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Transdermal delivery of tacrine: I. Identification of a suitable delivery vehicle

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Abstract

Peroral administration of tacrine, a drug approved for the treatment of Alzheimer's disease, is associated with low bioavailability (due to first-pass effect), short elimination half-life and reversible hepatotoxicity. Transdermal administration may reduce the degree of these problems. In this investigation the influence of three commonly used solvents (water, propylene glycol and ethanol), and their mixtures, on the in vitro permeation of tacrine through rat and human skin were evaluated. Maximum flux and permeability were observed from ethanol-propylene glycol and water-ethanol binary mixtures, respectively. The permeability of tacrine through rat skin was about 2.5 times higher than that through human skin. Excellent correlation between the rat and human skin data was observed. The flux from the ethanol-propylene glycol binary mixture was 98 $\mu g/cm^2$ per h through rat skin and was selected for in vivo transdermal administration. The observed in vivo tacrine plasma concentrations were in good agreement with the concentration-time profile simulated using in vitro flux and tacrine clearance in rat. Preliminary short-term (24 h) irritation studies did not indicate any irritation. The results from this investigation indicate that transdermal delivery of tacrine may be feasible and that the ethanol-propylene glycol (1:1) mixture appears to be a promising solvent system.

Keywords: Tacrine; Transdermal delivery; Rat; Human; Skin permeability

1. Introduction

Tacrine (9-amino-1,2,3,4-tetrahydroacridine), a reversible cholinesterase inhibitor, is used for treating the symptoms of mild to moderate Alzheimer's disease (AD) (Small, 1992). Summers et al. (1986) were the first to investigate the efficacy of tacrine in AD therapy and since then a number of clinical trials have been conducted (Kumar and Becker, 1989; Farlow et al., 1992; Small, 1992). Dose-dependent hepatotoxicity and peripheral cholinergic side effects have been observed in most of these clinical studies (O'Brien et al., 1991; Farlow et al., 1992). Tacrine appears to undergo extensive first-pass metabolism and is

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rapidly cleared from the systemic circulation. Short elimination half-life (1.38–3.6 h) and low peroral bioavailability (5.5–36%) have been reported in rat, dog and man (Young et al., 1988; Hartvig et al., 1990; Hussain et al., 1990; Ahlin et al., 1992; Telting-Diaz and Lunte, 1993). Several metabolites of tacrine, some possessing a fraction of its anticholinesterase activity, have been identified (Schwartz et al., 1990; Hsu et al., 1991). Transdermal delivery of tacrine may: (1) minimize first-pass metabolism; (2) provide fairly constant blood levels for extended period of time; and (3) reduce the incidence of gastrointestinal side effects and hepatotoxicity associated with peroral administration. It has also been postulated that maintenance of constant levels of tacrine in the brain may be required to maximize its effects on memory enhancement (Becker and Giacobini, 1988).

The purpose of this investigation was to evaluate the feasibility of developing a transdermal delivery system for tacrine. Selection of appropriate vehicle composition is an important step in optimizing the transdermal delivery. Vehicles modify topical bioavailability: (i) by virtue of a higher thermodynamic activity of the drug in the solvent and (ii) by affecting the barrier property of the skin or by changing its state of hydration. In this study, in vitro permeation of tacrine through rat and human skin was studied to identify a suitable delivery vehicle. A saturated solution of the drug in water (W), propylene glycol (P), and ethanol (E) and their mixtures (equal volume fractions) were evaluated.

2. Materials and methods

2.1. Short-term stability study

The stability of tacrine base (obtained from its HCl salt) and hydrochloride salt (Sigma Chemical Co., St. Louis, MO) was assessed in water and phosphate buffers of pH 1.3, 3.2, 5.5, 6.7, 7.8 and 8.0. The drug solution (1 μg/ml) was stored in tightly capped vials at room temperature and at 37°C. The solutions were analyzed by HPLC on different days for a period of 30 days.

2.2. Apparent partition coefficient (APC)

The APCs of tacrine HCl and base were determined in octanol/buffer pH 7.4, and mineral oil/water systems. The aqueous drug solution was equilibrated with the organic phase for 24 h at 32°C. The two phases were then separated and the aqueous phase was analyzed for drug content by UV spectroscopy at $\lambda_{max} = 240$ nm.

