ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1975, p. 518-525

Vol. 8, No. 5
Printed in U.S.A.

# Bacampicillin: a New Orally Well-Absorbed Derivative of Ampicillin

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Received for publication 16 June 1975

Bacampicillin (proposed international nonproprietary name), 1'-ethoxycarbonyloxyethyl 6-(D-α-aminophenylacetamido)penicillanate, is a new orally well-absorbed penicillin, highly active in vivo due to rapid transformation into ampicillin. The compound is stable in vitro at gastric pH and hydrolyzed slowly to ampicillin at neutral pH but very rapidly in the presence of biological fluids, e.g., tissue homogenates or serum. In vivo the transformation into ampicillin is so rapid that no unchanged compound could be detected in the blood after oral administration of bacampicillin to rats, dogs, and humans. On oral administration to mice, rats, and dogs, bacampicillin was found to be better absorbed than ampicillin, giving higher and earlier peak blood levels of ampicillin. The bioavailability of bacampicillin in rats and dogs was three to four times higher than that of an equimolar amount of ampicillin. On oral administration to rats, bacampicillin was found to give higher levels of ampicillin in organs such as the kidney, liver, and spleen than ampicillin itself. In "tissue cages" in rats, higher transudate levels of antibiotic were found after oral administration of bacampicillin than after ampicillin. On oral treatment of experimentally infected mice, bacampicillin was found to be more active than ampicillin.

Among the semisynthetic penicillins, ampicillin, 6-(D-α-aminophenylacetamido)penicillanic acid, has found widespread use owing to its broad-spectrum type of activity. It is orally absorbed, though only partially, to give urinary recoveries from 30 to 50% of the active compound (11). Certain esters of ampicillin, namely, pivampicillin (5) and talampicillin (4, 12), have been found to be well absorbed when given orally and undergo hydrolysis in the body to give peak levels of ampicillin higher than those obtained with ampicillin itself. These esters are analogues of acyloxyalkyl esters, first described by Jansen and Russell (8). We now describe a new type of hydrolyzable ester group containing a carbonate moiety with which it is possible to improve the oral absorption of ampicillin and other types of β-lactam antibiotics. Among a series of ampicillin esters containing this structure, one compound, 1'-ethoxycarbonyloxyethyl 6-(D-α-aminophenylacetamido)penicillanate (bacampicillin) (Fig. 1), was selected for clinical evaluation. This paper presents chemical and biological data, both in vitro and in vivo, for the preclinical assessment of this new antibacterial agent.

## MATERIALS AND METHODS

**Compounds.** Bacampicillin was synthesized at the Research and Development Laboratories, Astra Läkemedel, and Chemical Process Development Laboratory, Astra Pharmaceuticals, and was used as the hydrochloride ($[\alpha]_D^{20}$: + 173°), a white crystalline compound soluble in water. As the ester moiety contains a chiral center and is prepared from racemic starting material, the product is obtained as a mixture of two epimers with regard to this center. The individual epimers, A ($[\alpha]_D^{20}$: + 149°) and B ($[\alpha]_D^{20}$: + 199°), were obtained by selective crystallization. Ampicillin (trihydrate and sodium salt) of pharmaceutical quality (Astra Läkemedel) and pivampicillin (batch CH34, Leo Pharmaceuticals) were used as reference compounds in the studies.

[$^{35}$S]bacampicillin and [$^{35}$S]ampicillin were prepared from [$^{35}$S]benzylpenicillin obtained by fermentation and had specific activities of 18.8 $s^{-1}$ $nmol^{-1}$. Their radiochemical purities as checked by thin-layer chromatography were greater than 95%.

**Analytical methods.** Paper chromatograms were run in an *n*-butanol-ethanol-water (4:1:5, top layer, vol/vol/vol) system using microbiological detection with a *Bacillus cereus* strain. Thin-layer chromatography of the radioactive compounds was performed on silica gel plates (Merck) using an *n*-butanol-acetic acid-water (4:1:4, top layer) system. Detection was by autoradiography (Structuric X-ray film, Agfa-Gevaert).

Radioactivity was assayed using a liquid scintillation spectrometer (Packard Tri-Carb, model 3320). The scintillation liquid consisted of butyl 2-(4-*tert*-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole (7.0 g), ethylene glycol monoethyl ether (600 ml), and toluene (1,000 ml). The counting efficiency was esti-

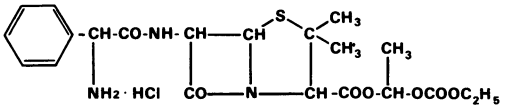

FIG. 1. *Structure of bacampicillin hydrochloride.*

mated by an external standard channels ratio procedure.

