Intraocular Penetration of Topically Applied [^{14}C]Fosfonet Sodium in Rabbits

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Abstract □ The intraocular penetration of [^{14}C]fosfonet was studied following topical application of 25 mg of an ointment containing 5% [^{14}C]fosfonet sodium onto the intact and abraded eyes of New Zealand white rabbits. Radioactivity penetrated rapidly through the abraded cornea and entered the anterior chamber. The concentration of fosfonet in the aqueous humor peaked at 7.2 μg/ml by 90 min. Assuming an aqueous humor volume of 300 μl, this level would correspond to approximately 0.2% of the applied dose. The highest concentration of fosfonet found in the abraded cornea was 0.3 μg/mg, or 0.4% of the applied dose. The half-lives for the elimination of fosfonet from the aqueous humor and cornea were about 2.5 and 2.7 hr, respectively. The fosfonet levels in the iris were extremely low throughout the 6-hr period. The penetration of [^{14}C]fosfonet through the intact cornea was considerably less than that found in the abraded eye. The peak concentrations of fosfonet in the aqueous humor and cornea of the intact eye were 0.26 μg/ml and 0.02 μg/mg, respectively, and occurred within 10 min of application of the ointment.

Keyphrases □ [^{14}C]Fosfonet sodium—intraocular penetration □ Ocular agents—[^{14}C]fosfonet, aqueous humor, ophthalmic bioavailability, rabbit eye □ Antiherpetic agent—effect of [^{14}C]fosfonet on rabbit cornea

Shipkowitz *et al.* (1) first reported that fosfonet sodium (disodium phosphonoacetate) inhibited the replication of herpes viruses both in tissue cultures and in experimental animal models. Fosfonet was effective in reducing the severity of the corneal lesions in rabbits when applied topically in concentrations of 0.5 to 5% starting 2 hr after virus inoculation. Subsequent studies demonstrated that 5% fosfonet, either as a solution or an ointment, was as effective as 0.5% idoxuridine in the treatment of established herpetic keratitis in rabbits (2–4) and was superior to idoxuridine in inhibiting the replication of the virus (3). Furthermore, it was found (4) that fosfonet was also effective against idoxuridine-resistant keratitis when applied topically on rabbit eyes and against herpetic iritis when administered to rabbits either intravenously or subconjunctively but not topically.

Fosfonet is relatively nontoxic to rabbit eyes. It was reported (4) that 5% fosfonet was not toxic to the corneal epithelium of rabbits when applied 6 times a day for 5 days. Similarly, rabbit eyes were treated with a 5% fosfonet ointment 4 times a day for 42 days and no evidence of toxicity in clinical and histopathological studies was found (3). Although fine punctate lesions of the superficial corneal epithelium were observed (2) when a 5% fosfonet solution was applied to normal rabbit eyes 8 times a day for 3 days, the lesions disappeared within 48 hr after treatment was stopped and were no more severe than those encountered with idoxuridine.

Because of its potential usefulness in treating herpetic keratitis, this study was performed to investigate the intraocular penetration of [^{14}C]fosfonet after topical application of an ointment containing 5% fosfonet sodium onto the intact or abraded eyes of rabbits. Since removal of the corneal epithelium frequently enhances the intraocular penetration of drugs and the integrity of the corneal epithelium may be disrupted in ocular herpes infections, it was of special interest to study the intraocular penetration of fosfonet in eyes with corneal abrasions.

Table I—Aqueous Humor, Cornea, and Iris Concentrations following Topical Application of a 5% [^{14}C]Fosfonet Sodium Ointment (25 mg) onto Abraded Eyes of New Zealand White Rabbits

