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Note

Rapid determination of amoxycillin (Clamoxyl®) and ampicillin (Penbritin®) in body fluids of many by means of high-performance liquid chromatography

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The determination in body fluids of antimicrobial drugs of biological origin is mainly carried out using microbiological techniques [1—6]. This guarantees the determination of the microbiologically active principles, including active metabolites. The relatively long time required for analysis by such techniques is a disadvantage when the results are urgently needed by clinicians. Frequently, in such cases more than one antibiotic drug is administered, which sometimes results in difficulties when measuring concentrations by means of the microbiological assay.

The analysis of amoxycillin and ampicillin by means of high-performance liquid chromatography (HPLC) was developed in order to obtain information about the pharmacokinetics and characteristics of penetration into tissue fluids. This type of analysis has been reported previously, but only for non-biological applications such as the quality control of pure substances [7].

Additional pharmacokinetic information on amoxycillin and ampicillin plasma concentrations will be useful in optimizing therapy with antibiotics for the treatment of patients with a variety of susceptible bacterial infections for which the minimum inhibitory concentration has been established. Such a study would serve in the formulation of guidelines for rational treatment. The HPLC method may be of use in pharmacokinetic studies, giving evidence that the antibiotic drug reaches the infected tissues in an adequate concentration.

EXPERIMENTAL

A spectra Physics 3500 B high-performance liquid chromatograph was used. The column (15 cm × 4.6 mm I.D.) was packed with LiChrosorb RP-8 (particle sice 5 μm) obtained from Chrompack (Middelburg, The Netherlands). An injection loop of 100 μl was used. Detection was effected at 225 nm.

The solvent for amoxycillin is a potassium dihydrogen phosphate buffer (Sörensen buffer, pH 4.6, 0.067 *M*) at a flow-rate of 1.2 ml/min. The solvent for ampicillin is a mixture of 425 ml of the KH_2PO_4 buffer (pH 4.6) and 75 ml of methanol at a flow-rate of 1.2 ml/min.

Drugs

Amoxycillin (100% chromatographically pure, 86% activity) and ampicillin (100% chromatographically pure, 86.5% activity) were obtained as pure compounds from Beecham Pharmaceuticals (Amstelveen, The Netherlands). 6-Aminopenicillanic acid and benzylpenicilloic acid were gifts from Gist-Brocades (Delft, The Netherlands).

Subjects

Amoxycillin and ampicillin were administered to volunteers from the Department of Clinical Pharmacy and patients from various departments of the St. Radboud Hospital. Blood samples of 0.2 ml were collected frequently by finger-tip puncture (Microlance No. 433, Becton Dickinson, U.S.A.). Urine was collected as spontaneously voided. The pH of the urine was kept alkaline in some subjects by the regular daily intake of 10 g of sodium bicarbonate. Saliva was collected at regular time intervals by spontaneous production.

Sample preparation

For both drugs the same procedure was followed for the analysis of plasma and saliva concentrations. 0.1 ml of plasma or saliva was mixed with 0.4 ml of perchloric acid (0.33 *N*) on a Vortex mixer. The mixture was centrifuged at ca. 2600 *g* for 5 min (Heraus Christ centrifuge). 100 μl of the clear supernatant were injected into the high-performance liquid chromatograph.

Urine was treated as follows. 10 μl of urine were mixed with 0.5 ml of perchloric acid (0.33 *N*) on a Vortex mixer. 100 μl were injected into the high-performance liquid chromatograph.

Before each series of determinations a calibration curve was constructed (peah height vs. concentration). The precision of the method was established as 100 ± 2%. The lowest concentration that can be measured accurately is 0.5 μg/ml. The total time required for one analysis is 15 min.

RESULTS

Ampicillin and amoxycillin are well separated from other endogenous compounds present in plasma, saliva and urine (Fig. 1A,B). Fig. 2 shows the pharmacokinetics of amoxycillin in a volunteer who took 750 mg of the antibiotic orally. The maximum plasma concentration was 14 μg/ml, this maximum being reached after 1.5 h. Amoxycillin was eliminated with a half-life of 1.1 h.

