Preclinical Investigation of the Topical Administration of Phenserine: Transdermal Flux, Cholinesterase Inhibition, and Cognitive Efficacy

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ABSTRACT

Phenserine (PS) was designed as a selective acetylcholinesterase (AChE) inhibitor, with a tartrate form (PST) for oral administration in mild to moderate Alzheimer’s disease (AD). Recent phase 3 trials of PST in Europe indicate that any clinically relevant activity of PST may be limited by its duration of action. Like many oral drugs, bioavailability and plasma concentrations of PST are regulated by hepatic and gastrointestinal first-pass effects. To minimize the kinetic limitations of first-pass metabolism, transdermal formulations of PS and PST (ointment/patch) were developed and characterized in vitro and in vivo. Initial in vitro kinetic characterization of PS or PST formulations used a diffusion cell chamber and skin samples isolated from hairless mice. Liquid paraffin and fatty alcohol/propylene glycol (FAPG) were found to be suitable vehicles for ointment formulation. Addition of a penetration enhancer, 1-[2-(decylthio)ethyl]azacyclopentane-2-one (HPE-101), improved stratum corneum permeability. Application of the optimal formulation of PS/HPE-101/FAPG to the shaved back of rats resulted in significantly lowered plasma and brain AChE activities and improved cognitive performance in animals with scopolamine-induced cognitive impairment. These results suggest that the transdermal application of AChE inhibitors may represent an effective therapeutic strategy for AD. Particular benefits over oral therapies might include avoiding first-pass metabolic effects and improved dosing compliance.

Accompanying the increased geriatric population of most industrialized countries is an upsurge in the prevalence of dementia, particularly Alzheimer’s disease (AD). AD is characterized by progressive neuronal loss leading to cognitive decline and a plethora of accompanying psychiatric problems. Pathologically, AD is characterized by the presence of 1) senile plaques, extracellular deposits primarily composed of β-amyloid (Aβ) being derived from amyloid precursor protein (APP) (Sambamurti et al., 2002; Selkoe, 2005); 2) neurofibrillary tangles of phosphorylated τ protein (Tanzi, 2005); and 3) cholinergic synaptic and neuronal loss, with associated brain atrophy (Whitehouse et al., 1982; Doucett et al., 1986).

Cholinesterase inhibitors (ChE-Is) attenuate the cholinergic deficit considered to underlie the dysfunctions in AD, and, to date, they represent the most widely used treatment strategy (Lahiri et al., 2004). Four CHE-Is are approved in the United States (tacrine, Cognex; donepezil, Aricept; riv-

ABBREVIATIONS: AD, Alzheimer’s disease; Aβ, β-amyloid; APP, amyloid precursor protein; ChE-I, cholinesterase inhibitor; AChE, acetylcholinesterase; PS, phenserine; PST, phenserine tartrate; BChE, butyrylcholinesterase; HPE-101, 1-[2-(decylthio)ethyl]azacyclopentane-2-one; FAPG, fatty alcohol propylene glycol; LSD, least significant difference; LP, liquid paraffin.
astigmine, Exelon; and galantamine, Reminyl). Whereas this drug class and the recently Food and Drug Administration-approved N-methyl-D-aspartate-receptor antagonist memantine (Namenda) are the only agents to have been consistently associated with improvements in cognitive function in AD (Leo et al., 2006), such improvements are, unfortunately, generally small. This modest efficacy has provided impetus to develop a new generation of ChE-Is with activity beyond symptomatic benefits and to maximize the efficacy of current agents based on a more complete understanding of time- and concentration-dependent enzyme/inhibitor interactions. Our research in both areas has focused on mechanisms that reduce levels of neurotoxic Aβ, in addition to cholinesterase inhibition.

