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Iontophoretic *in Vivo* Transdermal Delivery of β-Blockers in Hairless Rats and Reduced Skin Irritation by Liposomal Formulation

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Purpose. To demonstrate the *in vivo* transdermal delivery and establish the comparative pharmacokinetics of five β-blockers in hairless rat.

Methods. Intravenous dosing was initially done via jugular cannula. For iontophoretic delivery, current (0.1 mA/cm^2) was applied for 2 h through a drug reservoir patch containing the β-blocker (10 mg/ml). Blood samples were collected and analyzed by stereoselective HPLC assays. Any irritation resulting from patch application was quantified by a chromameter. Multilamellar liposomal formulation was prepared by the thin-film hydration method and converted to unilamellar liposomes by extrusion.

Results. With transdermal iontophoresis, therapeutically relevant amounts of propranolol (83.78 ± 7.4 ng/ml) were delivered within an hour and lasted for up to 4 h. C_{max} (185.1 ± 56.8 ng/ml) was reached at hour 3. A significantly higher amount ($p < 0.05$) of sotalol HCl was delivered compared to other β-blockers. There was no significant difference in the S/R ratio of AUC_{0-t} for enantiomers after both intravenous and transdermal delivery. Skin irritation was significantly reduced ($p < 0.05$) when a liposomal formulation of the propranolol base was used rather than the base itself.

Conclusions. The comparative pharmacokinetics of intravenous and transdermal iontophoretic delivery of five β-blockers in hairless rats was established. It was shown that there is no stereoselective permeation.

KEY WORDS: transdermal; iontophoresis; β-blockers; liposomes; propranolol; timolol.

INTRODUCTION

Transdermal delivery of cardiovascular drugs offers several advantages, and transdermal forms of nitroglycerin and clonidine have been marketed. Transdermal delivery of one of the common class of cardiovascular drugs, β-blockers, has also been investigated and can offer benefits. For example, the extensive first-pass metabolism of propranolol can be avoided. Because of large differences in first-pass effects for propranolol, plasma concentrations can vary widely between individuals following oral administration. Transdermal iontophoretic delivery would increase delivery to achieve therapeutic levels and might further allow modulation of delivery for individualized dosing. Iontophoresis involves the application of a small amount of physiologically acceptable direct current (d.c.) to drive ionic drugs into the body (1). Self-regulated systems based on iontophoretic delivery triggered by a blood pressure–sensing mechanism may also be possible. Most published studies are limited to investigation of passive transport or use of enhancers for delivery of propranolol (2–5).

The transdermal iontophoretic delivery of propranolol *in vitro* has been studied on membranes (6) and skins of different species including hairless mice (7), albino rats (8), and excised pig, rabbit, mouse, and human skin (9). In a clinical study (10), it was found that propranolol reduced the heart rate but was not able to decrease the force of contraction in 58 patients using the iontophoresis technique. *In vivo* pharmacokinetics of β-blockers in animal models and potential use of liposomes to minimize drug irritation have not been investigated. Also, results reported in literature relating to stereoselective permeation of drugs have been conflicting (11).

The broad aim of this research is to test the hypothesis that drugs such as β-blockers can be administered in a programmable fashion via electrically assisted transdermal delivery and that their irritation potential can be reduced by a liposomal formulation. This study investigated the *in vivo* iontophoretic delivery of five β-blockers (propranolol HCl, oxprenolol HCl, timolol maleate, metoprolol tartrate, and sotalol HCl) in hairless rats. A stereoselective assay was used to investigate if there is any stereoselective transdermal transport. Finally, liposomal formulation was used to investigate if it can reduce skin irritation.

MATERIALS AND METHODS

Materials

DL-Propranolol hydrochloride, timolol Maleate, (±) metoprolol tartrate, (±) sotalol hydrochloride, (−) menthyl chloroformate, (−) S-napthyl ethyl isocyanate, cholesterol, and stearylamine were obtained from Sigma (St. Louis, MO). (±) Oxprenolol hydrochloride was obtained from ICN Biochemicals Inc. (Aurora, OH). Hairless rats (350–500 g) were obtained from Charles River (Wilmington, MA). DSPC (1,2-disteoroyl-*sn*-glycero 3-phosphocholine) was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). Trans-Q® 1-GS iontophoretic drug delivery electrodes were obtained from Iomed Inc. (Salt Lake City, UT). All other chemicals and solvents (HPLC grade) were obtained from Fisher Scientific (Pittsburgh, PA).