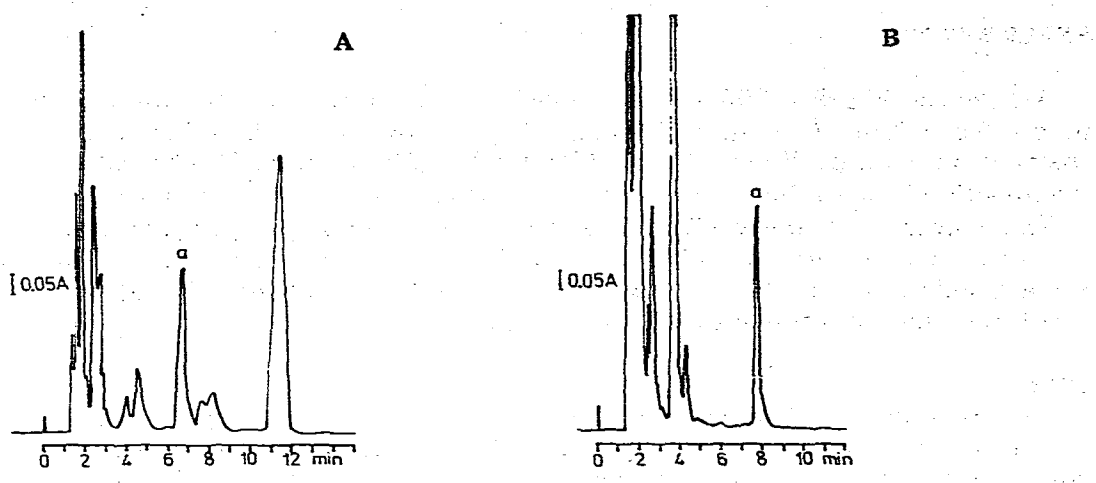


Fig. 1. A. Chromatogram of amoxycillin in human urine. The column used was LiChrosorb RP-8 (5 μm) and the solvent 0.067 *M* KH_2PO_4 (pH 4.6). The flow-rate is 1.2 ml/min. B. Chromatogram of ampicillin in human plasma (≈ 32 μg/ml). The column used was LiChrosorb RP-8 (5 μm) and the solvent a mixture of 425 ml of KH_2PO_4 buffer (pH 4.6) and 75 ml of methanol. Flow-rate, 1.2 ml/min.

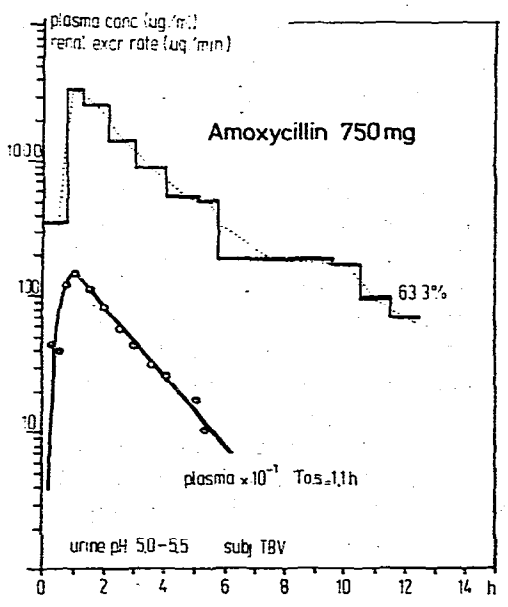
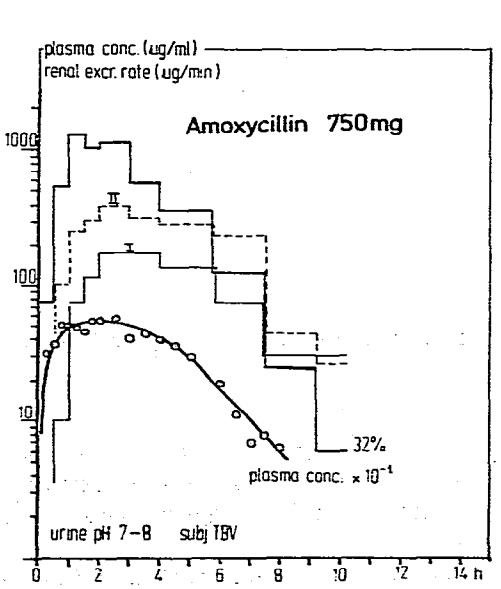


