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Clinical pharmacokinetic and pharmacodynamic evaluation of transdermal drug delivery systems of salbutamol sulfate

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Abstract

Transdermal drug delivery formulation containing 5 mg/patch of salbutamol sulfate (SS), providing an input rate of 100 μg/h of SS was formulated and subjected for pharmacokinetic and pharmacodynamic evaluation in moderately asthmatic patients ($n = 6$). A linear correlation was observed between cumulative amount of drug diffused in vitro and cumulative AUC_{0-t} of serum concentration–time curve ($R^2 = 0.99$). A steady-state serum concentration of 2.87 ± 0.1 ng/ml (per milligram dose) was attained after an initial lag period of 4.67 ± 1.03 h. The elimination half-life, clearance rate and elimination rate constant was 3.35 ± 1.07 h, 256.12 ± 3.55 ml/min and 0.24 ± 0.09 h^{-1}, respectively. The mean forced expiratory volume in one minute (FEV1) of the patients was 2.2 ± 0.14 l during steady state. The pharmacokinetic results correlated well with the FEV1 response of patients.

Keywords: Salbutamol sulfate; Transdermal delivery; FEV1; Bronchodilation

1. Introduction

Salbutamol sulfate (SS) is one of the widely used drugs for the treatment of bronchial asthma, chronic bronchitis and emphysema (Kelly and Murphy, 1992). The drug undergoes extensive first-pass metabolism and thus requires frequent administrations by oral route (Ahrens and Smith, 1984). The relatively short-term acting injectable and aerosol dosage forms of SS are recommended for instant relief in severe asthmatic attacks. Currently SS is available in the form of aerosols with strength of 100–200 μg. The recommended dose in adults and children is 2–3 inhalations every 4–6 h. More frequent administration is not recommended (Fishwick et al., 2001). A controlled release dosage form may be advantageous over conventional oral dosage forms and inhalers due to its ability to maintain prolonged therapeutic concentrations in the systemic circulation. Asthma being a chronic

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disease, and as most of the patients suffer from nocturnal attacks (Crescioli et al., 1996), there is need for drug delivery systems which maintains therapeutic concentrations for long duration. The aim of the present work was to study the possibility of transdermal controlled delivery of SS. Transdermal drug delivery systems, as compared to their corresponding classical oral, injectable and inhaler systems offer many advantages (Ranade, 1991). The most important advantages are improved systemic bioavailability of drugs because the first-pass metabolism by the liver and digestive system are avoided, and the controlled constant drug delivery profile (that is, controlled zero-order absorption). Other benefits include longer duration of therapeutic action from a single application, and reversible action. For example, patches can be removed to reverse any adverse effects that may be caused by overdosing.

Salbutamol sulfate has a pK_a and log P value of 9.2 and 0.11, respectively (Biddlecombe and Pleasance, 1999). However, appreciable transport of SS has been reported across different skin models (Badcock and Leach, 1989; Rajeev et al., 1982). The transport may be through the porous and parallel pathway of the skin.

The results of pharmacokinetic and pharmacodynamic studies of transdermal formulations of antiasthmatic drugs in animals have been published elsewhere (Murthy and Hiremath, 2001). The present study is an extrapolation of our previous studies to human volunteers. Although the pharmacokinetics of salbutamol from different dosage forms have been investigated in animal models and human volunteers (Powell et al., 1985; Shah et al., 1999), no work seem to have been published on clinical evaluation of the transdermal formulation of SS in patients.

2. Methods

Salbutamol sulfate (SS) was a gift sample from Astra Zeneca LTD, Bangalore, India; hydoxypropylmethyl cellulose (HPMC, 15 cps at 1%, w/v), polyisobutylene was a gift sample from JSRF, Bangalore (India), isopropyl myristate (IPM) and other chemicals were of high purity grade from Loba chemicals, Mumbai, India. The drug and the excipients of the formulation were of clinical grade.

2.1. Preparation of transdermal formulation

The polymer (HPMC, 2%, w/v), the plasticizer (PEG 400, 40%, w/w of polymer), IPM (2%, w/v) and SS (0.1%, w/v) were dissolved in distilled water and casted on a mercury pool and dried at 50 °C for 6 h in an air circulation drier. The film discs of 10 cm^2 area were placed at the center of an adhesive coated aluminium foil (backing layer) and was secured uniformly. Three millilitres of polyisobutylene solution in acetone (50%, v/v) was poured on to the dried films and a thin film of adhesive was formed by solvent evaporation. Each patch of 10 cm^2 area contained 5 mg/patch of drug. IPM was used to enhance the partitioning of drug between polymeric matrix and adhesive phase. It was also found to enhance the transdermal penetration of SS (Fig. 1).