**Hydrolysis in vitro. (i) Dilute serum.** The amounts of bacampicillin, and its two epimers and of pivampicillin hydrolyzed in 30 min were determined at 37 C in the following media: (i) phosphate buffer (Sörensen), pH 7.4; (ii) buffer with 10% pooled human serum added; (iii) buffer with 10% pooled rat serum added; (iv) synthetic gastric juice, pH 1.2 (USP XVIII). Amounts of the esters corresponding to 10 mg of ampicillin were dissolved in sterile distilled water and diluted in the respective media to a final concentration of 10 μg/ml (for gastric juice, 20 μg/ml). All media and accessories necessary for the procedure were preheated to 37 C, and test solutions were immediately incubated at 37 C. Zero samples were taken from the phosphate buffer solutions before incubation. After 30 min, aliquots (2 ml) of the solutions were shaken vigorously with their own volume of ethyl acetate to remove unhydrolyzed ester. The samples from the synthetic gastric juice were neutralized with 0.2 N disodium phosphate before extraction. After the extraction, the amount of ampicillin in the aqueous phase was determined microbiologically by an agar diffusion method with *Sarcina lutea* as the test organism (7).

**(ii) Whole blood and plasma.** To heparinized human blood or plasma (10.0 ml), or dog plasma (15.0 ml), at 37 C, [$^{35}$S]bacampicillin hydrochloride (2 and 3 μmol, respectively) was added, dissolved in preheated physiological saline (1.0 ml), to give a final concentration of about 100 μg/ml. Aliquots (1.0 ml) were taken from the incubation mixture at intervals up to 30 min, starting 1 min after mixing, and immediately chilled in an ice bath. In the case of whole blood the samples were hemolyzed with 1% saponin solution (1 ml) for 10 s. The unchanged drug was immediately extracted into methylene chloride (3.0 ml) by vigorous shaking for 2 min, followed by centrifugation. The clear organic phase was washed with phosphate buffer solution (1/15 mol/liter, pH 7.5; 1.0 ml) followed by centrifugation. For assay of the radioactivity, 1.0 ml of the clear organic phase was mixed with scintillation liquid (16 ml).

The extraction efficiency of bacampicillin from whole blood was determined to 100% (range 95 to 105%; $n = 6$). Ampicillin, added to a concentration of 100 μg/ml, was co-extracted into the organic phase to an extent of 0.23% (range 0.20 to 0.25%; $n = 5$).

First-order rate constants and half-lives were determined graphically from log concentration versus time plots.

**Hydrolysis in vivo. (i) Rats.** Two male SPF Sprague-Dawley rats (weighing 180 g) were given orally by stomach tube [$^{35}$S]bacampicillin hydrochloride (20 mg/kg, 40 μmol/kg) dissolved in physiological saline to a concentration of 4.0 mmol/liter. The radioactive dose was $7.5 \times 10^5$ $s^{-1}kg^{-1}$. Blood samples were taken 10 and 15 min, respectively, after administration and analyzed for unchanged ester. An aliquot (1.0 ml) of the ice-cooled heparinized whole blood was hemolyzed by addition of saponin solution (1 ml) containing unlabeled bacampicillin (100 μg). After 10 s, the unchanged drug was extracted into methylene chloride (3.0 ml) by vigorous shaking for 2 min, followed by centrifugation. The clear organic phase was washed with phosphate buffer solution (1/15 mol/liter, pH 7.5; 1.0 ml) by shaking and centrifugation. The radioactivity of the clear organic phase was determined after mixing an aliquot (1.0 ml) with scintillation liquid (16 ml).

**(ii) Dogs.** Blood samples were withdrawn from three female Beagle dogs, which had been given [$^{35}$S]bacampicillin hydrochloride (20 mg/kg, 40 μmol/kg) orally in absorption studies (vide infra). The samples were taken from one of the animals 5, 10, 20, 40, 60, 80, and 120 min after administration, and from the other two 20 min after administration, immediately chilled in an ice bath, and analyzed for the presence of unhydrolyzed ester as described above.

The detection limit for unchanged bacampicillin in the in vivo hydrolysis experiments was 0.02 μg/ml.

**Absorption studies. (i) Rats.** Two main groups of 55 male SPF Sprague-Dawley rats (weighing 190 ± 10 g) each divided into 11 subgroups, representing sampling times of 5, 15, and 30 min and 1, 2, 4, 6, 8, 24, 48, and 72 h, were starved overnight and given orally by stomach tube 40 μmol of [$^{35}$S]bacampicillin hydrochloride (20 mg/kg) and [$^{35}$S]ampicillin (14 mg/kg) per kg, respectively, in total radioactive doses of $7.5 \times 10^5$ $s^{-1}kg^{-1}$. The compounds were given as 4-mM solutions in physiological saline (phosphate buffer, 1/15 mol/liter, added to pH 8.0 to dissolve ampicillin). After dosing, the animals were kept by group in metabolism cages with free access to food and water. Blood samples were withdrawn by cardiac puncture and plasma was prepared. The urine and feces samples from each subgroup were pooled. All samples were stored at −25 C before analysis.

