Ocular Uptake and Kinetics of Orally Administered Isosorbide in Rabbits (36538)

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Isosorbide (Hydronol) (1,4:3,6-dianhydro-D-glucitol) is a dihydric alcohol formed by removal of two molecules of water from sorbitol. It has been tested extensively in animals and man as an orally administered osmotic agent, and has been found to decrease intraocular pressure (1–4).

Unlike mannitol, which is restricted to extracellular water, isosorbide in man equilibrates into a mean dilution space which approximates that of total body water (5). Isosorbide enters the aqueous humor, and, in man, aqueous concentrations reached 0.72 of that in plasma within 3 hr after a single dose (3). In rabbits, the concentration ratio between the aqueous and plasma reached unity in 5 hr.

In this study, we attempted to determine whether or not isosorbide was accumulated in the aqueous humor and other parts of the eye, particularly after repeated daily dosing. Further, the study evaluated the kinetics of isosorbide uptake into and efflux from the various ocular compartments.

Experimental. Animal dosing and sample collection. Male albino rabbits, 2.4–3.9 kg, were fasted overnight with water *ad libitum*. They were given isosorbide, 2 g/kg, in a 50% solution by oral intubation, either as a single dose or consecutive daily doses. This dose has been shown to lower intraocular pressure significantly in rabbits (3). At various times after one or the last of several doses, animals were anesthetized with pentobarbital sodium, 30 mg/kg, by iv infusion in the marginal ear vein. Aqueous humor was aspirated from the anterior chamber of both eyes, a heparinized blood sample was drawn by cardiac puncture, and one or both eyes were quickly excised and dissected. Animals were sacrificed by an injection of air. The cornea, iris, and lens were rinsed with distilled water, blotted, weighed, and along with aqueous and vitreous humors were refrigerated until analysis. Blood samples were centrifuged at 2000 rpm, and the plasma was retained for analysis.

Single dose studies. Rabbits were divided into three groups of six each. Animals were given isosorbide, 2 g/kg, by stomach tube and sacrificed at 1, 2, 4, 8, 12, or 24 hr following the dose. Aqueous humor was taken and both eyes were excised for analysis prior to sacrifice.

Multiple-dose studies. Rabbits were divided into four groups of three each. One group received three consecutive daily doses of isosorbide, 2 g/kg, by stomach tube and was sacrificed 24 hr after the last dose. The other three groups received four consecutive daily doses and were sacrificed 4, 8, or 24 hr after the last dose. All animals were fasted overnight prior to receiving the last dose. Only the left eyes were excised for analysis, although aqueous humor was aspirated from both eyes. Other groups of rabbits received either six or seven daily doses and were handled in the same manner as the three- or four-dose groups.

Damaged eye studies. Groups of rabbits had 0.1 ml of a detergent, "20 Volume Cream Peroxide," instilled in their left eyes. Right eyes served as controls. This detergent produced corneal opacity and other severe signs of eye inflammation within 24 to 48 hr which persist for more than 1 week. Eyes were graded for both severity and type of pathologic changes at 48 hr after detergent and 24 hr prior to isosorbide dosing; and only rabbits with severe corneal damage were used based on the grading system from Kay and Calandra (6). Six days after instillation of the detergent, isosorbide was given and the

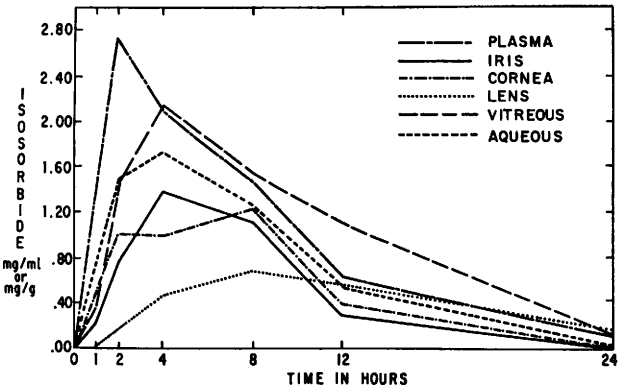

FIG. 1. Plasma and ocular levels of isosorbide in a rabbit after a single oral dose of 2 g/kg. This is a typical experiment with the average values from both eyes plotted per time point.

single-dose experiments were repeated as described. (These studies also included 3- and 18-hr time points.) Some of the eyes were fixed and examined grossly and microscopically for pathology.

Sample preparation and analysis for isosorbide. Corneas were cut into small pieces and incubated with 1.0 ml phosphate buffer, 0.1 *M* (pH 7.4) and 0.3 ml crude collagenase solution (Worthington Biochemical Corp.), 10 mg/ml, on a Dubnoff metabolic shaker at 37° for 18 hr. All samples were diluted with water and homogenized in a Potter-Elvehjem tube using a plastic pestle. An internal standard (isoidide) was added to an aliquot of the homogenate or fluid. Three times the volume of absolute ethanol was added to the aliquot to precipitate protein. After centrifugation for 15 min at 2000 rpm, an aliquot (0.2–1.0 μl) of the supernatant was injected into an F&M model 700 gas chromatograph with a flame ionization detector. The column was 3 ft, 1/4 in. copper packed with 5% Carbowax-20M on Anakrom A 90/100 mesh (Hewlett-Packard). The gases used were helium, 80 ml/min; hydrogen, 75 ml/min; and air at a ratio of 6 to 10 times greater than hydrogen. Temperatures were 285° in the injection port, 210° in the column, and 240° at the detector. Isosorbide concentrations were determined by the relative peak height method.