We designed and developed the acetylcholinesterase (AChE)-selective inhibitor phenserine [PS; (–)-phenylcarbamoyl eseroline] (Fig. 1), which possesses additional noncholinergic actions to lower the rate of APP synthesis and thereby reduce Aβ levels (Shaw et al., 2001; Greig et al., 2005b). Clinical trials to date with phenserine and the other approved ChE-Is have involved oral administration and water-soluble salt forms of the compounds, e.g., PS tartrate (PST). The oral route is most often used to administer therapeutics to humans, because it is convenient, safe, and inexpensive. However, oral administration has limitations associated with bioavailability loss through first-pass metabolic and transport effects in the intestinal wall and liver. To avoid these critical metabolic sites, alternative administration routes have been investigated.

Transdermal application provides a potential approach for efficient and effective administration. Although the transdermal route is most often used in local treatment, there may be therapeutic advantages for systemic therapy that include ready maintenance of steady-state drug levels, amelioration of peak concentration effects, and a lack of hepatic first-pass metabolism and gastrointestinal transport effects. Moreover, and of particular relevance for treatment of dementia, transdermal application may help achieve dosing compliance.

Transdermal delivery necessitates optimization of permeability by modulating the physicochemical characteristics of the therapeutic, such as solubility, diffusion, and enzymatic stability (Okuyama et al., 1999). It has been suggested that preferred characteristics of a therapeutic for transdermal application might include a molecular weight < 500 and a lipid/water partition coefficient (log P) value of approximately 2.5 (Ozawa et al., 1988).

PS is a crystalline compound developed through optimizing the structure/AChE-activity relationships of hexahydropyrrolo[2,3-b]indole carbamates (Greig et al., 2005b), the backbone of the classic anticholinesterase, and natural alkaloid physostigmine. The unsubstituted phenylcarbamate of eseroline, PS (C20H23N3O2/C4H6O6) is used biologically as a L(+)-tartrate salt to aid its aqueous solubility. The free base form of PS has a mol. wt. of 337.4 and is lipophilic, with a log P value of 2.2. It has a high brain penetration (brain/plasma ratio of 10:1), a moderately long duration of action in rodents (half-life, $t_{1/2}$ of 8.25 h), and a preferential selectivity for AChE versus butyrylcholinesterase (BChE) of approximately 70-fold (Greig et al., 2000). Such characteristics have been purported to make ChE-Is more tolerable in humans (Greig et al., 1995, 2005b), and furthermore, they make PS suitable for transdermal administration.

Clinical studies with phenserine have indicated that it is well tolerated by the oral route and dose-limited by classic cholinergically mediated adverse events, primarily nausea and vomiting (Greig et al., 2005a,b). The onset of AChE inhibition was rapid and occurred shortly after oral administration, reaching a maximum at 1.5 to 2.0 h and then declining relatively slowly, with a dose-dependent $t_{1/2}$ of 5 to 11 h. However, extensive metabolism occurred, with plasma drug levels falling rapidly beyond the $C_{\text{max}}$ which occurred at 1.5 h postoral administration (Greig et al., 2005a). These findings indicated that transdermal administration might provide pharmacokinetic advantages, both for maintenance of AChE inhibition and plasma drug concentrations to optimize noncholinergic actions on lowering Aβ. Therefore, stud-

![Fig. 1. A, diffusion cell setup. B, structure of PS.](https://example.com/f1.png)
ies were initiated to characterize the suitability of PS for transdermal formulation.

**Materials and Methods**

**Materials.** PS and PST (>99.9% optically and chirally pure) were synthesized as described previously (Yu et al., 2001). HPE-101 was obtained from Hisamitsu Pharmaceutical Co. (Saga, Japan). Other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO).

**Drug Formulation.** Solids of PS or PST were mixed with various ointment vehicles, as listed in Table 1. Fatty alcohol/proplene glycol (FAPG) ointment was prepared with various alcohols and propylene glycol. In brief, 9.5 g of stearyl alcohol, 8.0 g of cetyl alcohol, and 12.0 g of 1-docosanol were melted at 85°C and stirred into 70.5 g of propylene glycol at 90°C. During subsequent cooling, the agents were continuously mixed for 20 min to form a paste (FAPG ointment). The permeability enhancer HPE-101 was added to some formulations, with further mixing, at concentrations of 1 to 5% (w/w). Finely ground PS or PST was then blended into the FAPG ointment. The final concentration of PS or PST within vehicle was 1% (w/w) for in vitro studies and 10 or 20% (w/w) for in vivo studies.

