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Report

Systemic Delivery of Timolol After Dermal Application: Transdermal Flux and Skin Irritation Potential in the Rat and Dog

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Timolol, a beta-adrenergic antagonist, was evaluated for transdermal flux with rat skin *in vitro* and with the dog *in vivo*. Skin irritation after dermal application of timolol was assessed in the rat *in vivo*. Drug flux across rat skin *in vitro* ranged between 2 and 110 $\mu g\ cm^{-2}\ hr^{-1}$, dependent on the formulation. The transdermal flux of timolol in the dog was greater than 10 $\mu g\ cm^{-2}\ hr^{-1}$. This estimate was based on the degree of antagonism of isoproterenol challenge following transdermal administration of timolol relative to that obtained following intravenous administration of timolol. Irritation was observed in the rat after occluded dermal application of timolol free base but was not observed when the concentration of the drug in the formulation was decreased.

KEY WORDS: drug delivery, transdermal; skin permeation, rats, dogs; timolol, skin permeation; timolol, skin irritation.

INTRODUCTION

The successful development of dermally applied systems for systemic delivery of scopalamine and nitroglycerine has intensified interest in transdermal delivery of drugs. This mode of application for chronic, continuous therapy has certain obvious advantages in terms of controlled dosing, patient convenience, and safety. However, even with renewed interest, there are today only a handful of ethical transdermal products. The reasons for the product scarcity are simple. There are relatively few drugs that will penetrate the skin in therapeutic amounts when applied over a small surface area. Among those drugs that do penetrate, prolonged application often will produce an irritation response at the site of application.

Current research efforts have concentrated in large part on drugs of value in cardiovascular therapy, such as clonidine (1–3) and propranolol (4). Among these, the beta-adrenergic antagonists are considered to be of prime interest. It was anticipated that timolol, in its free-base form, may have sufficiently high skin permeability that it could be administered via the transdermal route, based on its physical–chemical properties. Timolol (mp, 61°C) has a partition coefficient of 1.9 (pH 7.3 buffer/*n*-octanol) and a pK_a of 9 and is soluble in both polar (water, 8 mg/ml at room temperature) and nonpolar solvents (hexane, 12 mg/ml at room temperature). Its solubility in triglycerides ranges from 10 to 20% by weight. The present report details some of the initial animal experiments that were designed to assess the feasibility of transdermal delivery of timolol. Several formulations of timolol were employed in these studies to investigate the effects of the amount of drug applied, pH, and concentration on transdermal permeation or irritation. The main aspects of this report describe the results from an *in vitro* transdermal flux model, an *in vivo* skin irritation model, and an *in vivo* model to measure beta blockade after transdermal application of timolol.

MATERIALS AND METHODS

In Vitro Diffusion Cell Model

Fuzzy rats, 8 weeks old and weighing an average of 250 g, were used to study *in vitro* dermal permeation (5) of timolol. The animals were obtained from Animal Services, Skin and Cancer Hospital, Temple University, 3222 Broad Street, Philadelphia, PA 19140. The diffusion cells consisted of a Plexiglas receptor chamber equipped with a sidearm to allow receptor phase sampling, a Teflon lid, and a Teflon-coated stirring bar (Kersco Engineering Consultants, Palo Alto, CA 94305). The receptor fluid was 45 ml of buffer solution consisting of 0.15 *M* NaCl, 0.47 m*M* NaH_2PO_4, 0.8 m*M* Na_2HPO_4, and 22 ppm gentamicin sulfate, adjusted to pH 7.0 with 0.067 *M* NaH_2PO_4 and/or 0.067 *M* Na_2HPO_4. Rats were sacrificed by injection of a euthanasia solution and the whole ventral and dorsal skin was removed. A section of skin was stretched over the lower opening of the Teflon lid and secured with a rubber gasket. The lid was placed firmly on the lower chamber of the diffusion cell and held in place with retaining bolts. The opening in the lid left

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an 8-cm^2 area exposed on the epidermal side through which permeation occurred.

