dilution in agar.

BACTERICIDAL ACTIVITY

Like ampicillin, amoxycillin was bactericidal in activity, and levels only slightly lower than the minimum inhibitory concentration resulted in a rapid reduction in the viable counts of the test organisms.

EFFECT OF SERUM

The antibacterial activity of amoxycillin was not reduced in the presence of 95% human serum. The extent of binding of amoxycillin to the protein of human serum as measured by ultrafiltration was found to be 17%, leaving 83% of the penicillin in serum as unbound active antibiotic. This degree of binding was similar to that of ampicillin (18% bound) and less than that of benzylpenicillin (59% bound).

SENSITIVITY DISCS

In general, amoxycillin sensitivity discs produced inhibition zones similar to those obtained with equivalent ampicillin discs. In tests, in which plates were flooded with a large inoculum of cells resulting in dense confluent growth, a 25-μg amoxycillin disc produced inhibition zones of 15 mm or greater in diameter with Gram-negative bacilli sensitive to 5 μg of amoxycillin/ml.

ABSORPTION AND EXCRETION IN FASTING SUBJECTS

The results in Table IV show the amoxycillin serum concentrations obtained in fasting volunteers after a single oral dose of 125, 250, 500, and 1,000 mg. The results are the summation of the mean values obtained in a number of separate studies carried out over a period of time. The number of studies and the total number of volunteers is indicated in the table. Data obtained with ampicillin, under the same experimental conditions, after a single oral dose of 250 and 500 mg are also shown in Table IV for comparison. It can be seen that increasing the dose resulted in a corresponding increase in the amoxycillin serum concentrations, and the mean peak serum concentrations, measured two hours after administration, were 2·7 μg/ml with the 125-mg dose, 5·1 μg/ml with the 250-mg dose, 10·8 μg/ml with the 500-mg dose, and 20·6 μg/ml with the 1,000-mg dose. The serum concentrations fell relatively rapidly from two hours onwards, reaching quite low levels at six hours.

In other studies, not included in Table IV, amoxycillin serum concentrations of 0·14 μg/ml at eight hours and 0·08 μg/ml at 11 hours were measured after a 250-mg dose to volunteer subjects. The amount excreted in the urine during the six-hour period after administration ranged from 58 to 68% of the dose administered, and bioautographic examination of the urine showed the presence only of unchanged amoxycillin. The average concentration of amoxycillin in pooled urine collected over the six-hour period after dosing was 580 μg/ml (range 50–1,600 μg/ml) for the 250-mg dose and 1,100 μg/ml (range 115–1,850 μg/ml) for the 500-mg dose.

The mean serum concentrations produced in these studies after a single 250-mg or 500-mg dose of amoxycillin were twice as high as those obtained with the same doses of ampicillin in fasting subjects in different studies done under the same experimental conditions, and the amounts of amoxycillin excreted in the urine during the six-hour period were significantly higher than those of ampicillin ($P < 0·001$) (Table IV). The absorption and excretion of amoxycillin and ampicillin in fasting subjects was also compared directly in cross-over studies in which the same groups of subjects were given single 250-mg and 500-mg oral doses of the penicillins. The results of these studies are

TABLE IV—*Absorption and Excretion of Amoxycillin after Oral Administration to Fasting Human Subjects*

Penicillin	Dose (mg)	No. of Studies	Total No. of Subjects	Mean Serum Concentration (μg/ml)					Excretion in Urine 0–6 hr % of dose
				½ hr	1 hr	2 hr	4 hr	6 hr	
Amoxycillin	125	4	38	0·7 (0·3–0·9)*	2·0 (1·6–2·5)	2·7 (2·4–3·0)	0·7 (0·5–0·8)	0·2 (0·16–0·24)	58 (52–64)
	250	7	76	2·1 (1·7–2·5)	4·6 (4·0–5·2)	5·1 (4·7–5·4)	1·1 (0·9–1·2)	0·4 (0·33–0·43)	64 (59–68)
	500	3	32	2·7 (1·7–3·7)	8·0 (6·4–9·7)	10·8 (9·6–12·0)	2·9 (2·4–3·4)	1·0 (0·8–1·3)	68 (63–73)
	1,000	2	22	4·2 (2·4–6·0)	11·9 (8·3–15·4)	20·6 (18·2–23·0)	5·8 (4·8–6·7)	1·7 (1·3–2·0)	67 (60–74)
Ampicillin	250	14	131	0·9 (0·7–1·0)	2·1 (1·8–2·3)	2·6 (2·4–2·8)	0·7 (0·67–0·8)	0·25 (0·22–0·28)	37 (34–40)
	500	12	123	1·6 (1·2–1·8)	4·0 (3·5–4·4)	5·2 (4·9–5·6)	1·6 (1·45–1·7)	0·56 (0·5–0·62)	48 (45–53)

*Figures in parentheses are 95% confidence intervals.