Time after Application	Aqueous Humor[a]			Cornea[a]		Iris[a]	
	%/ml	%	μg/ml	%	μg/mg	%	μg/mg
10 min	0.31 ± 0.05	0.09 ± 0.01	2.94 ± 0.47	0.40 ± 0.06	0.27 ± 0.04	<0.01 —	0.02 ± <0.01
20 min	0.40 ± 0.05	0.12 ± 0.02	3.76 ± 0.48	0.41 ± 0.05	0.27 ± 0.03	0.01 ± <0.01	0.02 ± <0.01
40 min	0.70 ± 0.08	0.21 ± 0.02	6.61 ± 0.79	0.41 ± 0.07	0.28 ± 0.04	0.01 ± <0.01	0.02 ± 0.01
1 hr	0.69 ± 0.10	0.21 ± 0.03	6.53 ± 0.91	0.30 ± 0.04	0.22 ± 0.02	<0.01 —	0.01 ± <0.01
1.5 hr	0.76 ± 0.10	0.23 ± 0.03	7.23 ± 0.91	0.34 ± 0.08	0.23 ± 0.05	0.01 ± <0.01	0.02 ± <0.01
2 hr	0.67 ± 0.08	0.20 ± 0.02	6.36 ± 0.79	0.28 ± 0.03	0.18 ± 0.02	<0.01 —	0.01 ± <0.01
3 hr	0.59 ± 0.09	0.18 ± 0.03	5.61 ± 0.87	0.21 ± 0.03	0.13 ±0.02	<0.01 —	0.01 ± <0.01
4 hr	0.42 ± 0.09	0.12 ± 0.03	3.97 ± 0.83	0.17 ± 0.02	0.10 ±0.01	<0.01 —	0.01 ± <0.01
6 hr	0.21 ± 0.03	0.06 ± 0.01	2.01 ± 0.31	0.12 ± 0.01	0.07 ± 0.01	<0.01 —	<0.01 —

[a] Mean ± standard error of 10–12 determinations, based on total radioactivity and expressed as micrograms of fosfonet, assuming a 25-mg dose of the ointment corresponding to 1250 μg of fosfonet sodium or 950 μg of fosfonet. Concentrations expressed per milliliter aqueous humor or milligram dry weight of cornea or iris. The percentage in aqueous humor was calculated from the %/ml, assuming a total aqueous humor volume of 0.30 ml.

EXPERIMENTAL

Materials—Fosfonet sodium was labeled with carbon 14 in the carboxylic position. Radiochemical and chemical purity was established by thin layer and anion exchange chromatography and mass spectrometry. A lanolin–petrolatum based ointment[1] containing 5% [^{14}C]fosfonet sodium was prepared and had a final activity of 0.16 μCi/mg ointment.

Animals—Male New Zealand white rabbits, weighing 2.3–4.0 kg, were used. Prior to use, the animals were maintained in standard laboratory animal cages and allowed food and water *ad libitum*. During the study, the rabbits were placed in restraining cages which held them in a normal upright position. All animals were acclimated to these conditions by being placed in the restrainers for successively longer periods of time twice a day for 3 days.

Corneal abrasions were made by anesthetizing the eye with 0.5% tetracaine hydrochloride[2] and then gently scraping the cornea with a scalpel blade to remove the epithelial surface. Fluorescein staining was performed in preliminary studies to assess the abrading technique. Extreme care was exercised to avoid damaging the corneal stroma or the conjunctiva, and any animal that had visible signs of damage (*e.g.*, bleeding) was not used in the study. About 1.5–2 hr elapsed between abrading and dosing the eye.

While holding the eyelids open and after the nictitating membrane had receded, the ointment (~25 mg) was applied onto the corneal surface and into the pocket formed by the lower lid and the globe. The eyelids were

[1] Composition of the ointment in % by weight: Solution containing 50% (w/v) fosfonet disodium monohydrate, 15.3%; anhydrous lanolin, 22.6%; 17% benzalkonium chloride, 0.01%; white petrolatum, 62.1%.

[2] Abbott Laboratories, North Chicago, Ill.