The total radioactivity of the blood, plasma, urine, and feces was determined according to a modification of the method of Mahin and Lofberg (9). An aliquot (about 100 mg) of the sample was dissolved in perchloric acid (0.20 ml) to which hydrogen peroxide (0.20 ml) was added. The samples, contained in capped counting vials, were placed in an oven at 70 C for 1 h. After cooling to −25 C, scintillation solution (16 ml) was added.

The concentration of penicillin in plasma and urine was determined by the cylinder plate method (7) using *S. lutea* as the test organism. The standard solutions for plasma were prepared in rat serum, and those for urine were prepared in Sörensen's phosphate buffer, pH 7.0. The samples were diluted with the same diluents.

**(ii) Dogs.** Three female Beagle dogs (weighing about 9 kg) received 40 μmol of [$^{35}$S]bacampicillin hydrochloride (20 mg/kg) and [$^{35}$S]ampicillin (14 mg/kg) per kg within an interval of 3 to 7 weeks. The radioactive dose was $7.5 \times 10^5$ $s^{-1}kg^{-1}$. The [$^{35}$S]bacampicillin hydrochloride was dissolved in physiological saline and the [$^{35}$S]ampicillin was dis-

solved in phosphate buffer solution (1/15 mol/liter, to a final pH of 8.0) to a concentration of 80 μmol/ml, and each was given orally in gelatine capsules. The dogs were housed in metabolism cages and given food once a day and had free access to water. Blood samples were withdrawn in heparinized tubes before, 5, 10, 20, 40, and 60 min, and 1.5, 2, 4, 8, 24, 48, 72, and 96 h after administration. Plasma was prepared. Urine was sampled by catheterization before and 2, 4, and 8 h after administration and collected, as was the feces, 24, 48, 72, and 96 h after administration. All samples were stored at −25 C before being analyzed as above.

**Penetration into tissue fluid.** The method used was a modification of that described by Gardner (6). White female SPF rats weighing about 100 g were anesthetized intraperitoneally with 50% pentobarbital in physiological saline (0.1 ml) and subcutaneously with 2% Xylocaine (0.5 ml) along the line of incision to be made. The backs were clipped and cleansed with 70% alcohol before a 2.5-cm longitudinal cut was made from the shoulders to the lumbar area. The skin was opened on both sides of the cut just enough to insert two sterilized coil springs (2 by 1 cm; 1-mm soft, stainless-steel wire) subcutaneously, one on each side of the spine. The incision was closed with sutures and covered with surgical tape to prevent infection. Each rat was placed in a separate cage and given food and water ad lib. After 3 weeks the rats were ready for the experiment.

Twenty-four fasting rats were each given orally equimolar amounts (270 μmol/kg) of sodium ampicillin (100 mg/kg) or bacampicillin hydrochloride (135 mg/kg) dissolved in distilled water (0.5 ml). Each animal also received heparin solution (0.5 ml) subcutaneously. At specific times, about 0.1-ml transudate samples were taken by needle aspiration from any one of the two coil springs of each rat, and immediately afterwards a 0.01-ml sample of blood was taken from the left orbital sinus by micropipette after puncture with a long needle. Six animals were used at each sampling time, and samples were taken from each animal on two consecutive occasions only. Transudate samples visibly contaminated with blood were discarded.

The blood samples were diluted 100 times in distilled water and assayed by the cylinder plate method (7) using *S. lutea* as the test organism and ampicillin as the standard penicillin. The sensitivity limit of the assay was 1 μg of undiluted sample per ml. The transudate samples were added dropwise to paper disks (6 mm; Grycksbo 33D), six disks for each sample, and assayed by the paper disk method (13) with *S. lutea* as test organism and ampicillin as standard penicillin. The standard disks were each pretreated with a drop of transudate from animals not treated with penicillin before applying the standard dilutions. The sensitivity limit of the assay was 0.1 μg/ml.

**Distribution into organs of the rat.** Female fasting SPF rats weighing about 100 g were given orally equimolar doses (270 μmol/kg) of bacampicillin hydrochloride (135 mg/kg) and sodium ampicillin (100 mg/kg) dissolved in distilled water. At specific times after administration, the animals were killed, four at each time, and the spleen, kidneys, and liver were taken out in that order and immediately placed in preweighed cylinders containing 3 ml (spleen and kidneys) or 10 ml (livers) of distilled water. All organs and samples were stored at −20 C until analyzed. The organs were homogenized by a high-speed stirrer (Ultra-Turrax) and assayed for their ampicillin content by the cylinder plate method using *S. lutea* as test organism. The liver and kidney homogenates were diluted 10 times with distilled water before analysis, whereas the spleen homogenates were analyzed undiluted.

