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FUNDAMENTAL AND CLINICAL STUDIES FOR OPHTHALMIC USE OF ASPOXICILLIN

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ABSTRACT

Fundamental and clinical studies of ASPC, a new penicillin antibotic, were performed in the field of ophthalmology, and the results obtained were as follows.

1) The bacteriological activities of ASPC were similar to that of ABPC, CBPC and PIPC. The distribution of the sensitivity for 20 clinical isolates of S. aureus was in the range of 0.78～12.5μg/ml, with peaks at 0.78 μg/ml and 3.13 μg/ml, and the number of each of the strains was 5 (25%) respectively. The strains of P. aeruginosa were sensitive at 25～> 100μg/ml and five of them were distributed under 50 μg/ml.

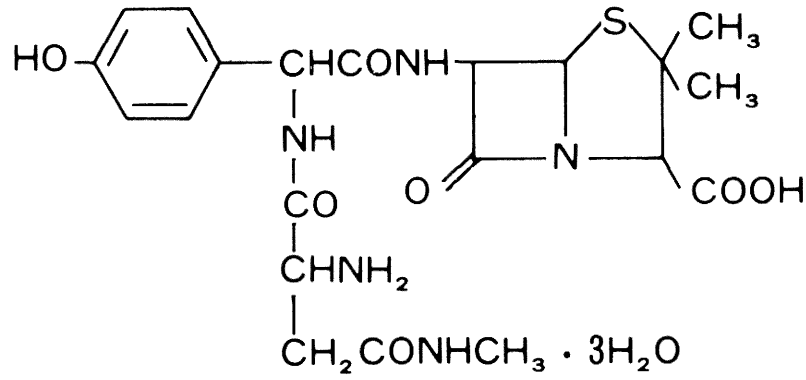
2) Ocular penetrations were examined in mature white rabbit eyes. After intravenous injection of 50 mg/kg, the aqueous level reached the peak of 6.0 μg/ml after 1/2 hours. Thereafter, it rapidly decreased and reached 0.7 μg/ml after 6 hours. Aqueous serum ratio at 30 minutes was 5.2%. The ocular tissue concentrations at 30 minutes after intravenous injection showed high levels in outer parts of the eye and relatively high levels in inner parts.

3) In the clinical study, the intravenous injection or drip infusion of 1.0 g or 2.0 g once or twice a day was performed in 12 cases, such as external hordeolum, acute dacryocystitis, corneal ulcer, corneal abscess, iridocyclitis purulenta and orbital infection. The results obtained were excellent in 3 cases, good in 8 cases and poor in 1 case.

4) No abnormal laboratory findings were recognized and no severe side effects were observed.

INTRODUCTION

Aspoxicillin (ASPC) is a penicillin antibiotic developed by Tanabe Seiyaku Co., Ltd., Japan, with a side chain of N^4-methyl-D-asparagine in the 6-position, and a chemical structure formula as follows:



Chemial structure formula

It has a broad antibacterial spectrum against both gram positive and gram negative bacteria, and has relatively strong bactericidal effects. Its curative efficacy was experimentally shown to be better vivo than in vitro.

Fundamental and clinical studies on ASPC for clinical application in cases of ocular infections have been performed.

In this paper, the results obtained are reported.

METHODS

1. Antibacterial activity

The minimum inhibitory concentrations (MIC) of ASPC against strains kept in our laboratory were determined according to the standard method specified by the Japan Society of Chemotherapy. Bacterial solutions left overnight in Tryptic soy broth were used as the inocula.

2. Sensitivity of clinical isolates

The sensitivity of 20 strains of S.aureus and 10 strains of P. aeruginosa to ASPC was determined by the same method as described above.

3. Ocular penetration of ASPC

The penetration of ASPC into the eye was examined using mature white rabbits weighing 2.5～3.0 kg. ASPC was intravenously injected in doses of 50 mg/kg; the

aqueous humor and blood were collected and changes of ASPC levels in these fluids with time were examined.

