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THE EFFECT OF SKIN PENETRATION ENHANCERS ON THE TRANSDERMAL DELIVERY OF PYRIDOSTIGMINE BROMIDE

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ABSTRACT

In vitro skin penetration studies of pyridostigmine bromide through human cadaver skin were conducted using a diffusion cell with constant hydrodynamic conditions. The results indicate that the drug penetrates poorly through human cadaver skin. However, skin penetration enhancers such as sodium oleate, sodium lauryl sulfate, n-decyl methyl sulfoxide, and N,N-dimethyldodecylamine-N-oxide substantially enhanced the permeability coefficient of the drug through human cadaver skin. The penetration enhancement of pyridostigmine bromide could be due to increased partitioning of the drug in the skin or due to the decreased tortuosity of the porous pathway in the stratum corneum.

INTRODUCTION

Drug penetration across the skin is usually by passive diffusion, and the stratum corneum usually limits the rate of drug transport[1]. There is considerable interest in the percutaneous administration of drugs which suffer from poor oral absorption and/or high oral first pass effect[2].

Pyridostigmine bromide, an anticholinergic drug, is poorly absorbed from the gastrointestinal tract[3]. Delivery of this drug via the transdermal route could be preferable; however, being a polar hydrophilic drug, pyridostigmine bromide is not likely to permeate at a high rate through the skin.

In this study, the permeability of pyridostigmine bromide through human cadaver skin was investigated. Several excipients including anionic and nonionic surfactants were studied as possible skin penetration enhancers.

EXPERIMENTAL

A. Materials:

1. Human Cadaver Skin: Split thickness (~300 μ) human cadaver skin was dermatomed within 48 hours of death and

further prepared as described under procedure for skin preparation.

2. Chemicals: Pyridostigmine bromide (Hoffmann-La Roche, Inc., New Jersey), n-decyl methyl sulfoxide (Columbia Organic Chemical Co., Inc., South Carolina), N,N-dimethyldodecylamine-N-oxide (Fluka AG., West Germany), sodium lauryl sulfate (Henkel KGaA., West Germany), sodium oleate (J. T. Baker Chemical Co., New Jersey), sodium chloride AR (EM Science, New Jersey), and 1,2-butanediol (Aldrich Chemical Co., Inc., Wisconsin) were obtained commercially.

3. Sodium Chloride Solution: A 0.9% sodium chloride solution (normal saline) in deaerated and deionized water was used as the receptor phase.

4. Drug Solution: A solution concentration of 100 mg/ml in normal saline was used.

5. Diffusion Cell: A two compartment diffusion cell prepared by Crown Glass Company, New Jersey, was used for the studies. Both compartments of the cell are jacketed for maintaining a constant temperature during the experiments. A temperature of 32°C was maintained

with a circulating water bath. The two cell halves were assembled using clamps and end screws, with the human cadaver skin sandwiched between the cells. The volume of each half cell was approximately 3.5 ml, and the effective diffusional area was approximately 0.7 cm^2. The receptor and the donor compartments contained normal saline and drug in normal saline respectively. The solutions in both compartments were kept stirred using magnetic stir bars at high and constant speed, and the receptor medium was kept under sink conditions during the experiment.

B. Procedure For Skin Preparation: The skin samples were dermatomed at 300 μ from the abdomen of human cadavers within 48 hours of death. The dermatomed skin samples were soaked in 15% glycerol containing 80 mg/ml gentamycin. The skin samples were stored at a suitable temperature (-30°C) until used. The skin samples were taken out of the freezer and allowed to thaw at room temperature. Once they thawed out, the skin samples were soaked in normal saline for 0.5 hour and carefully checked for any macroscopic damage using a magnifying glass. A square section of the skin (~3 cm^2) was positioned between the two half cells with the stratum corneum side facing the donor compartment. After the skin was mounted between half cells, the cells were made water tight by using a clamp and the excess skin trimmed off using scissors.

C. In Vitro Skin Penetration Test Procedure: A 3.5 ml volume of normal saline, and drug (with or without enhancers) in normal saline were introduced in the receptor and donor compartment, respectively. During the permeation studies a one milliliter aliquot was withdrawn from the acceptor compartment at regular time intervals and replaced with the same volume (1 ml) of normal saline at 32°C. The concentration of the drug in the receptor compartment was determined as a function of time using a HPLC assay procedure. Permeation studies were conducted at least in triplicate.