Intravenous Studies

A jugular cannula was placed into hairless rats (n = 4 for each group) under ketamine/xylazine anesthesia 1 day before intravenous dosing. Drug solutions (2 mg/ml) were prepared in sterile normal saline, and dosing was performed via the cannula. Dose administered was 1 mg/kg for timolol and 2 mg/kg for the other four β-blockers. Blood samples were withdrawn over 1 to 6 h, allowed to clot, and serum was separated by centrifugation.

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Transdermal Transport Studies

Hairless rats (n = 4 for each group) were used, and the research adhered to the principles of laboratory animal care. Rats were anesthetized by intraperitoneal injection of ketamine HCl (75 mg/kg) and xylazine (10 mg/kg). The abdominal area was cleaned, and baseline readings for skin irritation (erythema) were taken using a Chromameter (CR 300, Minolta, USA). A formulation containing 10 mg/ml of the β-blocker in 25 mM imidazole buffer (pH 6.2) was used. The formulation (1.0 ml) was added to the Trans-Q® 1-GS drug delivery reservoir. For iontophoresis experiments, a current of 0.1 mA/cm^2 was applied for 2 h using a Dupel® device (Empi Inc., MN). The pH of the formulation (pH 6.2) was selected relative to the pK_a of the β-blockers so that they are completely ionized and can be delivered under the anode. A reference electrode was placed next to the drug reservoir electrode and used as the cathode to complete the circuit. Blood samples were taken from the tail vein at specified intervals during and after the iontophoresis period. The patch was removed at the second hour after iontophoresis, and another chromameter reading was taken after cleaning the area. A one-way ANOVA was done to find the significant difference in the bioavailability by various routes of administration and skin irritation studies. Differences were considered to be significant at levels < 0.05.

Liposomal Formulation

Multilamellar liposomes (MLVs) were prepared by the thin film hydration method. The liposome composition used was DSPC: cholesterol: stearylamine (1:0.5:0.3 mole ratio). Stearylamine was used to induce a positive charge on the liposomes. Liposomes were prepared by dissolving DSPC, cholesterol, propranolol base, and stearylamine in chloroform. The organic solvent was removed under vacuum in a Rotavapor® R-3000 (Buchli, Switzerland), leaving behind a thin lipid film containing the propranolol base. The film was then kept under vacuum overnight to remove residual solvent. Nitrogen gas was then passed over the film for 30 min to remove the remaining traces of the organic solvent, after which the film was hydrated with imidazole buffer. The hydration was carried out at a temperature above the glass transition temperature of DSPC (>56°C). The mixture was then vortexed until the lipid film was completely hydrated to yield MLVs. The MLVs were converted into large unilamellar vesicles (LUVs) by being extruded through a 0.8-μm filter (polycarbonate membrane) followed by extrusion through a 0.4-μm filter (polycarbonate membrane) under positive pressure with nitrogen using an extruder (Lipex Biomembranes Inc., Vancouver, Canada). The production of LUVs by extrusion procedures has been described by Cullis *et al.* (12).

HPLC Assays

HPLC assays were performed using 25 cm long C-18 columns with particle diameter of 5 μm and pore size of 100 Å, unless specified otherwise. The mobile phase was filtered before use and sparged with helium gas at 15–30 ml/min during analysis (flow rate 1–2 ml/min). Calibration curves ranged from 10 to 1000 ng/ml. The r^2 was found to be 0.9927 or higher for each drug/enantiomer.

