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# Pharmacokinetics and *in vitro* and *in vivo* correlation of huperzine A loaded poly(lactic-co-glycolic acid) microspheres in dogs

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**Abstract**

The purpose of this study was to investigate the pharmacokinetics and *in vitro*/*in vivo* correlation (IVIVC) of huperzine A loaded poly(lactic-co-glycolic acid) (PLGA) microspheres in dogs. Several huperzine A loaded PLGA microspheres were prepared by an O/W method and three of them (single dose) were injected intramuscularly (i.m.) or subcutaneously (s.c.) to five beagle dogs, respectively. With the increase of the molecular weight of PLGA and the particle size of microspheres, the *in vitro* and *in vivo* release periods of huperzine A were prolonged. After s.c. injection, the release of huperzine A from microspheres was faster than that after i.m. injection. The IVIVC models of huperzine A loaded PLGA microspheres were established successfully and after i.m. administration the linear relationship between the *in vitro* and the *in vivo* releases was better than that after s.c. administration. It was also found when the particle size of the microspheres was smaller; the values of correlation coefficient were higher.


*Keywords:* Poly(lactic-co-glycolic acid); Microspheres; Huperzine A; Pharmacokinetics; *In vitro*/*in vivo* correlation

## 1. Introduction

Huperzine A, a lycopodium alkaloid isolated from the Chinese medicinal herb Huperzia serrata, is a reversible, potent, and selective inhibitor of acetylcholinesterase. Compared with other well-known acetylcholinesterase inhibitors, such as physostigmine, galanthamine, tacrine, and even donepezil, which have been approved for Alzheimer's disease (AD) in the United States and some European countries, huperzine A has better penetration through the blood-brain barrier, higher oral bioavailability, and longer duration of acetylcholinesterase inhibitory action (Tang and Han, 1999; Xiao et al., 1999; Zhu et al., 2004). In addition, huperzine A has antioxidant and neuroprotective properties, which suggests that it may be useful as a disease-modifying treatment for AD (Xiao et al., 1999; Wang et al., 2001; Zhang et al., 2002; Zhao and Li, 2002; Zhou and Tang, 2002). Currently, huperzine A is in phase II trials in the United States in elderly patients with age-associated memory loss. Results of clinical trials in China showed that huperzine A was efficient in treatment of patients with mild to moderate AD (Zhang et al., 2002).

Huperzine A is available currently in the market twice daily as tablet or capsule (200–400 μg/day) (Xu et al., 1995, 1997, 1999). Though this daily repeated oral administration is convenient for most of patients, it is very difficult for the advanced Alzheimer's patients who suffer heavy memory disorder, not to miss scheduled self-medication. Gastrointestinal side effects have also been reported, such as, nausea and anorexia (Sun et al., 1999; Jiang et al., 2002). Therefore, long term and parenteral formulations of huperzine A, which could ensure the therapeutic effects and make the care easier for caregivers, have an increasing importance for the treatment of Alzheimer's disease.

At present, many biodegradable poly(lactic-co-glycolic acid) PLGA depot products, especially microspheres or microparticles, containing various drugs become commercially available. Among these products, some were intended as intramuscular (i.m.) injection, such as Risperdal Consta (risperidone), Sandostatin LAR (octreotide acetate), Lupron depot (leuprolide acetate), Trelstar LA and Trelstar depot (triptorelin pamoate). Others were given as subcutaneous (s.c.) injection, for example,

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Nutropin Depot (human growth hormone) and Zoladex (goserelin acetate). Though enormous studies on PLGA depot products were reported, the investigation on the differences of plasma concentration–time profiles of these products administrated via i.m. and s.c. routes has been rarely conducted.

While the FDA *in vitro*/*in vivo* correlation (IVIVC) guidance is applicable only to oral dosage forms, the principles of this guidance can be used to develop IVIVC for non-oral products. However, few examples were given where *in vitro* dissolution in PBS can accurately predict the *in vivo* release profile for parenteral biodegradable depot systems (Negrin et al., 2001; Schliecker et al., 2004; Van Dijkhuizen-Radersma et al., 2004; Woo et al., 2004). Therefore, ongoing research is necessary in developing in IVIVC for these types of products (Uppoor, 2001).

This paper described pharmacokinetics and IVIVC after i.m. and s.c. injections of the microsphere formulations consisting of huperzine A and several PLGA polymers in dogs.