**Experimental infections.** The following bacteria were used: *Diplococcus pneumoniae* type II, *Listeria monocytogenes*, *Streptococcus pyogenes* C 203 M, *Streptococcus faecalis*, *Staphylococcus aureus* strain Smith, *Haemophilus influenzae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, and *Pasteurella multocida*. Master cultures were kept freeze dried. They were subcultured in tryptose phosphate broth (Difco) except in the case of *H. influenzae*, where brain heart infusion (Difco) with 1% supplement B (Difco) was used. Most of the bacteria were made virulent by passage in mice.

The determination of the median curative doses was performed by intraperitoneal injection of groups of 10 female white mice weighing 17 to 19 g (NMRI strain) with a dilution of the bacterial test strain that resulted in death of the control animals within 24 to 96 h. Bacterial suspensions for injection were prepared from overnight cultures diluted in broth, their virulence being checked by a 50% lethal dose control run to determine the dilution of the bacterial culture which gave 50% mortality in animals not treated with penicillin. Except in the case of *D. pneumoniae*, *Streptococcus pyogenes* C 203 M, *P. multocida*, and *L. monocytogenes*, 0.5 ml of 5% hog gastric mucin (Wilson type 1701-W) was given intraperitoneally at the same time as the infecting dose. Bacampicillin hydrochloride and sodium ampicillin dissolved in distilled water were administered to six groups of mice orally at the same time as the infecting organisms or, in one series of experiments, with *H. influenzae* 4 h afterwards. One group received a stock solution of the penicillins, and the other groups received consecutive fourfold dilutions. The two penicillins were tested in parallel in each experiment, using for both compounds the same challenge dose of the different bacteria. Median curative and median lethal doses were determined after 96 h by the method of Reed and Muench (10).

## RESULTS

**Hydrolysis.** The formation of ampicillin as the microbiologically active product of hydrolysis of bacampicillin both in vitro and in vivo could be demonstrated by chromatographic procedures. The compound was comparatively stable in synthetic gastric juice and in neutral

buffer solution, but the hydrolysis to ampicillin was greatly enhanced by dilute human or rat serum (Table 1). In the presence of 10% human serum, the rate of hydrolysis of bacampicillin was intermediate to those of its epimers, but all three products were more rapidly hydrolyzed than pivampicillin. Rat serum had a greater hydrolyzing power than human serum. Human blood and plasma and canine plasma in vitro at 37 C hydrolyzed bacampicillin approximately according to first-order kinetics, with half-lives of 5.2, 7.2, and 8.3 min, respectively (Fig. 2).

The hydrolysis of bacampicillin to ampicillin in vivo was investigated in rats and dogs given oral doses of 20 mg of [$^{35}$S]bacampicillin per kg. Although the analytical procedure used would allow detection of blood levels of bacampicillin as low as 0.02 $\mu$g/ml, i.e., about 0.5% or lower of the peak blood levels of ampicillin obtained, no evidence of the compound circulating in the blood was obtained, indicating that the hydrolysis of bacampicillin to ampicillin in vivo was very rapid and extensive and in effect occurred in connection with the oral absorption process.

**Oral absorption.** After oral administration to rats, bacampicillin was rapidly absorbed to give earlier and much higher peak levels of ampicillin compared to ampicillin itself (Fig. 3). After equimolar (40 $\mu$mol/kg) doses of [$^{35}$S]bacampicillin hydrochloride (20 mg/kg) and [$^{35}$S]ampicillin (14 mg/kg), peak plasma levels were 8.1 and 1.8 $\mu$g/ml, respectively. From linear plots of concentration of biological activity versus time the areas under the curves indicated a 3.7 times larger bioavailability of bacampicillin than of ampicillin. The better bioavailability of bacampicillin was also indicated by the recovery of 35% of the radioactive dose in the urine, against only 16% after administration of ampicillin.

In three dogs receiving oral equimolar (40 $\mu$mol/kg) doses of [$^{35}$S]bacampicillin (20 mg/kg) and [$^{35}$S]ampicillin (14 mg/kg) in a crossover fashion, the former was more efficiently and consistently absorbed, giving higher peak levels (Table 2). From linear plots of biological activity versus time, it was estimated that the areas under curves for bacampicillin were about three times higher than those for ampicillin. In the case of bacampicillin, an average 55% of the radioactive dose given was recovered in the urine compared to 27% in the case of ampicillin.