Several formulations of timolol were investigated in these *in vitro* studies in order to investigate the effect of pH and the amount of timolol base applied. Timolol maleate (Merck Lot 91481) was used as received. Timolol free base was prepared as a neat liquid phase by neutralization of an aqueous solution of timolol maleate with sodium carbonate and extraction of timolol base with diethyl ether, followed by removal of the ether under vacuum. This preparation was metastable in that it slowly crystallized after several weeks. Crystalline timolol free base (Merck Lot L-714,503-R14) was also obtained and used as received. The compositions of the specific preparations are as follows: (a) timolol base, neat (metastable) liquid phase; (b) crystalline timolol base solution in ethanol (57%); (c) crystalline timolol base solution in ethanol (10%); (d) crystalline timolol base solution in ethanol (1%); (e) timolol maleate (3.3%) in pH 5.0 citrate-buffered solution; (f) timolol maleate (3.3%) in pH 6.0 pyrophosphate-buffered solution; (g) timolol maleate (3.3%) in pH 7.0 phosphate-buffered solution, (h) timolol maleate (3.3%) in pH 8.0 pyrophosphate-buffered solution; and (i) timolol maleate (3.3%) in pH 8.8 pyrophosphate-buffered solution. Neat liquid (metastable) timolol base and alcoholic timolol base solution formulations were applied by droppers and were spread evenly over the skin surface with smooth glass rods. The amount of formulation applied to the skin was determined by volume or weight measurement. One-milliliter volumes of the buffered solutions were applied to the skin. The cells were occluded and placed in a 32°C incubator and stirred at 150 rpm. Periodically 0.10-ml, 1.0-ml, or 2.0-ml samples were taken over a 38-hr period and an equal volume of fresh buffer solution was added to the cell. The samples were stored frozen until assayed at the end of the experiment.

The concentration of timolol base was measured in each sample by a reverse-phase HPLC technique. A 20-μl injection of each sample and a timolol standard of similar concentration were made on the HPLC, which was equipped with a 10-cm Spheri-5 RP-18 column (Brownlee Labs) and a similar 4-cm guard column and uv detector set at 294 nm. The mobile phase was 9 m*M* H_3PO_4/5.5 m*M* tetramethylammonium hydroxide/32.5% CH_3CN or, alternatively, 50–60% CH_3CN containing 0.1% acetic acid and 0.1% sodium lauryl sulfate. The flow rate was 2.0 ml/min. The area of the timolol peak (at 2 to 4 min) was measured by an electronic integrator. The concentration of timolol base was calculated from the areas of the sample and standard peaks and the concentration of the standards using external standard procedure. The quantity of timolol base that had permeated across each fuzzy rat skin section was calculated from the sample concentration and diffusion cell volume, correcting for the amount removed from the cell.

Skin Irritation Study

Fuzzy rats (Temple University), 150 to 200 g, were used to study the dermal irritancy of timolol base in suspensions or solutions. Test preparations (50 mg) were applied to circular gauze pads, 1 mm thick and 16 mm in diameter, and affixed to the animals' dorsal surface with occlusive adhesive film (Adhesive Plaster for Patch Test, Kanebo, Ltd., Osaka, Japan). The occlusive dressings were removed after 3 or 7 days. Treated skin areas were then evaluated according to a modified Draize scoring method, and the irritation index was evaluated for each test site. The first or "primary irritation index" (PII) was an average value reflecting irritation both immediately after dressing removal and 72 hr later. The "secondary irritation index" (SII) was determined 7 days after dressing removal. The maximum possible PII or SII was 8, with a total possible score of 4 for erythema and a total possible score of 4 for edema. A PII or SII less than or equal to 2 indicted a mild irritant, a PII or SII greater than 2 but less than or equal to 6 indicated a moderate irritant, and a PII greater than 6 indicated a severe irritant.

The following test preparations were evaluated for dermal irritancy: (a) timolol base, neat (metastable) liquid; (b) crystalline timolol base solution (14.3%) in Captex 300 (medium-chain triglyceride oil, Capital City Products Co., Columbus, Ohio); (c) crystalline timolol base solution (5%) in Captex 300; (d) crystalline timolol base solution (2%) in Captex 300; (e) crystalline timolol base solution (0.5%) in Captex 300; and (f) Captex 300. This triglyceride was chosen to prepare simple formulations of timolol base because of its nonirritating properties and its good solvency for the drug. The solubility of crystalline timolol base in Captex 300 at room temperature was found to be approximately 15% by weight.