2.2. In vitro diffusion study

In vitro diffusion studies were carried out in Keshary–Chien diffusion cell (Chien, 1987). The receiver compartment was filled with phosphate buffered saline (PBS, pH 7.2). Freshly excised human cadaver epidermis (from the chest region of a male cadaver of age 46 years within 24–48 h post-mortem) isolated by heat separation method was used as the barrier (Berner et al., 1989). In brief, the full-thickness skin was exposed to 60 °C water for 80 s and the epidermis was peeled away from the dermis. The intactness of the epidermis was confirmed by resistance measurements (>20 kΩ/cm^2) and microscopic observation (Murthy et al., 2004). The patch was placed on the stratum corneum side of the epidermis and slight pressure was applied to adhere the patch on the surface uni-

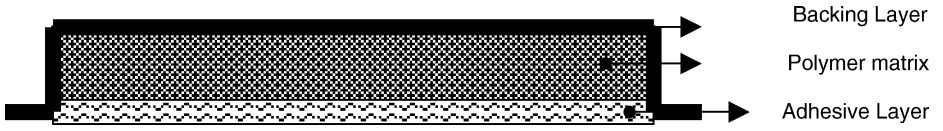

Fig. 1. Representation of different layers of transdermal patch formulation.

Table 1
Demographic details of the patients recruited for pharmacodynamics evaluation of transdermal drug delivery systems of salbutamol sulfate

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age (years)	26.4	30.5	33.8	32.1	28.6	30.8
Gender	M	M	M	M	F	F
Body weight (kg)	66.4	64.8	68.7	60.9	61.2	62.4
FEV1 prior to study	1.5	1.75	1.80	1.61	1.45	1.61
Co-medications prior to washout of 3 days	Theophylline (150 mg tablet once a day)	–	–	–	–	–

formly. The whole system was sandwiched between the donor and the receptor compartments and secured with a clamp. The agitation speed of 50 rpm and temperature of $37 \pm 1\,^{\circ}C$ were maintained during the experiment. The samples were withdrawn from the receiver compartment at 1-h interval up to 4 h and every 2-h interval then after up to 24 h. The samples were suitably diluted or concentrated and the drug concentration was measured by HPLC (Rajeev et al., 1982).

2.3. In vitro skin metabolism

Sections of freshly excised cadaver skin (from the chest region of a male cadaver of age 54 years within 2 h post-mortem) were homogenized (15%) in 1.15% potassium chloride solution (isotonic) using Omni tissue homogenizer. The temperature was maintained at $4\,^{\circ}C$ throughout the operation. The skin homogenates were centrifuged at $10{,}000\times$ g for 20 min at $4\,^{\circ}C$. The supernatant layer was separated and used immediately. Five millilitres of the pre-standardized drug solution of 25 μg/ml was mixed with 0.075 M of tris buffer (pH 7.5) at $37\,^{\circ}C$ for 3 min. Then 6 ml of the tissue homogenate supernatant was added and incubated at $37\,^{\circ}C$ for 1 h. One millilitre of this sample was transferred to a culture tube containing 2 M sodium hydroxide, to immediately quench any enzymatic reaction (Roy et al., 1994). The samples were then centrifuged at $1000\times$ g for 10 min to remove the precipitated proteins. The supernatant was separated and SS present in the supernatant was estimated. In the control experiment the tissue homogenate was replaced with 0.075 M tris buffer. The viability of skin was previously tested by studying the degradation of dexamethasone (Lopez et al., 2000). The enzymatic degradation rate constant of dexamethasone was found to be 5.94 ± 1.1/min at $37\,^{\circ}C$.

2.4. Patients

Six outpatients, (race: Asian (Indians)) otherwise healthy were recruited for the study. They were with a mean age of 30 ± 4 years. All the patients had diagnosed for moderate asthma with a mean duration of 3 years. The demographic details of the patients are given in Table 1. The subjects had been using β-adrenergic agonists for at least 1 year. Signed informed consent from the patients was taken. They were asked not to take any medication 3 days prior to the study and were strictly kept out of habits like smoking and alcohol. The patients had to demonstrate a FEV1 of ≥50–60% of predicted value everyday for about a week before the study (Verma et al., 2002; Marion et al., 2001). They were considered eligible for the study only if the increase in FEV1 was at least 15% with a minimum absolute increase of 200 ml within 15 min after inhalation of 0.5 mg terbutaline sulfate inhaler (which was confirmed a week before the study).