## 2. Materials and methods

### 2.1. Materials

Poly(D,L-lactide-co-glycolide) RG502H (lactide/glycolide ratio, 50/50; $M_n$, 5400) and RG503H (lactide/glycolide ratio, 50/50; $M_n$, 12 500) were supplied by Boehringer Ingelheim AG (Ingelheim, Germany); Huperzine A was obtained from Joyline & Joysun Pharmaceutical Stock Co. Ltd. (Zhengzhou, China).

### 2.2. Preparation of huperzine a microspheres

Huperzine A loaded microspheres were prepared using a modified O/W method. Huperzine A (6.5%, w/w, drug/polymer) and PLGA polymer or mixtures of PLGA polymers (20%, w/v) were dissolved in 50 mL dichloromethane. The solution was injected into 5000 mL 0.5% PVA aqueous solution at 6 °C under homogenization at various rate for 1 min and then the microspheres were solidified under mild mechanical stirring at 150 rpm with the temperature increased from 6 to 25 °C in the first 2 h and kept at 25 °C for 4 h. The solidified microspheres were filtrated with a 10 μm sieve and washed by distilled water for three times and then freezing dried (−20 to 25 °C). After passing through a 154 μm sieve, the microspheres were stored at 8 °C.

### 2.3. Characterization of the microspheres

#### 2.3.1. Particle size analysis

The particle size of the microspheres was determined using a laser particle size analyzer (LS230, Beckman Coulter Inc., Fullerton, CA, USA). Fifty milligrams of microspheres were suspended in 50 mL of distilled water and subjected to vortex mixing for 10 s before analysis.

#### 2.3.2. Scanning electron microscopy (SEM)

Microspheres were fixed on aluminum studs and coated with gold using a sputter coater. The samples were sputter-coated three times (2 min) under vacuum (0.1 mmHg) at a current intensity of 20 mA. Morphology of microspheres was then studied by scanning electron microscopy (JSM-840, JEOL, Tokyo, Japan).

#### 2.3.3. Determination of drug loading

Twenty milligrams of the microspheres was dissolved in 1 mL acetone in a 25 mL flask and 0.01 M HCl was added up to 25 mL under vigorous agitation. The precipitated polymer, in which no huperzine A was found, was removed by a 0.45 μm filter and the clear solution was used for analysis, which was carried out using HPLC connected with an UV detector in a mobile phase of the mixture of acetonitrile and 0.2% $H_3PO_4$ (25:75); flow rate, 0.7 mL/min; wavelength, 306 nm; column, ODS $C_{18}$ (250 mm × 4.6 mm i.d., 5 μm particle size); injection volume, 20 μL. The drug loading was then determined and the encapsulation efficiency were calculated using the following equation: Encapsulation efficiency (%) = 100 × drug loading/theoretical drug loading.

### 2.4. In vitro release

Five milligrams of microspheres was suspended in 3 mL of 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 0.01% Tween 80, 0.05% sodium azide and 0.04 M sodium chloride in a 5 mL plastic vial ($n = 3$), and then the suspension was placed in a shaking bath (HZS-H, DongLian Electronic Co., Harbin, China) at 40 rpm and 37 °C. Sink conditions were maintained during this study. At preset intervals, the vials were centrifuged at 3000 × *g* for 30 min and then 2 mL of the supernatant was drawn and replaced by fresh buffer. Huperzine A in supernatant was determined by HPLC method described above.

### 2.5. In vivo study

#### 2.5.1. Animals

Animal experiments were conducted according to protocols approved by the Animal Care and Use Committee of Shandong Engineering Research Center of Natural Drug. Five beagle dogs (male and female, 10.5 ± 0.4 kg), purchased from Kejin Laboratory Animal Co. Ltd. (Nanhai, Guangdong, china), were used in intravenous (i.v.), i.m. and s.c. administrations. During the whole study, uniform feed and free water were supplied. The process of blood sampling had a 2- or 4-week washout and recovery period.

#### 2.5.2. Drugs

Huperzine A solution (100 μg/mL) was prepared by dissolving huperzine A in 0.9% NaCl. Huperzine A loaded microspheres suspension (850 μg huperzine A/mL) was prepared by dispersing the huperzine A loaded microspheres in sterile water containing 1.5% carboxymethylcellulose sodium and 0.9% NaCl. The doses of the administrated huperzine A were designed according to the dose conversion factors based on body surface area between dog and human. The formula is that dose of μg/kg in dogs per day = dose of μg/kg in humans per day × 1.8 (FDA, 2002).