2.3. Experimental design

The three component simplex centroid mixture design (Cornell, 1981) was adopted with water (W), propylene glycol (P) and ethanol (E) as the three solvents. Seven distinct vehicle compositions (3 single, 3 binary and 1 ternary mixture) were evaluated and the following special cubic (polynomial) model was developed:

$$\eta_{ijk} = \beta_i x_i + \beta_j x_j + \beta_k x_k + \beta_{ij} x_i x_j + \beta_{ik} x_i x_k + \beta_{jk} x_j x_k + \beta_{ijk} x_i x_j x_k$$

where η_{ijk} is the response, $\beta_{i.}$ denotes the regression coefficient and x_{i-k} are the components of the solvent mixture. The single component coefficients are the mean response values. Significant positive or negative coefficients for the binary and the ternary mixtures indicate that the observed response deviates (i.e., is non-linear) from the simple average of the pure components (Cornell, 1981).

2.4. Solubility

The solubility of tacrine in the three solvents and their mixtures was determined, in triplicate, at room temperature. Excess amount of tacrine base was suspended in these solvents (mixtures) in screw-capped vials and equilibrated in a shaker bath at room temperature. After equilibration, vials were centrifuged and the supernatant was analyzed by UV spectroscopy.

2.5. In vitro permeation

Permeation characteristics of tacrine base were studied (in triplicate) using excised full-thickness human abdominal skin (obtained from the Department of Pathology, University of Cincinnati

Medical Center), and Sprague Dawley® rat skin from the dorsal region (Harlan, Madison, WI). Human skin samples from five different subjects (average age 72 years to reflect the Alzheimer's disease population, three females and two males, Caucasian) were used. A control (water-propylene glycol) solution was applied to all the five skin samples (different subjects) to estimate inter-subject variability, and the remaining six solutions were randomly assigned. Permeation studies were conducted in Franz diffusion cells (diameter, 1 cm; diffusional area, 3.14 cm^2; fill volume, approx. 8 ml) (Crown Glass Co., Sommerville, NJ). Deaerated phosphate-buffered saline (pH 7.4) with 0.02% sodium azide (as an antimicrobial agent) equilibrated to 32° C served as the receptor fluid. Saturated drug solutions (400 μl) were applied on the stratum corneum side. A pilot study to evaluate the effect of absorption enhancer was also conducted by incorporating 5% Azone® in propylene glycol. The donor compartment and the receptor port were both covered with Parafilm® to prevent evaporation. Receptor fluid samples were withdrawn periodically and analyzed by HPLC.

2.6. *In vivo transdermal studies*

Hair on the dorsal side of Sprague-Dawley® rats was clipped 18–24 h prior to the experiment. Tacrine was administered transdermally with the help of the 19 mm Hill Top Chamber® (Hill Top Co., Cincinnati, OH). The selected solution of tacrine was accurately measured by a microsyringe and loaded on to the webril pad of the Hill Top Chamber®. The patch was applied to the clipped area and was secured tightly with Elastoplast® (Beiersdorf, Inc., Norwalk, CT) bandage around the body to ensure adequate contact between the saturated gauze and the skin. The patch was applied for 24 h and blood samples were withdrawn periodically. Serum was separated from the blood and was analyzed by HPLC.

2.7. *HPLC analysis (reversed phase)*

A modification of the method described by Forsyth et al. (1988) on a Beckman HPLC system (Model no. 334, Beckman Instruments Inc., Arlington Heights, IL) was employed. The sample was eluted through a Supelcosil LC 18 DB (25 × 4.6 mm, 5 μm) column (Supelco Park, Bellefonte, PA) by a mobile phase composed of 20, 10, 1 and 69 volumes of methanol, acetonitrile, triethylamine and water, respectively, adjusted to a pH of 6.5 with phosphoric acid, at a flow rate of 1.1 ml/min. The column effluent was monitored at 240 nm by a variable wavelength UV detector.

2.8. *Serum extraction*

To 100–400 μl serum sample, 100 μl of 0.1 N NaOH and 100 μl of 1 μg/ml of the internal standard (1,2,3,4-tetrahydro-9-acridanone) were added and extracted with methylene chloride. The organic phase was separated from the aqueous layer and was evaporated under a stream of nitrogen. The evaporated phase was reconstituted in mobile phase and an aliquot was used for analysis. Standard curves were prepared according to the same procedure using serum spiked with tacrine and its metabolite.

