[Chem. Pharm. Bull. 36(4)1463—1468(1988)]

Rectal Absorption of Bacampicillin in Rabbits[1)]

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(Received July 6, 1987)

In this study, the rectal absorption of bacampicillin hydrochloride (BAPC) in rabbits as a model was investigated with the aim of preparing efficacious suppositories. First, analytical methods, were developed to determine penicillins in plasma for evaluating the absorption of BAPC. When BAPC was mixed in the plasma, BAPC was not detected only ampicillin (ABPC) derived from BAPC was detected by high performance liquid chromatographic analysis. The bioavailability of BAPC was studied following oral and rectal administration in rabbits. Administration of a BAPC suppository formulated with Witepsol H-15 produced absorption at a level 80% of that found after oral dosing, whereas no measurable level was seen following rectal administration of an ABPC suppository corresponding to the BAPC dose. Further, rectal absorption of BAPC increased with increasing dose and a prolonged plasma level was observed.

Keywords——bacampicillin; rectal administration; bioavailability; Witepsol H-15 suppository; mean residence time; residence time variance; ampicillin; bacampicillin absorption evaluation

β-Lactam antibiotics are generally thought not to be absorbable rectally because they have a carboxyl group in their structure and are hydrophilic. Thus, many workers have studied promotors of permeation through the rectal membrane.[2-4)] Enamine derivatives of ABPC are of interest because of their absorbability and absorption promoting effect.[5)] They have highly lipophilic characteristics despite having a hydrophilic carboxyl group.

Bacampicillin (BAPC) is a prodrug of ampicillin (ABPC) and is well absorbed following oral administration because of its lipophilicity.[6)] Thus, in the present work, we investigated the rectal absorption of BAPC in rabbits with the aim of preparing a suppository. An assay system of BAPC in plasma was established for this purpose.

Experimental

Materials——BAPC (659 μg/mg as ABPC, Yoshitomi Pharmaceutical Ind., Itd.) was used as received. ABPC Na (Meiji Seika Kaisha Ltd.) and Witepsol H-15 (Maruishi Pharmaceutical Co., Ltd.) were the normal commercial preparation. All other reagents were of the highest commercial grade and were used without further purification.

Preparation of Suppository——Suitable amounts of ABPC Na or BAPC were mixed in a melted suppository base (45 °C) and then dispersed well by sonication with an ultrasonic cleaner (Branson, 220J) for 5 min at 40 °C. The melted base containing antibiotics was poured into steel molds and allowed to solidify at room temperature. The actual content of the ingredient in the suppositories was determined after dissolving a suppository in water to make 100 ml at 40 °C. No effect of Witepsol H-15 on I_2-colorimetry[7)] was observed. In each suppository, the found value of BAPC was in good agreement with the theoretical one. Further, as the content was homogeneous throughout a suppository, no sedimentation of the ingredient occurred during solidification in the mold.

Stability Assay of Antibiotics in Suppository Base——The time course for BAPC or ABPC Na, which was dispersed in the melted Witepsol H-15 by sonification, was studied at 45 °C. The determination of the antibiotics was done on a solution prepared by dissolving *ca.* 0.1 g of the suspension into water to make 20 ml. A storage test of suppositories prepared with the same content was done by storage in a refrigerator (4 °C).

Analytical Method——(i) I_2-colorimetry. The assay procedure was the same as described previously.[7] (ii) Bioassay. Antibacterial activity was assayed by the disk method with *Streptococcus mutans* as a test organism using brain heart infusion (Difco) agar. (iii) High performance liquid chromatography (HPLC) analysis. An HPLC apparatus (Shimadzu, LC-5A) equipped with a detector (Shimadzu, SPD-2A) was used. The conditions for analysis were as follows: column, 15 cm × 4 mm i.d.; packing, TSK gel ODS-80TM (Toyo Soda Manufacturing Co., Ltd.); mobile phase, 0.01 M phosphate buffer (pH 6.0)–MeOH (70:30) for ABPC, (40:60) for BAPC; flow rate, 1.0 ml/min; wavelength, 225 nm; sensitivity, 0.02 a.u.f.s. (iv) Measurement of ABPC in plasma. For all treatments, 0.5—1.0 ml blood samples were collected from the marginal ear vein of rabbits before and at suitable intervals after heparin (100 unit/kg) administration. Samples were centrifuged at 2000 rpm for 20 min immediately after collection. The plasma samples were diluted to 5—10 times with water, followed by ultrafiltration using an Air-PRESS-30 (Toyo Soda Manufacturing Co., Ltd.). The resulting filtrates were subjected to bioassay and/or HPLC analysis.

