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Pharmacokinetics of Huperzine A after transdermal and oral administration in beagle dogs

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Abstract

Comparison of single and multiple dose pharmacokinetics between patches and conventional tablets of Huperzine A (Hup-A) was performed in beagle dogs to evaluate the patches' controlled drug release characteristics *in vivo*, a newly developed transdermal system for treatment of Alzheimer disease. Results showed that transdermal administration of Hup-A prolonged T_{max} value (24 h vs. 3 h, $P<0.01$), lowered C_{max} value (3.4 ± 0.2 ng mL^{-1} vs. 9.8 ± 1.0 ng mL^{-1}, $P<0.01$), and produced a relatively constant serum concentration within 84 h after a single transdermal dose of 4 mg/20 cm^2 Hup-A patches. Following application of the patches, Hup-A serum concentrations increased for approximately 12–24 h, reaching an average C_{max} of 3.4 ± 0.2 ng mL^{-1}. Thereafter, a serum concentration of at least 2.1 ng mL^{-1} was maintained for up to 84 h. The serum concentration was maintained within the range of 2.4–4.3 ng mL^{-1} during 2-week wearing period after multiple dosing, and the degree of fluctuation at the steady state of td and po administration was significantly different (0.51 vs. 1.99, $P<0.01$). This study indicates that Hup-A patches exhibited good controlled-release properties *in vivo*, maintained a relatively constant serum concentration within 3.5 d after wearing, and are suitable for twice-weekly application.

Keywords: Huperzine A; Dogs; Pharmacokinetics; Transdermal administration

1. Introduction

Huperzine A (Hup-A, Fig. 1), a natural alkaloid isolated from the Chinese herb *Huperzia serrata*, is a potent, reversible acetylcholinesterase inhibitor (AChEI), which crosses the blood–brain barrier smoothly, and shows high specificity for acetylcholinesterase (Wang and Tang, 2005; Liang and Tang, 2004). It has been approved as a drug for treatment of Alzheimer disease (AD) in China, marketed as a dietary supplement (Jiang et al., 2003) and is currently in phase II trials in USA (http://www.clinicaltrials.gov/ct/show/NCT00083590). Clinical trials conducted in China demonstrated that Hup-A induces significant improvement in the memory of elderly people with AD and vascular dementia (Wang et al., 2006; Zhang et al., 2002), and also enhances the memory and learning performance of adolescent students (Sun et al., 1999). In addition, Hup-A has non-cholinergic neuroprotective effects beyond its AChEI and has been considered for use as a protective agent against organophosphate nerve agent intoxication (Zhang et al., 2007; Gordon et al., 2005). Currently, the marketed pharmaceutical forms of Hup-A are oral (po) given immediate-release tablets and capsules, which have to be given 2–3 times per day. AD is a chronic progressive disease, and patients with AD have decreased memory, so long-term therapy with Hup-A is hard to persist, and overdosing resulting in adverse effects or underdosing so that a therapeutic level is not reached can also easily occur. The transdermal delivery system provides smoother, continuous drug delivery and steadier plasma levels, which may reduce the incidence of side effect associated with conventional dosage forms of the drug (Xu et al., 1999), thus making optimal therapeutic doses easier to attain and potentially improving treatment efficacy and compliance. Recently, our lab (Institute of Materia Medica, Zhejiang Academy of Medical Sciences) developed Hup-A transdermal patches, which

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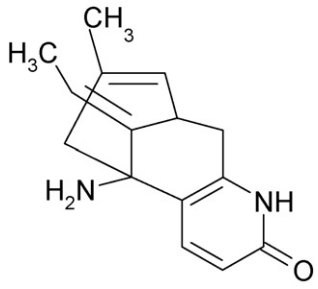

Fig. 1. Chemical structure of Huperzine A.

was designed to deliver Hup-A continuously and constantly over 3–4 d intervals after application to intact skin, to overcome the disadvantage of products given po. The average daily dose of Hup-A absorbed from the 20 cm^2 patch (containing 4 mg of Hup-A) is 456 μg (±90 μg) within 3.5 d (data not shown here). This result was obtained from analysis of residual Hup-A contents of patches worn over a continuous 3.5 d period during 48 separate occasions in 12 healthy volunteers; this analysis was performed according to the U.S. Food and Drug Administration's Guidance for Industry (topical dermatological drug product NDAs and ANDAs: *in vitro* bioavailability, bioequivalence, *in vitro* release, and associated studies, www.fda.gov).