2.9. *Preliminary irritation study*

A pilot irritation study was conducted in three white New Zealand rabbits. Hair on the dorsal skin were clipped and three sites (approx. 10 cm^2) were marked 24 h prior to the study. 25 mm Webril pads of the Hill Top® chambers were saturated with tacrine HCl and base solutions in propylene glycol (250 mg/ml) and pure propylene glycol (control). The pads were placed on the marked sites and secured with Elastoplast® bandage. After 24 h the bandages and the Hill Top® chambers were removed and the sites examined for any irritation.

3. Results and discussion

3.1. *Short-term stability*

Tacrine HCl and base were both found to be stable (no detectable loss of drug) at room temperature and at 37°C in all the buffers, for the

Table 1
Solubility and permeation properties, flux, permeability coefficient (K_p) and t_{lag} of tacrine base (mean ± SD; $n = 3$) through rat and human skin

Solvent (mixtures)	Solubility (mg/ml)	Flux ($\mu g/cm^2$ per h)		K_p ($\times 10^{-4}$) (cm/h)		t_{lag} (h)	
		Human	Rat	Human	Rat	Human	Rat
W	0.248 ± 0.02	0.016 ± 0.01	0.023 ± 0.01	0.587 ± 0.13	0.993 ± 0.04	13.6 ± 4.4	6.78 ± 1.84
E	137.1 ± 15.6	33.90 ± 4.81	95.88 ± 0.57	2.600 ± 0.36	7.080 ± 0.04	22.19 ± 1.88	12.13 ± 0.46
P	252.6 ± 20.2	13.73 ± 1.95	22.50 ± 2.01	0.618 ± 0.087	0.800 ± 0.07	35.32 ± 5.0	21.3 ± 1.45
W:E 1:1	30.96 ± 2.41	30.06 ± 5.40	68.91 ± 8.54	9.720 ± 1.75	22.58 ± 2.80	12.6 ± 2.16	5.96 ± 1.58
W:P 1:1	8.69 ± 0.35	2.440 ± 1.15 [a]	4.510 ± 0.46	2.850 ± 1.39 [a]	5.450 ± 0.55	25.12 ± 6.80 [a]	12.84 ± 5.14
E:P 1:1	250.3 ± 12.7	59.20 ± 7.94	98.67 ± 14.1	2.560 ± 0.35	3.890 ± 0.58	26.6 ± 2.1	13.77 ± 0.31
W:E:P 1:1:1	65.35 ± 4.45	16.22 ± 2.22	54.01 ± 5.71	2.770 ± 0.40	9.120 ± 0.96	20.85 ± 1.12	10.53 ± 1.35

W, water; E, ethanol; P, propylene glycol.
[a] $n = 5$.

period tested. This study was conducted to ensure the stability of the drug under experimental conditions encountered in this study.

3.2. APC and solubility

The APCs of tacrine HCl and the base in octanol/buffer pH 7.4 were 2.04 and 2.24, respectively, and 0.79 and 0.86, respectively, in mineral oil/water. From the octanol/buffer partition coefficient (base), the true partition coefficient was calculated to be log PC = 2.46. The solubilities of tacrine base in the solvents and their mixtures are listed in Table 1.

The special cubic polynomial model parameters for log(solubility) as a function of the solvent composition are listed in Table 2 and the response surface shown as a contour plot in Fig. 1. The solubility of tacrine could be rank ordered as: propylene glycol > ethanol ⋙ water. The solubility (log-transformed) was significantly higher (than the average of the solubilities in the pure components) in the three ethanol-containing mixtures (WE, EP and WEP). This probably reflects some interaction (probably, hydrogen bonding) between ethanol and tacrine.

Several empirical models have been proposed to predict the permeability of chemical compounds from saturated aqueous solutions, through human skin using the molecular weight and the partition coefficient of the compound. These models should be useful in predicting the permeability behavior of a new chemical entity. The predicted K_p of tacrine using the model proposed by Potts and Guy (1992) was 1.04×10^{-4} cm/h, while the model of Michaels et al. (1975) predicted 1.70×10^{-4} cm/h. These predictions were within one order of magnitude of experimental value (range: $0.33–0.85 \times 10^{-4}$ cm/h).