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Table II—Aqueous Humor and Cornea Concentrations following Topical Application of a 5% [^{14}C]Fosfonet Sodium Ointment (25 mg) onto Intact Eyes of New Zealand White Rabbits

Time After Application	Aqueous Humor[a]			Cornea[a]	
	%/ml	%	μg/ml	%	μg/mg
10 min	0.03 ± 0.01	0.008 ± 0.002	0.26 ± 0.08	0.04 ± 0.01	0.02 ± <0.01
20 min	0.01 ± <0.01	0.002 ± <0.001	0.06 ± 0.01	0.02 ± <0.01	0.01 ± <0.01
40 min	0.01 ± <0.01	0.004 ± 0.001	0.12 ± 0.03	0.03 ± <0.01	0.01 ± <0.01
1 hr	0.01 ± <0.01	0.003 ± <0.001	0.08 ± 0.01	0.02 ± <0.01	0.01 ± <0.01
1.5 hr	0.02 ± 0.01	0.006 ± 0.002	0.20 ± 0.07	0.02 ± 0.01	0.01 ± <0.01
2 hr	0.02 ± 0.01	0.005 ± 0.003	0.16 ± 0.10	0.01 ± <0.01	0.01 ± <0.01
3 hr	0.02 ± <0.01	0.005 ± <0.001	0.16 ± 0.02	0.02 ± <0.01	0.01 ± <0.01
4 hr	0.02 ± 0.01	0.005 ± 0.002	0.15 ± 0.05	0.01 ± <0.01	0.01 ± <0.01
6 hr	0.02 ± 0.01	0.005 ± 0.002	0.16 ± 0.05	0.01 ± <0.01	<0.01 —

[a] Mean ± standard error of six determinations, based on total radioactivity and expressed as micrograms of fosfonet, assuming a 25-mg dose of the ointment corresponding to 1250 μg of fosfonet sodium or 950 μg of fosfonet. Concentrations expressed per milliliter aqueous humor or milligram dry weight of cornea. The percentage in aqueous humor was calculated from the %/ml, assuming a total aqueous humor volume of 0.30 ml.

then released and gently manipulated to spread the ointment over the cornea. Generally both eyes of each rabbit were used, but the dosing schedule was adjusted so that the eyes represented two different, but not successive, time points. At selected times after dosing, the rabbits were sacrificed by injecting an overdose of a solution[3] containing sodium pentobarbital, sodium secobarbital, and mephenesin into the marginal ear vein. The cornea and sclera were thoroughly rinsed with physiological saline[4] and gently wiped dry with a tissue. An aqueous humor sample (0.1–0.2 ml) was withdrawn from the anterior chamber. The optic nerve and blood vessels were then clamped with a hemostat, and the corneal surface was again rinsed with saline. The cornea and iris were removed by cutting around the corneal-scleral limbus, and the iris was separated from the cornea. Both tissues were thoroughly rinsed in saline, allowed to dry for several days at room temperature, and weighed.

In some animals, ointment was applied to both eyes at the same time, and blood samples were withdrawn from the central artery of an ear at selected times after dosing. The heparinized blood samples were centrifuged and the plasma was saved for radioassay.

Radioassay—An aliquot of the aqueous humor samples was applied to an absorbant paper disc[5] encased in a cellophane combustion envelope[6]. The tissue and plasma samples were placed in combustion cones[7] containing cellulose powder[8]. All samples were then burned in a sample oxidizer[9], and radioassay was accomplished by liquid scintillation spectrometry[10]. Correction for quenching was made by automatic external standardization. All levels were based on total radioactivity and were expressed as micrograms of fosfonet. To correct for the small and statistically insignificant differences in the milligrams of ointment applied, the results were expressed as adjusted concentrations based on a 25.0-mg dose.

RESULTS

The drug concentrations in the aqueous humor, cornea, and iris at various times after topical application of the [^{14}C]fosfonet sodium ointment onto the abraded eyes of New Zealand white rabbits are presented in Table I. The radioactivity appeared to penetrate rapidly through the abraded cornea and enter the aqueous humor. The concentration of fosfonet in the aqueous humor ~2.9 μg/ml by 10 min, reached 6.6 μg/ml by 40 min, and peaked at 7.2 μg/ml by 90 min. Thereafter, the levels in the aqueous humor declined slowly, with a half-life of ~2.5 hr, as determined by linear regression analysis. The peak concentration of fosfonet in the abraded cornea was ~0.3 μg/mg dry weight and remained relatively constant through at least 40 min. The half-life for the disappearance of radioactivity from the cornea was ~2.7 hr. The levels of fosfonet in the iris were very low throughout the study, never exceeding 0.02 μg/mg.