In Vivo Dog Model

A male beagle dog (15 kg) was placed in a restrainer and both forelegs were prepared with iv catheters. The dog was wired for EKG recording and the heart rate was recorded as a function of time. During a test period the dog was challenged with a series of iv bolus injections of Isoprel (isoproterenol hydrochloride, 0.2 mg/ml, in lactate buffer) using 0.050 ml per challenge. The average increase in heart rate from the initial series of challenges served as the baseline response in this test. A percentage reduction of 25% or less was considered nondetectable. The dog was calibrated using an iv steady-state infusion of timolol maleate to block the isoproterenol-induced tachycardia. A dose–response relationship was obtained by comparing percentage blockade versus timolol infusion rate. The dog was prepared for dermal application of test systems by closely clipping the hair on the mid dorsal surface.

The following test preparations were evaluated for inhibition of isoproterenol-induced tachycardia: (a) Silastic patch—a thin, circular, dermal patch, 8 cm^2 in area, was made from two sections of 0.127-mm-thick silicone rubber sheeting (Silastic sheeting, nonreinforced, Dow Corning Corporation, Midland, MI) which were bonded together at the edges with Silastic Type A medical adhesive (Dow Corning Corporation, Midland, MI) and filled by injection with 0.20 g timolol base, neat (metastable) liquid, and backed with foil; and (b) ethanol solutions of timolol base which were prepared at concentrations of 10, 20, or 100 mg/ml.

The *in vitro* release rate of timolol from a similar Silastic patch in 900 ml of water at 37°C, stirred at 100 rpm, was measured by monitoring the uv absorbance of the resultant timolol solution at 294 nm. The release rate was found to be 175 μg timolol base hr^{-1} cm^{-2}.

Table I. *In Vitro* Permeation of Timolol Through Fuzzy Rat Skin from Nonaqueous Formulations

Time (hr)	Cumulative timolol (mg) permeated for formulation			
	a	b	c	d
13	0.92	1.0	2.6	0.016
21	4.6	3.3	7.1	0.050
28	9.2	6.6	11	0.059
38	18	14	13	0.18
Amount of formulation applied	133 mg	240 mg	0.16 ml	0.20 ml
Total timolol applied	133 mg	137 mg	16.2 mg	2.04 mg

The Silastic patch was overlaid with adhesive film and retained for a period of 7 days. The ethanol solutions of timolol base were nonocclusive. The solutions were applied over a test area of either 5 or 50 cm^2 and the ethanol was allowed to evaporate. The test sites contained 10 mg after application. The dog was challenged at least once daily with a series of isoproterenol injections during the period of application and, after removal of the test patch, until the baseline response to isoproterenol was restored.

RESULTS

Table I summarizes the results obtained from single *in vitro* measurements for formulations a to d. All are essentially nonaqueous in nature. The data shown are the cumulative amounts of timolol, as the base, that had permeated through the rat skin as a function of time over the 38 hr period. Also shown are the amount of formulation applied and the amount of timolol applied. The amount of timolol that permeated through the skin increased from the 2-mg application to the 16-mg application; further increases in the amounts of timolol applied to the skin did not lead to increases in the amount of timolol permeated. A maximum skin flux of 110 $\mu g\ cm^{-2}\ hr^{-1}$ was observed. Figure 1 summarizes the results obtained for duplicate *in vitro* measurements of timolol permeation for formulations e through i and their range. All are essentially aqueous in nature. The permeation rate of timolol increased from 2 $\mu g\ cm^{-2}\ hr^{-1}$ at pH 5–6 to 50–60 $\mu g\ cm^{-2}\ hr^{-1}$ at pH 8.8.

As shown in Table II placebo applications of Captex 300 caused no irritation in the fuzzy rat skin irritation tests. Captex 300 solutions of 0.5, 2, and 5% also caused no irritation. Timolol base 14.3% solutions in Captex 300 caused mild irritation which did not persist for 7 days. Timolol base neat caused moderate irritation which persisted for more than 7 days.

Table III summarizes the results from the *in vivo* dog model. The Silastic patch maintained a pharmacologic response between 68 and 85% of maximum from day 2 through day 7 after application. Decay of the response required only 2 days after removal of the test system. Nonocclusive applications of ethanolic solutions of timolol base produced a more pronounced pharmacologic effect when applied over 5 cm^2 compared to the same amount of timolol applied over 50 cm^2.