A Waters® LC-Module I system, a Waters® 2475 fluorescence detector, and a HP-3396 Series II integrator (Fisher Scientific, USA) were used. A method modified from Prakash *et al.* (13) was used to analyze (±) propranolol. (+) Flecainide was used as the internal standard (IS). The excitation and emission wavelengths on the detector were set at 228 and 340 nm, respectively. The mobile phase consisted of methanol and

Table I. Pharmacokinetic Parameters for β-Blockers in Hairless Rats following Intravenous Bolus Delivery

Parameter	Isomer	Propranolol HCl	Oxprenolol HCl	Timolol maleate	Metoprolol tartrate	Sotalol HCl
k_e (1/min)	R	0.25 ± 0.01	0.06 ± 0.01	—	0.03 ± 0.01	0.01 ± 0.001
	S	0.01 ± 0.01	0.05 ± 0.01	0.01 ± 0.002	0.02 ± 0.004	0.01 ± 0.001
	R+S	0.02 ± 0.01	0.05 ± 0.01	—	0.02 ± 0.01	0.01 ± 0.001
$t_{1/2}$ (min)	R	32.7 ± 15.4	11.7 ± 3.1	—	28.3 ± 8.1	112 ± 10.7
	S	45.6 ± 14.8	14.0 ± 1.3	104 ± 29	31.5 ± 5.2	117 ± 11.6
	R+S	45.5 ± 18.7	12.94 ± 2.0	—	31.2 ± 10.2	114 ± 9.3
$AUC_{0-\infty}$ (min · μg/ml)	R	22.5 ± 7.6	19.5 ± 9.1	—	7.0 ± 1.2	48.7 ± 3.9
	S	27.7 ± 20.8	14.8 ± 7.4	16.5 ± 0.4	5.6 ± 0.8	55.1 ± 4.9
	R+S	34.7 ± 14.8	34.2 ± 16.4	—	12.7 ± 2.2	104 ± 0.9
$AUMC_{0-\infty}$ (min · min · μg/ml)	R	754 ± 520	290 ± 72	—	289 ± 113	7,778 ± 1,267
	S	1,263 ± 243	285 ± 128	1,964 ± 472	220 ± 70	9,040 ± 996
	R+S	1,673 ± 1,103	573 ± 195	—	528 ± 217	16,790 ± 2219
C_0 (ng/ml)	R	1,383 ± 620	1,398 ± 1,053	—	241 ± 43	502 ± 116
	S	233 ± 46	814 ± 594	281 ± 39	274 ± 39	475 ± 84
	R+S	1644 ± 627	2,205 ± 1,641	—	512 ± 80	977 ± 200
V_d (ml)	R	515 ± 286	555 ± 421	—	2,804 ± 207	1,427 ± 237
	S	2,292 ± 1,889	862 ± 554	4,197 ± 1,323	3,967 ± 593	1,320 ± 142
	R+S	1,072 ± 758	689 ± 470	—	3,374 ± 384	1,363 ± 177
Cl (ml/min)	R	15.1 ± 13.1	30.3 ± 14.9	—	71.9 ± 16.2	8.8 ± 0.7
	S	16.2 ± 14.2	41.8 ± 23.9	27.9 ± 3.2	88.4 ± 16.5	7.8 ± 0.5
	R+S	11.9 ± 9.9	35.1 ± 18.4	—	78.8 ± 17.5	8.3 ± 0.6

water (890:110, v/v). For (±) oxprenolol HCl, a method modified from Laethem *et al.* (14) was used. *RS*-propranolol was used as the IS, and methanol–tetrahydrofuran–0.2 M acetate buffer (pH 3.6) (51:14:35, v/v/v) as the mobile phase. The separations were performed at 30°C using a column heater (Timberline Instruments, Boulder, CO). Fluorescence was monitored at 226 nm for excitation and at 333 nm for emission. A method modified from Kubota *et al.* (15) was used for timolol. Timolol maleate is marketed only as the *levo*-enantiomer. A UV detector was used for this analysis with the wavelength set at 295 nm. The mobile phase was acetonitrile–water–triethylamine (18:81:1), v/v/v), and the pH was adjusted to 3.0 with phosphoric acid. For (±) metoprolol tartrate, a method modified from Bhatti and Foster (16) was used. A stainless-steel 15-cm-long YMC Pack SIL (YMC Inc., Milford, MA) column, with a particle diameter of 3 μm and a pore size of 100 Å was used. The mobile phase consisted of hexane–chloroform–methanol (85:14:1, v/v/v). Fluorescence was monitored at 222 nm for excitation and at 337 nm for emission. (±) Propranolol HCl was used as the IS. (±) Sotalol HCl was analyzed using a method modified from Fiset *et al.* (17). A 25-cm-long Microsorb C-8 column (Rainin Instruments, Emeryville, CA) was used. The excitation and emission wavelengths were set at 235 and 300 nm, respectively. The mobile phase consisted of methanol–water–acetonitrile (50:35:15). Tris buffer (2 M) was adjusted to pH 9.0 with 12 M hydrochloride acid and kept at 4°C. *S* (−) Atenolol was used as the IS. Chiral reagent solution was prepared on the day of the analysis by dissolving 400 μL of (−)-methyl chloroformate in 10 ml of acetonitrile.