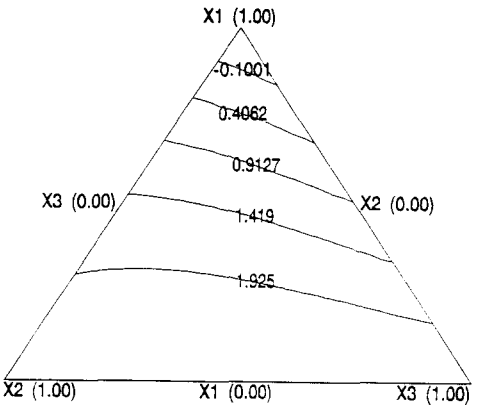

Fig. 1. Log solubility (mg/ml) of tacrine as a function of vehicle composition (X1, water; X2, ethanol; X3, propylene glycol).

3.3. In vitro permeation

The in vitro permeation parameters, flux (slope, $\mu g/cm^2$ per h) and t_{lag} (x-intercept, h) were estimated from (the linear portion of) the cumulative amount permeated (per unit area) vs

Table 2
Regression parameters of the characterized response surfaces

Response	Parameters							
	W	E	P	WE	WP	EP	WEP	r^2
Log(solubility) (mg/ml)	−0.607	2.135	2.401	2.903 [a]	0.167	0.519 [a]	2.856 [a]	0.999
Flux ($\mu g/cm^2$ per h)								
Rat	0.023	95.80	22.46	83.93 [a]	−26.91	142.1 [a]	−204.0	0.979
Human	0.016	33.86	13.73	52.48 [a]	−20.64	141.6 [a]	−511.0 [a]	0.969
K_p ($\times 10^{-4}$) (cm/h)								
Rat	0.99	7.30	0.80	73.75 [a]	18.22 [a]	−0.64	−109.4 [a]	0.981
Human	0.59	2.60	0.62	32.50 [a]	5.47 [a]	3.78	−84.90 [a]	0.954
t_{lag} (h)								
Rat	6.760	12.13	21.33	−13.96 [a]	−4.830	−11.80	−14.23	0.866
Human	13.60	22.20	35.30	−21.20 [a]	2.640	−8.600	4.440	0.903

[a] Significant interaction ($\alpha < 0.05$).
W, water; E, ethanol; P, propylene glycol.

time plots. The permeability coefficient (K_p, cm/h) was defined as the flux normalized to the initial concentration of the drug in the vehicle.

Human abdominal skin samples were obtained from five different subjects. Due to the small size of the skin samples all the seven vehicles could not be evaluated on each sample of skin. We expected large intersubject variations, therefore the permeability of tacrine from one selected solvent mixture (WP) was determined for all five skin samples. The other six vehicles were randomly assigned. A high variability (coefficient of variation of about 50%) in the flux values from the W and WP mixture was observed. Since these vehicles with high variability also had the low flux values, this may reflect analytical variability at low concentrations rather than intersubject variability.

The in vitro permeation data (flux, permeability and t_{lag}) are summarized in Table 1. The vehicle influence is summarized as polynomial regression models in Table 2, and as contour plots (human data only) in Fig. 2 and 3. The rat skin was found to be about 2.5 times more permeable than the human skin, however, excellent correlations were observed between the two skin models. Linear regression analysis revealed a highly significant ($p < 0.05$) correlation for both K_p ($r^2 = 0.957$) and t_{lag} ($r^2 = 0.990$) and therefore the following discussion is based on the human data.

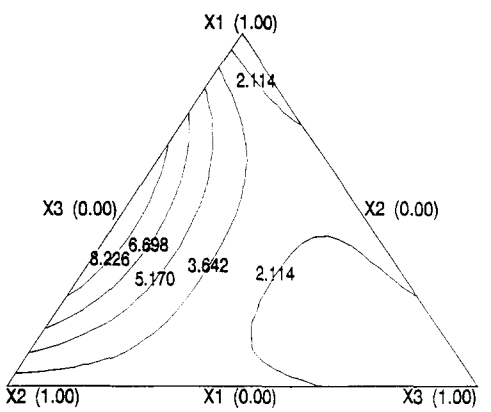

Fig. 2. Permeability of tacrine (K_p; cm/h, $\times 10^{-4}$) through human skin as a function of vehicle composition (X1, water; X2, ethanol; X3, propylene glycol).