Further, the eyeballs were removed at the time of the peak levels in the aqueous humor, and the ocular tissue levels vere determined. To determine the ocular tissue levels,M.luteus ATCC 9341 was used as the test bacterium, and the thin layer cup plate method with Tryptic soy broth was employed. To dilute the specimen and to prepare the standard curve, a phospate buffer solution, pH 7.0 was used.

4. Clinical trials

A clinical trial was performed on a total of 12 cases with the following diseases: external hordeolum (1 case), acute dacryocystitis (1 case), corneal ulcer (5 cases), corneal abscess (2 cases), purulent iridocyclitis (2 cases) and orbital infection (1 case). In these cases, 1 or 2 g of ASPC was given twice a day by intravenous injection or drip infusion. The clinical efficacy was then examined. In the cases of corneal infections, a 1% solution of ASPC was concomitantly instilled in the eye.

An intracutaneous reaction of ASPC was negative in all of the cases.

RESULTS

1. Antimicrobial spectrum

Table 1 shows the results. The numbers there give the MIC found. MICs were 1.56 μg/ml for H.aegyptius, 0.19 μg/ml for M.lacunata. 0.78 to 1.56 μg/ml for S. pneumoniae, 0.78 to 1.56 μg/ml for C. diphtheriae, 0.19 μg/ml N. gonorrhoeae, 0.19 μg/ml for S. hemolyticus, 3.13 to 6.25 μg/ml for S. viridans, 0.78 to 6.25 μg/ml for S. aureus, 25 to 50 μg/ml for P. aeruginosa, and 0.78 μg/ml for S. aureus 209p.

Table 1. Antimicrobial spectrum (μg/ml) (10^8cells/ml)

Organisms	No. of strains	ASPC	ABPC	CBPC	PIPC
H. aegyptius (K-W)	4	1.56	2.5	3.12	6.25
M. lacunata (M-A)	7	0.19	<0.19	<0.19	0.39~0.78
S. pneumoniae	8	0.78~1.56	<0.19~0.78	0.19~25	1.56~6.25
C. diphtheriae	4	0.78~1.56	0.19~0.39	0.78~1.56	0.78~6.25
N. gonorrhoeae	1	<0.19	0.01	0.02	0.19
S. hemolyticus	2	0.19	0.05	0.19	0.78, 1.56
S. viridans	2	3.13, 6.25	1.56	25	1.56, 6.25
S. aureus	4	0.78~6.25	0.38~100	0.39~6.25	0.78~6.25
P. aeruginosa	2	25, 50	>100	25, 50	6.25, 25
S. aureus 209 P	1	0.78	0.39	0.39	0.39

The antibacterial activity of ASPC was almost the same as that of aminobenzyl PC (ABPC),[1] Carbenicillin (CBPC)[2], and Piperacillin (PIPC),[3] also shown in Table 1. The activity of ASPC against P. aeruginosa was the same as that of CBPC.

2. Sensitivity of clinical isolates

Table 2 shows the results.

Sensitivity of S. aureus to ASPC was distributed in a range from 0.78 to 12.5 μg/ml. There were peaks involving 5 strains each at 0.78 and at 3.13 μg/ml ; each of these values accounted for 25% of the total 20 strains. MICs for the ten strains of P. aeruginosa were

Table 2. Sensitivity distribution of clinical isolates ASPC

Organsms	No. of strains	≦0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
S. aureus	20			5	3	5	3	4				
P. aeruginosa	10								1	4	2	3

