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CONTROLLED TRANSDERMAL MUCOLYTIC DELIVERY SYSTEM : IN VIVO PERFORMANCE

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ABSTRACT

A multicomponent transdermal bromhexine delivering system was developed. The drug permeation across the human cadavar skin from either sexes was determined and found to be dependent on age, sex and site of skin. The system that could control the the delivery of the drug to allow it to permeate across the skin at a rate that is required to achieve the therapeutic concentration was selected and chosen for in vivo performance evaluation. Prior to in human volunteers availability testing the designed system was evaluated in rabbits and the system exhibiting desirable performance was redesigned for evaluation in human volunteers. $T.P.S_0$ system delivering 1.991 ± 0.116 mg bromhexine/cm^2/hr with a 453 $\mu g/cm^2$ priming dose was tested in human volunteers and found to maintain bromhexine blood level at or around peak plasma level for 20-24 hrs. The study reveals that bromhexine holds potential for transdermal delivery with a need of further clinical and pharmacodynamic studies.

INTRODUCTION

The ideal way to determine the Transdermal delivery potential of a compound in human being is to perform actual studies in humans. The mechanism and parameters of transdermal delivery elucidated in in vivo with human skin are most relevant to clinical studies (1). Lipophilic drugs such as indomethacin, nitroglycerine and isorbide dinitrate are frequently being used for development of their transdermal therapeutic systems because of their intrinsic high across the skin permeability (2,3). The prolonged and steady state plasma level of lipophilic drug, nitroglycerine could be achieved and maintained for a duration of 24 hours to 32 hours by controlled drug delivery through its transdermal therapeutic systems (4).

The object of the treatment with Bromhexine is to reduce airways resistance. It helps in the chronic bronchitis and chronic asthma by thining bronchial mucus which leads to clearance of the airways by facilitating expectoration. Bruce and Kumar (5) have discussed the therapeutic indications of the drug attributed to aforementioned characteristics, in the management of bronchopulmonary diseases. The absorption, excretion and metabolism of Bromhexine in man after oral and I.V. administration showed that bromhexine disappeared from the blood rapidly after i.v. injection. This was explained by the lipophilic properties of bromhexine that is noted to form depot in deep tissues. On oral administration, maximum plasma concentration is achieved in one hour thereafter comparatively higher level of drug is maintained as compared to the drug plasma levels produced following intravenous administration (6).

The incorporation of surface active agents is reported to improved drug stability, clinical potency, absorption and drug toxicity (7). Two transdermal systems, i.e. film system and pseudolatex system were prepared. The pseudolatex system containing surfactant was choosen for in vivo characterization.

The transport of drug can vary not only with sex age but also with the skin site of application (8). Thus a multicomponent system with a rate controlling membrane that limits and regulate the amount of drug delivered from the dosage form was prepared and evaluated. The release rate controlling membrane maintains the drug release at a desired rate that is below the inherent drug skin permeation rate.

MATERIALS AND METHODS

Bromhexine hydrochloride (IPCA Laboratories Bombay, India), Bisolvon Tablets (German Remedies, Bombay, India), Heparin (Sigma Chemical Company, St. Louis MO, U.S.A.). All the solvents and chemicals used were of Analar grade as supplied by E.Merck India Ltd.,

In-vitro Skin Permeation Kinetics Across Cadaver Skin :

Preparation of Skin : Full thickness (500-600 μm) abdominal and hip human cadaver skin from either sexes was used (Medical College, Indore, M.P. India) within 48 hours of death. The skin was stored in deep freezer until used. Prior to utilization the skin was thawed out by soaking into the normal saline at 37 ± 1°C for 0.5 hours.

In-vitro Skin Permeation Experiments :

The in vitro transdermal transport of the drug has been estimated using vertical Franz diffusion cell. Male/female human cadaver skin was interposed between delivery system and receptor compartment. The buffer (7.4 pH) was continuously perfused via peristaltic pump set at a rate 10 ml/hr. The aliquots from receiver compartment were collected at an hourly intervals for 24 hours. The contents of receiver compartment were stirred with a teflon coated magnetic bar. The experiment was carried out for 24 hours while the temperature of diffusion cell was maintained at 37 ± 1°C. The results are presented as a mean of 3 experiments (Table I).

