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Report

Transdermal Delivery of Isoproterenol HCl: An Investigation of Stability, Solubility, Partition Coefficient, and Vehicle Effects

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Effects of solubility, partition coefficient, and selected adjuvants (propylene glycol and Azone) on percutaneous penetration of isoproterenol HCl have been investigated using human cadaver skin. Isoproterenol was found to be stable (<1% decomposition) for 24 hr at 22 ± 0.5°C in the pH range 1 to 7 in the following solvents: water, normal saline, propylene glycol and a series of propylene glycol–water mixtures (10, 20, 40, and 60%; v/v); however, decomposition was significant beyond pH 8. In normal saline, the rate of decomposition increased significantly with an increase in temperature to 37°C. The solubility of isoproterenol HCl decreased and its skin/vehicle partition coefficient increased with increasing proportions of propylene glycol in the vehicle, while the product of the solubility and partition coefficient appeared to plateau at 20% propylene glycol in water. Optimal penetration enhancing effects of Azone were seen when incorporated at a concentration of 1% (v/v) in the 20% (v/v) propylene glycol–water blend and, more significantly, when skin was pretreated with pure Azone for 60 min prior to application of the drug formulation.

KEY WORDS: isoproterenol HCl; stability; solubility; partition coefficient; percutaneous transport; effect of Azone.

INTRODUCTION

Isoproterenol hydrochloride (4-{1-hydroxy-2[(1-methylethyl)amino]ethyl}-1,2-benzenediol hydrochloride), a direct-acting sympathomimetic agent in use as a bronchodilator and cardiac stimulant, is known to exhibit erratic and unreliable absorption following oral or sublingual delivery (1,2). It is highly metabolized following oral or sublingual administration and has a very short biological half-life, ranging from a few minutes to 2–4 hr depending upon the route of administration (3,4). The intravenous dose of isoproterenol HCl is small; 20–60 μg is given initially, followed by 10–200 μg as needed to elicit the desired response. The recommended oral or sublingual dose ranges from 10 to 15 mg four times per day but not more than 60 mg per day. These characteristics provided a strong incentive for investigating transdermal delivery of isoproterenol HCl. Transdermal delivery would eliminate first-pass metabolism in the gut and the liver and the variability in absorption associated with gastrointestinal transit while improving patient compliance. In addition, isoproterenol HCl offered an excellent opportunity to test the percutaneous penetration enhancing effects of Azone on an ionizable compound.

This study examines the stability, solubility, skin–vehicle partition coefficient, and *in vitro* percutaneous penetration of isoproterenol HCl from 0, 10, 20, 40, 60, and 100% (v/v) propylene glycol in distilled water across human cadaver skin from the abdominal region. The skin-penetration enhancing effect of Azone on isoproterenol HCl dissolved in 20% (v/v) propylene glycol–water has also been investigated.

MATERIALS AND METHODS

Analyses of samples for isoproterenol HCl (Sigma Chemical Co., St. Louis, Mo.) content were carried out by ion-pair high-performance liquid chromatography at room temperature by the method of Ghanekar and Das Gupta (5) and also spectrophotometrically (6) at 280 nm (Spectronic 710 spectrophotometer, Bausch and Lomb, Rochester, N.Y.). The retention time of isoproterenol HCl was 6.25 min and the assay was capable of separating the parent drug from its decomposition products (5).

The chromatograph (Waters Associates, Milford, Mass.) was equipped with a 6000-psi pump, a variable wavelength detector, a loop injector, and an automated integrated system. A 20-μl sample was injected into a 250-mm-long, 4.6-mm-diameter stainless-steel column (Nucleosil C_{18}, Alltech Associates Inc., Deerfield, Il.) and eluted with a 20% (v/v) solution of methanol (J. T. Baker Chemical Co., Phillipsburg, N.J.) in water containing 2% acetic acid with 0.005 *M* sodium 1-heptane sulfonate (K and K Laboratories, Plainview, N.Y.) at pH 2.6.

The buffering system employed throughout this investigation was composed of varying proportions of citric acid (Amend Drug and Chemical Co., Irvington, N.J.), boric

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acid, and tribasic sodium phosphate (both from J. T. Baker Chemical Co., Phillipsburg, N.J.).

Determination of Stability

The 24-hr stability of isoproterenol HCl solutions in distilled water was determined in triplicate by storing buffered solutions ranging from pH 1.93 to pH 10.1 at 22 ± 0.5°C and analyzing for drug content. The stability of isoproterenol HCl in a series of buffered propylene glycol–water mixtures (0, 10, 20, 40, and 60%; v/v) and in normal saline was determined at 22 ± 0.5°C over a pH range of 1.93–7.38 in a similar manner.