In rats as well as in dogs, the levels of radioactivity in plasma and urine were higher for both compounds than those of biological activity. This was due to biotransformation of ampicillin, leading mainly to antibacterially inactive penicilloate. The recovery of radioactivity was complete for both compounds. The amounts not accounted for in the urine were excreted with the feces.

**Distribution.** Since only ampicillin, not ba-

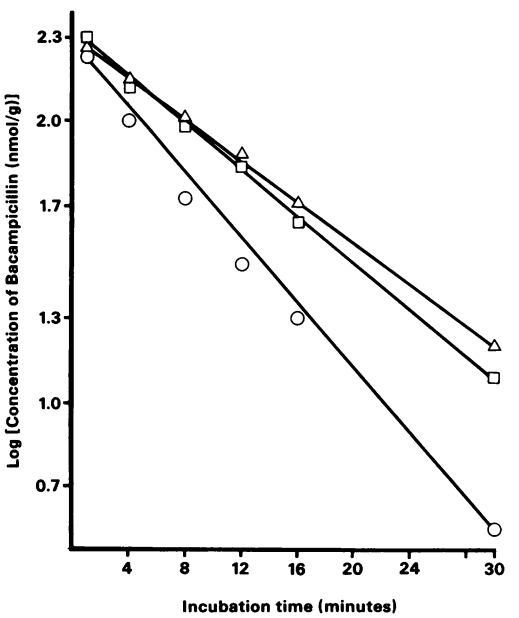


FIG. 2. *In vitro hydrolysis at 37 C of bacampicillin in heparinized human blood* (○), *human plasma* (□), *and canine plasma* (△).

TABLE 1. *Hydrolysis of bacampicillin and pivampicillin in various media*

| Compound | Hydrolysis to ampicillin in 30 min at 37 C (%)[a] | | | |
|---|---|---|---|---|
| | Phosphate buffer (pH 7.4) | Phosphate buffer + 10% human serum | Phosphate buffer + 10% rat serum | Synthetic gastric juice (pH 1.2) |
| Bacampicillin | 3.2 ± 0.7 | 54.4 ± 5.8 | 95.0 ± 7.4 | 0.24 ± 0.02 |
| Bacampicillin, A epimer | 3.0 ± 0.2 | 67.6 ± 5.0 | 100 ± 6.7 | 0.27 ± 0.05 |
| Bacampicillin, B epimer | 2.9 ± 0.2 | 32.4 ± 2.1 | 106 ± 5.0 | 0.28 ± 0.03 |
| Pivampicillin | 5.4 ± 0.3 | 10.2 ± 1.4 | 101 ± 5.8 | 0.24 ± 0.04 |

[a] Mean of 10 observations plus standard error of the mean.

campicillin, could be found circulating in the blood after oral administration of the latter, the antibiotic being distributed in the body must be ampicillin. The penetration of it into organs and tissues after oral administration of bacampicillin and ampicillin was compared in two studies in rats receiving equimolar amounts (270 μmol/kg) of the two compounds. The peak blood levels obtained with this dose again appeared higher after bacampicillin than after ampicillin (Table 3; Fig. 4), and for both compounds they were in good agreement with those to be expected from the plasma levels of the previous study with a lower dose (Fig. 3).

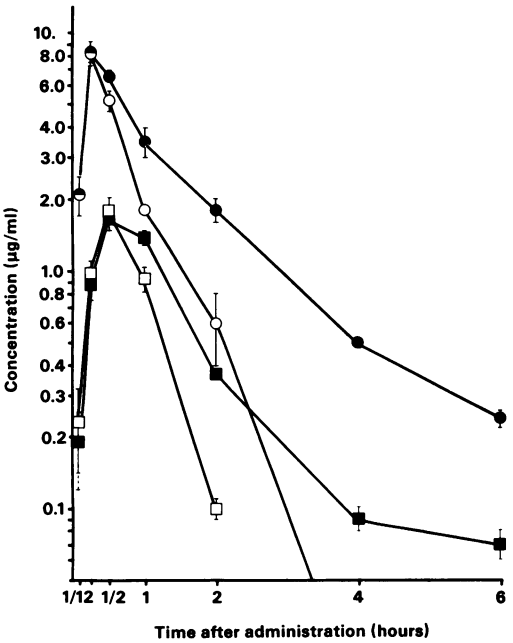


FIG. 3. *Plasma concentrations of total radioactivity and biological activity in rats after single oral, equimolar doses (40 μmol/kg) of [$^{35}S$]bacampicillin hydrochloride (20 mg/kg, ○, ●) and [$^{35}S$]ampicillin (14 mg/kg, □, ■). Each point represents the mean value of five animals. Bars indicate the standard error of the mean. Filled symbols, Total radioactivity; open symbols, biological activity.*