The penetration of fosfonet through the intact cornea (Table II) was considerably less than that found in the abraded eyes. The peak levels in both the aqueous humor and cornea of the intact eyes were reached by 10 min but were only 0.26 μg/ml and 0.02 μg/mg, respectively. The concentrations in the iris were consistently less than 0.01 μg/mg.

The systemic absorption of fosfonet appeared to be quite limited. In rabbits with an intact corneal epithelium, the plasma concentrations at times ranging from 10 min to 24 hr never reached 0.01 μg/ml, while the levels in the rabbits with abraded corneas averaged 0.01–0.02 μg/ml.

DISCUSSION

The results of this study were based on total radioactivity but were expressed as micrograms of fosfonet. Since previous studies[11] in these laboratories had indicated that intravenously administered [^{14}C]fosfonet was excreted unchanged in the urine of rabbits, it is reasonable to assume that most of the radioactivity did represent the parent drug. It was also assumed that the radioactivity found in each eye came from transcorneal penetration and not from the systemic circulation. The low plasma levels of radioactivity suggested that this assumption was also valid. The plasma levels appeared to be slightly higher in the rabbits with abraded corneas than those with an intact corneal epithelium, possibly due to the greater penetration of fosfonet into the abraded eye. However, it was more striking that the drug levels in the systemic circulation were exceedingly low.

Generally, less than 1% of the instilled dose of most drugs crosses the cornea and enters the anterior chamber (5). For example, it was reported (6) that ~0.1–0.2% of an instilled dose of a tritiated pilocarpine nitrate solution was found in the aqueous humor of intact rabbit eyes. If the total volume of aqueous humor in a rabbit eye is assumed to be 300 μl (7, 8), then ~0.23% of the applied fosfonet dose would be present in the aqueous humor of the abraded eyes at the time of the peak concentration (1.5 hr). In contrast, less than 0.01% of the dose would have penetrated through the intact cornea and entered the aqueous humor. Comparison of the areas under the aqueous humor concentration–time curves indicated that the amount of drug penetrating through the intact cornea was only about 3% of that found in the aqueous humor of the abraded eyes. Removal of the corneal epithelium enhances the rate and degree of penetration, especially with water soluble or polar compounds (9). Therefore, the marked difference in the penetration of fosfonet through the intact and abraded corneas is not surprising. However, it should be noted that the integrity of the corneal epithelium is one factor that might markedly affect the intraocular penetration and possibly the therapeutic efficacy of fosfonet.

Most drugs achieve peak concentrations in the cornea and aqueous humor in a short time, thus appearing to penetrate rapidly into the ocular tissues. However, recent studies with pilocarpine nitrate have shown that the short time required to reach the peak concentrations is primarily dependent on the rapid parallel elimination of the drug from the precorneal area and that the actual absorption rates for the cornea and aqueous humor are lower than expected from the concentration–time profiles (10–13). With fosfonet, the peak concentrations in both the intact and abraded corneas were reached within 10 min, the shortest time interval studied. The peak concentration in the aqueous humor of the intact eye was also reached within 10 min, but there was considerable fluctuation in the aqueous humor levels during the first two hr. In contrast, the aqueous humor concentrations in the abraded eye peaked considerably later (1.5 hr). The reasons for the difference in the peak concentration times are not known. More work is required to determine fosfonet pharmacokinetics in intact and abraded eyes and to assess other factors that might influence the time needed to reach peak concentrations.

It was found (4) that fosfonet was effective in treating experimental herpetic iritis in rabbits when administered systemically or subconjunctively but not topically. The low fosfonet levels in the iris were consistent with the lack of beneficial effects of topically applied fosfonet in herpetic iritis in rabbits. However, stromal disease and iritis are difficult to treat effectively with the topical application of most other antiviral agents as well (14).