Drug Administration——(i) Rectal administration. White male rabbits weighing from 2.5 to 3.5 kg were fasted for 24 h prior to the experiments, but were allowed free access to water. The suppository was used after reducing its size by cutting off the upper parts according to the body weight of the rabbit and was positioned 3 cm into the rectum. To prevent expulsion of the suppository, a clip was used to hold the anus closed for 4 h after dosing. (ii) Oral and intravenous administration. The oral BAPC dose (17 mg/kg) consisted of 2 ml of solution and was introduced directly into the stomach *via* a catheter followed by flushing with 2 ml of water. The intravenous administration was carried out by injection of 2 ml of ABPC Na solution into a marginal ear vein.

Pharmacokinetic Analysis——The data were analyzed according to the model-independent moment analysis.[8] The last determined plasma concentration was extrapolated to infinite time by using the terminal slope of the log-time disposition curve. The value of the area under the plasma concentration–time curve ($[AUC]_0^\infty$) was calculated from the time course of plasma concentration by means of the trapezoidal rule. The mean residence time (MRT) and the variance of residence time (VRT) of the drug in the body were calculated by the method of Yamaoka *et al.*[9]

BAPC Release Test of Suppositories——The test of BAPC release from the suppository was performed by using an instrument (Toyama Sangyo Co., Ltd., Model TMS-103) as reported by Muranishi *et al.*[10] Normal saline (300 ml) was used as the test solution. Each suppository was placed on an artificial membrane (0.45 μm) in a cylindrical plastic cell. This cell, containing 7 ml of normal saline, was immersed in the test solution, which was stirred at 100 rpm; the inner stirrer was rotated at 50 rpm. The temperature was controlled at 37 ± 0.5 °C. A 5 ml aliquot of the solution was taken out, followed by addition of 5 ml of normal saline to maintain a constant volume. Relaeased BAPC was assayed by I_2-colorimetry.

Results and Discussion

Stability of BAPC and ABPC in Witepsol H-15

Many suppository bases do not seem to be adequate for the preparation of suppositories of β-lactam antibiotics because of considerable degradation during storage.[5] Table I shows the time courses of BAPC or ABPC Na in the bases at 45 °C. The initial concentrations of BAPC and ABPC Na were 25 mg/g base. When similar suppositories were kept for two weeks at 0 °C, the drug contents were: BAPC, $100 \pm 3.1\%$; ABPC, $99.7 \pm 2.3\%$, $n = 10$. Thus, suppositories were used for experiments within two weeks after preparation.

Analysis of BAPC and ABPC by Bioassay or HPLC

The determination of ABPC in aqueous solution by bioassay and HPLC assay gave as good linear correlation ($y = 0.993x - 0.080$, $r = 0.9995$). Further, no effect of the pretreatment

Table I. Stability of BAPC and ABPC in Witepsol H-15 at 45 °C

Time (h)	BAPC content (mg/g·base)	ABPC content (mg/g·base)
0	24.9	25.3
4	26.0	24.7
26	25.3	25.0
45	25.5	24.8

No degraded products were detected in BAPC or ABPC suspension. Each value represents the mean of two experiments.