2.5. Protocol

The study was conducted at Bowring and Lady Curzon Hospital, Ministry of health, Government of Karnataka, Bangalore, India, after the approval of the protocol was by the ethics committee. The patients underwent an initial screening visit that included physical examination including vital signs and ECG, an hour before the study. The patients were rested for10 min before taking the base line FEV1 and withdrawal of first blood sample (2 ml) from a suitable vein in the forearm. The transdermal patch was applied onto the anterior surface of the other forearm near the elbow (the transport of SS across chest and forearm regions' skin were not significantly different ($P = 0.062$, unpublished in vitro diffusion data)). Subsequent FEV1 measurements were done and the blood samples were withdrawn at

every 2-h interval up to 30 h. The blood samples were centrifuged immediately after withdrawal at 5000× g and the serum was separated and stored at −20 °C for further analysis. The volunteers were instructed not to remove the patch and also to observe for any sign of irritation at the site of application. The patch was removed after 24 h and the area of application was washed with 1× 3 ml of deionized water and the washings were collected. The amount of drug present in the washings was below the detection level in three patients and was 24, 71 and 43 ng in the other patients, respectively. At the conclusion of the study, physical examination including vital signs and ECG were performed. The patients were discharged after suitable medication to restore reasonably safe FEV1 and they were asked to continue with their regular medication.

2.6. Total transdermal dose

The total dose of SS absorbed transdermally was determined by measuring the remaining drug in the formulation, which had been removed from each patient after 24 h, and unused formulation from the same batch. The error due to the residual drug left on the skin after removal of the patch was considered as unabsorbed fraction during calculation. Formulations either used or unused were first dissolved in 3 ml of hydroalcoholic mixture (1:1). Ten microlitres of this solution was taken into 10 ml of distilled water and the amount of drug present was estimated by HPLC. The recovery efficiency of this method was ∼96%.

2.7. Sample analysis

The method reported by Rajeev et al. (1982), was used for extraction of SS from serum and estimation by HPLC. In brief, the mobile phase was methanol:0.05 M pentanesulfonic acid, (6:94 v/v, pH 2.5). The flow rate of the mobile phase was 1 ml/min and injection volume was 100 μl. CN column (Zobrax; Dupont Wilmington, DE; 6-μm particles, 25 cm × 4.6 mm i.d.) was used for separation and fluorescence detection at 280 nm on excitation at 225 nm. The standard curve was linear over a working range of 2–200 ng/ml and gave an average correlation coefficient of 0.986 during validation. The R.S.D. ranged from 2.2 to 2.8% for inter-day and from 1.8 to 2.9% for intra-day.

2.8. Statistical analysis

The pharmacokinetic parameters were obtained using the non-compartment method of analysis. The curve fitting and statistical analysis were carried out using KINETICA-version 4 software. Area under the curve was calculated by trapezoidal rule. Steady-state serum concentration (C_{ss}) was determined by dividing $AUC_{0-\infty}$ by the sampling interval. Time to reach maximum steady-state serum concentration (T_{max}) was obtained by direct observation of subject's data. Clearance (Gibaldi, 1986) was calculated by transdermal flux (μg/h)/C_{ss}.

The data in the graphs represents the mean readings of six patients with the error bars representing the standard deviation. The *t*-test was selected as the test for significance and *P*-value less than 0.05 was considered statistically significant.

3. Results and discussion

The desired input rate was calculated from the product of clearance rate of SS (ml/h) and the required serum concentration (Ghosh et al., 1992). It was reported that from a single dose of inhaled salbutamol the resulted C_{max} was 2.3 ± 1 ng/ml, which was found to improve the FEV1 in patients with mild to moderate asthma (Kelly and Murphy, 1992). In a similar study, SS given via turbuhaler and Diskus dry powder resulted in a C_{max} of 4.04 and 3.21 ng/ml, respectively, in healthy volunteers (Lipworth and Clark, 1997). Based on these results, we assumed of achieving a serum concentration of 5 ng/ml of SS at steady state.

The formulation was optimized by varying different parameters such as drug, polymer, plasticizer, penetration enhancers and the adhesive concentration with the aim of achieving an input rate of ∼100 μg/h from a 10 cm^2 transdermal formulation. At doses less than 5 mg/patch the transdermal flux was significantly less than the required flux. The actual in vitro diffusion flux from the formulation with 5 mg of drug was 108.89 ± 5.83 μg/h ($n = 6 \pm$ S.D.). The in vitro release profile of the formulation is shown in Fig. 2.The lag time to achieve the steady-state flux was 2.5 ± 0.5 h ($n = 6 \pm$ S.D.).