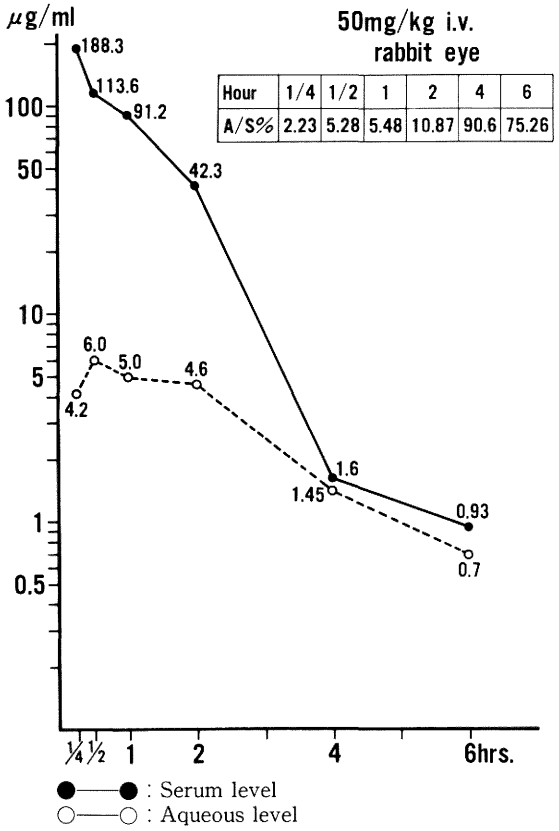


Fig. 1. (Ooishi and associates). Aqueous and serum level of ASPC

from 25 to over 100 μg/ml. For 5 of the strains (50%) the MIC was 50 μg/ml or less.

3. Ocular penetration of ASPC

Results are expressed as the average ASPC level in two eyes.

1) Aqueous humor levels of ASPC (Fig. 1)

ASPC level in the aqueous humor was 4.2μg/ml at 0.25 hr after injection. The peak in the aqueous humor, 6.0 μg/ml, was at 0.5 hr after injection, and thereafter, the level decreased gradually as follows: 5.0 μg/ml at 1 hr, 4.6 μg/ml at 2 hr, 1.45 μg/ml at 4 hr, and 0.7 μg/ml at 6 hr after injection,

The peak serum level, 188.3 μg/ml, was at 0.25 hr after injection; thereafter, the level decreased relatively rapidly, reaching 0.93 μg/ml at 6 hr after injection. At 0.5 hr after injection, the ratio of the level in the aqueous humor to that in the serum was 5.28%.

2) Ocular tissue levels of ASPC

Figure 2 shows the ocular tissue levels of ASPC at 0.5 hr after injection.

ASPC levels in the eyelid and bulbar conjunctiva in the outer parts of the eye were quite high, about 50 μg/g. Levels in the extraocular muscles, sclera, and cornea decreased, in that order. The ASPC level in the iris and ciliary body was fairly high, 29.49 μg/g. The level in the retina and choroid was also somewhat high, at 11.86 μg/g. The level in the vitreous body was 0.43 μg/ml; penetration into the crystalline lens did not occur.