Details of derivatization and extraction varied for each drug. To 100 to 200 μl of serum, IS and NaOH (perchloric acid for sotalol) were added, and serum extracted with 1% 1-butanol in *n*-hexane (propranolol), dichloromethane (oxprenolol and timolol), chloroform (metoprolol), or chloroform-2-propanol (3:1) (sotalol). The organic phase was separated following shaking and centrifugation and evaporated to dryness under a stream of nitrogen at 25 to 50°C in a water bath (Organomation, N-Evap™ 112, Nitrogen Evaporator, Berlin, MA). For propranolol, the residue was then dissolved in a 100-μl volume of 0.4% triethylamine in acetonitrile and methanol (50:50, v/v), and 50 μL of a 0.023 M solution of (−) MCF in acetonitrile was then added as the derivatizing agent. After thorough mixing for 15 min, an aliquot of the reaction mixture was injected onto the HPLC for propranolol enantiomer quantification. For oxprenolol, the dry residue was dissolved in 100 μl of dichloromethane, vortex-mixed for 5 s, and finally, 10 μl of a 0.01% (v/v) *S*-NEIC solution was added. The samples were then placed in a shaker maintained at 37°C for 2 h, after which the excess reagent was removed by the addition of 20 μl of tertiary butylamine (TBA). Excess TBA was evaporated under a nitrogen stream. The residues were dissolved in 50 μl of the mobile phase, and aliquots of 10 μL

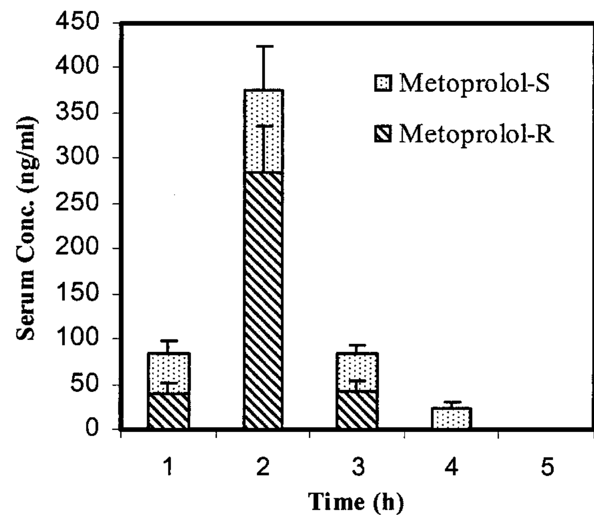

Fig. 2. Mean serum concentrations of (*R*)- and (*S*)-metoprolol enantiomers after transdermal iontophoretic delivery (0.1 mA/cm^2 for 2 h) of (±)-metoprolol tartrate to hairless rats. The data are expressed as mean ± SD for n = 4.