TABLE I

Formulation Composition and Bromhexine hydrochloride Permeation across Cadaver Skin of both the sexes (Male and Female)

Product	Content	Skin permeation^{n} mg/cm^{2}/day Male Cadaver Skin^{N}	Female Cadaver Skin^{N}
T.P.S$_0$	EURL-100:PVP:Drug 20:80:0.15	1.991 ± 0.116	1.989 ± 0.123
T.P.S$_1$	EURL-100:PVP:Drug 50:60:0.15	0.968 ± 0.061	0.969 ± 0.066
T.P.S$_2$	EURL-100:PVP:Drug 60:40:0.15	0.846 ± 0.052	0.844 ± 0.056
T.P.S$_3$	EURL-100:PVP:Drug 80:20:0.15	0.658 ± 0.039	0.656 ± 0.042

N = number of different skin samples tested : 8,
Age: Male 45 - 68 ± 8.6, Female 45 - 65 ± 8.0 years

n = number of perfusate sampling intervals (25-30 minutes) per skin sample : 6.

In-vivo Studies

Animal Specification : Male and female white rabbits weighing 2.5 to 3.5 kg were used as test animals for in vivo performance and evaluation of the developed system.

(a) Oral Administration : Bisolvon , a marketed product was given to rabbits at a dose of 0.6 mg/kg body weight in an aqueous dispersion form through a stomach feeding tube no. 2. Blood samples were collected periodically for 24 hours after dosing and stored frozen till estimated for drug concentration. In the collected samples the drug content was determined after separation of plasma using gas liquid chromatography method.

(b) Transdermal Administration : The hair of the back area of the animals were removed with electric clipper without damaging stratum carneum. The trilaminated 3.0 cm^2 dosage form was designed using the product T.P.S$_1$ (Fig. 1). The formulation was applied on dorsal region 24 hours after hair removal. The designing of product used in in vivo study was the same as discussed for one that was used in in vitro permeation

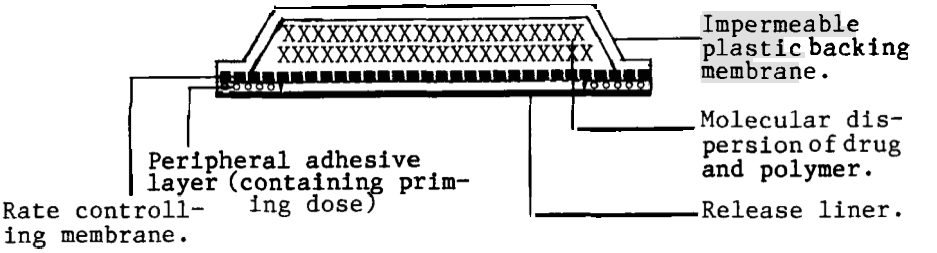

Fig 1 : Transdermal Pseudolatex System (T.P.S.)

studies except an adhesive rim loaded with the priming dose of Bromhexine hydrochloride. The formulation remained in contact with the skin for 24 hours.

Drug Estimation :

Bromhexine in plasma samples (1 ml) was determined by the method of Leehneer et al. by Gas Liquid Chromatography (9) using Hewlett Packard Gas Chromatograph (Model No. 5710).

Transdermal Bioavailability of Drug in Human Volunteers :

(a) Oral Administration : Tablet Bisolvon was administered to 10 healthy male and female human volunteers having no premedication history, of 30 to 38 years age and 60 to 72 kg body weight.

(b) Transdermal Application : The abdomen region was selected as the site for application. Transdermal formulation $T.P.S_0$ (8 cm^2) was applied for 24 hours then after it was removed.

(c) Drug Estimation : The blood samples (5 ml) were taken from median cubital vein after addition of 1 drop of heparin solution (5000 unit/ml in 0.4% physiological saline). The collected samples were centrifuged for 15 min at 5000 rpm. Drug concentrations was estimated in plasma by gas liquid chromatography.