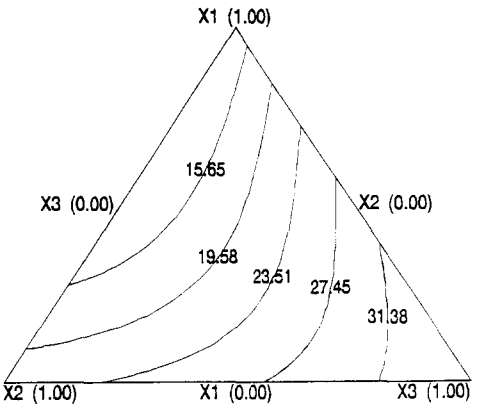

Fig. 3. Diffusion lag-times (h), human skin, as a function of vehicle composition (X1, water; X2, ethanol; X3, propylene glycol).

Of the three selected solvents, the use of E resulted in higher ($p < 0.05$) K_p when compared to W and P. However, the t_{lag} values could be rank ordered as: P > E > W. For the binary mixtures, K_p values were in the order: WE > WP = EP with significant positive interactions in both WE and WP vehicles. The t_{lag} values were found to be in the order: WP = EP > WE. Significant negative interaction was observed only for the WE vehicle. Based on these observations it could be concluded that WE possesses significant 'absorption promoter' activity (NB: the thermodynamic activity of the drug was assumed to be constant in all the vehicles; saturated solutions). For the four E vehicles, the following relationships were apparent for K_p; WE > WEP = EP = E; and for t_{lag}; E = EP > WEP > WE. In vehicles containing less than 50% E, significant loss of absorption promoter activity was apparent. Also, the K_p was significantly lower for EP than the WE mixture.

Permeation enhancement of several non-polar and ionic solutes by water-ethanol systems have been studied extensively and transport of ethanol has been demonstrated to correlate linearly with the drug transport (Ghanem et al., 1987; Berner et al., 1989a,b; Kurihara-Bergstrom et al., 1990; Liu et al., 1991). Berner et al. (1989a,b) demonstrated the transport of ethanol to be a function of its activity in the donor solution. It was postulated that in the smaller ethanol volume fractions

(< 0.8), permeation enhancement due to ethanol dominates, and at large volume fractions (> 0.8), dehydration effect is dominant. Stratum corneum dehydration and decrease in ethanol flux and K_p with pure ethanol have also been observed (Scheuplein and Blank, 1973; Ghanem et al., 1987; Berner et al., 1989c; Kurihara-Bergstrom et al., 1990). These observations suggest that decrease in K_p for tacrine from pure E is probably due to skin dehydration.

The K_p values were similar for W and P ($p > 0.05$) and significantly higher ($p < 0.05$) for WP (Table 1). Also, t_{lag} increased linearly ($r^2 = 0.996$ and 0.999 for rat and human skin, respectively) with increases in volume fractions of propylene glycol. Based on the observation that propylene glycol and the drug permeate simultaneously, a carrier mechanism has been postulated in which propylene glycol partitions into the skin and thereby promotes the movement of the drug into and through the skin (Polano and Ponec, 1976; Mollgaard and Hoelgaard, 1983; Hoelgaard and Mollgaard, 1985; Kaiho et al., 1987). Similar observations were made by Ostrenga et al. (1971), and they also suggested that the increase in t_{lag} is due to change in barrier diffusivity of the skin as a result of the incorporation of large quantities of glycol which leads to dehydration. The presence of water therefore probably explains the higher K_p observed in the case of water propylene glycol mixture.

3.4. In vivo transdermal study in rat

The highest fluxes through both the rat and human skin were observed in the E–P region. Therefore, the EP binary vehicle (rat flux = 98.67 $\mu g/cm^2$ per h; human flux = 59.2 $\mu g/cm^2$ per h) was selected for further in vivo studies.

The observed in vivo plasma concentrations (symbols) and simulated concentration profile (line) are shown in Fig. 4. The simulated concentrations were calculated by assuming an in vivo t_{lag} of 2 h and using the in vitro flux as the rate of input and the total body clearance value as the rate of output. The total body clearance of 40.29 ml/min per kg was determined from an intravenous pharmacokinetic study (Sathyan, 1993).