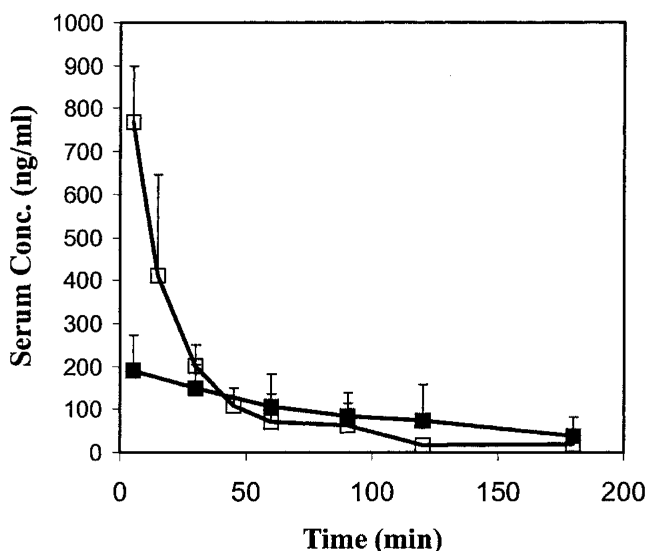

Fig. 1. Mean serum concentrations of (*R*)- and (*S*)-propranolol enantiomers after intravenous administration of (±)-propranolol HCl (2 mg/kg) to hairless rats. The data are expressed as mean ± SD for n = 4. Key: (□) *R*-propranolol; (■) *S*-propranolol.

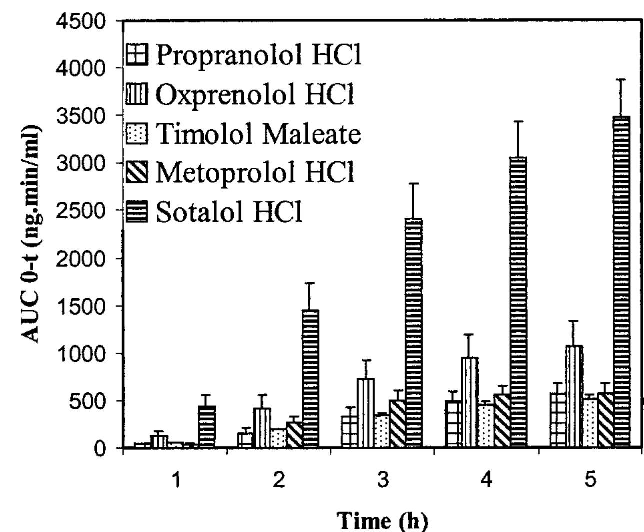

Fig. 3. Transdermal delivery of β-blockers (AUC 0-t) in hairless rats after application of iontophoretic current (0.1 mA/cm^2) for 2 h. The data are expressed as mean ± SD for n = 4.

were injected into the HPLC system. For timolol, the residue was reconstituted with 100 μl of mobile phase and filtered with an ACRO LC3S disposable filter assembly (0.45 μm, Fisher Scientific, Pittsburgh, PA), and 30 μL of the filtered solution was injected onto the HPLC system. For metoprolol, 100 μl of the *S*-NEIC solution [0.05% (v/v) in chloroform] was added to the residue. The solution was again vortex-mixed for 30 s, and an aliquot of 50 μl was injected onto the HPLC system. For sotalol, 200 μl of a saturated sodium carbonate solution followed by 200 μl of (−)-methyl chloroformate solution were added to the residue; samples were vortex-mixed for 30 s. Water (1 ml) and 2 ml of chloroform were added to samples, which were again vortex-mixed for 1 min. Following centrifugation at 1500 *g* for 5 min, the aqueous layer was discarded, and the organic phase was evaporated to dryness. The residue containing diastereoisomeric derivatives of sotalol enantiomers and of *S* (−) atenolol was reconstituted with 100 μl of mobile phase and centrifuged for 5 min. A 20-μl aliquot was injected into the HPLC system.

RESULTS AND DISCUSSION

Hairless rats were used for these studies. Hairless animals still have rudimentary follicles with a hair follicle density much closer to that of human skin as compared to regular hairy animals. This becomes important for iontophoretic delivery, as transport pathways are predominantly appendageal (1). All five β-blockers were initially administered intravenously to calculate primary pharmacokinetic parameters in hairless rats and calculate clearance, which was used later for calculation of transdermal bioavailability. These parameters are not reported in the literature for hairless rats. Pharmacokinetic parameters were obtained from the serum concentration vs. time profiles by noncompartmental analysis using linear/log trapezoidal method model (NCA Model 201, WinNonlin® version 3.1, Pharsight, CA) and are listed in Table I. Serum concentration vs. time profiles for *R* and *S* forms of propranolol are shown in Fig. 1. The profiles were similar for timolol (only *levo*-form), oxprenolol, metoprolol, and sotalol (data not shown).