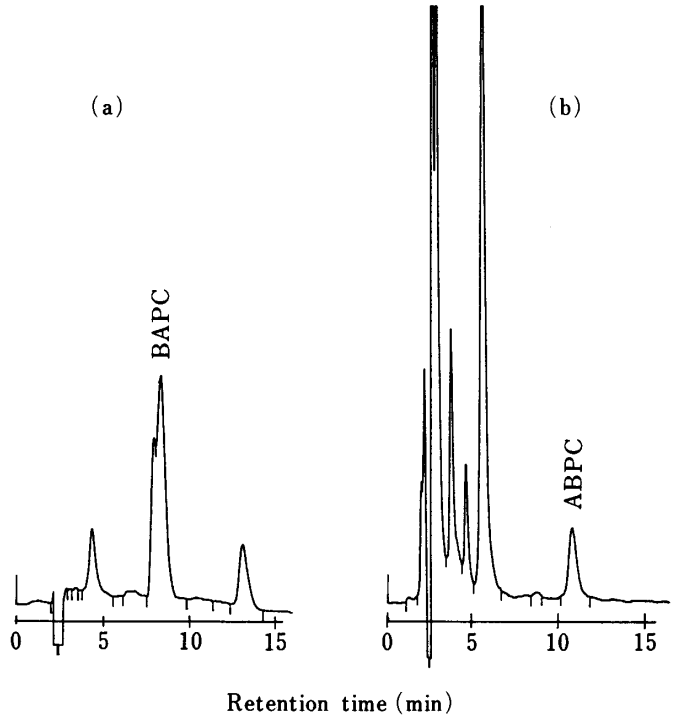

Fig. 1. Chromatogram of BAPC in Aqueous Solution and ABPC in Plasma

Analytical conditions: see text.

for deproteinization on the determination of ABPC was observed. HPLC analysis of BAPC, however, revealed two peaks under the conditions described in Experimental (Fig. 1a). These peaks were supposed to represent that of epimers of the BAPC ester moiety,[11] and the calibration curve between the total area of these peaks and the concentration of BAPC was found to show good linearity. On the other hand, BAPC showed very little biological activity (below one tenth of that of ABPC).

Determination of BAPC and ABPC in Plasma

A mixture of ABPC solution with rabbit plasma was assayed by bioassay and HPLC (Fig. 1b). ABPC added was accurately determined by bioassay, while at above 4 μg/ml, HPLC assay gave lower values than bioassay. As no ABPC was adsorbed by the filter, this seemed to be due to binding with plasma protein. Total ABPC in plasma could be determined without any effect of protein binding by diluting a sample solution 5—10 fold.

Boudin *et al.*[6] reported that only ABPC was detected in the blood following oral administration of BAPC, presumably because BAPC was hydrolyzed easily by esterases in the blood or during the absorption from the intestinal tract. However, the behavior of BAPC in the blood during and after absorption from the rectum is unknown. Thus, various concentrations of BAPC mixed with plasma at 0 and 37 °C were analyzed by bioassay and HPLC assay, and ABPC in the samples was determined simultaneously by HPLC assay.

It was consequently proved that BAPC in the plasma could be completely determined as equimolar ABPC, and free BAPC was not detected in these solutions. To confirm this, antibacterial activities and intact ABPC (by HPLC) after rectal administration of BAPC (17 mg/kg) were measured. The found values at each time agreed very closely with each other. Further, no BAPC was detected in these peripheral blood samples. Thus, the presence of BAPC in the bloodstream need not be considered at the low dose of 17 mg/kg, even if BAPC itself was absorbed from the rectum. At such a low dose, hydrolysis of BAPC seems to be performed during passage through the rectal membrane or in the bloodstream.

Consequently, it had become apparent that the determination of ABPC makes it possible to evaluate BAPC absorption, because BAPC administered through the rectum is quickly converted into ABPC in the same manner as after oral administration.