*2.5.3. Dosing and sample collection*

*2.5.3.1. i.v. administration of huperzine A solution.* Five beagle dogs were injected i.v. with a single dose of huperzine A (10 μg/kg). Blood samples (3 mL) were collected into heparinized tubes before and at 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after drug administration. Plasma was separated by centrifugation for 10 min at 3000 × *g* and stored at −20 °C until analysis.

*2.5.3.2. i.m. and s.c. administrations of huperzine A loaded microspheres.* Five beagle dogs were injected i.m. or s.c. with a single dose of huperzine A loaded microspheres suspension at dose of 170 μg/kg. Blood samples were similarly collected at 0.5, 1, 2, 3, 6, 8 h, 1–15 days after drug administration. Plasma was separated by centrifugation for 10 min at 3000 × *g* and stored at −20 °C until analysis. Huperzine A was analyzed using LC–MS/MS, according to the previous method (Wang et al., 2004).

*2.5.4. Pharmacokinetic analysis*

The terminal elimination rate constants ($K_e$) after i.v. administration were estimated with least-squares regression of values in the terminal log-linear region of plasma concentration–time curves at the time points above. The terminal elimination half-life ($T_{1/2}$) was calculated as $0.693/K_e$. The areas under the curve from time zero to last sampling time ($AUC_{0-t}$) after drug administration were determined by the linear trapezoidal rule. The area under the curve from time zero to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/K_e$, where $C_t$ is the last detectable plasma concentration and $t$ is the time at which this concentration occurred. The mean plasma concentration–time data after i.v. administration of the huperzine A solution was used to obtain the best fitted compartmental model by Drug and Statistics (DAS) version 2.0 (Anhui Provincial Center for Drug Clinical Evaluation, China) program. Model-dependent pharmacokinetic parameters of $K_{10}$, $K_{12}$ and $K_{21}$ were also obtained. All data were expressed as mean ± standard deviation (S.D.).

*2.6. IVIVC*

The data generated in the pharmacokinetic study were used to develop the IVIVC (Level A). The relationship between percent *in vitro* dissolution in PBS at 37 °C and the fraction of drug absorbed *in vivo* ($F_a$) was examined. The $F_a$ was determined using the Wagner–Nelson method (WN) by the following equation: $F_a = (C_t/K_e + AUC_{0-t})/AUC_{0-\infty}$ (Wagner and Nelson, 1963) and the Loo–Riegelman method (LR) by the following equation: $F_a = [C_t/K_{10} + AUC_{0-t} + (X_p)_t/(V_c \times K_{10})]/AUC_{0-\infty}$ (Loo and Riegelman, 1968). The relationship between percent *in vitro* dissolution in PBS at 37 °C and percent AUC ($AUC_{0-t}/AUC_{0-\infty}$) was also examined. Linear regression analysis was applied to the IVIVC plots. The values of correlation coefficient ($R^2$), slope and intercept were calculated, respectively.

## 3. Results

*3.1. Characterization of the microspheres*

Formulations A–G in Table 1 represented the huperzine A loaded microspheres prepared with various PLGA or homogenization speeds. The characterizations of the microspheres showed that the encapsulation efficiency of huperzine A was influenced significantly by the molecular weight of PLGA and was increased from 24.4% in the case of 503H to 64.2% of 502H. This indicated that an increased content of the carboxylic terminal group of PLGA polymers due to a reduction of the molecular weight could improve the encapsulation of huperzine A which had a primary amino group (Gao et al., 2006). When the homogenization speed was reduced from 2000 to 1000 rpm in the case of the preparation of the microspheres using a mixture of PLGA (502H/503H, 1/1), the encapsulation efficiency and the mean particle size were increased from 38.4 to 45.3% and from 12.7 to 72.3 μm, respectively. Formulation F was obtained by the same preparation method as formulation E except that the concentration of PLGA in dichloromethane was 25% (w/v). Compared with formulation E, formulation F had an about 1.5 times larger particle size, but only displayed a slightly higher encapsulation efficiency. SEM analysis showed a spherical, nonporous and solid morphology of the microspheres. The *in vitro* releases of huperzine A from all microspheres in PBS of pH 7.4 at 37 °C were shown in Fig. 1. The release rates were reduced by the increases of the molecular weight of PLGA and of the particle size.