Determination of Solubility

The solubility of isoproterenol HCl in propylene glycol–water mixtures and in Azone was determined in triplicate at 22 ± 0.5°C by agitating an excess amount of drug in an appropriate solvent until equilibration (24 hr) and analyzing the filtered solution.

Determination of Partition Coefficients

The skin/vehicle partition coefficients (P) were determined in triplicate at 22 ± 5°C by agitating whole-skin (epidermis and dermis) preparations of known weight (100–200 mg) with known amounts of solutions of the drug in selected propylene glycol–water mixtures in amber-colored flasks under nitrogen until equilibration (72 hr). The skin samples were washed once with 5 ml of distilled water and the isoproterenol content of the skin was determined by homogenizing it with a known volume of distilled water and assaying the filtrate (0.22-μm filter). The total recovery of isoproterenol HCl from the wash, the filtrate, and the original solution was about 95%. The skin/vehicle partition coefficients were calculated from Eq. (3) (Ref. 7) derived as follows:

$$P = C_s/C_v = \frac{M_s/V_s}{M_v V_v} \quad (1)$$

where C_s and C_v are the drug concentrations in the skin and in the aqueous solution, respectively, at equilibrium; M_s and M_v are the masses of isoproterenol HCl in the skin and in the vehicle respectively, at equilibrium; and V_s and V_v are the volumes of the skin and the vehicle, respectively. M_s may be expressed in terms of M_v and M_v°, the total mass of drug initially present in the aqueous solution:

$$M_s = M_v^\circ - M_v \quad (2)$$

Therefore Eq. (1) may be rearranged to give

$$P = C_v^\circ - C_v/C_v \quad (3)$$

where C_v° is the initial concentration of drug in the vehicle.

Skin Diffusion Studies

All of the penetration experiments reported here utilized human abdominal skin obtained at autopsy, kept frozen (8,9), and used within 3 months. The whole-skin samples (epidermis and dermis) were prepared and diffusion studies were conducted in duplicate using a special glass diffusion cell as reported previously (10). The appropriate formulation (1.5 ml) was applied to the exposed skin surface (2.01 cm^2). Normal saline maintained at 37 ± 0.5°C was used as the receptor fluid. The entire skin cell assembly was kept wrapped with aluminum foil to protect it from light. At selected time intervals (1, 2, 4, 6, 8, 10, 12, and 24 hr) the receptor solution was completely withdrawn with a disposable syringe and immediately assayed for the drug content spectrophotometrically. The plots of the amount of drug penetrated versus time revealed that steady state was attained in less than 4 hr. Random samples from each diffusion study were also assayed by high-performance liquid chromatography (HPLC) to monitor any unexpected decomposition of isoproterenol HCl even though preliminary trials were satisfactory. An observed variation of about 4% between the results of the two methods accounted for the experimental error and any metabolism during skin diffusion.

RESULTS AND DISCUSSION

The stability of isoproterenol HCl is distilled water, normal saline, and 0, 10, 20, 40, and 60% (v/v) propylene glycol in water at pH values ranging from 1.9 to 7.4 at 22 ± 0.5°C is shown in Table I. Almost 99% of the amount of the drug present initially remained intact at the end of 24 hr. However, in a separate 24-hr study at the same temperature, only 94.07 ± 0.01, 50.81 ± 0.02, and 30.61 ± 0.01% of the initial amount of isoproterenol HCl was present in aqueous solutions at pH 8.0, 9.0, and 10.1, respectively. In normal saline at 37 ± 0.5°C, only 50.33 ± 0.08% of the initial amount remained intact after 24 hr, although imperceptible degradation was noted at the end of 1.5 hr. Incorporation of ethylenediaminetetraacetic acid at 0.01 and 0.02% concentration levels in the normal saline solutions improved the 24-hr stability of intact isoproterenol HCl to only 60.1 ± 0.13 and 61.73 ± 0.016%, respectively. In view of these observations, receptor fluid in the skin diffusion cells was re-

Table I. Stability of isoproterenol HCl in Water, Normal Saline, and Propylene Glycol–Water Mixtures

