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EFFECT OF pH ON ENHANCEMENT OF IN VITRO PERCUTANEOUS TRANSPORT OF ISOPROTERENOL HCL BY AZONE

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ABSTRACT

This in vitro study examined the effect of Azone on skin permeability of isoproterenol HCl at pH of 2.0, 8.0, 8.5 and 9.0. Azone was found to enhance the percutaneous transport of the drug from an aqueous vehicle under the pH conditions studied. The flux across human cadaver skin increased with increasing vehicle pH for Azone-treated and untreated skin with an observed maximum at pH 9.0.

INTRODUCTION

The passive diffusion process across the skin favors neutral molecules or the uncharged form of ionizable molecules due to their lipophilicity [1,2,3] Therefore the transdermal flux of the

ionizable drugs can be affected by changing the pH of the diffusional environment and the resulting alteration in the ratio of uncharged to charged species. In an earlier study[4], it was shown that Azone enhances the penetration of isoproterenol HCl across human cadaver skin. Pretreatment of the skin with Azone prior to application of the drug formulation was shown to be more effective in facilitating drug diffusion than incorporating Azone in the formulation. In the present study, effect of pH on the transdermal diffusion of isoproterenol HCl from an aqueous vehicle across Azone-treated and untreated excised human skin was examined at pH 2.0, 6.0, 8.5, and 9.0 in order to gain further insight into percutaneous penetration enhancing effect of Azone on various forms (ionic vs. nonionic) of this compound and to define optimal conditions for transdermal delivery of isoproterenol HCl.

MATERIALS AND METHODS

Assay Method

Analyses of samples for isoproterenol HCl 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, NY). The chromatograph (Waters Associates, Milford, MA) was equipped with a 6000-psi pump, a variable wavelength detector, a loop

injector and an automated integrator 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 20% v/v solution of methanol (J.T. Baker Chemical Co., Phillipsburg, NJ) in water containing 2% acetic acid with 0.005 M sodium 1-heptane-sulfonate (K and K Laboratories, Plainview, NY) at pH of 2.6.

Skin Diffusion Studies

The human cadaver skin samples were prepared [7,8] and diffusion studies were conducted in duplicate using a special glass diffusion cell [9] wrapped in aluminum foil in order to protect from light . Appropriate formulation (1.5 ml) was applied to the exposed skin surface (2.01 cm^2). Normal saline maintained at 37° ± 0.5° was used as the receptor fluid. At selected time intervals (1, 2, 4, 6, 8, 10 and 12 h) the receptor fluid was completely withdrawn and immediately assayed spectrophotometrically for the drug content. Random samples were also assayed by HPLC to monitor any unexpected decomposition of isoproterenol HCl.

a. **Effect of the pH of the Vehicle on Excised Human Skin:**

Twelve diffusion cells prepared from two separate pieces of skin were divided into three groups of four cells each. The mean flux from suspensions of isoprot-

erenol HCl in distilled water was determined for each group after a 12-h study. Then the donor and receptor chambers were washed with distilled water and the receptor chambers were refilled with fresh normal saline. The donor chambers were treated for 12 h as follows: Group 1 with distilled water (control), group 2 with buffered solution of pH 2, and group 3 with buffered solution of pH 10. Then again the donor and receptor chambers were washed with distilled water and the skin diffusion study was conducted with aqueous suspension of isoproterenol HCl. The mean flux values calculated for each group were compared with flux values for the same skin sample prior to exposure to respective buffer. The data were analyzed statistically by ANOVA. This experimental design was dictated by the size of the available skin samples.

b. Effect of pH on Percutaneous Penetration of Isoproterenol HCl:

Five sets of penetration experiments were carried out in order to have meaningful results. In each set, 10 diffusion cells were prepared using abdominal skin samples from the same site of the same donor - two for each buffered solution (pH 2.0, 8.0, 8.5, and 9.0) containing known amount of isoproterenol HCl and two for control (suspension of isoproterenol in distilled water). The skin diffusion study was conducted and the

mean flux values were calculated after a 12-h study. Then the donor and receptor chambers were rinsed with distilled water and normal saline respectively and the epidermal side was exposed to neat Azone for an hour, washed with ethanol (5 x 1 ml) and then distilled water (5 x 1 ml) to remove excess Azone. The donor side was then refilled with same (freshly prepared) buffered formulations and the diffusion study was repeated. Mean flux for each buffered vehicle across Azone-pretreated skin samples were calculated and compared with flux values from untreated skin.