With transdermal iontophoresis, therapeutically relevant amounts of propranolol (83.78 ± 7.4 ng/ml) were delivered within an hour and lasted for up to 4 h. C_{max} (185.1 ± 56.8 ng/ml) was reached at hour 3. A somewhat similar profile was seen for metoprolol, but with a C_{max} at hour 2 (Fig. 2), and for the other β-blockers (data not shown). Serum concentration vs. time profiles of *R*- and *S*-enantiomers were measured for all β-blockers (except timolol, which was in *levo* form only. A comparison of delivery for various β-blockers is shown in Fig. 3. A significantly higher amount ($p < 0.05$) of sotalol HCl was delivered compared to other β-blockers. All the β-blockers selected (pK_a 9.21–9.65) carry a positive charge at pH 6.2 (pH of the buffer), at pH 5.5 (pH at the surface of the skin), and pH 7.4 (pH at the viable epidermis). At the skin surface and in the buffer, the degrees of ionization of all the β-blockers selected are close to 100% (including sotalol at pK_a 9.65). Hence, all the drugs are expected to be pushed by the electrode until the drug reaches the viable epidermis. However, once the drugs reach the viable epidermis (pH 7.4), sotalol will have a higher amount of unionized fraction because of its second pK_a value (8.15). The latter in turn would lead to better passive transport through the viable epidermis into the blood circulation. Pharmacokinetic profiles observed for various β-blockers following transdermal iontophoretic administration are listed in Table II.

Of the β-blockers used, propranolol is very lipophilic; oxprenolol, timolol, and metoprolol are lipophilic; and sotalol is hydrophilic. The corresponding partition coefficients are

Table II. Pharmacokinetic Parameters for β-Blockers in Hairless Rats following Transdermal Iontophoretic Delivery

Parameter	Isomer	Propranolol HCl	Oxprenolol HCl	Timolol maleate	Metoprolol tartrate	Sotalol HCl
k_e (1/h)	R	0.8 ± 0.3	0.8 ± 0.3	—	1.9 ± 0.3	0.25 ± 0.09
	S	0.4 ± 0.1	0.6 ± 0.2	0.3 ± 0.08	0.6 ± 0.3	0.8 ± 0.2
	R+S	0.5 ± 0.2	0.7 ± 0.2	—	1.4 ± 0.1	0.4 ± 0.1
$t_{1/2}$ (h)	R	1.0 ± 0.4	0.9 ± 0.2	—	0.4 ± 0.05	3.1 ± 1.1
	S	1.6 ± 0.8	1.3 ± 0.4	2.2 ± 0.6	1.2 ± 0.4	1.0 ± 0.3
	R+S	1.4 ± 0.6	1.1 ± 0.3	—	0.5 ± 0.04	1.7 ± 0.3
$AUC_{0\text{-}\infty}$ (hr · μg/ml)	R	0.5 ± 0.1	0.6 ± 0.2	—	0.3 ± 0.05	2.9 ± 0.7
	S	0.3 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.2 ± 0.04	1.6 ± 0.2
	R+S	0.6 ± 0.3	1.2 ± 0.2	—	0.5 ± 0.1	4.3 ± 0.7
$AUMC_{0\text{-}\infty}$ (hr · hr ·tμg/ml)	R	1.4 ± 0.3	1.6 ± 0.4	—	0.7 ± 0.1	15.9 ± 7.8
	S	1.1 ± 0.2	1.9 ± 0.2	3.1 ± 1.0	0.7 ± 0.1	4.6 ± 0.9
	R+S	2.2 ± 1.2	3.4 ± 0.6	—	1.2 ± 0.2	14.9 ± 3.7
C_{max} (ng/ml)	R	158.7 ± 53.4	184 ± 35.3	—	283.6 ± 52.8	575 ± 88
	S	76.4 ± 38.6	164 ± 24.1	170.5 ± 17.5	92.6 ± 49.1	565 ± 72.3
	R+S	181 ± 113	348.3 ± 59.3	—	376 ± 95	1,140 ± 155
$V_{d/F}$	R	15,298 ± 6,651	12,631 ± 6,208	—	15,405 ± 2,383	7,528 ± 1,756
	S	60,394 ± 12,961	16,583 ± 1,257	41,934 ± 5,940	22,474 ± 4,626	3,979 ± 666
	R+S	27,161 ± 13,106	14,580 ± 6,919	—	19,210 ± 3,502	5,826 ± 952
Cl/F (ml/h)	R	11,288 ± 2,235	9,128 ± 2,876	—	15,405 ± 2,383	1,767 ± 352
	S	23,909 ± 7,068	8,432 ± 1,257	13,565 ± 2,093	22,474 ± 4,626	2,876 ± 357
	R+S	15,239 ± 3,226	8,792 ± 2,038	—	19,210 ± 3,502	2,371 ± 357
F	R	0.08	0.2		0.3	0.3
	S	0.04	0.3	0.12	0.2	0.2
	R+S	0.05	0.24		0.25	0.2

