Ocular penetration of cephalixin in the rabbit

WALTER E. GAGER, FRED J. ELSAS, AND J. LAWTON SMITH

From the Department of Ophthalmology, University of Miami School of Medicine, Miami, Florida

The cephalosporin group of antibiotics has gained widespread use in combating resistant infections. Because of the necessity for intravenous administration of cephalothin, and the intramuscular route for cephaloridine, the development of a new member of this series which is effective orally prompted study of its penetration into the eye. This is the first reported study of the passage of cephalixin into the aqueous humour.

Material and methods

Cephalixin was provided in pure powdered form by the manufacturer through the courtesy of Dr. L. D. Bechtold. Preliminary experiments performed in the Medical Research Laboratory of the Eli Lilly Company had shown that in man nearly 100 per cent. of orally administered cephalixin is absorbed from the gastrointestinal tract within 60 to 90 minutes, and that 98 per cent. is excreted unconjugated in the urine after 6 hours. For the present study, doses of 125 mg. powdered cephalixin were dissolved in sterile distilled water and administered by gastric lavage to rabbits. Healthy adult rabbits were weighed, and only animals of the same weight range selected, so that the same dosage was given to all animals—50 mg./kg. as a single oral dose. Samples of blood and aqueous humour were then collected at intervals of from 1 to 8 hours, and were frozen within 20 minutes after being obtained. Non-toxic pink-topped Vacutainer tubes (NT3206) were used for collection and freezing of the blood samples. After topical 0.5 per cent. proparacaine hydrochloride anaesthesia, disposable sterile tuberculin syringes with No. 27 gauge needles were used for anterior chamber paracentesis. Anterior chamber taps were performed only on white and quiet (*i.e.* non-inflamed) eyes. No paracentesis was repeated on an eye within 48 hours of a previous tap. The specimens were packed in dry ice and shipped *via* air express to the Lilly Medical Research Laboratories for bioassay. Control sera and aqueous humour samples obtained from normal animals were sent with each shipment. The specimens forwarded to the laboratory were identified by code number only, so that those performing the bioassay determinations had no information concerning time, dose, or which were controls.

Results

This study consisted of seventy specimens (35 each of serum and matched aqueous humour) from rabbits after oral cephalixin, and eight specimens (4 each of serum and matched aqueous humour) from healthy control rabbits. A total of 78 specimens was thus studied—39 serum and 39 aqueous humour. The *Sarcina lutea* disc-plate technique of bioassay was employed at the Lilly Laboratory, and the results were reported in µg./ml. No activity was found in any of the eight control specimens. The other data are summarized in the (Table, overleaf) and shown graphically in (Fig. 1, overleaf).

Received for publication October 28, 1968

Address for reprints: Walter E. Gager, M.D., Department of Ophthalmology, Marquette School of Medicine, Milwaukee, Wisconsin 53226, U.S.A.

This work was supported in part by grants Ro1 CC 00411-01 and 5 Ro1 CC 00144-03, Communicable Disease Center, 5 Ro1 NB06308-03, National Institute of Neurological Diseases and Blindness, and by Research to Prevent Blindness

Table Passage of oral cephalixin across blood-aqueous barrier in the rabbit (dose 50 mg./kg.)

Time (hrs)	1	2	3	4	5	6	7	8
No. of rabbits tested	5	6	8	6	4	3	2	1
Serum level (µg./ml.)	0-28 16-14	5-1-12-5 6-24	1-1-11-2 6-50	0-31-20-6 5-16	0-76-5-3 2-0	0-57-1-25 0-89	0-0-57 0-28	0-0-57 3-6
Aqueous humour level (µg./ml.)	0-4-4 2-35	1-25-3-0 1-95	0-36-2-25 1-24	0-44-1-6 0-87	0-26-0-70 0-51	0-23-0-43 0-33	0-0-46 0-23	0-0-46 0-0
Aqueous/serum ratio (µg./ml.)	0-14	0-31	0-19	0-15	0-25	0-37	1-2	0-0

FIG. 1 Serum and aqueous humour levels of cephalixin in the rabbit after a single oral dose of 50 mg./kg.

Discussion

Cephalixin is the generic name for 7-(D- α -amino- α -phenyl) acetamido-3-methyl-3-cephem-4-carboxylic acid. It is a white crystalline compound of molecular weight 347-4. The structural formula is seen in Fig. 2. Cephalixin is a zwitter ion, *i.e.* it has both a basic and an acidic group in the molecule. Because of the presence of both groups, it exists essentially as an inner salt at physiological pH levels. Cephalixin is stable in both crystalline form and in solution. It is soluble in water to the extent of 13 mg./ml. at pH 4-3, the isoelectric point. Data from the Lilly Laboratory indicate that cephalixin is comparable to cephaloglycin in activity against *Staphylococcus aureus* and beta

FIG. 2 Structural formula of cephalixin

haemolytic streptococci, but less so than cephalothin or cephaloridine. It compares favourably with cephalothin against *E. coli*, *Salmonella*, and *Shigella*. Cephalixin is less potent against *Proteus mirabilis* than the other cephalosporins, but this organism would be considered to come within its spectrum. The enterococci, other species of *Proteus* and the *Pseudomonas* organisms are relatively resistant to all the cephalosporins.