**In Vitro Transdermal PS Permeability.** A diffusion cell chamber (flow-through cell; Fig. 1) was used (Addicks et al., 1987; Scalfani et al., 1993) to assess the availability of PS from vehicle, and subsequent transdermal permeability. Because the stratum corneum has been characterized as a major barrier to transdermal permeability (Sheuplein and Blank, 1973; Smith et al., 1982; Madison et al., 1987), permeabilities were investigated through both intact and tape-stripped skin. Intact skin or tape-stripped skin was isolated from the back of hairless mice and placed between the donor and the acceptor phase of the flow-through cell, allowing a permeation surface area of 1 cm². Stripped skin without the stratum corneum was prepared by stripping with Scotch tape (Scotch 845) over some 30 occasions. Formulated ointment was placed on the skin within the donor phase. Thereafter, saline (37°C) was circulated within the acceptor phase at a rate of 5 ml/h. Serial 2-ml samples were collected from the acceptor phase at specified times. PS concentrations were quantified by high-performance liquid chromatography under the following conditions: column, octadecyl silica gel reversed phase (C18; 5 μm; 4 mm i.d. × 150 mm); mobile phase, methanol/water [9:1 (v/v)]; flow rate, 0.2 ml/min; and detection, UV at 246 nm. The cumulative permeated amount of PS (i.e., micrometers per square centimeter) and steady-state flux rate (μg/cm² per hour) were determined from PS concentrations of each fraction (i) as follows: Q(ti) = Q(ti - 1) + (C(ti) × V(ti)(A) and flux(ti) = [Q(ti) - Q(ti - 1)]/(l(ti) - (ti - 1)), where i is number of fractions, ti is sampling time (hours), Q(ti) is cumulative permeated amount of PS at ti (micrometers per square centimeter), C(ti) is concentration of PS in sample at ti (micrometers per milliliter), V(ti) is sampling volume between ti - 1 and ti (milliliters), and A is area available for permeability (square centimeters), and flux(ti) is flux at ti (hours).

Further in vitro studies investigated the effects of modulating the surface area available for transfer of the PS in the diffusion cell. This was necessary before undertaking in vivo efficacy studies to ensure that sufficient transdermal PS flux occurred to sustain systemic levels within the previously established therapeutic range for the rat.

**In Vivo Transdermal PS Permeability and Efficacy on Cognitive Performance and AChE Activity.** Animals were lightly anesthetized (fluorothane) and carefully shaved to the skin in the mid-riff region of their back, facilitating the attachment of transdermal patches. FAPG ointments (≥ 2 PS) were applied to the shaved backs of adult (4-month-old) male Fischer-344 rats (Charles River Laboratories, Inc., Wilmington, MA) using two pads (250 mg of ointment per 4 cm² surface area pad; 500 mg of total ointment in 8 cm² surface area for each animal). Ointments were formulated with PS at 10% (w/w) with and without HPE-101 (5% w/w). Plasma AChE activity was then monitored in tail-blood, plasma samples for 8 h. At the end of the experiment, animals were killed by fluorothane overdose. Brain and plasma samples were collected, and they were immediately frozen at −70°C for later determination of AChE activity by the Ellman method (Ellman et al., 1961). Plasma was additionally analyzed for biochemical markers of hepatic, renal, and muscle function (aspartate aminotransferase, alanine aminotransferase, leucine aminopeptidase, leucine aminopeptidase, lactate dehydrogenase, alkaline phosphatase, albumin, creatine kinase, total protein, serum creatinine, blood urea nitrogen, triglycerides, total and free cholesterol, and phospholipids).