These results show that timolol base, applied over as little skin area as 5 cm^2, will penetrate the skin of the beagle dog at a rate sufficient to block isoproterenol-induced tachycardia. The systemic effect is sustained for several days after a single, nonoccluded application of the active principle in a volatile solvent.

Although the percentage inhibition of the isoproterenol-induced tachycardia after dermal dosing was greater than that observed for the intravenous dosage range investigated,

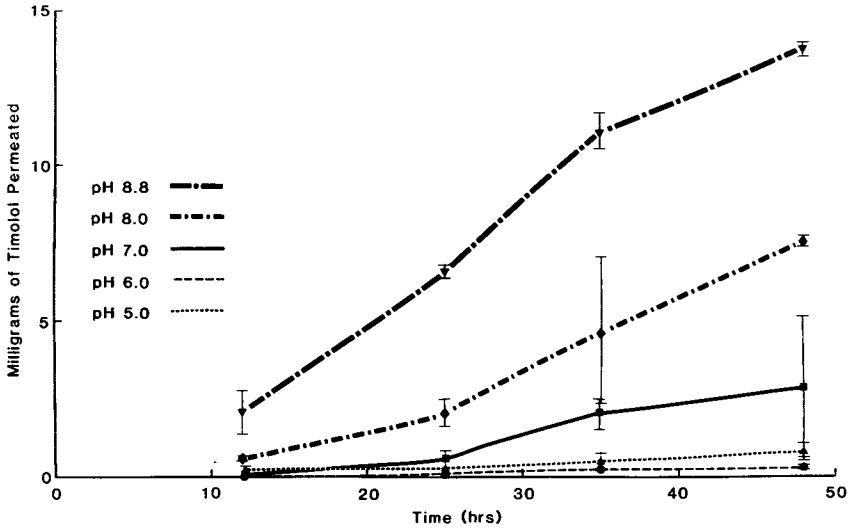

Fig. 1. Cumulative amounts of timolol (as the free base) permeated through 8 cm^2 of fuzzy rat skin, *in vitro*, versus time in hours for formulations e (pH 5.0), f (pH 6.0), g (pH 7.0), h (pH 8.0), and i (pH 8.8). The points shown are average values for duplicate cells with the ranges indicated by vertical bars. (e) Timolol maleate (3.3%) in pH 5.0 citrate-buffered solution; (f) timolol maleate (3.3%) in pH 6.0 pyrophosphate-buffered solution; (g) timolol maleate (3.3%) in pH 7.0 phosphate-buffered solution; (h) timolol maleate (3.3%) in pH 8.0 pyrophosphate-buffered solution; (i) timolol maleate (3.3%) in pH 8.8 pyrophosphate-buffered solution.

Table II. Fuzzy Rat Skin Irritation (3- and 7-Day Occlusion)

Sample (~50 mg applied over 2-cm² surface)	Primary irritation index,[a] $T_0 + T_{72}$ hr[b]		Secondary irritation index,[a] T_7 days[b]	
	3-day application	7 day	3-day application	7 day
Timolol base (neat)	2.0 ± 1.5 (9)	3.0 ± 2.0 (5)	0.5 ± 1.0 (9)	2.0 ± 2.0 (5)
Timolol base, 14.3%, in Captex 300	0.5 ± 0.5 (2)	0 (2)	0 (2)	0 (2)
Timolol base, 5%, in Captex 300	0 (2)	0 (2)	0 (2)	0 (2)
Timolol base, 2%, in Captex 300	0 (2)	0 (2)	0 (2)	0 (2)
Timolol base, 0.5%, in Captex 300	0 (2)	0 (2)	0 (2)	0 (2)
Captex 300 (control)	0 (2)	0 (2)	0 (2)	0 (2)

[a] Irritation index ± SD (*N*); data rounded to nearest 0.5.
[b] T_0 is time at which occlusive application is removed.

the data in Table III can be utilized to estimate an approximate dermal absorption rate for timolol base. A dermal timolol absorption rate of 10 μg cm^{-2} hr^{-1} would be required to generate a 50% response for an 8-cm² area, based on the iv data. Thus, it can be concluded that the dermal permeation rate of timolol base was somewhat greater than approximately 10 μg cm^{-2} hr^{-1} for both the Silastic patch application and the application of ethanolic timolol solution on 5 cm² since the response was greater than 50% inhibition.