Fig. 2. Plasma concentration—time curve and renal excretion rate of amoxycillin in a human volunteer who took 750 mg of the drug orally. The maximum plasma concentration is 14 μg/ml. The urine pH was 5.3 ± 0.2 throughout the experiment.

The renal excretion rate reached a maximum value of 3500 μg/min. The excretion curve is parallel to the plasma concentration—time curve. The renal clearance constant was calculated to be 250 ml/min. 63.3% of the dose administered was excreted in the urine as the parent drug. No influence of the urinary flow-rate (ml/min) on the renal excretion rate was observed. The pH of the urine of the volunteers was measured and found to be acidic (pH 5.3 ± 0.2 throughout the experiment). The subjects experienced excitement and nervousness. The same effects are observed after the intake of high amounts of ammonium chloride for the purpose of rendering the urine acidic (8 g/day). Amoxycillin is an acidic drug, the pK_a values of the COOH, NH_2 and OH group are 2.4, 7.4 and 9.6 respectively, and a solution of 0.2% (w/v) of the drug in CO_2-free water has a pH of 3.5—5.5 [8].

Fig. 3 shows the pharmacokinetics of amoxycillin in the same volunteer after intake of 750 mg of the drug. During the experiment the urine pH was kept alkaline (pH 7.5—8.2) by the regular intake, four times a day, of 2.5 g of sodium bicarbonate. The maximum plasma concentration was only 7.5 μg/ml. The maximum remains for a longer period than with acidic urine (Fig. 4). The half-life of elimination was measured again as 1.1 h. The maximum renal excretion rate was lower (1400 μg/ml). The total percentage of the drug



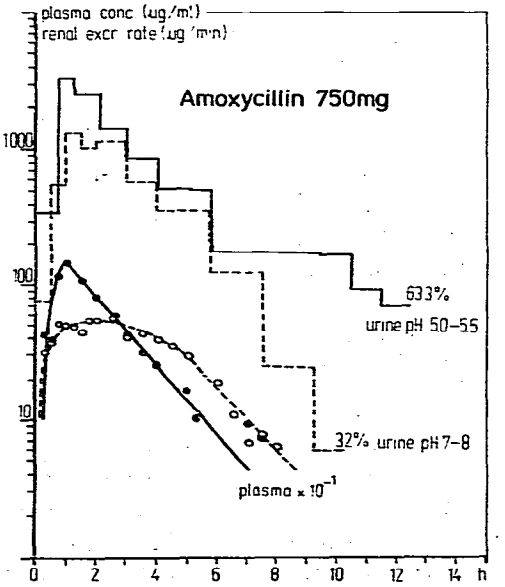


Fig. 3. Plasma concentration—time curve and renal excretion rate of amoxycillin in a human volunteer who took 750 mg of the drug orally. The urine is kept alkaline (pH 7.5—8.2). The maximum plasma concentration is 7.5 μg/ml. The compounds I and II are unidentified (see Table I).

Fig. 4. Plasma concentration—time curve and renal excretion rate of amoxycillin in the same volunteer under different urinary pH conditions (acidic and alkaline). 63.3% of the drug was excreted unchanged with acidic urine while 32% was excreted unchanged under alkaline conditions. The area under the plasma concentration—time curve under alkaline conditions is 87% of that obtained with acidic conditions.

excreted as the parent drug appeared to be considerably lower, 32%.