The purpose of the present study was to evaluate Hup-A patches' controlled drug release characteristics *in vivo* by characterizing the Hup-A concentration–time profiles and pharmacokinetics following single and multiple doses transdermal administration. The conventional tablets were taken as a reference product. In addition, in vitro skin permeation was also studied to support the choice of a suitable td dose and trial condition. The similar skin permeability of Hup-A in dog and human beings support the use of the dog as a species for evaluating the preclinical pharmacokinetics of transdermally administered Hup-A (Ye and Chen, 2007).

2. Material and methods

2.1. Materials

Hup-A was provided by the Wen Ling Pharmaceutical Co. (Zhejiang, China), and has 99.6% purity verified by the Zhejiang Provincial Institute for Drug Control. Hup-A patches is a thin, 20-cm^2, adhesive matrix type system containing 4 mg Hup-A, developed by the Institute of Materia Medica, Zhejiang Academy of Medical Sciences (lot 030208). Hup-A tablets (50 μg/tablet) were obtained from the Shanghai Hongqi Pharmaceutical Co. (Lot 020801). CoTran™ 9720 drug-free backing layer and Scotchpak™ 1020 Polyester protective release liner were purchased from 3M Co. (USA). Polyacrylate pressure sensitive adhesive was obtained from National Starch & Chemical Co. (USA). The internal standard, mebendazole, was obtained from the National Institute for the Control of Pharmaceutical and Biological Products of China. All other chemicals or solvents were analytical reagent or chromatographic grade and purchased from commercial sources.

2.2. Preparation of Hup-A transdermal patches

The transdermal patch is composed by three layers: a drug-free backing layer, a layer of adhesive containing Hup-A and skin penetration enhancers, and a protective release liner. Accurately weighed Hup-A were dissolved in ethyl acetate along with penetration enhancers Azone and 1,2-propylene glycol. This solution was then added to polyacrylate pressure sensitive adhesive matrix and stirred continuously to get uniform solution. The final solution was made by adding ethyl acetate to suitable viscous consistency. Definite weight of the above solution was then coated on backing layer, dried at temperature 70 °C for 30 min, covered by release liner and finally cut to a size of 20 cm^2 by cutting machine. The patches were then subjected to further evaluation before use.

2.3. Animals

Six purebred beagle dogs, weighing 18.6 ± 1.5 kg, half male and half female, were obtained from the Yu Hang Animal Center (Hangzhou, Zhejiang, China). Throughout the experiment, dogs were housed, 1 dog per cage, in a room temperature maintained at 25 ± 2 °C, for at least 10 d before the experiment. The Zhejiang Academy of Medical Sciences Institutional Animal Care and Use Committee (Certificate No. IACUC-03-001) approved this animal study.

2.4. Study design

The marketed oral products of Hup-A in China are twice-a-day tablets and capsules. Hup-A is a biologically potent molecule (the maximum recommended human daily dose: 450 μg) and thus has very low blood concentration. So, the dose design is also based on this consideration that the trough Hup-A concentrations must be above the limit of quantitation of the analytic method. In addition, *in vitro* dog skin permeation study and pharmacokinetic pilot experiment were performed to support the choice of a suitable td dose and trial condition. Accordingly, the doses of the Hup-A patches and tablets were chosen as 20 cm^2 (containing 4 mg of Hup-A) per 3.5 d and 500 μg once a day, respectively. Dogs were divided into 2 groups ($n = 3$), fasted for at least 12 h prior to experiments, and were given water freely. The study design was a randomized 2-period crossover with a 1-week washout period between treatments. The dogs underwent 4 different study sessions: tablet single dose, tablet multiple dose, patch single dose and patch-multiple dose. Two single doses were studied at the first 2 groups.

Hup-A tablets were embedded in steamed stuffed buns and fed to dogs. The 20 cm^2 patches of Hup-A were applied to an area of clean and dry skin on the back of the dogs immediately after removal of the protective liner. The site of skin was depilated using 5% sodium sulfide ointment 12 h ago before application of the patch.