D. Analytical Method: A liquid chromatograph equipped with a Varian model 5000 pump, Varian UV 200 variable wavelength detector, Varian 8500 auto sampler, and an electronic integrator was used in the study.

Drug analysis was accomplished using a reversed phase Hamilton PRP-1 column, 10 μm particle size, and a mobile phase consisting of 15% acetonitrile, 84.5% water and 0.5% acetic acid at a flow rate of 1.5 ml per minute. The drug was detected using a UV detector at 269 nm. The retention time of the drug, under these conditions, was approximately 4.5 minutes.

Drug concentration in the sample solution was determined by comparing the peak area of the drug to a calibration curve

constructed from the peak area of a series of standard solutions.

RESULTS AND DISCUSSION

The cumulative amount (μg) of the drug penetrated through unit surface area at different times was calculated. The permeability coefficients of the drug in the presence and absence of the skin penetration enhancers were calculated using the steady state portion of the curve and are given in Table I.

Pyridostigmine bromide being a polar hydrophilic molecule showed poor permeability across human cadaver skin. However, the permeability coefficient of the drug increased substantially (50-200 fold) in the presence of sodium lauryl sulfate in the donor compartment of the penetration cell. An increase in the permeability coefficient of pyridostigmine bromide was also demonstrated with sodium oleate. A maximum permeability coefficient of the drug of 355.1×10^{-5} cm/hr was obtained at a sodium oleate concentration of 10 mg/ml. The permeability coefficient decreased when the concentration of sodium oleate was raised from 10 to 50 mg/ml. This could be attributed to micelle formation at a sodium oleate concentration of 50 mg/ml.

In vitro skin penetration studies were also conducted with excipients such as n-decyl methyl sulfoxide, N,N-

TABLE I

Effect of Skin Penetration Enhancer on Pyridostigmine Bromide Permeability Across Human Cadaver Skin

Concentration of pyridostigmine bromide (mg/ml)	Enhancer	Concentration of enhancer (mg/ml)	$P \times 10^5$ (cm/hr) mean ± standard deviation
100	--	--	2.08 ± 0.99
100	Sodium lauryl sulfate	1	110 ± 55.3
		1.5	438 ± 297
100	Sodium oleate	1	24.6 ± 9.01
		5	235 ± 53
		10	355.1 ± 89.8
		50	81.2 ± 23.2
100	n-decyl methyl sulfoxide	1	74.62 ± 30.5
		2	110.83 ± 12.3
		5	881 ± 281
		10	1482 ± 563
		20	1363 ± 272
100	N,N-dimethyldodecyl-amine-N-oxide	1	102 ± 2.52
		2	283 ± 62
		10	396 ± 247
100	1,2-butanediol	50	2 ± 1

dimethyldodecylamine-N-oxide, and 1,2-butanediol. The permeability coefficient of the drug was increased with increasing concentrations of n-decyl methyl sulfoxide up to 10 mg/ml; however, no further increase in the permeability coefficient was achieved at 20 mg/ml of n-decyl methyl sulfoxide. The permeability coefficient of pyridostigmine bromide in the presence of 20 mg/ml of n-decyl methyl sulfoxide was 1367 x 10^{-5} cm/hr, an increase of 681 fold over the control containing no penetration enhancer.

A similar large increase (197 fold) in the permeability coefficient was achieved when N,N-dimethyldodecylamine-N-oxide was used as a penetration enhancer.

At a concentration of 50 mg/ml, 1,2-butanediol did not increase the permeability coefficient of the drug.

In conclusion, sodium lauryl sulfate, n-decyl methyl sulfoxide, N,N-dimethyldodecylamine-N-oxide, and sodium oleate increased the permeability of pyridostigmine bromide through human cadaver skin. Pyridostigmine bromide may form nonpolar ion-pairs with nonionic surfactants such as sodium lauryl sulfate or sodium oleate. The ion-pairs could partition better in the stratum corneum due to high stratum corneum/water partition coefficient resulting in increased permeability of the drug.

The penetration enhancement of pyridostigmine bromide in the presence of surfactants through the skin could also occur due to the opening up of the pores in stratum corneum (4). In essence, the tortuosity of the porous pathway could be significantly decreased by polar surfactants resulting in higher permeability of the drug.

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