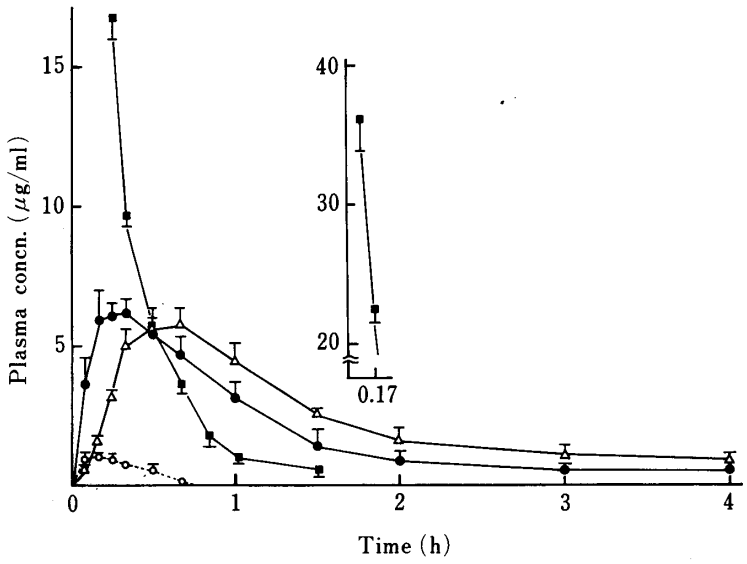

Fig. 2. A Comparison of ABPC Plasma Levels after Rectal (●) or Oral (△) Administration of BAPC (17 mg/kg), and Rectal (○) or Intravenous (■) Administration of ABPC (12.6 mg/kg) to Rabbits

Each point represents the mean ± S.D. for three or four rabbits.

BAPC Absorption from the Rectum in Rabbits

The time courses of the concentrations of the antibiotics in blood were studied following oral, rectal or intravenous administration of ABPC (12.6 mg/kg) or BAPC (17 mg/kg) to rabbits (Fig. 2). The absorption of BAPC from the rectum was rapid compared to oral absorption, but the concentration of the resulting ABPC in plasma after rectal administration was similar to that after oral administration. The elimination of ABPC from blood after rectal and oral administration of BAPC was slower than that after intravenous administration of ABPC, which may be due to the prolonged absorption, and was almost completed in 4 h. The mean maximum plasma concentration (C_{max}) of ABPC after rectal administration of BAPC was 6.95 ± 0.25 μg/ml at 17.1 ± 4.1 min (time to peak, t_{max}), while that after oral absorption was 6.33 ± 0.37 μg/ml at 33.3 ± 9.4 min, as shown in Table II.

Some workers have reported that the rectal absorption of various drugs is more rapid[12] or slower[13] than its oral absorption, but it is not clear which is generally the case. In the case of oral drug administration, physiological variables such as coprophagy in rabbits may affect the absorbability of the drug. Maeda *et al.*[14] reported that the peak time and plasma pattern after oral administration to conventionally fasted rabbits were different from those in stomach-emptying-controlled rabbits, suggesting slower absorption in conventionally fasted rabbits. Because the rabbits used in this study were conventionally fasted rabbits, the slower oral absorption of BAPC compared to the rectal absorption may be a result of such physiological variables.

The rectal absorption of ABPC (equimolar with BAPC), on the other hand, was very poor (C_{max} was 0.98 ± 0.19 μg/ml), while the oral absorption was similar to that of BAPC. This suggests the importance of lipophilicity for the rectal absorption of antibiotics.[15] Thus, BAPC seems to be well absorbed from the rectum, as from the intestinal tract, because of its lipophilicity.

The bioavailability parameters such as $[AUC]_0^\infty$, MRT and VRT obtained from the results in Fig. 2 are summarized in Table II. In order to calculate the apparent bioavailabilities of antibiotics after oral and rectal administration of BAPC (or ABPC), ABPC was administered into the vein of rabbits at a dose of 12.6 mg/kg (equimolar dose 17.0 mg/kg of

TABLE II. Bioavailability Parameters after Rectal, Oral and Intravenous Administration of Antibiotics in Rabbits

Drug	Route of administration	Dose (mg/kg)	$[AUC]_0^\infty$ a) ($\mu g \cdot h/ml$)	C_{max} a) ($\mu g/ml$)	t_{max} a) (min)	MRT (h)	VRT (h^2)	BAPC/base (w/w %)	EBA b) (%)
BAPC	supp.	12.5	3.60±1.19	5.50±1.60	17.5± 2.5	0.39	0.22	7	27.3
		15.5	5.24±1.23	5.84±1.23	13.8± 6.6	0.84	0.52	9	31.8
		17.0	8.00±0.74	6.95±0.25	17.1± 4.1	1.42	3.14	10	44.2
		31.3	17.01±1.47	7.01±1.42	21.0± 6.8	2.38	5.63	18	51.1
		40.0	24.68±5.20	8.56±1.48	33.3±11.5	4.71	36.09	23	58.1
	p.o.	17.0	10.11±2.70	6.33±0.37	33.3± 9.4	1.77	2.85		55.9
ABPC	supp.	12.6	0.73±0.25	0.98±0.19	10.0± 2.0	0.66	0.36	7	4.0
	p.o.	12.6	9.15±2.64	4.60±1.71	25.0±10.0	1.80	2.03		50.6
	i.v.	12.6	18.08±7.17						