Adult (4-month-old) male Fischer-344 rats (Harlan, Indianapolis, IN) were also used to assess the in vivo efficacy of transdermal PS on cognitive performance and cholinesterase activities in plasma and brain (cerebral cortex). Using previously described protocols, we investigated the ability of transdermal PS to correct learning impairment induced pharmacologically with scopolamine, and we assessed in a 14-unit T-maze (Ingram 1988). Animals were first trained to criterion in one-way active avoidance in a straight runway. Thereafter, trials that involved negotiation of five maze segments to avoid footshock were conducted in a 14-unit T-maze. On day 1, each rat was trained in a straight runway to move from a start box to a goal box (~1 m) within 10 s while avoiding a mild footshock (0.8 mA). Training success was indicated by 13 of 15 correct avoidance (maximal trials 30). Training ensured that the animals had learned footshock avoidance before the maze learning trials.

The respective Animal Care and Use Committee’s of the Intramural Research Program, National Institute on Aging, and Kumamoto University approved the experimental protocols used in compliance with the guidelines for animal experimentation of the National Institutes of Health (Department of Health, Education, and Welfare publication 85-23, revised, 1995).

**Drug Treatment.** On day 2, animals were lightly anesthetized (fluorothane) and carefully shaved to the skin in the mid-riff region of their back, facilitating the attachment of transdermal patches. Thereafter, animals were randomly assigned to one of three treatment groups: 1) control, physiologic saline (i.p., 1 ml/kg body weight) + transdermal vehicle ointment; 2) scopolamine treatment, scopolamine (0.75 mg/kg i.p. in 1 ml/kg physiological saline) + transdermal vehicle ointment; and 3) scopolamine and PS cotreatment: scopolamine (0.75 mg/kg i.p. in 1 ml/kg physiological saline) + transdermal PS ointment. Two adhesive bandages (3.5 × 3.5 cm with a 2.0- × 2.0-cm gauze pad), containing 250 mg of PS ointment or vehicle, were applied to the shaved area on the back of each rat. Rats in the PS treatment group received 50 mg of PS formulated into vehicle ointment, as described above. Three hours before maze testing on day 3,
the adhesive bandages were removed and replaced with bandages containing fresh applications of PS ointment or vehicle. Following transfer to the testing room, and 30 min before testing in a 14-unit T-maze, i.p. injections of either physiological saline or scopolamine were administered.

**Cognitive Performance and Cholinesterase Inhibition.** All rats performed 20 trials in a 14-unit T-maze with an intertrial interval of 2 min. The performance measure was the number of errors committed on each trial. Immediately following the final maze run, animals were killed by fluorothane overdose. Plasma and brain samples were collected, and they were immediately frozen at −70°C. Cholinesterase levels were later determined by the Ellman method (Ellman et al., 1961).

**Data Analysis.** All data are reported as means ± S.E.M. of at least four trials or animals. Statistical significance was determined by analysis of variance with post hoc LSD test, Dunnett’s test, and unpaired t tests as appropriate.

**Results**

**Permeability of PS and PST in Vitro.** Phenserine was used as free base (PS) and tartrate (PST) forms in studies to identify the most suitable ointment vehicle (Table 1) and active agent for a transdermal formulation. The actions of the penetration enhancer HPE-101 were also assessed. PS or PST [1% (w/w)] was formulated in various vehicles containing 0 to 5% (w/w) HPE-101.

Figure 2 shows the permeation profiles of PS from various vehicles containing PS or PST through intact skin from the hairless mouse into receptor phase. Only the LP and FAPG vehicles allowed sufficient permeation of PS through the intact dermal barrier. Cumulative amounts of PS from the PS-containing vehicle recovered during the 8 h of the study were greater than those from the PST-containing vehicle, although the flux of PS for the PST-containing vehicle began earlier than that for the PS-containing vehicle. Subsequent studies focused on PS formulated within LP and FAPG vehicles.