TABLE V—*Effect of Food on the Absorption of Amoxycillin in Human Subjects*

Dose (mg)	No. of Subjects	Penicillin	State	Mean Serum Concentration (μg/ml)				
				½ hr	1 hr	2 hr	4 hr	6 hr
250	12	Amoxycillin	Non-fasting	1·2	4·3	4·7	1·6	0·5
			Fasting	2·0	4·8	5·2	1·1	0·4

shown in Figs. 2 and 3, which confirm the data in Table IV—namely, that the mean serum concentrations obtained with amoxycillin were about twice as high as those with ampicillin.

Administration of 1 g of probenecid before dosing with amoxycillin resulted in increased and prolonged antibiotic serum concentrations and in a reduction in urinary excretion, which is characteristic of the effect of probenecid on the absorption and excretion of penicillins in general.

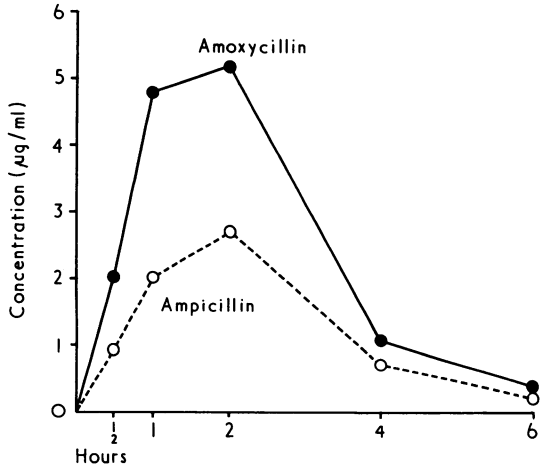


FIG. 2—Mean serum concentrations of amoxycillin and ampicillin after a single 250-mg oral dose in a cross-over study in 12 fasting subjects.

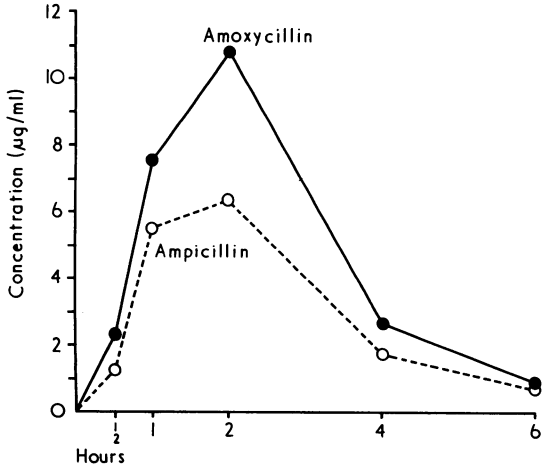


FIG. 3—Mean serum concentrations of amoxycillin and ampicillin after a single 500-mg oral dose in a cross-over study in 12 fasting subjects.

EFFECT OF FOOD

Mean serum concentrations obtained in a cross-over study after a 250-mg dose of amoxycillin in fasting and non-fasting volunteers are shown in Table V. In this study the non-fasting volunteers ate a normal breakfast one hour before taking the penicillin. It will be seen that the levels of amoxycillin achieved in serum after food were very similar to those obtained in the fasting state.

Discussion

The results presented here show that the antibacterial spectrum and level of in-vitro activity of amoxycillin is similar to that of ampicillin. Likewise, amoxycillin is not highly bound to serum protein and its activity is not reduced in the presence of serum. Amoxycillin is evidently well absorbed when given to fasting and non-fasting subjects and produces serum concentrations twice as high as those produced with equivalent doses of ampicillin. Increasing the dose of amoxycillin results in a corresponding increase in serum concentrations, which is characteristic of penicillins. The urinary excretion of amoxycillin is significantly higher than that of ampicillin and 60–70% of an oral dose of the compound appears unchanged in the urine. The taking of food does not significantly alter absorption of amoxycillin, which may be an important benefit, and the serum concentrations of amoxycillin are markedly increased after administration of probenecid.

The antibacterial activity of amoxycillin and its favourable absorption and excretion characteristics are also reflected in animal studies, and amoxycillin has been shown to be superior to ampicillin in the treatment of experimental infections (Acred *et al.*, 1971). The properties of amoxycillin suggest this new semi-synthetic penicillin may have clinical advantages over ampicillin.

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