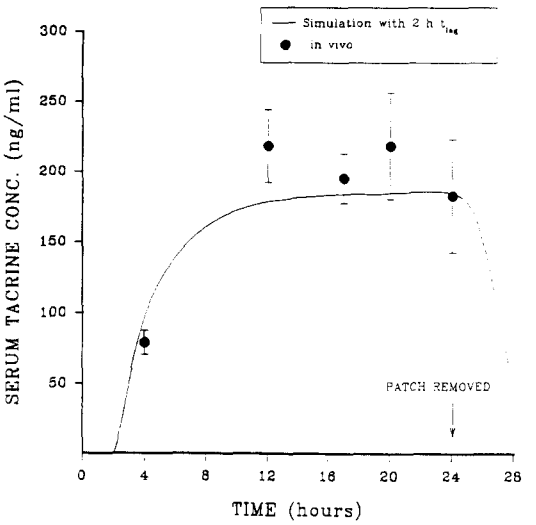

Fig. 4. Simulated (using in vitro flux, 98.67 $\mu g/cm^2$ per h and Cl_{tot}, 40.29 ml/min per kg) and experimental in vivo serum tacrine concentration following transdermal application of ethanol-propylene glycol mixture in rat (area of patch = 1.13 cm^2).

The excellent agreement between the observed and the simulated curve implies that the in vitro t_{lag} of 14 h does not emulate the in vivo condition. It has been postulated that this inconsistency results from the presence of the microcirculation under the epidermis in intact living skin (Chien et al., 1989). Also, the large in vitro t_{lag} for tacrine may indicate significant interaction with the dermal tissue.

To maintain an average therapeutic concentration (C_{ssav}) of 10 ng/ml (range: 10–70 ng/ml; Park et al., 1986) in a 70 kg patient the desired input rate (R_o) can be calculated as follows:

$$R_o = C_{ssav} \cdot Cl_{tot} = 1.47 \text{ mg/h}$$

where Cl_{tot} = 2.1 l/h per kg (Hartvig et al., 1990). The required R_o may be achieved using the EP solvent system (flux = 59.2 $\mu g/cm^2$ per h) applied over a delivery area of about 25 cm^2. A plot of simulated C_{ssav} values as a function of the solvent composition, for a delivery area of 25 cm^2, was constructed using the special cubic model (Table 2) for the human flux and is shown as Fig. 5. It appears that the desired plasma concentration of 10 ng/ml is achieved only by the

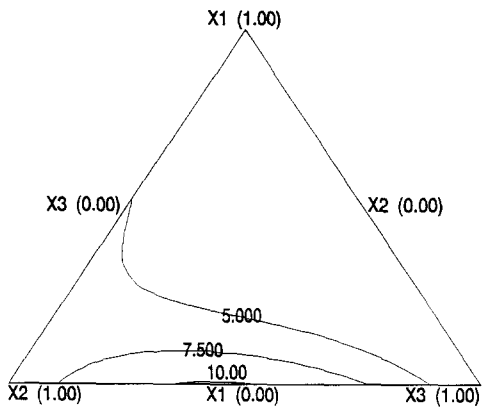

Fig. 5. Simulated steady-state plasma tacrine concentrations (ng/ml) in humans (body weight = 70 kg, Cl_{tot} = 2.1 l/h per kg) after applications of saturated solutions over an area of 25 cm^2 (X1, water; X2, ethanol; X3, propylene glycol).

EP solvent system. However, it should be noted that the cubic interaction term was significant at the $p < 0.05$ level (see Table 2) for the human flux values and the experimental design used did not allow for testing 'lack-of-fit' of the selected model (additional ternary mixtures should have been evaluated for this purpose). If a ternary mixture composition capable of providing a flux equivalent to that of EP exists it is most likely to be in the region formed by the 7.5 ng/ml curve (Fig. 5).

The delivery area may be reduced (or the desired blood level increased) by incorporating a more potent absorption enhancer such as Azone. Our results from a preliminary study (using rat skin) with 5% Azone in propylene glycol support this possibility. The flux, K_p and t_{lag} from the Azone containing vehicle were: 195 ± 10.42 $\mu g/cm^2$ per h, $8.0 \pm 0.4 \times 10^{-4}$ cm/h and 4.88 ± 1.06 h, respectively.

Preliminary irritation study conducted in the rabbit model and the single application study in rat did not indicate any irritation following 24 h application, however, long-term irritation studies would be necessary to confirm the low irritation potential of tacrine. Based on the data presented in this communication, transdermal delivery of tacrine appears to be a feasible alternative to peroral administration.

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