There were no deviations from the pharmacokinetic/pharmacodynamic study protocol. No patients

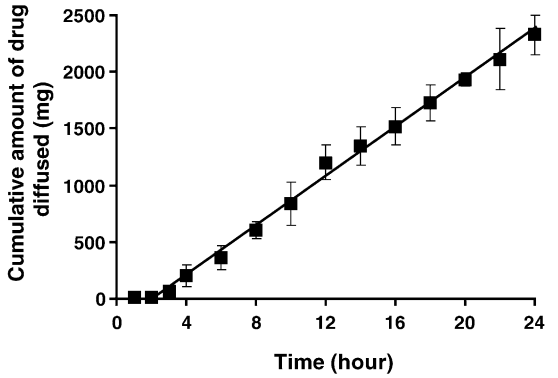

Fig. 2. In vitro diffusion profile of SS from transdermal device (area 10 cm^2) across human cadaver epidermis. The data points represents an average of $n = 6 \pm$ S.D.

experienced any untoward effect or discomfort during and up to a week after the study period. No signs of skin reactions were seen at the site of application in any of the patients. The mean serum concentration–time curve of SS is shown in Fig. 3. The mean pharmacokinetic parameters normalized to per milligram dose absorbed are listed in Table 2.

The steady-state serum concentration of SS administered by transdermal formulation was 2.87 ± 0.1 ng/ml/mg after an initial lag time of 4 h. Upon removal of the formulation, a mild reservoir effect was observed for 2 h, followed by normal elimination. The reservoir effect may be due to SS retained in the stratum corneum lipids due to the positive charge of the drug. The calculated mean C_{max} was 3.9 ± 0.4 ng/ml/mg at mean time of 4.66 ± 1.03 h. In the in vitro metabolism studies, the recovery of SS from the solution incubated with the tissue homogenate was about $94 \pm 4.9\%$. This is indicative that the drug did not undergo degradation due to dermal metabolism. The mean dose of drug absorbed was 2.48 ± 0.32 mg within 24 h as determined by mass balance method. A linear correlation exists between the cumulative drugs diffused in vitro versus cumulative AUC_{0-t} of serum concentration–time curve (Fig. 4, $R^2 = 0.99$). The elimination half-life 3.34 ± 1.07 h coincides well with the previously reported 3.86 ± 0.83 h determined after intravenous administration in healthy volunteers (Fairfax et al., 1980). The clearance rate of 256.12 ± 3.55 ml/min and elimination rate constant $0.24 \pm 0.09\ h^{-1}$ was obviously consistent with the previous reports (Murthy and Hiremath, 2001; Morgan et al., 1986).

Table 2
Pharmacokinetic parameters (normalized to per milligram dose) of salbutamol sulfate in moderately asthmatic patients on transdermal delivery

C_{max} (ng/ml)	3.90 ± 0.40
T_{max} (h)	4.66 ± 1.03
C_{ss} (ng/ml)	2.87 ± 0.1
AUC_{0-24} (ng ml^{-1} h^{-1})	68.99 ± 2.41
$AUMC_{0-24}$ (ng ml^{-1} h^{-2})	870.36 ± 29.67
K_{el}	0.23 ± 0.09
Elimination $T_{1/2}$	3.35 ± 1.07
Clearance (ml/min)	256.12 ± 3.55

$n = 6 \pm$ S.D.

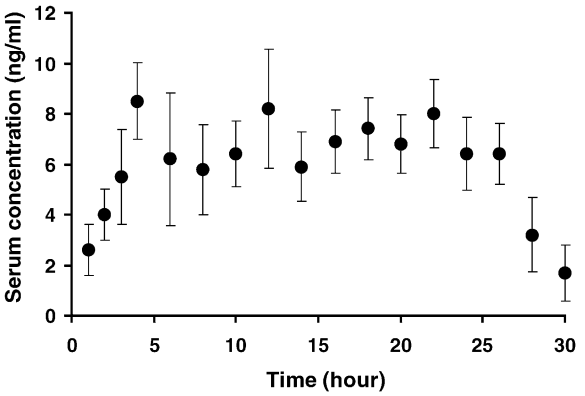

Fig. 3. Mean ($n = 6 \pm$ S.D.) serum concentration–time profile of SS in patients applied with the transdermal device (5 mg of SS/10 cm^2) for 24 h.