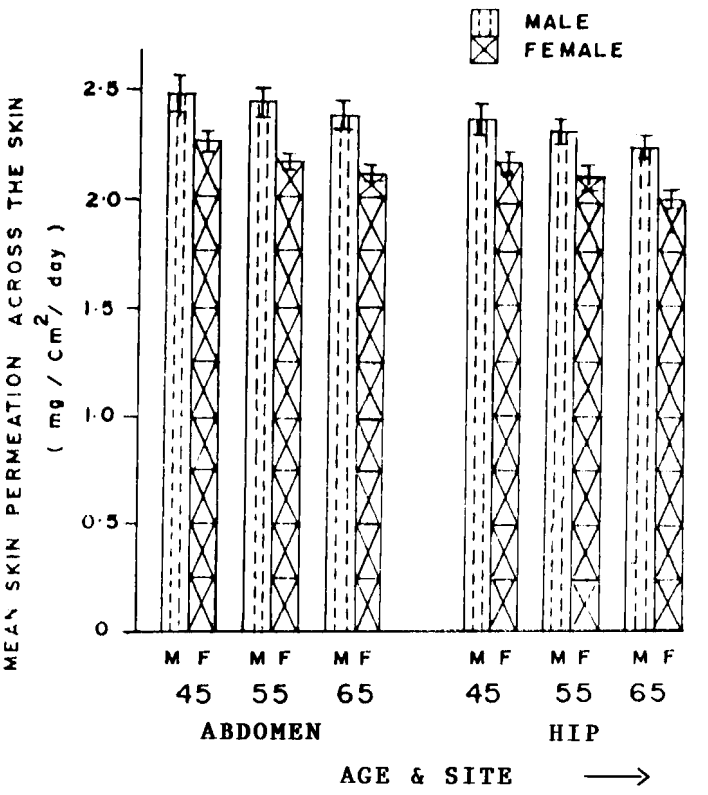

Fig 2 : Effect of age, skin site and sex on the in vitro transdermal flux of Bromhexine hydrochloride (at 37 ± 1°C) provided by pseudolatex system (T.P.S_0 polymeric composition) without rate controlling membrane. Each bar represent the mean ± S.D. skin permeation (mg/cm^2/day). The age groups for males 45 - 65 ± 8.6 and for females 45 - 65 ± 8.0 years.

RESULTS AND DISCUSSION

The effect of age, skin site and source of skin on drug permeation obviously demand the need of developing a family of transdermal systems that could eliminate the effects of aforementioned variables and vis a vis delivers the drug at a defined rate in a controlled manner. The effect of variables on drug permeation was observed in in vitro studies with the pseudolatex system. Bar diagram (Fig. 2) shows that the effect of age and

site on in vitro permeation of drug was significant especially in the case of skin collected from female cadavers as compared to the male cadavers skin. Two sites were selected for the studies i.e., abdomen and hip. However, significant Inter-subject variation was also noted in male subjects as well ($P < 0.05$).

The prepared multicomponent transdermal drug delivery systems were investigated for the effect of above variables. The results reveal (Table 1) no significant change in in vitro across the skin permeation rate of drug, irrespective of source of skin. This behaviour could be attributed to the constructive features of the developed system where the rate controlling membrane limits and controls the amount of drug delivered by the formulations at required permeation rate that is well below the inherent drug across the skin permeation rate (0.098 $mg/cm^2/hr$).

The trial transdermal drug delivery system $T.P.S_0$ and $T.P.S._1$ of the polymeric combinations were selected for the preparation and in vitro permeation study across the skin. The in vitro evaluation of $T.P.S_1$ shows 0.968 ± 0.06 $mg/cm^2/day$ in in vitro permeation rate and found suitable for being closer to the calculated required rate to achieve the Cp 0.30 µg (0.504 mg/day). Three cm^2 patch releases the drug across the skin at the rate 0.597 ± 0.37 mg/day. The priming dose (114 $\mu g/cm^2$) calculated on the basis of pharmacokinetic parameters and drug solubility in the skin was incorporated in the peripheral adhesive layer.