Determination of kinetic values. Times of peak isosorbide levels in plasma and the components of the eyes were estimated by fitting two intersecting straight lines of log plasma concentration on time by least squares procedures to all data points simultaneously to achieve minimal residual variance. Similarly, the excretion half-life ($T_{1/2}$) was estimated from the linear regression fitted by least squares across all data points for both single and multiple dose experiments from 8 to 24 hr.

Results. Single-dose studies. The shapes of the plots of isosorbide concentration on time after dose were similar for all tissues, particularly in the downward slopes, with the exception of the lens. A typical experiment is plotted in Fig. 1. Plots of log tissue concentrations exhibited the same similarities. Highest drug levels were attained in the plasma and the more aqueous components of the eye. Plasma and aqueous humor levels compared favorably with those reported by Becker, Kolker and Krupin (3) for rabbits although they measured isosorbide levels only through 5 hr. In this study, average drug levels in plasma and ocular tissues had declined to less than 0.20 mg/ml or mg/g at 24 hr (Fig. 1).

Multiple-dose studies. The three- and four-dose experiments were considered jointly with the six- and seven-dose studies since the results of both were very similar. Figure 2 shows the efflux curves for the three and four-dose animals based on the average values for three rabbits. Isosorbide levels and efflux curves of the plasma and different components of the eye were comparable to

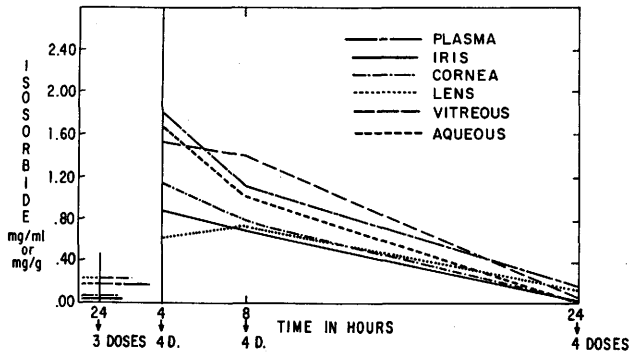

FIG. 2. Plasma and ocular levels of isosorbide in rabbits after 3 or 4 daily oral doses of 2 g/kg. The plotted values are means from 3 animals/time point.

the single-dose experiments (Fig. 1). Further, drug levels 24 hr after the last of four or seven doses were not different from those after three or six doses, and all were essentially identical to levels obtained 24 hr after a single dose, *i.e.*, < 0.20 mg/ml or mg/g.

Damaged eye studies. Peak levels of isosorbide and the shapes of the concentration plots on time for control and damaged eyes were quite similar, with the exception of the lens, where there appeared to be a more rapid uptake in the damaged eyes. Histopathologic examination showed the presence of keratitis, corneal edema, and hypopyon. Thus, corneas from damaged eyes had an average net weight more than double that in control eyes; 148 mg vs 71 mg. There were no weight differences between the other parts of the control and treated eyes.

Pharmacokinetic analysis of isosorbide uptake and excretion in rabbit eyes. Since there were no differences in normal eyes between frequency of dosing groups, the data for plasma and each eye component were combined for calculation of kinetic values. Table I shows that peak isosorbide levels in aqueous, vitreous, iris, and cornea were attained only slightly later than in plasma, indicating rapid equilibration with plasma. However, the time to peak concentration in the lens was two- to threefold greater. The excretion half-life ($T_{1/2}$) for isosorbide in plasma was significantly greater than for the ocular tissues except lens (slopes $p < .01$).

Although detergent-treated eyes showed definite pathologic changes, there were minimal differences between them and the control eyes in the times of peak drug levels except in the lens where the mean time was reduced from 3.5 hr in control eyes to 2.3 hr in damaged eyes (Table II). Concurrently, the $T_{1/2}$ for lens was significantly shortened from 5.4 to 3.7 hr in damaged eyes. Thus, with the notable exception of the lens, this type of eye damage did not alter the kinetics of isosorbide.

Discussion. In terms of drug distribution, accumulation of a drug at a target site may be beneficial, however, accumulation of isosorbide in the eye probably would not be desirable. In treatment of increased intraocular pressure, isosorbide may be given repeatedly at high doses, and, in view of its

TABLE I. Pharmacokinetic Values for Isosorbide in Plasma and Eyes After Oral Doses to Rabbits.[a]

Tissue	Peak drug level (hr)	Excretion half-life (hr)
Plasma	1.05 (55)[b]	5.0 (4.1–6.7)[c] (30)[d]
Aqueous	1.3 (57)	2.7[e] (2.3–3.2) (21)
Vitreous	1.5 (57)	3.7[e] (3.0–4.6) (29)
Cornea	1.3 (54)	3.1[e] (2.8–3.6) (29)
Iris	1.3 (60)	3.6[e] (3.1–4.2) (27)
Lens	3.5 (60)	5.4 (4.2–7.7) (29)

[a] Single and multiple dosing combined.