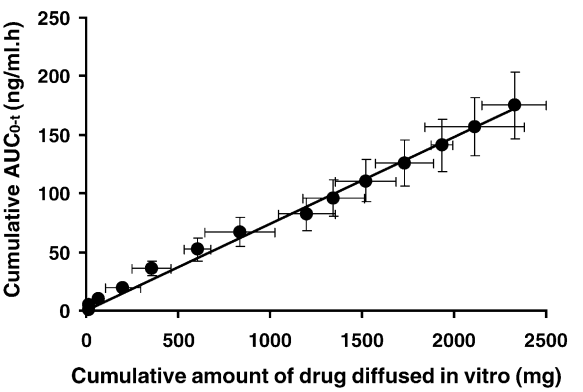

Fig. 4. In vitro–in vivo correlation of SS delivered from transdermal device. The correlating parameters are cumulative in vitro release (mg) and cumulative AUC_{0-t} (ng ml^{-1} h^{-1}).

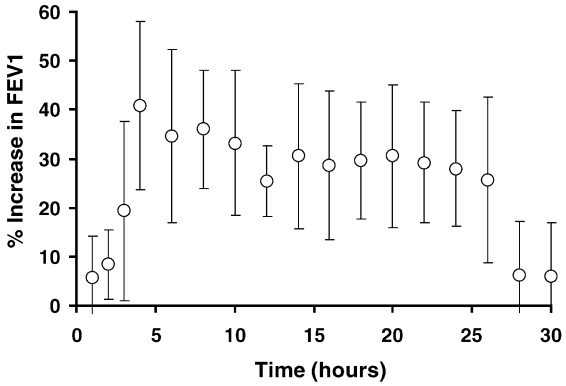

Fig. 5. Mean percentage increase of FEV1 in six moderately asthmatic patients on application of transdermal device (5 mg of SS/10 cm^2) for 24 h.

The mean FEV1 of the patients was 2.2 ± 0.14 l. Fig. 5 represents the baseline-adjusted mean FEV1 (% of baseline) as a function of time. Both, serum concentration–time and FEV1 response plots showed similar pattern. The peak concentration and peak FEV1 were coinciding (∼4 h). The FEV1 was still steady up to 2 h after removing the patch, which may be due to the delayed absorption of residual drug from the stratum corneum. The correlation coefficient between (area under the response curve) $AURC_{0-t}$ and AUC_{0-t} was 0.99 (Fig. 6).

4. Conclusions

The study demonstrates the feasibility of formulating the transdermal drug delivery systems of SS for treatment of bronchitis. The transdermal formulation used in the present study could maintain steady-state concentration well above the minimum desired concentration for duration up to 24–26 h minimizing the number of administrations as in oral or inhalers. It is possible to design transdermal formulations providing different input flux to suit the patient needs. The response in terms of FEV1 correlated well with the pharmacokinetics of SS. The transdermal formulations were found to be safe and non-reactive. Transdermal delivery of SS appears to be relatively a better route for patients who respond reasonably to the β-agonists. In the light of the results of the present work, we intend to further improvise the formulation for transdermal delivery of SS for durations up to several days.

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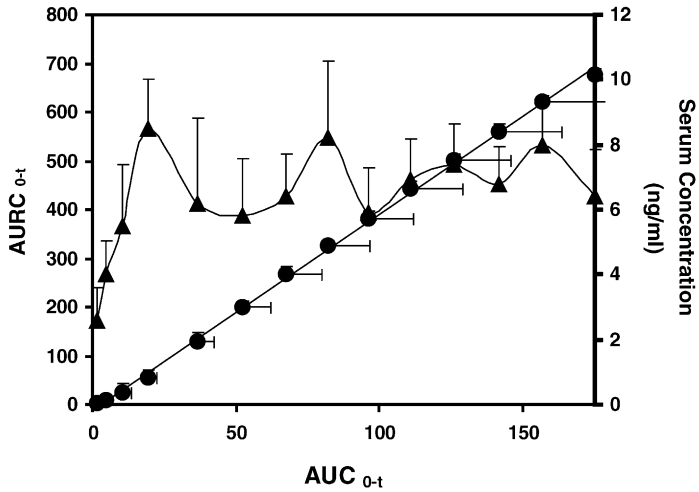

Fig. 6. Correlation between AUC_{0-t} (ng ml^{-1} h^{-1}) and $AURC_{0-t}$ in patients applied with the transdermal device (5 mg of SS/10 cm^2). The serum concentration is shown in the second *Y*-axis.

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