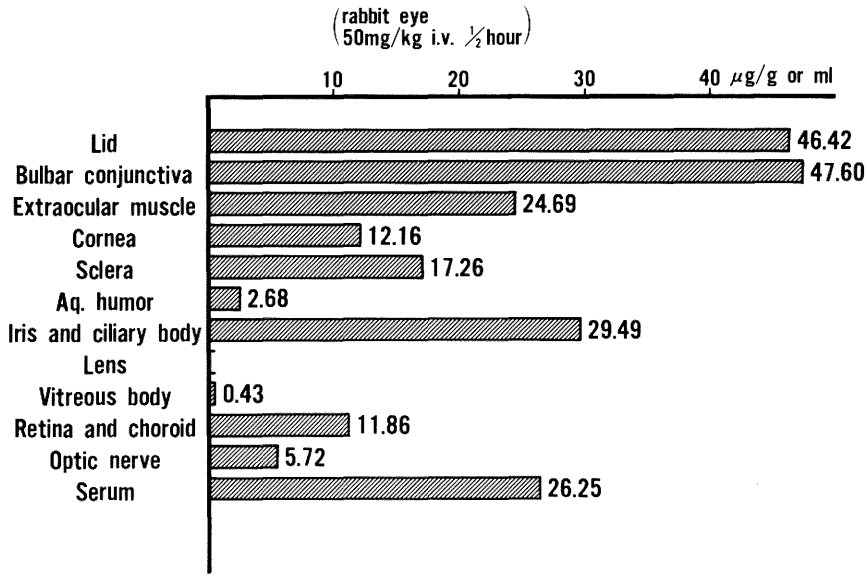


Fig. 2. (Ooishi and associates). Ocular tissue concentration of ASPC

4. Clinical results

Table 3 shows the clinical results of ASPC

Table 3. Clinical Results of ASPC

No.	Age Sex	Diagnosis	Eye	Organisms	Daily dosis (g×times, route)	Days of administration	Total dosis (g)	Effect	Side effects
1	27 ♂	External hordeolum	OD	*S. aureus*	1.0×1 I. V.	3	3.0	+	−
2	46 ♀	Acute dacryocystitis	OS	GNR ◦ *S. epidermidis*	1.0×1 I. V.	4	4.0	+	−
3	68 ♀	Corneal ulcer	OD	*S. epidermidis*	1.0×2 D. I.	7	14.0	++	−
4	63 ♀	Corneal ulcer	OS	*S. pneumoniae*	2.0×1 D. I.	5	10.0	+	−
5	78 ♀	Corneal ulcer	OD	Non fermentative GNR	2.0×1 D. I.	6	12.0	+	−
6	66 ♂	Corneal ulcer	OD	GNR	2.0×2 D. I.	5	20.0	++	−
7	40 ♂	Corneal ulcer	OS	GNR	2.0×2 D. I.	5	20.0	−	−
8	47 ♂	Corneal abscess	OS	GNR	1.0×1 I. V.	4	4.0	+	−
9	62 ♂	Corneal abscess	OD	GPR	2.0×1 D. I.	7	14.0	++	−
10	58 ♂	Iridocyclitis purulenta	OS	*S. epidermidis*	1.0×2 I. V.	7	14.0	+	−
11	47 ♂	Iridocyclitis purulenta	OD	*S. epidermidis*	1.0×2 D. I.	7	14.0	+	−
12	58 ♀	Orbital infection	OS	◦ *S. nonhemolyticus* ◦ *S. epidermidis*	2.0×2 D. I.	5	20.0	+	−

The patient with external hordeolum complained of severe reddening, swelling, and spontaneous pain in the right eyelid, and S. aureus was detected when a pus culture was made by puncture drainage. After the intravenous administration of 1 g of ASPC per day for 3 days (for a total of 3 g) , the purulent inflammation was cured.

In the case of acute dacryocystitis, the complaints of redddning, swelling, and pain in the tear sac area were observed. After the intravenous injection of 1 g of ASPC per day for 4 days (for a total of 4 g), subjective and objective symptoms improved.

In the cases of corneal ulcer, S. pneumoniae. S. epidermidis, glucose nonfermentative GNR, and another GNR were detected when smears from the corneal ulcer were cultured. A 1% ASPC solution was instilled into the eyes 4 times a day along with 1% Atropine. In addition. 1 or 2 g of ASPC was given intravenously by drip infusion, 1 or 2 times a day. A total of 10 to 20 g of ASPC was administered, over 5 to 7 days. The size of the ulcers decreased and disappeared in 4 of the 5 cases. The clinical efficacy of ASPC was rated in these cases as excellent or good.

In the cases of corneal abscess, GNR or GPR was detected. In one case , an intravenous drip infusion of 2 g of ASPC was performed daily for 7 days, (a total of 14g).

The abscess rapidly disappeared, and the clinical efficacy was rated as excellent. In the other case, 1 g of ASPC was injected daily, for a total of 4 g in 4 days. The size of the abscess decreased, and the clinical efficacy of ASPC in this case was rated as good.

In both cases of purulent iridocyclitis, the disease occured after intraocular foreign body, and corneal injury. Marked turbidity of the aqueous humor was present, and S. epidermidis was detected in secretions of the conjunctival sac. One gram of ASPC was given intravenously by drip infusion or intravenous injection, twice a day. The turbidity of the aqueous hnmor disappeared by the 7th day of administration, and the clinical efficacy of ASPC was rated as good.