Vehicle	pH	Amount left (%)[a]	pH	Amount left (%)	pH	Amount left (%)	pH	Amount left (%)
Distilled water	1.9	98.95 ± 0.91	3.8	98.78 ± 4.59	5.2	98.29 ± 2.43	6.5	98.61 ± 1.99
Normal saline	1.9	98.86 ± 1.30	3.7	96.81 ± 2.04	5.3	98.23 ± 2.04	6.5	99.54 ± 0.59
10% (v/v) PG/DW	2.0	99.24 ± 0.83	3.6	98.7 ± 0.80	5.9	98.72 ± 1.46	6.6	98.75 ± 1.32
20% (v/v) PG/DW	2.0	99.77 ± 0.52	3.8	99.67 ± 0.42	6.3	99.16 ± 0.90	6.7	98.41 ± 1.96
40% (v/v) PG/DW	2.1	99.72 ± 0.06	3.6	98.53 ± 0.83	6.3	101.65 ± 1.09	7.2	98.66 ± 2.19
60% (v/v) PG/DW	2.2	100.24 ± 0.67	3.7	98.28 ± 2.54	7.1	99.92 ± 0.25	7.4	98.85 ± 0.98

[a] Values were determined after storage at room temperature (22 ± 0.5°C) for 24 hr and are expressed as the mean of three samples ± SE.

Table II. Solubility and Skin/Vehicle Partition Coefficient of Isoproterenol HCl at 22 ± 0.5°C

Vehicle[a]	N	pH[b,c]	Solubility (C) (mg/ml)[b]	Partition coefficient (P)[b]	C × P
Distilled water	6	3.9 ± 0.1	389.0 ± 8.5	0.546 ± 0.083	212.5
10% PG/DW	2	3.9 ± 0.3	357.8 ± 5.6	0.465 ± 0.067	166.5
20% PG/DW	2	4.0 ± 0.1	335.1 ± 10.3	1.247 ± 0.067	418.1
40% PG/DW	2	4.1 ± 0.3	288.3 ± 2.2	1.438 ± 1.314	414.4
60% PG/DW	2	4.3 ± 0.4	243.3 ± 2.0	1.527 ± 0.353	371.6
100% PG	6	3.9 ± 0.1	91.6 ± 3.4	5.066 ± 1.341	464.1
Azone	3		8.7 ± 0.1		

[a] All percentages are expressed as v/v.
[b] The tabulated values are expressed as the mean ± SE.
[c] Corning Model 125 pH meter with glass electrode and Ag/AgCl internal reference electrode.

plenished with fresh normal saline every 2 hr or less except for the 24-hr data point and assayed immediately for isoproterenol HCl content.

The solubilities of isoproterenol HCl in distilled water, propylene glycol, Azone, and 10, 20, 40, and 60% (v/v) propylene glycol in water at 22 ± 0.5°C are shown in are Table II. The solubility of isoproterenol HCl in propylene glycol–water systems was found to follow the following relationship:

$$\text{solubility (mg/ml)} = 394.39 - 2.8\ (\%\ \text{PG}) \quad (4)$$

The skin/vehicle partition coefficient of isoproterenol HCl was found to increase as its solubility decreased with increasing percentages of propylene glycol in the vehicle (Table II). For the formulations tested, the flux, J, would be directly proportional to the product of the drug solubility in the vehicle, C, and its partition coefficient, P, as predicted by Eq. (5) if the skin thickness, h, and diffusion coefficient, D, remained constant.

$$J = D \cdot P \cdot C/h \quad (5)$$

As shown in Table II, the product $P{\cdot}C$ dropped initially and then increased dramatically as the propylene glycol concentration reached 20%. Further increases in the propylene glycol concentration did not show as significant an improvement in $P{\cdot}C$ values, although the maximum $P{\cdot}C$ value was recorded for the pure propylene glycol vehicle. The observed flux values (Table III) are consistent with the $P{\cdot}C$ data in that fluxes increased initially and then remained essentially constant for formulations containing 20 to 100% propylene glycol. It would appear that the increased partitioning of isoproterenol HCl with increasing propylene glycol concentrations and previously reported (11,12) decrease in the diffusional resistance of the skin by propylene glycol did not adequately compensate for the decreased solubility.

Table III. Effect of Propylene Glycol on *in Vitro* Percutaneous Penetration of Isoproterenol HCl

Propylene glycol (%, v/v)	Flux (J) (μg/cm²/hr)[a]	Permeability coefficient (K_p) (cm/hr × 10^{-3})[b]
0	14.70 ± 1.01	0.378
20	21.56 ± 0.78	0.643
60	21.59 ± 2.55	0.887
100	19.70 ± 1.91	2.150

[a] The tabulated flux values are reported ±95% confidence limits; correlation coefficient, $r > 0.997$.
[b] Calculated from $J = K_p C$.