2.5. In vitro skin permeation study

The dogs' dorsal skin, treated in the same way for *in vitro* permeation study and *in vivo* pharmacokinetics study, was depilated

using 5% sodium sulfide ointment, and then washed away the ointment with water. For *in vitro* skin permeation study, fresh skin was excised from the dorsal region by killing the dog 12 h later after skin depilation, dermatomed to a thickness of approximately 300 μm, and washed with normal saline before being placed on a diffusion cell. At the same time, dorsal skin without treatment using sodium sulfide ointment was prepared by cutting hair with scissors, for use as a control skin to evaluate damage brought by 5% sodium sulfide.

In vitro dog skin permeation study of Hup-A patches was conducted at 37 °C across the excised dorsal skin mounted on the receptor compartment of a 2-chambered Valia-Chien glass diffusion cell (fabricated by Zhejiang University Glass Company), while stirring at a constant rate of 500 rpm. Valia-Chien cells were composed of a receptor compartment with a volume of 4 mL, and an effective diffusion area of approximately 0.72 cm^2. Phosphate buffered saline (0.05 M, pH 7.4) was used as a receptor medium. At specified intervals, 4 mL samples were withdrawn from the receiver compartment, and an equivalent amount of receptor medium was added to maintain the constant volume. The samples were assayed using a validated reverse-phase high performance liquid chromatography method (Ye et al., 2006).

2.6. Blood sample collection

In the single dose study, the patch was worn for 84 h (i.e. 3.5 d). Blood samples were collected from each dog by puncturing the femoral vein pre-dose (0 h), and at 4 h, 6 h, 8 h, 10 h, 12 h, 24 h, 32 h, 48 h, 56 h, 72 h, 84 h (↓, the paths withdrawn), 96 h and 104 h after wearing td, pre-dose (0) and at 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h and 24 h after being given po, respectively. Blood (4–5 mL) was processed for serum by centrifugation at 3000 × *g* for 10 min. Serum samples were immediately frozen and maintained at −20 °C until analysis.

The multiple dose study was carried out in another separate session after a 1 week washout from the single dose study. Hup-A patches were applied twice a week for 2 weeks (4 times). After 84 h of dosing, the patches were removed and replaced by new ones at a new back site. Blood samples were taken at 24 h and 84 h after the first 3 times dosing and were then taken, as described in the single dose study, following the last dosing. Tablets were given daily for 5 consecutive days. Blood samples were taken at 3 h and 24 h after the first 4 d and were then taken, as described in the single dose study, after the last dosing.

2.7. Serum Hup-A concentration analysis

Hup-A concentrations in serum were measured using a validated ion-pair reverse-phase HPLC method (Ye et al., 2005). The pH of 2.5 mL serum was adjusted to 9.5 by adding 1 mL of Borax–sodium carbonate buffer. Mebendazole (35 μL) was added as the internal standard (I.S.). Hup-A and I.S. were extracted with 3.0 mL chloroform–isopropanol (95:5, v/v) by vortex-mixing for 2 min. The organic layer was separated by centrifugation at 3000 × *g* for 10 min, transferred to a clean spiky bottom tube and evaporated to dryness under a gentle stream of nitrogen at 50 °C. The extraction procedure was performed twice. The residue was dissolved with 100 μL of mobile phase and then centrifuged at 2000 × *g* for 2 min to precipitate solid impurity of the residue. An aliquot of 50 μL of the resulting solution was injected into the HPLC system. The assay was linear over the concentration range of 1–12 ng mL^{-1} and intra- and inter-day precision over this range was not more than 12.8%. The limit of quantification in serum was 1 ng mL^{-1}.

2.8. Pharmacokinetic analysis

Pharmacokinetic analysis was performed by analyzing individual data with a CRFBU program (patches) and a 3P97 program (tablets) on a computer (the programs were provided by China Academy of Sciences). The pharmacokinetic parameters of Hup-A patches were compared with that of conventional Hup-A tablets using an analysis of variance model, with factors including formulation, subject and period. Statistical analysis of the data was performed using the two-one-side test and 90% confidential interval. *P*-values of less than 0.05 indicated statistical significance.

In the single dose study, the parameters calculated and reported were T_{max}, C_{max}, the terminal disposition half-life ($t_{1/2}$) and trapezoidal AUC (i.e. AUC_{0-t}). Maximum measured serum concentrations (C_{max}) and the corresponding times (T_{max}) were taken directly from the raw data.