Figure 3 and Table 2 present data regarding the effects of the penetration enhancer HPE-101 [0–5% (w/w)] on total flux and steady-state flux rates of PS, from LP and FAPG formulations, through intact skin. Permeabilities of PS were markedly improved by the addition of HPE-101. Maximum benefits of HPE-101 addition were observed between 3 and 5% (w/w) HPE-101 concentrations.

Because the stratum corneum is an impediment to transdermal permeability in intact skin and its integrity may be reduced by age, we also examined PS permeability from formulated LP and FAPG ointments using stripped skin from the hairless mouse. Figure 4 shows the permeation profiles of PS from these vehicles through both intact and stripped skin. Cumulative flux of PS was increased in stripped versus intact skin, by 2.9- and 1.8-fold for LP and FAPG ointments, respectively.

**Fig. 2.** In vitro permeation profiles of PS from vehicles containing PS [1% (w/w)] or PST [1% (w/w)] supplemented with HPE-101 [3% (w/w)], through 1-cm² intact skin into saline at 37°C. A, cumulative amounts of PS. B, flux of PS. ○, PST from FAPG; □, PST from LP; ▲, PST from PG; △, PST from hydrophilic ointment; ×, PST from hydrophilic petrolatum; ■, PS from FAPG; ◀, PS from LP; ●, PS from PG; ○, PS from hydrophilic ointment; and *, PS from hydrophilic petrolatum. Each point represents mean ± S.E.M. (n = 4).
Steady-state fluxes and cumulative amounts of in vitro transdermal permeation of PS [1% (w/w) in vehicle] across 1-cm² intact skin into saline at 37°C. Transdermal preparations were formulated with LP or FAPG vehicles and HPE-101 at 0 to 5% concentrations (w/w) (n = 4).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Steady-State Flux</th>
<th>Cumulative Amount for 12 h</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>µg/h/cm²</td>
<td>µg</td>
</tr>
<tr>
<td>PS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>0</td>
<td>5.2 ± 41.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>37.0 ± 15.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>95.6 ± 14.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>122.3 ± 19.4</td>
</tr>
<tr>
<td>FAPG</td>
<td>0</td>
<td>16.6 ± 10.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>112.3 ± 19.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>117.3 ± 21.9</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>83.3 ± 13.2</td>
</tr>
</tbody>
</table>

Fig. 3. In vitro permeation profiles of PS from LP and FAPG vehicles containing PS [1% (w/w)] supplemented with HPE-101 [0–5% (w/w)] through 1-cm² intact skin into saline at 37°C. □, from LP with HPE [5% (w/w)]; ○, from LP with HPE [3% (w/w)]; △, from LP with HPE [1% (w/w)]; ▲, from LP without HPE; ■, from FAPG with HPE [5% (w/w)]; ◀, from FAPG with HPE [3% (w/w)]; ◇, from FAPG with HPE [1% (w/w)]; and ◤, from FAPG without HPE. Each point represents mean ± S.E.M. (n = 4).

Fig. 4. In vitro permeation profiles of PS from LP and FAPG vehicles containing PS [1% (w/w)] supplemented with HPE-101 [3% (w/w)] through 1-cm² intact or stripped skin into saline at 37°C. ■, FS from LP through stripped skin; □, PS from LP through intact skin; ○, PS from FAPG through stripped skin; and ◇, PS from FAPG through intact skin. Each point represents mean ± S.E.M. (n = 4).