In the chromatogram of the urine two components (I and II) could be measured. The renal excretion rate of both unidentified compounds showed a pharmacokinetic behaviour, i.e. increase and decrease of concentration in the time course of the experiment as a result of amoxycillin intake (Fig. 3). The compounds elute under different conditions from the RP-8 column (Table I), thus the structures are different from 6-aminopenicillanic acid and benzylpenicilloic acid. The renal excretion rates of these two compounds, characterized by the initials I and II referring to their retention times and given in Table I, are shown in Fig. 3. The kinetic profile of these metabolites under acidic urine conditions is different, the renal excretion rates of both compounds are almost identical.

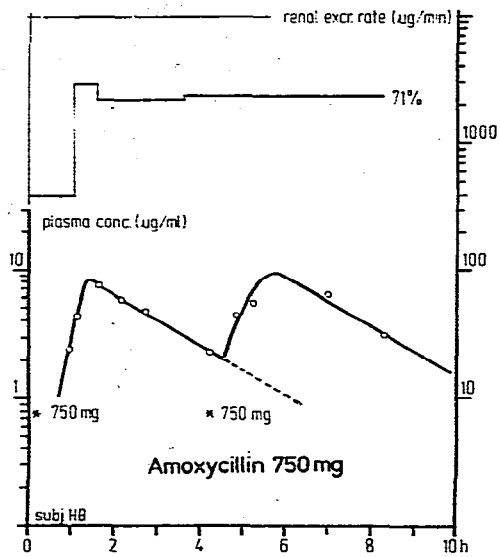


Fig. 5. Plasma concentration—time curve and renal excretion rate of amoxycillin at the start of treatment of 750 mg orally, twice daily.

Fig. 5 shows the start of a plasma concentration—time profile of a patient who took amoxycillin orally twice a day (750 mg twice daily). The maximum concentration appears to be 8.5 μg/ml, the maximum being reached after 1.75 h. The elimination half-life is found to be 1.5 h.

Ampicillin taken by three volunteers reached a maximum plasma concentration of 11 μg/ml after an oral dose of 1250 mg and shows a half-life of 1.5 h. 15% of the oral dose was recovered as the parent compound in the urine.

No ampicillin or amoxycillin could be detected in saliva.

DISCUSSION

The HPLC method for ampicillin and amoxycillin presented here is rapid, sensitive and reproducible. The results are comparable with those obtained with the microbiological methods, as reported earlier [9,10]. Both methods

TABLE I

RELATIVE RETENTION TIMES OF AMOXYCILLIN AND RELATED COMPOUNDS

Compound	Relative retention time	
	Solvent: KH_2PO_4	Solvent: KH_2PO_4 + methanol
Amoxycillin	1.00	0.22
Ampicillin	—	1.00
I in urine	0.52	—
II in urine	0.63	—
6-Aminopenicillanic acid	0.35	0.01
Benzylpenicilloic acid	—	0.01

are complementary to each other, one measuring the drug as a chemical entity, the other as the active principle.

As an aid to therapy, the HPLC method may be the preferred technique when the bacteria have been identified and the appropriate drug has been chosen. The low renal excretion of amoxycillin under alkaline conditions is not yet fully understood and is the subject of further research. A possible explanation might be that the phenolic hydroxyl group is the dominating group in the regulation of the renal excretion. The lower maximum plasma concentration of amoxycillin under alkaline conditions is compensated by the extended time course of the plasma concentration. The area under the plasma concentration—time curve with alkaline conditions is only 87% of that obtained with acidic conditions, which implies that about the same amount of drug is absorbed. The differences in the absorption kinetics are too small to be responsible for the big differences in renal excretion rates as shown in Fig. 4.

Ampicillin and amoxycillin, as acidic compounds, are excreted in the saliva at extremely low rates. This behaviour has been reported earlier for acidic compounds [11].

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