In the multiple dose studies, the parameters calculated and reported were T_{max}, C_{max}, C_{min}, $AUC^{ss}_{0-\tau}$, C_{av} and DF. C_{max} and T_{max} were obtained directly from the raw data after the last dose being given. C_{min} represented drug concentrations at the end of each dosing interval during the steady state; $AUC^{ss}_{0-\tau}$ represented the area under the serum concentration–time curve from time zero to time τ over a dosing interval at the steady state, where τ is the length of the dosing interval; C_{av} represented average drug concentration at the steady state, where

$$C_{av} = \frac{AUC^{ss}_{0-\tau}}{\tau}$$

DF represented the degree of fluctuation at the steady state was obtained according to the following equation:

$$DF = \frac{C_{max} - C_{min}}{C_{av}}$$

3. Results and discussion

3.1. In vitro skin permeation study results

The cumulative Hup-A amount permeated from 20 cm^2 patches through the dog skin was approximately 1800 μg (Fig. 2), and the average daily dose was 501 μg (±103 μg, $n = 6$) within 3.5 d, approximately equivalent to both the maximum po dose of 450 μg per day for humans and the in vitro daily permeation amount across human cadaver skin from 20 cm^2 patches (Ye and Chen, 2007). Combined with preliminary pharmacokinetic study, 20 cm^2 patches/3.5 d and 500 μg tablets/d was chosen as administered dose. In addition, skin depilated

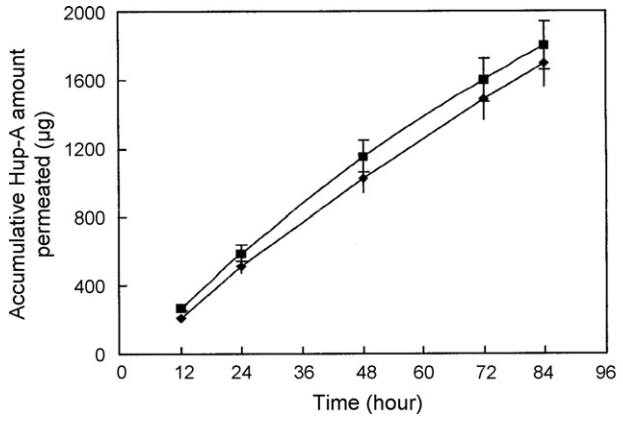

Fig. 2. Profile of mean (±S.D., $n=6$) cumulative amount of Huperzine A permeated from 20 cm^2 patches across excised dorsal skin of beagle dog. (■) Skin depilated using 5% sodium sulfide ointment; (♦) skin, hair cut with scissors.

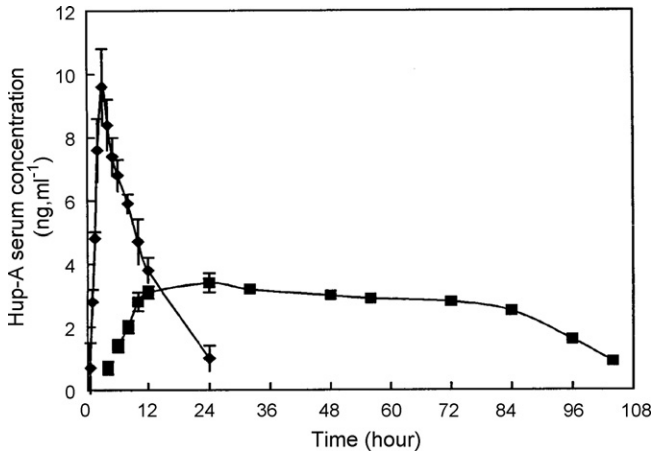

Fig. 3. Profiles of mean (±S.D., $n=6$) serum concentration vs. time after single dose percutaneous and po administration in 6 dogs. (■) Hup-A patches 4 mg/20 cm^2; (♦) Hup-A tablets 500 μg.

using 5% sodium sulfide ointment resulted in slight permeation increase in the first 24 h (Fig. 2), but accumulative permeation amounts in 84 h was not of statistically significant difference through skins with or without depilating treatment.