Bisolvon tablet weighing 0.6 mg/kg body weight was given to the animals through oral route to compare the relative availability of the drug with the dermal route. The C_{max} following the oral administration was recorded in 2.5 hours, whilst in the case of transdermal application it was achieved in 4.0 hours. The drug plasma profile following oral and transdermal treatments showed (Fig 3) that the drug plasma concentration t_{max} in the case of oral treatment was 21.9 ± 1.09 ng/ml which was lower than the concentration recorded at t_{max} in the case of transdermal

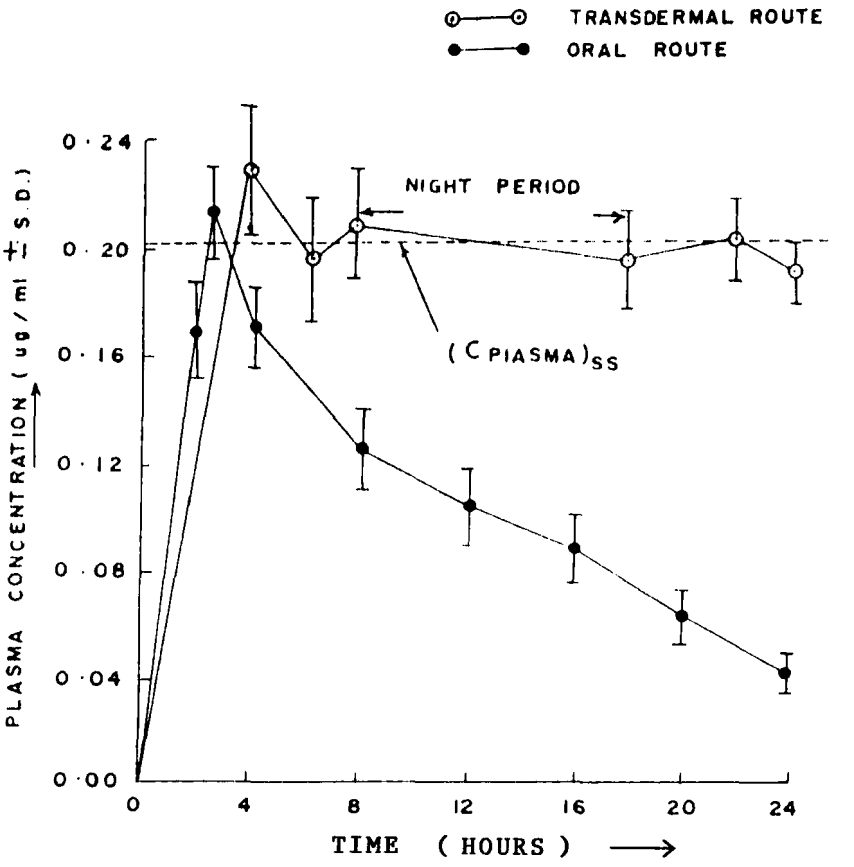

Fig 3 : Plasma profile of Bromhexine hydrochloride in 12 rabbits following oral administration 0.6 mg/kg and transdermal application (3 cm^2).

treatment (23.0 ± 1.11 ng/ml). The steady state concentration was calculated (Cpss 21.013 ± 0.501 ng/ml). It was noted that the attained C_{max} (21.9 ng/ml ± 1.19) in oral treatment persisted for a short time and then the plasma drug concentration continued to decline whilst in the case of transdermal treatment it was almost maintained above MIC for 20 hours with minimum turfs. The other derived pharmacokinetic parameters are recorded in table II.

TABLE II

Pharmacokinetic Parameters of Bromhexine hydrochloride after Oral and Transdermal Administration in Rabbits

Parameters	Oral	Transdermal
C_{max} (ng/ml)	21.90 ± 1.09	23.00 ± 1.11
T_{max} (h)	2.50 ± 0.50	3.70 ± 0.30
Auc (μg.h/ml)	2.47 ± 0.39	4.53 ± 0.85
Duration[(a)]	5.10 ± 0.45	20.00 ± 1.33

a = Time for which effective concentration was maintained (15 to 24 ng).