*3.2. Pharmacokinetic study*

The mean plasma concentration–time curve of huperzine A after single i.v. administration of the huperzine A solution at the dose of 10 μg/kg in dogs was shown in Fig. 2. Huperzine

Table 1
Preparation of huperzine A loaded microspheres

| Formulation | PLGA | $M_n$ (GPC) | Homogenization speed (rpm) | Encapsulation efficiency (%) | Particle size (μm) |
|---|---|---|---|---|---|
| A | 502H | 5400 | 1000 | 64.2 | 51.6 |
| B | 502H/503H (1/1) | 8200 | 2000 | 38.4 | 12.7 |
| C | 502H/503H (1/1) | 8200 | 1500 | 41.1 | 35.8 |
| D | 502H/503H (1/1) | 8200 | 1200 | 43.9 | 55.1 |
| E | 502H/503H (1/1) | 8200 | 1000 | 45.3 | 72.3 |
| F[a] | 502H/503H (1/1) | 8200 | 1000 | 47.4 | 113.2 |
| G | 503H | 12500 | 1000 | 24.4 | 80.2 |

[a] Concentration of PLGA in dichloromethane was 25% (w/v).

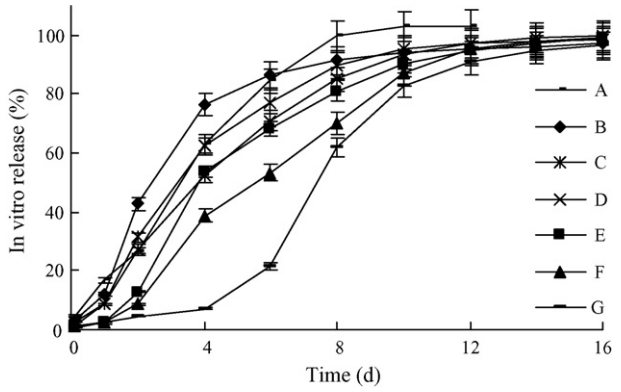


Fig. 1. *In vitro* releases of huperzine A loaded microspheres A–G in PBS at 37 °C. Each point represents mean ± S.D. ($n = 3$).

A was rapidly eliminated with the $K_e$ of 0.14 ± 0.01 /h and the $T_{1/2}$ of 5.12 ± 0.31 h and well fitted to a two-compartment model ($1/c^2$) with the $K_{10}$, $K_{12}$ and $K_{21}$ of 0.35 ± 0.07, 0.81 ± 0.81 and 0.40 ± 0.26 /h, respectively.

The mean plasma concentration–time curves of huperzine A following i.m. and s.c. injections of microspheres E at the dose of 170 μg/kg in dogs were shown in Fig. 3a.

After low initial bursts, the PLGA microspheres sustained the releases of huperzine A within 12–13 days. The mean cumulative releases calculated by the methods mentioned above showed that 3.7–5.8% of huperzine A following i.m. administration or 3.5–7.3% of huperzine A following s.c. administration was delivered within 24 h, respectively. In the case of s.c. administration, the plasma concentration of huperzine A in the first few hours was lower than that of i.m. administration but surpassed in a short time and reached a $C_{max}$ of 3.4 ± 1.2 ng/mL at day 3, which was higher than that (2.3 ± 0.4 ng/mL) of i.m. administration. At day 4 the plasma concentration was lower again than that of i.m. route and slowly fell until the drug was exhausted at day 12 with the $AUC_{0-\infty}$ of 252.2 ± 28.1 ng h/mL, which was similar to that (263.7 ± 31.2 ng h/mL) of i.m. administration.