3.2. Pharmacokinetics study results

The drug serum concentration–time course of Hup-A tablets groups conformed to 1 compartment model with first order absorption. Pharmacokinetic parameters after being given po and td are shown in Table 1. In the single dose study, the values of C_{max} (3.4 ± 0.2 ng mL^{-1} vs. 9.8 ± 1.0 ng mL^{-1}) and T_{max} (24 h vs. 3 h) were significant different ($P < 0.01$, Table 1) after being given td and po. Hup-A patches resulted in longer T_{max}, lower C_{max}, and produced a relatively constant serum concentration within 84 h after wearing. The mean serum concentration–time profiles (Fig. 3) showed that Hup-A serum concentrations increased for approximately 12–24 h following the application of the patches, then reached average maximum concentrations of 3.4 ± 0.2 ng mL^{-1}, thereafter, a serum concentration of at least 2.1 ng mL^{-1} (the lowest concentration at 84 h of a dog) was maintained for up to 84 h. Following removal, serum Hup-A concentrations decline gradually and fall approximately 36% in 20 h.

The half-life ($t_{1/2}$) was 13.4 ± 2.9 h and 5.9 ± 1.3 h, respectively, after being given td and po (Table 1). Prolonging the elimination of half-life is a common phenomenon in transdermal bioavailability studies (Murthy and Hiremath, 2001; Mosser, 1992; Ubaidulla et al., 2007). Continued absorption of the Hup-A within the skin accounts for the slower disappearance of Hup-A from the serum than is seen after being given po. However, it is not certain whether the long half-life observed after patch application, to some extent, might really reflect a slow absorption rate constant. The AUC of Hup-A patches at multiple doses were slightly higher than those at single doses, with a multiple to single ratio of 1.18 ($AUC^{ss}_{0-inf}/AUC_{0-inf}$) (Table 1). In addition, the ratio of C_{min} at the steady state and C_{min} after the first dose at multiple doses (84 h) was 1.13. Those phenomena indicated that slight drug accumulation may occur during multiple applications. The accumulation might be related to the "reservoir effect" of drugs in epidermis stratum corneum (Chen et al., 1996).

Huperzine A has a rapid and nearly complete oral absorption (Chu et al., 2006). The relative bioavailability of Hup-A patches was 32% calculated according the dose-calibrated equation from the multiple dose study. Low bioavailability is a common characteristic of the transdermal system (Taburet et al., 1995; Zhang et al., 2004). Adequate drugs are needed as permeation forces, and great amounts are still left after wearing.

The mean C_{min} and C_{max} of Hup-A after percutaneous and po multiple doses administration are shown in Fig. 4. The serum concentrations maintained within the range of 2.4–4.3 ng mL^{-1} during 2-week wearing periods. After patches application, a steady-state serum concentration was reached quickly and maintained well during subsequent applications. The degree of fluctuation at the steady state of td and po administration was significant different (0.51 vs. 1.99, $P < 0.01$). In addition, the application of Hup-A patches for 3.5 consecutive days was well tolerated, and local skin reactions were generally minimal and

Table 1
Pharmacokinetic parameters after being given td and po to 6 beagle dogs

Parameters	Patches	Tablets
Single dose		
T_{max} (h)[b]	24.0 (7.2)[a]	3.0 (0.6)
C_{max} (ng mL^{-1})	3.4 ± 0.2[a]	9.8 ± 1.0
$t_{1/2}$(h)	13.4 ± 2.9	5.9 ± 1.3
AUC_{0-inf} (ng h mL^{-1})	325.6 ± 32.0	109.1 ± 12.8
Multiple dose		
T_{max} (h)[b]	17.0 (10.0)[a]	3.0 (0.8)
C_{max} (ng mL^{-1})	4.4 ± 0.2[a]	10.1 ± 1.1
C_{av} (ng mL^{-1})	3.3 ± 0.1	4.5 ± 0.4
$AUC^{ss}_{0-\tau}$ (ng h mL^{-1})	277.4 ± 10.3 (0 −84 h)	108.5 ± 8.8 (0 −24 h)
AUC^{ss}_{0-inf} (ng h mL^{-1})	380.1 ± 43.1	118.4 ± 11.0
DF	0.51 ± 0.1[c]	1.99 ± 0.2

Mean ± S.D. $n=6$.
[a] $P < 0.01$ vs. tablets.
[b] Median (interquartile range) values for T_{max}. DF, degree of fluctuation.