a) Values reported are means±S.D. ($n=3$—4). *b*) Extent of bioavailability:

$$\frac{[AUC]_0^\infty \text{ supp./dose supp.}}{[AUC]_0^\infty \text{ i.v./dose i.v.}}, \quad \frac{[AUC]_0^\infty \text{ } p.o./\text{dose } p.o.}{[AUC]_0^\infty \text{ i.v./dose i.v.}}.$$

The dose of BAPC for calculating EBA was converted to the dose of ABPC. The abbreviations used are as follows: supp., suppository; *p.o.*, oral administration; i.v., intravenous injection.

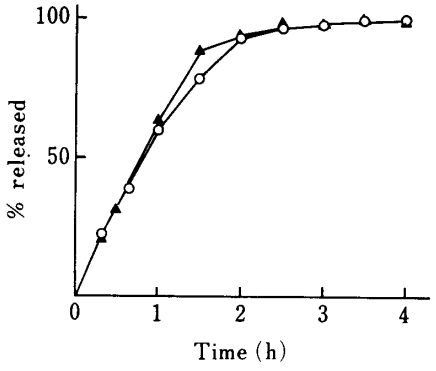

Fig. 3. Release of BAPC from Suppository at 37 °C

○, 184 mg/g·base; ▲, 100 mg/g·base.

BAPC). Its $[AUC]_0^\infty$ was 18.08±7.17 $\mu g \cdot h/ml$ (Table II). The extents of bioavailability (EBA) after rectal and oral administration, calculated based on $[AUC]_0^\infty$ of ABPC intravenous injection, are also summarized in Table II. As shown in Table II, adjusting for the dose administered, the EBA values of the BAPC oral and the BAPC rectal administrations were 55.9% and 44.2%, respectively. The relative bioavailability of the rectal administration of BAPC in Witepsol H-15 suppository, was about 80% with respect to oral administration, while that of ABPC was only 7.9%. Thus, the absorbability of BAPC from the rectum without any absorption promoter is fairly good compared to that of ABPC with an absorption promoter.[16] The EBA by rectal administration of various doses of BAPC was determined in a similar manner. Both C_{max} and t_{max} increased with increasing dose. In general, the permeation of the drug through the rectal membrane is thought to be passive.[17] The values of $[AUC]_0^\infty$, however, were not linearly correlated with the dose, but were higher than expected (Table II).

The release rate profiles of BAPC from suppositories are shown in Fig. 3. It was found that the release rate of BAPC from each suppository was constant regardless of the content of BAPC in the base. The absorption behavior *in vivo* can not always be evaluated from the release rate profiles *in vitro*. However, since the release rate was proved not to be affected by the content of suppository base, the increase of $[AUC]_0^\infty$ with increase of the dose shown in Table II is assumed to be not only due to the increase of BAPC concentration in the rectal secreting fluid, but also due to the diffusion of the secreting fluid, which is increased with

to the diffusion of the secreting fluid, which is increased with the enhanced osmolarity arising from drug dissolution,[18] into the rectum, suggesting an increase of distribution area of BAPC in the rectum. This is consistent with the changes of MRT and VRT.

The results of this study indicate that BAPC, a prodrug of ABPC, is suitable for rectal administration, but further work on the choice of suppository bases and BAPC concentration in the base should be carried out to improve the bioavailability.

Acknowledgements We are grateful to Yoshitomi Pharmaceutical Co., Ltd. for providing BAPC, and to Misses R. Kikuchi and M. Yamashita for their technical assistance.

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