[3] Repose; Diamond Laboratories, Des Moines, Ia.
[4] Abbott Laboratories, North Chicago, Ill.
[5] Schleicher and Schuell, Keene N.H.
[6] Ivers-Lee Co., West Caldwell, N.J.
[7] Packard Instrument Co., Downers Grove, Ill.
[8] Whatman, Inc., Clifton, N.J.
[9] Model 306 Sample Oxidizer, Packard Instrument Co., Downers Grove, Ill.
[10] Model 3380 Liquid Scintillation Spectrometer, Packard Instrument Co., Downers, Grove, Ill.

[11] Dr. G. Ikeda, Abbott Laboratories, North Chicago, Ill. (unpublished results).

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COMMUNICATIONS

Unusual Cholesterol Solubility in Water/Glyceryl-1-monooctanoate Solutions

Keyphrases □ Cholesterol—unusual solubility in water/glyceryl-1-monooctanoate solutions □ Glyceryl-1-monooctanoate—aqueous solutions, unusual solubilities of cholesterol □ Gallstones—cholesterol, solubility in water/glyceryl-1-monooctanoate solutions

To the Editor:

Glyceryl-1-monooctanoate (monooctanoin) (I) has been recently used in humans for dissolution of cholesterol gallstones in the common bile duct (1, 2). The solvent is slowly infused into the bile duct for several days, usually *via* a T-tube left in place following cholecystectomy. The high cholesterol solubility in I, 11.7% (w/v) at 37°, was reported in a systematic study of cholesterol solubility in organic solvents by Flynn *et al.* (3). Optimum cholesterol solubility appeared to occur when the solvent (*n*-alkanols or fatty acid ethyl esters) had a total carbon chain length of about seven atoms.

The present study was initiated to determine if cholesterol was involved in formation of liquid crystalline phases in aqueous I solutions. Larsson found that highly purified I and water formed a lamellar liquid crystalline phase at 37° when the water content was between 8 and 45% (4). Such equilibria could be important in gallstone dissolution since I would be in contact with bile during the infusion procedure and with moisture during handling. But when water/I mixtures were prepared with the same type of I used in the reported gallstone dissolution studies[1], only isotropic phases were observed by polarizing microscopy[2]. This apparent discrepancy is thought to be caused by the presence of about 30% of the corresponding diglyceride in the commercial material (1, 5). Diglycerides or triglycerides are more hydrophobic and do not form lyotropic mesophases (4). The solubility of water in the commercial sample of I was determined visually to be ~18–20% (w/w) at 37°. An exact value is not meaningful, since each batch will vary somewhat in its fatty acid distribution and diglyceride content. Above this concentration simple emulsions were formed rather than liquid crystalline phases.

Cholesterol is known to crystallize in anhydrous and monohydrate forms (6) and the anhydrous form is ~50% more soluble in aqueous bile salt solutions (7). Since either of these crystalline forms could possibly exist in aqueous I solutions, the cholesterol solubility in such solvent mixtures was determined (Fig. 1). Suspensions of anhydrous cholesterol[3] or cholesterol monohydrate (recrystallized from aqueous ethanol) were prepared in aqueous I solutions and equilibrated using a vibratory mixer[4] in a constant-temperature bath. The suspensions at equilibrium were observed with the polarizing microscope and quickly filtered through 0.45 μm membranes[5] which had been equilibrated at the test temperature. The two crystal forms were microscopically identified by their characteristic habits (6). The filtrates were analyzed for cholesterol by HPLC (8) with detection at 205 nm and for water content by Karl Fischer titrimetry[6].

Cholesterol solubility increased to a maximum and then decreased over the range of water concentration studied. The solubility was independent of the sampling time and crystal form initially present, indicating that equilibrium had been attained. An explanation cannot be offered for the higher solubility found in the present investigation compared to previous reports (3).

Immediate microscopic inspection of the suspensions showed that at water concentrations below the apparent

[1] Capmul 8210, Capitol City Products, Columbus, Ohio.
[2] Zetopan, Reichert, Vienna, Austria.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Vibromixer E1, Chemapec, Woodbury, N.Y.
[5] Millipore, Bedford, Mass.
[6] Auto-aquatrator, Precision Scientific, Chicago, Ill.

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