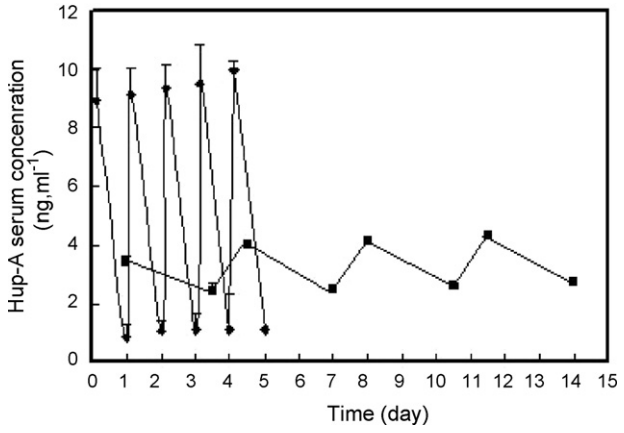

Fig. 4. The mean C_{min} and C_{max} of Huperzine A (Hup-A) after multiple dose td and po administration. (■) Hup-A patches 4 mg/20 cm^2; (♦) Hup-A tablets 500 μg.

self-limited. Those results suggested that smooth, continuous release of Hup-A from the patch has the potential to maintain drug levels in the optimal therapeutic window, reducing fluctuation between peaks and troughs that might be associated with side effects and reduced efficacy, respectively. Because of this improved tolerability, patients are more likely to attain optimal doses and continue treatment for longer periods, with the potential to achieve greater and more sustained clinical benefits.

The po dose of 500 μg per day for the dog is approximately 2.2 times the maximum recommended human dose according to the dose conversion factors based on body surface area between dog and human. The concentration of Hup-A (2.1–3.4 ng mL^{-1}) at 12–84 h after single dose td administration was almost comparable with that (3.8 ng mL^{-1}) at 12 h after single po administration. The latest report (Li et al., 2007) is that C_{max} of Hup-A was 2.47 ± 0.49 ng mL^{-1} vs. 2.51 ± 0.51 ng mL^{-1} after a single 200 μg dose of 2 kind conventional tablets in 18 healthy male Chinese volunteers. However, it still could not be concluded from this that the concentrations achieved by the td doses for the dog would be an adequate amount to treat a patient. Preclinical pharmacodynamics study showed that Hup-A transdermal patches had a prolonged pharmacological response and good learning and memory improvement in normal and memory deficits mice (Shi et al., 2002). The patches have now been approved for phase I/II clinical trials by the State Food and Drug Administration of China. Based on the results acquired from the dogs' model, human pharmacokinetics studies are further needed during clinical study. As for the transdermal system, the amount of Hup-A released from each system is proportional to the surface area. So far, 2 designed patch sizes are 10 cm^2 and 15 cm^2, which can be converted to other sizes, for example, of 20 cm^2, 25 cm^2, and 30 cm^2. The different patch sizes allow the dose to be adjusted according to clinical responses and offer dosing flexibility.

In conclusion, the pharmacokinetic study showed that transdermal administration of Hup-A prolonged T_{max}, lowered C_{max}, and reduced fluctuations in serum concentrations. The Hup-A patches exhibited good controlled-release properties *in vivo* within the 84 h wearing period, and are suitable for twice-weekly application.

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References

Chen, G.S., Zhang, M.Y., Wang, Q., Ye, J.C., 1996. Pharmacokinetics of weekly transdermal estradiol controlled delivery system in postmenopausal Chinese. Zhongguo Yao Li Xue Bao 17, 407–410.

Chu, D., Liu, W., Li, P., Gu, J., Liu, K., 2006. Pharmacokinetics of huperzine A in dogs following single intravenous and oral administrations. Planta Med. 72, 552–555.

Gordon, R.K., Haigh, J.R., Garcia, G.E., Feaster, S.R., Riel, M.A., Lenz, D.E., Aisen, P.S., Doctor, B.P., 2005. Oral administration of pyridostigmine bromide and huperzine A protect S human whole blood cholinesterases from ex vivo exposure to soman. Chem. Biol. Interact. 157–158, 239–246.