The penetration into tissue fluid was studied in an experimental model comprising "tissue cages" formed by implantation of coil springs in the backs of the rats (1, 6). The serous fluid, transudate, formed in similar cages has been assumed to be the equivalent of the interstitial fluid and in communication with it (3). In the experiment which comprised observations on four groups of animals for each compound, the mean transudate levels were consistently found to be higher after bacampicillin than after ampicillin (Table 3; Fig. 4). The difference was two- to threefold, but it was statistically significant ($P < 0.05$) only at 2 h after administration. The results further indicated a longer duration of the transudate than of the blood levels for both compounds, with a statistically significant difference ($P < 0.05$) in the case of bacampicillin 4 h after administration.

Analysis of homogenates of kidney and liver showed three to four times higher levels of ampicillin after administration of bacampicillin than after ampicillin (Table 4). In the spleen bacampicillin gave slightly higher levels.

**Experimental infections.** Orally administered bacampicillin showed good activity against experimental infections in mice caused by various gram-positive and gram-negative bacteria. When the compounds were given directly after the animals had been infected, bacampicillin was found to be as active as, or more active than, ampicillin against nine of the eleven organisms tested (Tables 5 and 6).

In one series of experiments with *H. influenzae*, the penicillins were, however, not given until 4 h after infection. It is likely that under such conditions the infection will be more severe as the bacteria are given time to establish themselves in the tissues of the animals. In

TABLE 2. *Plasma concentrations of ampicillin by microbiological assay after oral administration of equimolar (40 μmol/kg) amounts of bacampicillin hydrochloride (20 mg/kg) and ampicillin (14 mg/kg) to three dogs in a crossover experiment*

| Dog no. | Compound | Plasma concn (μg/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 min | 10 min | 20 min | 40 min | 1 h | 1.5 h | 2 h | 4 h | 8 h |
| 1 | Bacampicillin | 0.13 | 1.0 | 2.5 | 4.0 | 2.8 | 2.5 | 1.3 | 0.71 | 0.13 |
| | Ampicillin | 0.17 | 0.76 | 2.2 | 2.3 | 1.9 | 1.5 | 0.69 | 0.18 | 0.02 |
| 2 | Bacampicillin | 0.11 | 0.89 | 4.2 | 6.8 | 6.6 | 4.7 | 3.4 | 0.60 | 0.10 |
| | Ampicillin | 0.02 | 0.03 | 0.11 | 0.48 | 0.49 | 1.8 | 3.3 | 3.3 | 0.14 |
| 3 | Bacampicillin | 0.02 | 0.09 | 0.65 | 5.4 | 3.9 | 2.6 | 2.5 | 0.70 | 0.13 |
| | Ampicillin | 0.02 | 0.12 | 0.79 | 1.1 | 0.94 | 0.68 | 0.45 | 0.11 | 0.02 |

accordance with this, much higher doses of the penicillins were needed to cure the animals of the infection (Table 6). However, it was also found that bacampicillin in this case was relatively more effective than ampicillin than when the animals were treated directly after the infection.

## DISCUSSION

Bacampicillin is rapidly converted into ampicillin by biological fluids. The apparently complete and immediate hydrolysis in vivo of the ester group observed in rats and dogs has also been demonstrated in humans. In volunteers receiving 400 mg of $^{35}$S-labeled bacampicillin orally, no unchanged ester could be found circulating in the blood (Å. Swahn, Ph.D. dissertation, Karolinska Institutet, Stockholm, 1974).

Other studies have shown that the ester group, both on chemical and enzymatic hydrolysis, forms products of a physiological nature, i.e., acetaldehyde, ethanol, and carbon dioxide. Biological specimens of various kinds, such as human intestinal fluid, rat liver, and intestinal mucosa (Swahn, Ph.D. dissertation), and canine cerebrospinal fluid have been found to catalyze the hydrolysis of the ester group of bacampicillin. The enzyme systems catalyzing the hydrolysis have not yet been identified. Bovine carbonic anhydrase (EC 4.2.1.1) was found to be inactive, whereas hog carboxylic-ester hydrolase (EC 3.1.1.1) hydrolyzed bacampicillin very readily. This type of enzyme, also known as aliesterase, is a general esterase with widespread distribution in the mammalian body.

In the absorption experiments carried out bacampicillin, according to all criteria used, i.e., blood levels and, urinary and fecal recovery, was found to be more efficiently absorbed orally than ampicillin. The results also suggested a different absorption profile for the former, with earlier and much higher peak blood levels than obtained with the latter.