In the case of orbital infection, severe reddening and swelling of the eyelid and chemosis were noted, and orbital cellulitis was found. S. epidermidis and S. non-hemolyticus were detected by culture of the secretions. Two grams of ASPC was given intravenously by drip infusion, twice a day. A total of 20 g of ASPC was administered for 5 days. Subjective and objective symptoms were alleviated, and by the 10th day after the start of the administration, the inflammation disappeared.

No allergic reaction as a side effect occurred in any case. Blood and renal hepatic function tests revealed no abnormal values either before or after the administration of ASPC.

DISCUSSION

The effects of ASPC on ocular infections were compared with those of conventional penicillins, in order to examine the clinical utility of ASPC.

The antimicrobial spectrum of ASPC is similar to those of ABPC,[1] CBPC,[2] and PIPC.[3] In particular, the effects of ASPC and CBPC against P. aeruginosa are very similar. Our results agree with results reported at the Symposium on New Drugs at the 30th Annual Meeting of the Japan Society of Chemotherapy.[4]

The MICs of ASPC for clinically isolated S. aureus was from 0.78 to 12.5 μg/ml, and for P. aeruginosa, from 25 to over 100 μg/ml. In a previous report,[2] the MICs of CBPC for 100 strains of S. aureus were from 0.5 to 25 μg/ml, and those for 15 strains of P. aeruginosa were from 25 to over 100 μg/ml. Thus, the antibacterial activity of ASPC and of CBPC against these two bacteria was almost the same. Some strains of S. aureus were less sensitive to ASPC than to ABPC. Some strains of P. aeruginosa were more sensitive to ASPC than to ABPC, and less sensitive to ASPC than to PIPC . These results concurred with the report given at the Symposium on New Drugs.[4]

We now recognize that ASPC has a broad antimicrobial spectrum against gram positive and gram negative organisms and also some antibacterial activity against P. aeruginosa.

Reports on the ocular penetration of ASPC have not yet appeared. In the present study , the peak level of ASPC in the aqueous humor of rabbits was 6.0 μg/ml at 0.5 hr after the intravenous injection of 50 mg/kg. The ratio of the aqueous level to serum

level was 5.28%. In our previous report,[5] the peak level of CBPC was 4.5 μg/ml at 0.5 hr after the intravenous injection of the same dose and the aqueous humor/serum ratio, 6.2%. Thus, the ocular penetration of ASPC was close to that of CBPC. ASPC levels in the tissue of the eye were highest in the outer and inner parts of the eye. In general, the penetration of this drug into the ocular tissues was high. The ocular tissue levels were almost the same as those achieved with the intravenous injection of CBPC at the same dose level.[5]

Results of the basic study here suggest that clinical efficacy of ASPC can be expected. The ocular penetration of ASPC was satisfactorily effective after the intravenous injection in cases of ocular infections caused by gram positive and gram negative cocci.

The clinical efficacy of ASPC was rated as excellent or good in all, except one case, of the internal infections of the eye (e. g., purulent iridocyclitis) and of the exterrnal infections of the eye (e. g., hordeolum, dacryocystitis and corneal ulcer) where both gram positive cocci (S. aureus, S. epidermidis, S.pneumoniae, and S.nonhemolyticus) and GNR and nonfermentative GNR where detected. As for dosages, in a usual case, administration of 1.0 or 2.0 g of ASPC once or twice daily by intravenous injection or drip infusion was considered to be appropriate, and in severe cases, 2.0 g twice a day.

Side effects worthy of mention were not seen, nor were there any abnormal test results in any of the following laboratory tests: blood (RBC, Ht, Hb, WBC, Plate, Eosino.), liver function (GOT, GPT, AL-p), and renal tests (BUN, S-Cr).

We can conclude that ASPC is a clinically useful synthetic penicillin in treating various types of ocular infections caused by either gram positive cocci or gram negative bacilli.

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