[b] Numbers in parentheses represent all the data points over the time span of observation.

[c] Range—based on the 95% confidence limits of the slope fitted for the 8- to 24-hr values.

[d] Numbers in parentheses represent all data points at times of 8 hr or longer.

[e] $p < .01$ when compared with plasma half-life.

TABLE II. Pharmacokinetic Values for Isosorbide in Normal and Damaged Eyes of Rabbits.[a]

Tissue	Peak drug level (hr)		Excretion half-life (hr)	
	Normal	Damaged	Normal	Damaged
Aqueous	1.3 (57)[b]	1.1 (26)	2.7 (2.3–3.2)[c] (21)[d]	2.8 (2.3– 3.9) (12)
Vitreous	1.5 (57)	1.3 (27)	3.7 (3.0–4.6) (29)	3.6 (2.7– 5.4) (13)
Cornea	1.3 (54)	1.2 (27)	3.1 (2.8–3.6) (29)	2.9 (2.3– 4.0) (13)
Iris	1.3 (60)	1.05 (21)	3.6 (3.1–4.2) (27)	4.5 (2.7–13.1) (12)
Lens	3.5 (60)	2.3 (26)	5.4[e] (4.2–7.7) (29)	3.7 (2.6– 6.1) (12)

[a] Values for normal eyes are from single and multiple dose groups combined.
[b] Numbers in parentheses represent all the data points over the time span of observation.
[c] Range—based on the 95% confidence limits of the slope fitted for the 8- to 24-hr values.
[d] Numbers in parentheses represent all data points at times of 8 hr or longer.
[e] Normal vs damaged ($p < .01$).

ability to cross cell membranes, possible accumulation in the eye had to be investigated.

The studies described in this paper indicate that isosorbide levels do not accumulate in rabbit eyes, even after seven consecutive daily doses of 2 g/kg. In view of the estimated excretion half-lives of less than 6 hr for all tissues, accumulation upon daily dosing is not to be expected. Furthermore, in damaged eyes, the times to peak level and $T_{1/2}$ were not different from undamaged eyes except for the lens where these observations were shorter. The lower kinetic values for the lens in damaged eyes possibly could be accounted for by increased vascularity and/or permeability of the adjacent eye compartments such as the cornea. Thus, to maintain effective ocular drug concentrations for therapeutic effects in humans, it is likely that a more frequent dosage interval than 24 hr would be necessary. Further, though it is often difficult to extrapolate metabolic fate data from lower animals to man, plasma kinetic constants for isosorbide in the rabbit are relationally similar to those reported by Modi, Rhoades, and Nodine (5) for man; *i.e.*, time to peak level—1.05 hr in rabbit vs 1.46 hr in man; estimated $T_{1/2}$—5.0 hr in rabbits vs 7.13 hr in man.

Finally, of the various components of the eye, only the lens appears to differ in its kinetics. The lower and later peak levels of isosorbide in the lens may be due to the absence of vascularity, and the lens is dependent on the transfer of solutes from the aqueous and vitreous humor for its nutrition (7). Thus, the time lag in reaching a peak level could be due to the delay in diffusion from aqueous or vitreous humors to the lens with slow equilibration also occurring because of the density and size of the structure. Efflux back into the plasma could be delayed until drug levels had decreased in the aqueous and vitreous humors. Most important, though, the drug concentration in lens does decline to the same low concentration as in the plasma and other components of the eye by 24 hr postdose, with the pattern of efflux the same after single and multiple doses.

Summary. Isosorbide is an oral osmotic agent which lowers intraocular pressure in rabbits and man. To determine its possible accumulation in the eye after repeated administration, male rabbits were orally dosed with isosorbide, 2 g/kg, daily for from 1 to 7 days. Plasma and ocular components were removed from 1 to 24 hr after the last dose. After single or multiple doses of isosorbide, times to peak level and excretion half-lives ($T_{1/2}$) were similar for all tissues except the lens. Times to peak drug levels were: plasma, 1.1 hr; aqueous, vitreous, iris, and cornea, 1.3–1.5 hr; lens, 3.5 hr. Plasma $T_{1/2}$ (5.0 hr) was not different from that for lens (5.4 hr) but was significantly longer than $T_{1/2}$ for other ocular tissues. Further, average drug levels in plasma and all ocular tissues declined to the same low levels (< 0.20 mg/ml or mg/g) 24 hr after either a single dose or the last of several doses; thus, indicating no accumulation with 24-hr dose intervals. There were no differences in drug kinetics

between normal eyes and eyes damaged with detergent treatment except that lens $T_{1/2}$ was shorter and isosorbide reached a peak level sooner in lenses from damaged eyes.

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