DISCUSSION

The results obtained in these *in vitro* studies show that timolol very rapidly permeated fuzzy rat skin when applied in formulations containing timolol, at least partially in the free-base form. The results obtained at various pH values are consistent with permeation of timolol as the free-base species. The combined effects of concentration and pH were observed to influence the permeation rate over several orders of magnitude. Since no acid–base equilibria were involved with the nonaqueous formulations which were investigated, the principal effects observed on the permeation rates were related to the amount of timolol applied and specific solution effects.

The results reported here are highly encouraging with respect to the feasibility of systemic delivery of timolol by the transdermal route. In fact, the flux of timolol through the skin is remarkably high, both in the rat skin *in vitro* model and in the dog *in vivo* model. It is also notable that timolol does cause irritation after prolonged application in the fuzzy rat in the skin irritation model when the free-base form is applied neat or as a nearly saturated solution in Captex 300. Application of less concentrated solutions of timolol in Captex 300 did not cause irritation in this model. More detailed studies involving equal dosing of timolol on a per-area basis in different vehicles would be necessary to evaluate further if the irritation can be limited by the use of timolol formulations at a low thermodynamic activity. It is not known whether the irritation was caused by the alkaline nature of timolol free base, its beta-adrenergic activity (6,7), or both. A multiday system for human therapy is conceivable if the delivery rate through the skin can be controlled in such a way that irritation is minimized. Preliminary human studies (8) with transdermally administered timolol indicate that models such as these are useful screens for transdermal drug delivery candidates.

REFERENCES

1. S. Popli, G. Stroke, T. S. Ing, J. T. Daugirdas, M. J. Norusis, J. E. Hano, and V. C. Gandhi. *Clin. Ther.* 5:624–628 (1983).

Table III. Percentage Inhibition of Isoproterenol-Induced Tachycardia After Dermal Application of Timolol Base to a Beagle Dog

Timolol dosage	Baseline increase in heart rate after isoproterenol	Average inhibition of isoproterenol-induced tachycardia after application of timolol[a]									
		iv (30 min)	Dermal (days)[b]								
			1	2	3	4	5	6	7	8	9	10
iv, 0.79 μg kg^{-1} hr^{-1}	129	3										
iv, 1.71 μg kg^{-1} hr^{-1}	129	30										
iv, 4.77 μg kg^{-1} hr^{-1}	129	51										
Dermal, Silastic patch (7 day), 8 cm²[c]	129		64	68	67	—	85	75	81	49	8	0
Dermal, 10 mg on 5 cm² (via ethanol solution)	108		91	84	54	18	—	3	0			
Dermal, 10 mg on 50 cm² (via ethanol solution)	108		26	38	12	8						

[a] Values reflect percentage reduction in baseline heart rate.
[b] Days are defined as Day 1 = 0–24 hr; Day 2 = 24–48 hr; etc.
[c] Contained 200 mg timolol base.

2. W. J. Mroczek, M. Ulrych, and S. Yoder. *Clin. Pharmacol. Ther.* **31**:252 (1982).
3. M. A. Weber, J. I. Drayer, F. G. McMahon, R. Hamburger, R. Shah, and L. N. Kirk. *Arch. Intern. Med.* 144:1211–1213 (1984).
4. C. Behl, J. Pei, N. Bellantone, and M. Matluck. Presented at the 130th American Pharmaceutical Association Meeting, April 9–14, New Orleans, La. (1983).
5. J. J. Windheuser, J. L. Haslam, L. Caldwell, and R. D. Shaffer. *J. Pharm. Sci.* 71:1211–1213 (1982).
6. R. H. Shereff, W. Harwell, P. Lieberman, E. W. Rosenberg, and H. Robinson. *J. Allergy Clin. Immunol.* **52**:328–333 (1973).
7. N. Lamkin, P. Lieberman, R. Shereff, E. W. Rosenberg, and H. Robinson. *J. Allergy Clin. Immunol.* 57:449–453 (1976).
8. P. H. Vlasses, L. G. T. Ribeiro, H. H. Rotmensch, J. V. Bondi, A. E. Loper, M. Hichens, M. C. Dunlay, and R. K. Ferguson. *J. Cardiovasc. Pharmacol.* 7:245–250 (1985).