The concentrations found in the kidney and liver homogenates suggest that the higher initial blood levels obtained with bacampicillin cause a correspondingly higher tissue penetration. In the spleen the levels were lower, although here too they appeared slightly higher after bacampicillin than after ampicillin. The relation to the blood levels was, however, less evident.

The results obtained with the "tissue cage" model also indicate that bacampicillin gives a better tissue penetration than ampicillin itself,

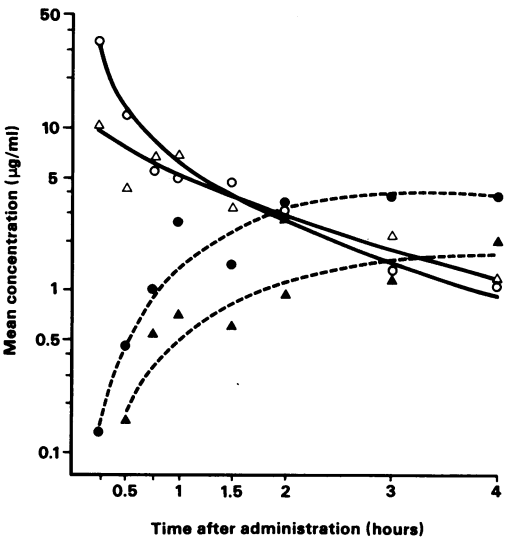


FIG. 4. *Blood and transudate concentrations of ampicillin after oral administration of equimolar amounts (270 μmol/kg) of bacampicillin hydrochloride (135 mg/kg, ○, ●) and sodium ampicillin (100 mg/kg, △, ▲) to rats with subcutaneous coil springs. Open symbols, Blood concentrations; filled symbols, transudate concentrations.*

TABLE 3. *Blood and transudate concentrations of ampicillin after oral administration of equimolar amounts (270 μmol/kg) of bacampicillin hydrochloride (135 mg/kg) and sodium ampicillin (100 mg/kg) to rats with subcutaneous coil springs*

| Compound | Concn of ampicillin (μg/ml)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.25 h | 0.5 h | 0.75 h | 1 h | 1.5 h | 2 h | 3 h | 4 h |
| **Blood** | | | | | | | | |
| Bacampicillin | 33.83 ± 16.5 | 12.2 ± 4.0 | 5.38 ± 0.53 | 4.99 ± 0.83 | 4.61 ± 0.88 | 3.10 ± 0.68 | 1.35 ± 0.12 | 1.10 ± 0.35 |
| Ampicillin | 10.4 ± 5.60 | 4.28 ± 0.94 | 6.42 ± 1.01 | 5.16 ± 0.65 | 3.13 ± 0.62 | 2.85 ± 0.61 | 2.12 ± 1.06 | 1.19 ± 0.74 |
| **Transudate** | | | | | | | | |
| Bacampicillin | 0.14 ± 0.05 | 0.45 ± 0.12 | 1.00 ± 0.30 | 2.63 ± 1.14 | 1.44 ± 0.34 | 3.43[b] ± 0.65 | 3.82 ± 1.24 | 3.99 ± 0.86 |
| Ampicillin | ND[c] | 0.16 ± 0.10 | 0.55 ± 0.13 | 0.72 ± 0.09 | 0.59 ± 0.12 | 0.95 ± 0.20 | 1.20 ± 0.24 | 1.97 ± 0.21 |

[a] Mean of four to six observations plus standard error of the mean.
[b] Mean of three observations.
[c] ND, Not detectable.

TABLE 4. *Ampicillin concentrations in organ homogenates after oral administration of equimolar amounts (270 μmol/kg) of bacampicillin hydrochloride (135 mg/kg) and sodium ampicillin (100 mg/kg) to rats*

| Compound | Tissue | Concn of ampicillin (μg/g)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.5 h | 1 h | 1.5 h | 2 h | 3 h | 4 h |
| Bacampicillin | Kidney | 31.1 ± 3.7 | 35.2 ± 8.2 | 35.7 ± 9.4 | 43.0 ± 23.7 | 22.0 ± 11.3 | 14.5 ± 4.7 |
| | Liver | 27.8 ± 3.3 | 26.1 ± 2.2 | 25.9 ± 3.3 | 14.9 ± 2.4 | 7.9 ± 1.3 | 3.9 ± 0.4 |
| | Spleen | 1.7 ± 0.3 | 1.1 ± 0.1 | 1.6 ± 0.3 | 1.0 ± 0.1 | 0.5 ± 0.1 | 0.3 ± 0.1 |
| Ampicillin | Kidney | 9.9 ± 1.2 | 12.1 ± 4.5 | 9.3 ± 1.7 | 11.9 ± 5.1 | 7.7 ± 2.2 | 3.6 ± 1.1 |
| | Liver | 7.3 ± 0.9 | 7.0 ± 1.0 | 5.7 ± 0.6 | 5.0 ± 0.3 | 4.3 ± 0.6 | 2.1 ± 0.3 |
| | Spleen | 1.2 ± 0.6 | 1.3 ± 0.7 | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.03 | 0.4 ± 0.3 |

[a] Mean of four observations plus standard error of the mean.