The history of the cephalosporins is of interest. In July, 1945, Prof. Giuseppe Brotzu conducted a search for antibiotic-producing micro-organisms off the coast of Sardinia in the Mediterranean sea. A micro-organism known as *Cephalosporium acremonium* was found to produce an antibiotic substance in the broth where it was grown. Brotzu (1948) reported his results, and subsequently forwarded the organism to Oxford, England. Abraham, Newton, Schenck, Hargie, Olson, Schuurmans, Fisher, and Fusari (1955) reported the isolation of three different antibiotics from the culture media on which the cephalosporin was produced: a penicillin (cephalosporin N), a series of antibacterial steroids (cephalosporin P), and a new antibiotic (cephalosporin C). Chauvette, Flynn, Jackson, Lavagnino, Morin, Mueller, Pioch, Roeske, Ryan, Spencer, and van Heyningen (1962) began to attempt semi-synthetic improvement of the effectiveness and spectrum of cephalosporin C, and the series of semi-synthetic cephalosporins resulted. Cephalothin has been available commercially as Keflin for several years. Cephaloridine has more recently been released as Loridine. Cephaloglycin is now being studied in the United States; preliminary studies by Dr. Robert Sexton (personal communication) are said to have shown reasonable penetration of the aqueous humour by cephalothin, and it was therefore thought reasonable to study this aspect of cephalixin.

Review of the data presented reveals that in the rabbit after a single oral dose of cephalixin 50 mg./kg., there is a prompt rise in the serum level of the antibiotic, which is highest at one hour, when it averaged 16 µg./ml. The level fell during the second hour to approximately 6 µg./ml., and remained at about this level for the next 2 hours. After the 6th hour the serum levels fell sharply.

Similarly, cephalixin appeared promptly in the aqueous humour in this species, and maximum levels were found 1 to 2 hours after administration, with an average level of 2 µg./ml. This fell during the third hour, and was very low after the fourth hour. However, it is seen that cephalixin does enter the aqueous humour in the non-inflamed rabbit eye, and averages about 15–20 per cent. of the serum level. This is a relatively good passage of an antibiotic across the blood-aqueous barrier, and especially in an agent given by the oral route. No side-effects were observed in the rabbits studied.

It may be mentioned at this point that preliminary investigation of cephalixin in the owl monkey (*Aotus trivirgatus*) in this laboratory has shown an interesting species difference from the rabbit. In the owl monkey, there is little entry of cephalixin into the aqueous humour during the first hour, but it is seen after 2 hours and appears to be increasing between the third and fifth hours. This was seen although the serum levels had fallen notably between the fourth and fifth hours.

The results obtained in two patients given a single oral dose of cephalixin before ocular surgery might be mentioned. One patient was given 0.5 g. cephalixin by mouth and simultaneous samples of serum and aqueous humour were obtained 2½ hours later. The serum level was 8.20 µg./ml. and the aqueous humour level was 0.70 µg./ml. Another patient received a single 1.0 gram oral dose of cephalixin, and specimens obtained 3½ hours later revealed a serum level of 6.20 µg./ml. and an aqueous humour level of 0.78 µg./ml. Thus, in two patients with white and quiet eyes, about 12 per cent. of the serum level was found in the aqueous humour.

Because of the reported efficacy of cephaloridine in the treatment of patients with early syphilis (Glicksman, Short, and Knox, 1968), cephalixin was given orally to an owl monkey with a histologically proven late syphiloderm and produced prompt healing of a lesion which had been present in a chronic state for over 8 months before therapy. It is thought that further investigation of cephalixin for use in ophthalmology is indicated.

Summary

Cephalixin is a new semi-synthetic cephalosporin derivative which possesses the advantage over cephalothin and cephaloridine of being effective when taken by mouth. It is rapidly absorbed and appears promptly in the aqueous humour of the non-inflamed rabbit eye. Aqueous humour levels were obtained averaging 15–20 per cent. of the serum level. Cephalixin merits further investigation in ophthalmology.

Grateful acknowledgment is given to Dr. L. D. Bechtol, Medical Research Division, Eli Lilly and Company, for his help and co-operation with this project.

Dr. W. E. Gager performed this work under a Special Traineeship, National Institute of Neurological Diseases and Blindness.

References

- ABRAHAM, E. P., NEWTON, G. C. F., SCHENCK, J. R., HARGIE, M. P., OLSON, B. H., SCHUURMANS, D. M., FISHER, M. W., and FUSARI, S. A. (1955) *Nature (Lond.)*, **176**, 551
 BROTZU, G. (1948) *Lavori Inst. Igiene Univ. Cagliari*, 1948–54
 CHAUVETTE, R. R., FLYNN, E. H., JACKSON, B. G., LAVAGNINO, E. R., MORIN, R. B., MUELLER, R. A., PIOCH, R. P., ROESKE, R. W., RYAN, C. W., SPENCER, J. L., and VAN HEYNINGEN, E. (1962) *J. Amer. chem. Soc.*, **84**, 3401
 GLICKSMAN, J. M., SHORT, D. H., and KNOX, J. M. (1968) *Arch. intern. Med.*, **121**, 342