To study the in vivo efficacy of transdermal PS, it was necessary to ensure sufficient total transdermal flux of PS to achieve systemic levels within the therapeutic window for PS in the rat. It has been reported that the therapeutic window for PS in rat plasma for maintenance of brain AChE inhibition is in the region of 50–100 nM (Greig et al., 2000), which is in accord with its brain AChE IC₅₀ value of 36 nM (Greig et al., 2005b). To reach this therapeutic concentration with transdermal application, the optimal flux of PS was calculated using various pharmacokinetics parameters assessed in previous studies using intravenous administration of PS. The optimal flux rate was calculated using flux = kₑ × Vₑ × Cₛ/ₐ, where kₑ, Vₑ, Cₛ, and A are rate constants for elimination, distribution volume, systemic PS level at steady state, and area available for transfer, respectively. Based on approximately 200 g body weight, kₑ and Vₑ were calculated to be 3.3 h⁻¹ and 1103.2 ml, respectively. To achieve the necessary systemic PS level, a flux rate of 60 to 120 µg/h was calculated to be necessary. Data presented above (Table 2) show that the experimental fluxes of PS in the series of experiments using a 1-cm² area of intact skin for permeation within the diffusion cell, were approximately 5-fold lower than that required to achieve systemic levels of PS within the therapeutic range. However, the flux rate could theoretically be increased by providing a greater surface area for absorption.

Having determined the need for a flux of 60 to 120 µg/h to achieve appropriate systemic PS levels, we investigated PS fluxes using a diffusion cell with 5-cm² area of intact skin for permeation to compare with 1-cm² data reported above (Malcolm and Thomas, 1995). Formulations also contained 5% (w/w) HPE-101. Figure 5 shows permeation profiles of PS from LP and FAPG vehicles for 1- and 5-cm² diffusion cell areas using intact hairless mouse skin. The 5-fold increase in available diffusion area resulted in 4.3- and 8.2-fold increases in total accumulated transdermal flux over a 12-h period, for LP and FAPG formulations, respectively. Figure 6 shows that fluxes of PS from both vehicles entered the range of 60 to 120 µg/h with 5-cm² diffusion areas of intact skin, in the presence of 5% (w/w) HPE-101. It should be noted that the time necessary to reach the sufficient flux of PS from the FAPG vehicle was shorter than that from the LP vehicle. This would be theoretically sufficient to support systemic PS levels within the therapeutic dose window.

The in vitro findings suggest that LP and FAPG vehicles have almost the same performance for delivering PS through hairless mouse skin and into the systemic circulation. To develop a final dosage form for transdermal PS, the LP vehicle requires an additional pharmaceutical processing. In
In contrast, the FAPG vehicle enables us to prepare the ointment of PS without any processing. Based on the aforementioned consideration, we decided on the continued use of FAPG vehicle for in vivo studies.

**In Vivo Transdermal PS Permeability and Efficacy Effects on Cognitive Performance and AChE Activity.** FAPG vehicle application alone, with or without HPE-101, did not affect plasma AChE activity compared with untreated animals (data not presented). As shown in Fig. 7, plasma AChE activity was significantly reduced by 30% after treatment with PS ointment containing HPE-101 [5% (w/w)] versus controls receiving vehicle ointment alone. Concomitant inhibition of AChE activity in brain at 8 h was 60% (p < 0.05) after treatment with PS ointment containing HPE-101 [5% (w/w)] versus controls. PS ointment without HPE-101 resulted in reductions in plasma AChE activity versus controls that did not achieve statistical significance. Plasma levels of biochemical markers of hepatic, renal, and muscle function assessed at 1 and 8 h remained within their biological range for both PS treatments and controls (data not shown).

Figure 8 shows the effects of transdermal PS on cognitive impairment (errors in trial negotiation) induced with scopol

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**Fig. 5.** In vitro permeation of PS from LP and FAPG vehicles containing PS [1% (w/w)] supplemented with HPE-101 [5% (w/w)] through 1- or 5-cm² intact skin into saline at 37°C. ●, from LP through 5-cm² intact skin; ○, from LP through 1-cm² intact skin; ▲, from FAPG through 5-cm² intact skin; and Δ, from FAPG through 1-cm² intact skin. Each point represents mean ± S.E.M. (n = 4).

**Fig. 6.** In vitro flux of PS from LP and FAPG vehicles containing PS [1% (w/w)] supplemented with HPE-101 [5% (w/w)] through 1- or 5-cm² intact skin into saline at 37°C. ●, from LP through 5-cm² intact skin; ○, from LP through 1-cm² intact skin; ▲, from FAPG through 5-cm² intact skin; and Δ, from FAPG through 1-cm² intact skin. Each point represents mean ± S.E.M. (n = 4).