Effect of Azone

Comparison of flux values obtained from suspensions of 1, 5, and 10% (v/v) Azone and excess drug in 20% (v/v) propylene glycol in water revealed no statistically significant differences, although the suspension formulation with 1% Azone had the maximum flux. When the skin diffusion study was conducted after gelling the same suspension formulations with 4% (w/w) hydroxypropyl cellulose, in the absence of Azone, gelling the suspension decreased the flux as revealed by comparison of fluxes for control 1 and control 2 (Table IV). Furthermore, the incorporation of Azone in the gel formulations more than compensated for the increased diffusional resistance of the gel, resulting in an overall increase in flux. As in the case of the suspension formulation, the gel with 1% Azone was most effective. In order to ex-

Table IV. Effect of Azone on *in Vitro* Percutaneous Penetration of Isoproterenol HCl from Gels

Azone concentration (%, v/v)	Flux (J) (μg/cm²/hr)[a]	Test for parallelism	
		Control 1	Control 2
Control 1[b]	42.93 ± 6.56	—	—
Control 2[c]	13.64 ± 1.84	—	—
1[d]	104.16 ± 4.38	$P < 0.01$	$P < 0.01$
5[d]	95.11 ± 4.44	$P < 0.025$	$P < 0.025$
10[d]	86.69 ± 10.29	$P < 0.05$	$P < 0.05$

[a] The tabulated flux values are reported ±95% confidence limits; correlation coefficient, $r > 0.991$.
[b] Suspension of isoproterenol HCl in 20/80 PG/water without Azone.
[c] Suspension of isoproterenol HCl in 20/80 PG/water gelled with 4% (w/w) hydroxypropyl cellulose.
[d] Control 2 formulation with 1, 5, and 10% (v/v) Azone, respectively.

Table V. Effect of Azone Pretreatment on *in Vitro* Percutaneous Penetration of Isoproterenol HCl

Pretreatment time (min)	Flux (J) ($\mu g/cm^2/hr$)[a]	Test For parallelism
Control[b]	12.97 ± 3.78	—
3	12.22 ± 2.25	$P > 0.35$
30	23.16 ± 3.94	$P < 0.025$
60	23.85 ± 0.89	$P < 0.025$
720	19.13 ± 1.12	$P < 0.01$

[a] The tabulated flux values are reported ±95% confidence limits; correlation coefficient, $r > 0.988$.
[b] Untreated skin.

amine whether the observed enhancement of drug penetration by Azone was due to lowered skin resistance to drug diffusion, another study was conducted. In this study the skin was pretreated with Azone for periods of 3, 30, 60, and 720 min, prior to the application of a suspension of isoproterenol HCl in 20% propylene glycol in water. The mean steady-state flux values of each treatment period were compared with that of untreated control skin from the same donor. The results (Table V) revealed that fluxes across 30- and 60-min pretreated skin were highest and were significantly different ($P < 0.025$) from the untreated control, in agreement with the work of BenKorah *et al.* (13) on benzocaine. When a suspension of excess isoproterenol HCl in 20% propylene glycol–water was applied to 60-min Azone-pretreated skin and untreated skin and a similar formulation with 1% Azone was applied to another untreated skin from the same site of the same donor (Fig. 1), the mean steady-state fluxes were, respectively, 23.85 ± 0.89 (r = 0.9999), 12.97 ± 3.78 (r = 0.9877), and 16.15 ± 2.41 (r = 0.9943), confirming that pretreatment of skin was more effective in enhancing the penetration of isoproterenol HCl through human cadaver skin than the incorporation of Azone in the formulation and strongly suggesting a direct effect of Azone on the skin barrier.

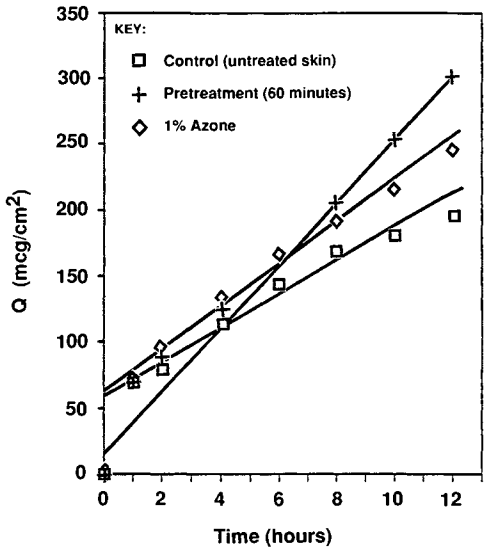

FIG. 1. Effect of Azone on *in vitro* percutaneous absorption of isoproterenol HCl.

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