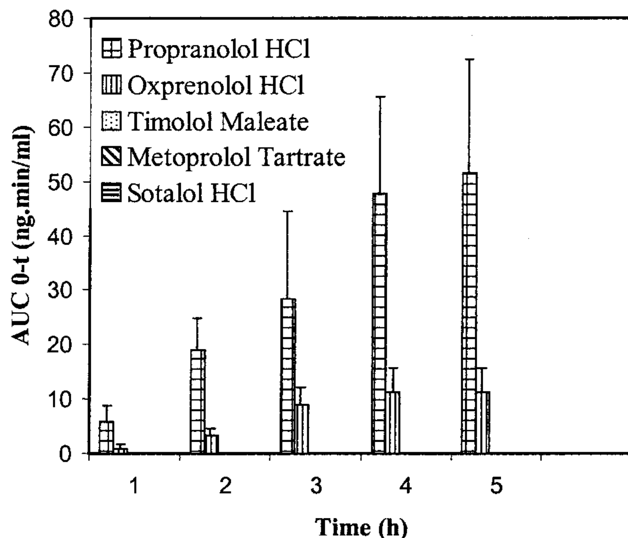

Fig. 4. Transdermal delivery of β-blockers (AUC 0-t) in hairless rats after application of passive patch (no current). Only propranolol and oxprenolol had a measurable response. The data are expressed as mean ± SD for n = 4.

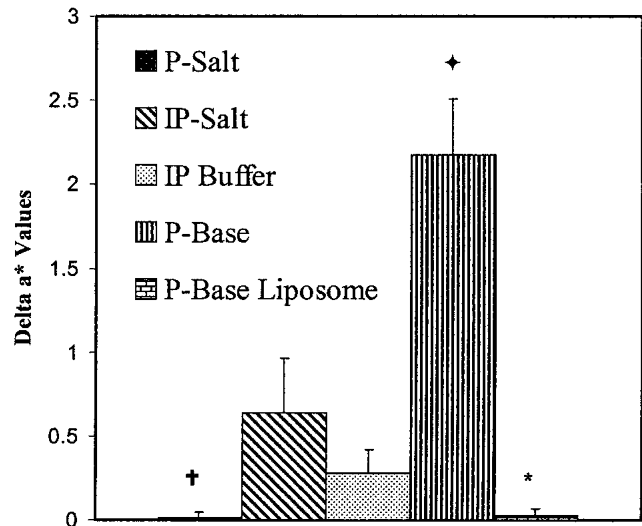

Fig. 5. Skin irritation produced by propranolol on hairless rats under different study conditions. The data are expressed as mean ± SD for n = 4 rats. Key: P, passive (passive application of salt form had an undetectable response); IP, iontophoresis; † significant difference ($p < 0.05$) between passive and iontophoresis of propranolol HCl; *significant difference ($p < 0.005$) between propranolol base and liposome formulation of the base; ✦significant difference ($p < 0.05$) between propranolol base and propranolol HCl iontophoresis, and between propranolol base and imidazole iontophoresis.