**Fig. 7.** Left, in vivo, rat plasma AChE activity during 8-h transdermal administration of FAPG ointment containing PS [10% (w/w)], supplemented with or without HPE-101 [5% (w/w)]. ■, untreated control; ○, FAPG ointment with PS [10% (w/w)]; ●, FAPG ointment with PS [10% (w/w)]; and HPE-101 [5% (w/w)]. ●, p < 0.05; **, p < 0.001 versus untreated control. Each point represents mean ± S.E.M. (n = 5). Right, brain AChE activity at 8 h in control rats and those administered FAPG ointment with PS [10% (w/w)] and HPE-101 [5% (w/w)] (p < 0.05 PS treatment versus control).
AChE and BChE activities in plasma and cerebral cortex of rats treated with and without scopolamine (i.p.) and cotreated with PS ointment

TABLE 3

AChE and BChE activities in plasma and cerebral cortex of rats treated with and without scopolamine (i.p.) and cotreated with PS ointment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma</th>
<th>Cortex</th>
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<tr>
<td></td>
<td>AChE</td>
<td>BChE</td>
</tr>
<tr>
<td>Control (with and without scopolamine)</td>
<td>1754 ± 62 (14)</td>
<td>1006 ± 43 (14)</td>
</tr>
<tr>
<td>PS</td>
<td>551 ± 65** (8)</td>
<td>822 ± 32** (8)</td>
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<tr>
<td>Inhibition</td>
<td>68%</td>
<td>18%</td>
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** Dunnell’s t test; p < 0.01.

Discussion

Data from these investigations are supportive of the potential future use of PS as a transdermally applied therapeutic for AD and other conditions that feature a central cholinergic deficit. Initial in vitro studies indicated the suitability of PS for transdermal application, as would be predicted from its physicochemical characteristics (Ozawa et al., 1988; Yu et al., 2001).

We examined PS and PST permeabilities through both intact skin and tape-stripped skin isolated from hairless mice, and we demonstrated the suitability of LP and FAPG as vehicles in which the agent was formulated. For intact skin, in vitro total fluxes were greater for PS than PST, with release from FAPG being superior to that from LP. Furthermore, the time course profile of flux rates through intact skin (Fig. 2B) clearly shows a superior, more predictable release of PS from vehicle compared with PST. It would be expected that the free base (PS) would be readily released from vehicle compared with the more water-soluble tartrate salt (PST). In the stripped skin studies, the formulation vehicle seemed to have little impact. It has been widely reported that the stratum corneum represents a major barrier to transdermal permeability (Scheuplein and Blank, 1973; Smith et al., 1982; Madison et al., 1987), and as expected, transdermal fluxes of PS were greater through stripped skin than intact skin. Because the barrier integrity of the stratum corneum may be compromised with age and because AD is an age-associated disease, the former was assessed to provide an estimate of maximal permeability. However, the goal of developing a clinical application system, precluded the further investigation of a delivery system reliant on the absence of the stratum corneum. It has also been reported that the use of an albumin solution in the receptor phase of the diffusion cell, to
better mimic subdermal physiological conditions, may increase drug permeabilities by greater than 5-fold, compared with flux into pH-adjusted saline solutions as in our studies (Surber et al., 1991). Therefore, it may have been possible to achieve enhanced permeabilities through the skin of the hairless mouse in vitro by modification of the experimental conditions.

Several studies have shown that penetration enhancers are effective to improve fluxes and subsequent bioavailability related to poor kinetic profiles of the transdermal administration route (Aungst et al., 1986; Tezuuki et al., 1988). In these studies, we examined the effectiveness of HPE-101, a well studied penetration enhancer (Nakashima et al., 1996; Yasuno et al., 2001), to optimize the permeability of PS in vitro. HPE-101 seems to improve permeability through hydrophilic routes of the stratum corneum without significant toxicity (Yano et al., 1992, 1993). Data indicated that the optimal concentration of HPE-101 to increase PS flux, was in the range 3 to 5% (w/w).