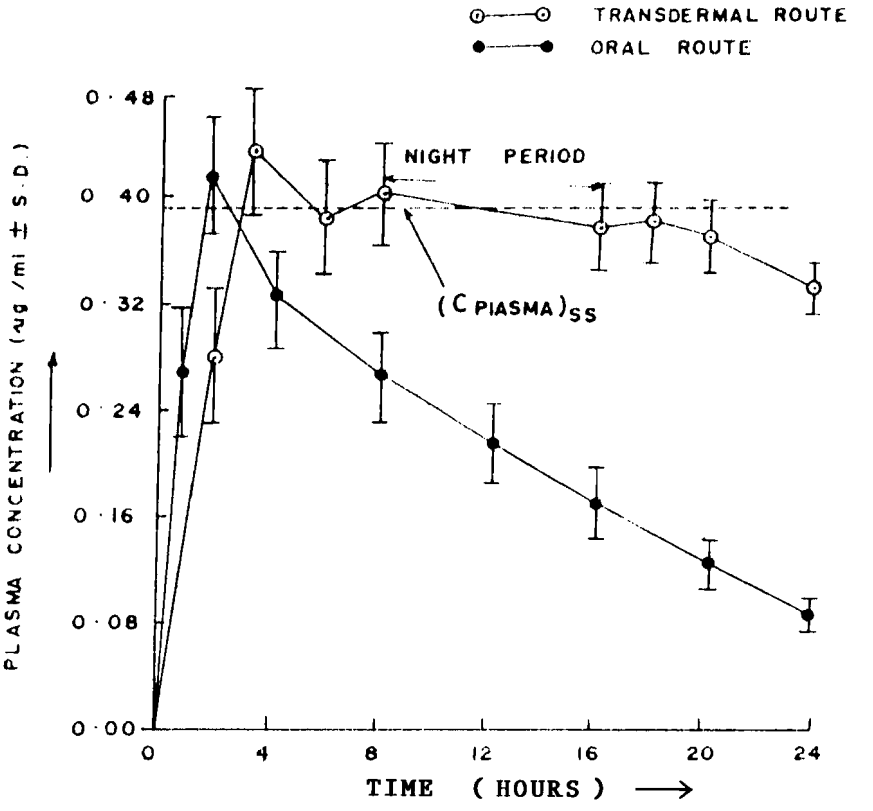

Fig 4 : Plasma profile of Bromhexine hydrochloride in 10 human volunteers following oral administration (8 mg tablet) and transdermal application (8 cm^2).

TABLE III

Pharmacokinetic Parameters of Bromhexine hydrochloride after Oral and Transdermal Administration in Human Volunteers.

Parameters	Oral	Transdermal
C_{max} (ng/ml)	42.10 ± 3.30	43.50 ± 4.20
T_{max} (h)	1.50 ± 0.29	3.00 ± 0.21
Auc (μg.h/ml)	5.07 ± 1.30	9.76 ± 1.85
Duration [(a)]	8.00 ± 2.93	22.00 ± 1.97

a = Time for which a therapeutically effective concentration was maintained (20 to 45 ng).

Formulations $T.P.S_0$ and $T.P.S_1$ exhibited promising *in vivo* performance with regard to plasma profile in test animals was further studied *in vivo*. The required rate calculated for 70 kg human volunteer to achieve 0.40 $\mu g.ml^{-1}$ effective plasma concentration(cp) was found to be 15.76 mg/day. The $T.P.S_0$ 8 cm^2 patch that delivers 15.72 mg of drug in 24 hours, with 453 $\mu g/cm^2$ Bromhexine as priming dose in the contact peripheral adhesive layer was choosen.

The system was applied on the abdomen region of six human volunteers for 24 hours. The Bisolvon tablets weighing 8 mg were given orally to the another ten human volunteers. The periodically collected blood samples were estimated for drug content and drug plasma profile were constructed. The generated plasma profiles (Fig. 4) on comparing reveals that in the case of human volunteers the faster absorption could be established irrespective of the route of administration when compared with rabbits. In the case of oral treatment the C_{max} 42.1 ± 5.3 ng/ml was achieved in 1.5 hours while it was achieved in 3 hours following transdermal application. Once the C_{max} was achieved in case of transdermal route it was maintained for 20 hours around 30 ng/ml that is above the minimum effective concentration. The steady state

concentration (Cpss) was calculated (38.93 ± 1.03 ng/ml). The other derived pharmacokinetic parameters are recorded in table III.

These results indicate that Bromhexine hydrochloride holds promise for transdermal formulation. The multicomponent controlled release systems should be preferred for the lipophilic drugs over other simple T.D.D.S. The feasibility of the drug as transdermal drug delivery system for asthmatic patients needs further clinical and pharmacodynamic studies.

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