Jiang, H., Luo, X., Bai, D., 2003. Progress in clinical, pharmacological, chemical and structural biological studies of huperzine A: a drug of traditional Chinese medicine origin for the treatment of Alzheimer's disease. Curr. Med. Chem. 10, 2231–2252.

Li, W., Li, J., Hu, Q., 2007. Determination of huperzine A in human plasma by liquid chromatography-electrospray tandem mass spectrometry: application to a bioequivalence study on Chinese volunteers. Biomed. Chromatogr., Oct 15 (Epub ahead of print).

Liang, Y.Q., Tang, X.C., 2004. Comparative effects of huperzine A, donepezil and rivastigmine on cortical acetylcholine level and acetylcholinesterase activity in rats. Neurosci. Lett. 361, 56–59.

Mosser, K.H., 1992. Transdermal fentanyl in cancer pain. Am. Farm. Phys. 47, 729–730.

Murthy, S.N., Hiremath, S.R., 2001. Physical and chemical permeation enhancers in transdermal delivery of terbutaline sulphate. AAPS PharmSciTech, 2, E-TN1.

Shi, H., Ye, J.C., Zhang, W.G., Chen, G.S., 2002. The improvement functions of Huperzine-A transdermal on learning and memory in mice. Chin. JMAP 19, 276–277.

Sun, Q.Q., Xu, S.S., Pan, J.L., Guo, H.M., Cao, W.Q., 1999. Huperzine-A capsules enhance memory and learning performance in 34 pairs of matched adolescent students. Acta Pharmacol. Sin. 20, 601–603.

Taburet, A.M., Singlas, E., Glass, R.C., Thoma, F., Leutenegger, E., 1995. Pharmacokinetic comparison of oral and local action transcutaneous flurbiprofen in healthy volunteers. J. Clin. Pharm. Ther. 20, 101–107.

Ubaidulla, U., Reddv, M.V., Ruckmani, K., Ahmad, F.I., Khar, R.K., 2007. Transdermal therapeutic system of carvedilol: effect of hydrophilic and hydrophobic matrix on in vitro and in vivo characteristics. AAPS Pharm. Sci. Technol. 8, 2.

Wang, R., Tang, X.C., 2005. Neuroprotective effects of huperzine A. A natural cholinesterase inhibitor for the treatment of Alzheimer's disease. Neurosignals 14, 71–82.

Wang, R., Yan, H., Tang, X.C., 2006. Progress in studies of huperzine A, a natural cholinesterase inhibitor from Chinese herbal medicine. Acta Pharmacol. Sin. 27, 1–26.

Xu, S.S., Cai, Z.Y., Qu, Z.W., Yang, R.M., Cai, Y.L., Wang, G.Q., Su, X.Q., Zhong, X.S., Cheng, R.Y., Xu, W.A., Li, J.X., Feng, B., 1999. Huperzine-A in capsules and tablets for treating patients with Alzheimer disease. Zhongguo Yao Li Xue Bao 20, 486–490.

Ye, J.C., Chen, G.S., 2007. Skin permeation of Huperzine A in different skin species. Chin. Pharm. J. 14, 1095–1097.

Ye, J.C., Chen, G.S., Zeng, S., Zhang, W.G., 2006. HPLC method for the determination of Huperzine A and related substances in transdermal patches. Chin. Pharm. J. 20, 1589–1591.

Ye, J.C., Zeng, S., Zhang, W.G., Chen, G.S., 2005. Ion-pair reverse-phase high performance liquid chromatography method for determination of Huperzine-

A in beagle dog serum. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 817, 187–191.

Zhang, Z., Wang, X., Chen, Q., Shu, L., Wang, J., Shan, G., 2002. Clinical efficacy and safety of huperzine Alpha in treatment of mild to moderate Alzheimer disease, a placebo-controlled, double-blind, randomized trial. Chin. Med. J. 82, 941–944.

Zhang, Y., Xu, D.H., Ma, Z., Chen, Y., Zhao, J.J., Xu, S.B., 2004. Preparation and evaluation in vivo and in vitro of glimepiride gel-matrix controlled-release patch. Yao Xue Xue Bao 39, 640–644.

Zhang, H.Y., Yan, H., Tang, X.C., 2007. Non-cholinergic effects of Huperzine A: beyond inhibition of acetylcholinesterase. Cell Mol. Neurobiol., Jul 27 (Epub ahead of print).