RESULTS AND DISCUSSION

The effects of 12-h exposure of human cadaver skin to buffered solutions of pH 2 and pH 10 *per se* were examined by comparing the flux values of isoproterenol HCl (suspension in distilled water) before and after treatment of the skin sample with a solution of respective pH. The data (Table I) showed that there was no significant difference ($P > 0.05$) in the flux values as checked by testing the parallelism of the steady-state regions of the two curves (before and after respective treatment), although there was significant difference in the amount penetrated per time interval as tested by paired t-Test. Having established the effect of pH 2 and 10 buffer and duration of study on the skin, the same experimental design was used to study the effect

TABLE I

Effect of pH of the Vehicle on Human Cadaver Skin

Time, h	Q (mcg/cm^2)					
	Group I		Group 2		Group 3	
	Untreated	Treated, pH 6.9	Untreated	Treated, pH 2	Untreated	Treated, pH 10
0	0.00	0.00	0.00	0.00	0.00	0.00
1	77.20	23.15	88.68	12.00	67.74	13.64
2	168.24	75.27	134.80	20.93	91.17	21.22
4	321.11	168.90	190.62	43.02	114.71	30.24
6	491.57	320.19	259.22	94.90	135.26	53.10
8	682.79	470.47	340.89	161.87	155.24	75.67
10	849.21	628.68	435.72	238.64	176.85	100.55
12	1020.25	818.16	523.16	321.65	190.00	129.18
Flux (J), $mcg/cm^2/h$	86.22	80.35	39.41	35.05	10.83	12.27
95% C.L.	± 3.22	± 7.20	± 4.97	± 5.65	± 1.58	± 1.14
Correlation Coefficient, (r)	0.9996	0.9988	0.9959	0.9962	0.9921	0.9987
Test for Parallelism		P > 0.05		P > 0.05		P > 0.1
Paired t-Test[a]		P < 0.001		P < 0.001		P < 0.001

[a] Paired t-Test was applied on mean amount penetrated at each time interval.

TABLE II

Effect of pH on In Vitro Percutaneous Penetration of Isoproterenol HCl

pH	N[2]	Mean Flux[1] (mcg/cm²/hr) Untreated Skin	Azone-Treated Skin	T Test
2.0	5	0.0136(0.0040)[3]	0.0200(0.0053)	P>0.3
6.0	4	0.0204(0.0030)	0.0227(0.0025)	P>0.5
8.5	5	0.0193(0.0036)	0.0422(0.0101)	P>0.05
9.0	4	0.0219(0.0049)	0.0724(0.0109)	P<0.01

[1] Normalized for a concentration of 100 mg/ml
[2] Number of Experiments
[3] The numbers in parentheses represent the standard error of the mean.

of Azone on skin permeability towards various ionic and nonionic forms of isoproterenol HCl under varying conditions of pH. Each experimental set included two penetration studies on the same sample of skin at each pH (2, 8, 8.5, and 9) before and after treatment with Azone enabling comparison of fluxes at four pH values with each other. Since initial drug concentrations in various formulations were not identical but approximately similar within each study, the fluxes were normalized for comparison. As shown in Table II, the flux improved with increasing pH for both, the untreated skin and the azone-pretreated skin. The formulations with pH 9 exhibited the highest flux across the untreated and azone-treated skin. These results are in agreement with the theoretical plot of concentration of

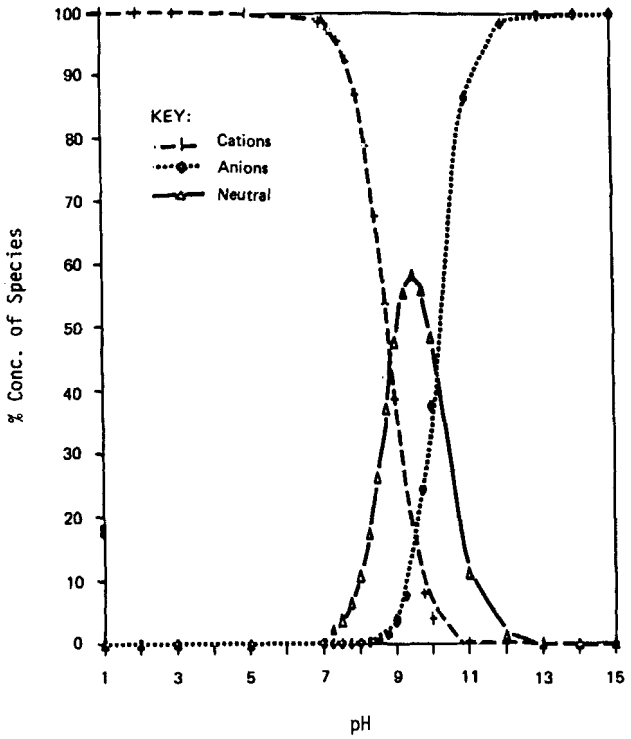

FIGURE 1
The Concentration of Nonionic and Ionic Forms of Isoproterenol HCl as a Function of Vehicle pH

various species of isoproterenol as a function of pH illustrated in Figure 1. Isoproterenol at any given pH exists as an equilibrium mixture of cationic, anionic, uncharged and zwitterionic forms. The concentration of neutral (uncharged and zwitterionic) species would be maximum around pH of 9 and the concentration of anions would begin to predominate beyond this pH value. The penetration enhancement effect of Azone was most pronounced at pH 9.0 (Table II). Previous investiga-

tions of BenKorah et. al. on percutaneous absorption of benzocaine in presence of Azone[10] and others[11,12] have strongly suggested that Azone enhances percutaneous penetration by lowering the skin's resistance to diffusion of penetrant molecules possibly by increasing the fluidity of stratum corneum lipids.

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