1640, 235, 82, 76, and 0.24, respectively (18). For passive permeation, a lipophilic molecule is generally expected to permeate the skin better, as it can partition more easily into the skin. The passive permeation of β-blockers in this study was in general low compared to iontophoretic delivery. Comparative data for all β-blockers are shown in Fig. 4. As expected, the most lipophilic molecules, propranolol and oxprenolol, were found to have the highest permeation.

During iontophoretic delivery, the serum concentrations of *R*-isomer were higher at initial time points (Fig. 2) but then reduced during the elimination phase. This may be because of possible differences in the disposition of the enantiomers. It has been reported (19) that disposition of propranolol is stereoselective in rats, and stereoselectivity varies with the route of administration. Plasma levels of the active *S*-enantiomer were reported to be only 27% of the total (*S*+*R*) plasma concentrations after IV and 18% after oral dose. However, some level of complexity may be involved because it has been reported that the disposition kinetics may be stereoselective only at high doses; at lower doses, the liver overshadows these differences because the hepatic clearance of (*S*)-(−)-propranolol is not saturated (20). In our studies, there was no significant difference in the *S/R* ratio of total amount delivered ($AUC_{0\text{-}t}$) after both intravenous and transdermal iontophoretic delivery (Table III). This suggests that there is no stereoselective permeation of (*R*) and (*S*) isomers across the

Table III. Comparison of Ratios of Enantiomers of β-Blockers as Determined by Stereoselective HPLC Essays following Intravenous or Transdermal Iontophoretic Delivery

	S/R ratio	
β-Blockers	Transdermal/Iontophoretic	Intravenous
Propranolol HCl	0.37 ± 0.06	0.44 ± 0.1
Oxprenolol HCl	0.96 ± 0.08	0.8 ± 0.09
Metoprolol tartrate	0.63 ± 0.06	0.73 ± 0.06
Sotalol HCl	0.93 ± 0.06	1.1 ± 0.1

skin, and any differences seen result from differences in disposition kinetics.

There are several reports of skin irritation produced by β-blockers (21–24). These reports have typically used base forms of β-blockers. Because the base form is highly lipophilic (compared to propranolol HCl), it can easily permeate across the stratum corneum. However, it may not easily diffuse into the more hydrophilic viable epidermis and thus can form a depot in the skin, which in turn becomes a chemical irritant leading to erythema. Kobayashi *et al.* (22) have established a linear relationship between the amount of propranolol permeating and the intensity of erythema. Although iontophoresis also causes irritation, this irritation is significantly reduced at low current densities, such as the one used in this study (0.1 mA/cm^2 of skin). The erythema resulting from skin irritation can be measured using a chromameter. The chromameter used uses a three-dimensional coordinate system with a brightness axis (L*), a red–green axis (a*), and a blue–yellow axis (b*). The true color of the skin is determined by all these values, and a composite skin irritation index can be calculated. However, for this study, only the a* observations were used because they provide a measure of erythema. The difference in the a* values taken before patch application and after patch removal (Δa*) were used to quantify the erythema resulting from the propranolol formulations. In the present study, the salt forms of β-blockers used were found to produce very mild or no irritation on application to the skin. When these salt forms were delivered iontophoretically through the skin, the irritation increased but was still relatively low compared to that produced by the passive application of the base form of the β-blocker on the skin. The data are shown for propranolol in Fig. 5. The use of a liposomal formulation of propranolol base was then investigated to determine if liposomes can reduce or eliminate this irritation.

As can be seen in Fig. 5, the skin irritation was significantly reduced ($p < 0.05$) when a liposomal formulation of the propranolol base was used rather than the base itself. Our results seem to support the observation made in some clinical studies that liposomal formulations of econazole, a topical antifungal, have a higher patient acceptance than the cream formulation because of lack of irritation (25). Reduced irritation by liposomes was also observed for retinoids. This reduced skin irritation by liposomes apparently results because the "free" or unentrapped form of the "irritant" drug is always lower at any given time in a liposomal formulation as compared to conventional vehicles (26).

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