Using the in vitro permeability findings as a basis for the design of in vivo efficacy studies, we modulated the area available for transdermal flux so that the in vitro model predicted the achievement of systemic, in vivo concentrations of PS within the calculated therapeutic window for efficacy in AChE inhibition. This optimization was also necessitated by reports of significant reductions of in vivo absorption rate (flux) in rats and humans compared with in vitro permeability data from hairless mice (Tregear, 1966; Iwasaki et al., 1994). Treatment with scopolamine induces rapid and significant reductions in rat brain cholinergic activity and causes learning impairment as assessed in the 14-unit T-maze (Spangler et al., 1986; Iijima et al., 1993).

In our studies, the transdermal administration of PS significantly reduced the number of cognitive errors induced by scopolamine. In a sequence of 20 trials (four blocks × five trials), significant improvements in cognitive performance of the PS treated animals were observed beginning at trial 13. This compares favorably with our previous systemic treatment with PS (1.5–10 mg/kg i.p.) using the same experimental paradigm, which resulted in significant effects occurring slightly earlier in the trials sequence; beginning at trial 8 (Iijima et al., 1993; Patel et al., 1998). The delay in onset of the PS effects in the transdermal model might be caused by the lag-time of PS release from the ointment and/or slow kinetics associated with transdermal absorption and systemic assimilation.

The improvements in maze performance were accompanied by selective inhibition of AChE activities in plasma and brain as measured in tissues collected at the end of the series of maze trials. BChE activities in plasma and brain were less affected by administration of transdermal PS. PS has previously been characterized as having a 70:1 preference for inhibition of AChE compared with BChE (Greig et al., 2005b). It has been reported in AD patients that improvements in cognition, and particularly short-term memory, correlate with the extent of plasma AChE inhibition, within the range of 0 to 50% inhibition (Becker et al., 1991; Greig et al., 1995). The data from this series of investigations clearly suggest that transdermal treatment with an appropriately formulated ointment of PS could have the potential to achieve levels of AChE inhibition that would provide similar clinical benefits.

In addition to a central cholinergic deficit that may be partly ameliorated by AChE inhibition with agents such as PS, AD is further characterized by the presence of central extracellular plaques, primarily composed of Aβ (Sambamurti et al., 2002). These deposits are considered to be intimately involved in the pathology of AD; although exact mechanisms remain to be elucidated (Selkoe, 2005). PS possesses additional noncholinergic actions to reduce Aβ levels through lowering the rate of APP synthesis (Shaw et al., 2001; Greig et al., 2005b). Therefore, it is likely that PS delivered via the transdermal route, as in this series of studies, would exert beneficial effects on APP processing in addition to anticholinesterase activity. Further studies will be undertaken to elucidate these probable effects of transdermal PS on Aβ kinetics in this animal model. The transdermal application of agents such as PS may also provide real benefits with respect to treatment compliance. In a disorder such as AD where the ability to accept oral medications often progressively declines, the option of a therapeutic patch for drug delivery is especially attractive.

We conclude that these investigations demonstrate that PS may be a suitable candidate for transdermal administration; achieving transdermal flux of sufficient magnitude to sup-
port cognitive efficacy in an accepted animal model of cognitive impairment. Furthermore, the transdermal route avoids concerns of immediate hepatic toxicity and other deleterious consequences of first-pass metabolism. The slow release of active agent from ointment vehicle in a transdermal (patch) setting may also prove beneficial in sustaining the duration of action of PS, which has been suggested to be a limiting factor for clinical efficacy. These findings allow us to speculate that PS thus may be formulated to provide clinical benefits when developed transdermally that may help to reduce the cholinergic deficit of AD and possibly influence APP processing (Lahiri et al., 2007).

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