TABLE 5. *Activity of bacampicillin hydrochloride and ampicillin against experimental infections in mice when administered orally immediately after infection*

| Infecting organism | Challenge ($LD_{50}$)[a] | $CD_{50}$ (mg/kg)[b] | |
|---|---|---|---|
| | | Bacampicillin hydrochloride | Ampicillin |
| *Diplococcus pneumoniae* type II | 8.1 | <0.6 | 0.8 |
| | 12.0 | 0.9 | 1.4 |
| | 23.0 | 0.6 | 1.1 |
| | 32.0 | 3.6 | 3.0 |
| | 38.6 | 6.1 | 6.5 |
| | 40.0 | 8.1 | 3.5 |
| *Listeria monocytogenes* | 24.0 | 13.7 | 14.0 |
| | 28.0 | 12.2 | 13.5 |
| | 48.0 | 16.7 | 19.0 |
| *Staphylococcus aureus*, Smith | $3.3 \times 10^4$ | 0.6 | 0.8 |
| | $>10^5$ | <0.6 | 1.0 |
| | $>10^5$ | 1.3 | 0.8 |
| *Streptococcus faecalis* | 4.3 | 48.8 | 58.0 |
| | 4.3 | 44.8 | 52.0 |
| | 5.5 | 17.1 | 46.5 |
| *Streptococcus pyogenes* | 143.0 | <0.1 | 0.5 |
| | 647.0 | 0.4 | 0.8 |
| | $>10^5$ | 1.2 | 3.6 |
| | $>10^5$ | 4.6 | 11.0 |
| *Escherichia coli* | 2.6 | 3.0 | 10.0 |
| | 2.8 | 9.5 | 12.0 |
| | 3.2 | 12.9 | 6.5 |
| *Klebsiella pneumoniae* | 26.0 | 12.9 | 35.5 |
| | 60.0 | 6.5 | 10.5 |
| | 100.0 | 4.9 | 9.0 |
| *Pasteurella multocida* | $7.9 \times 10^4$ | 13.5 | 9.5 |
| | $>10^5$ | 12.5 | 21.0 |
| | $>10^5$ | 23.6 | 11.5 |
| *Proteus mirabilis* | 226 | 8.5 | 9.5 |
| | 270 | 4.0 | 12.5 |
| | 316 | 5.2 | 13.5 |
| *Proteus vulgaris* | 1.1 | 214 | 105 |
| | 1.4 | 170 | 125 |
| | 2.8 | 214 | 315 |

[a] One $LD_{50}$, The dose of bacteria required to kill 50% of the mice.

[b] $CD_{50}$, Median curative dose.

TABLE 6. *Activity of bacampicillin hydrochloride and ampicillin against experimental H. influenzae infections in mice when administered immediately or 4 h after infection*

| Challenge ($LD_{50}$)[a] | $CD_{50}$ (mg/kg)[b] | | | |
|---|---|---|---|---|
| | Penicillin immediately after infection | | Penicillin 4 h after infection | |
| | Bacampicillin hydrochloride | Ampicillin | Bacampicillin hydrochloride | Ampicillin |
| 38.3 | 3.0 | 3.5 | | |
| 631 | 3.0 | 3.0 | | |
| 3,100 | 4.5 | 4.5 | | |
| 26,300 | | | 56 | 195 |
| 2,000 | | | 25 | 125 |
| 266 | | | 12.5 | 17.5 |
| 1,000 | | | 24.0 | 113 |
| 310 | | | 19.5 | 105 |

[a] One $LD_{50}$, The dose of bacteria required to kill 50% of the mice.

[b] $CD_{50}$, Median curative dose.

due to the high peak blood concentrations. This is in agreement with another study with a different experimental model demonstrating that high peak blood levels give better tissue penetration than lower but more sustained ones (2). Of further interest is that, for both compounds, the levels in the cages appear more sustained than the blood levels, indicating a diffusion barrier surrounding the cages. Similar barriers are likely to occur around infectional foci in tissues, and the results thus suggest that bacampicillin, due to better oral absorption than ampicillin, might have pharmacokinetic advantages of importance for the dose to be used and for the dosage interval.

ACKNOWLEDGMENTS

We are grateful to B. Pring for linguistic corrections of the manuscript.

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