The mean plasma concentration–time curves of huperzine A after i.m. administrations of microspheres *A* and *F* at the dose of 170 μg/kg in dogs were shown in Fig. 3b. As expected, the initial release of huperzine A from the microspheres was also low with the mean cumulative release of 6.4–9.6% for formulation A and

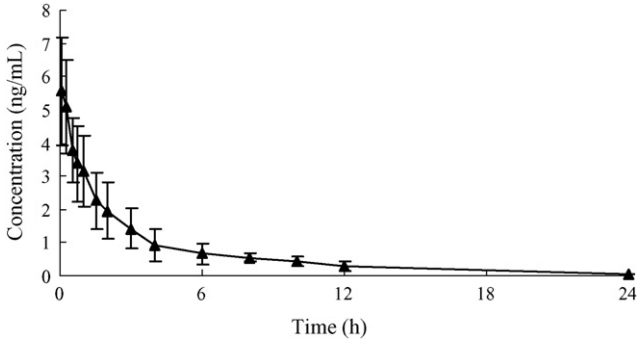


Fig. 2. Plasma concentration vs. time curve for huperzine A after single i.v. administration of the huperzine A solution at the dose of 10 μg/kg in dogs. Each point represents mean ± S.D. ($n = 5$).

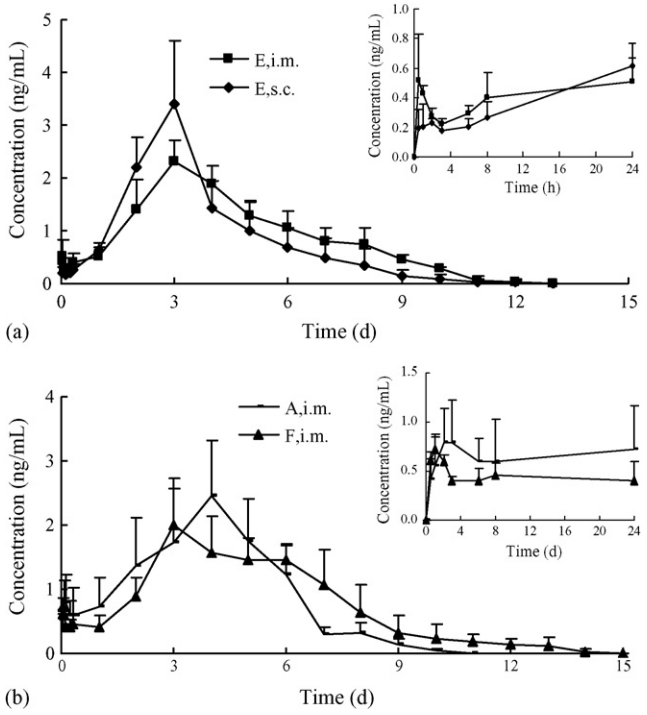


Fig. 3. Plasma concentration vs. time curves for huperzine A after (a) i.m. and s.c. administrations of the microspheres E and (b) i.m. administrations of the microspheres A and F at the dose of 170 μg/kg in dogs. The insert shows the plasma concentration vs. time curves for huperzine A until 24 h after drug administration. Each point represents mean ± S.D. ($n = 5$).

4.2–5.6% for formulation F within the first day, respectively. The plasma concentration of huperzine A reached the $C_{max}$ of 2.5 ± 0.9 ng/mL at day 4 for formulation A and 2.0 ± 0.7 ng/mL at day 3 for formulation F, respectively. And then the plasma concentration slowly fell until the drug was exhausted at day 11 with the $AUC_{0-\infty}$ of 248.1 ± 18.2 ng h/mL for formulation A and at day 15 with the $AUC_{0-\infty}$ of 256.9 ± 23.4 ng h/mL for formulation F, respectively.

### 3.3. IVIVC

The data generated in the pharmacokinetic study after the i.v. administration of the huperzine A solution at the dose of 10 μg/kg and after the i.m. administrations of the microsphere formulations A, E and F at the dose of 170 μg/kg to dogs were used to develop the IVIVC. The Wagner–Nelson procedure and the Loo–Riegelman method with the linear trapezoidal rule were used to obtain an *in vivo* cumulative release profiles. The relationship between percent *in vitro* dissolution of and percent $AUC_{0-\infty}$ of the huperzine A loaded microspheres was also established.

The *in vitro* and *in vivo* cumulative release profiles and percent $AUC_{0-\infty}$ of the huperzine A loaded microspheres were shown in Fig. 4. The percent drug absorbed and percent $AUC_{0-\infty}$ versus the amount of drug released *in vitro* plots were shown in Fig. 5. Table 2 showed a good linear regression relationship between the percent *in vitro* dissolution in PBS at 37 °C and the percent absorption ($R^2 = 0.974$–$0.990$,

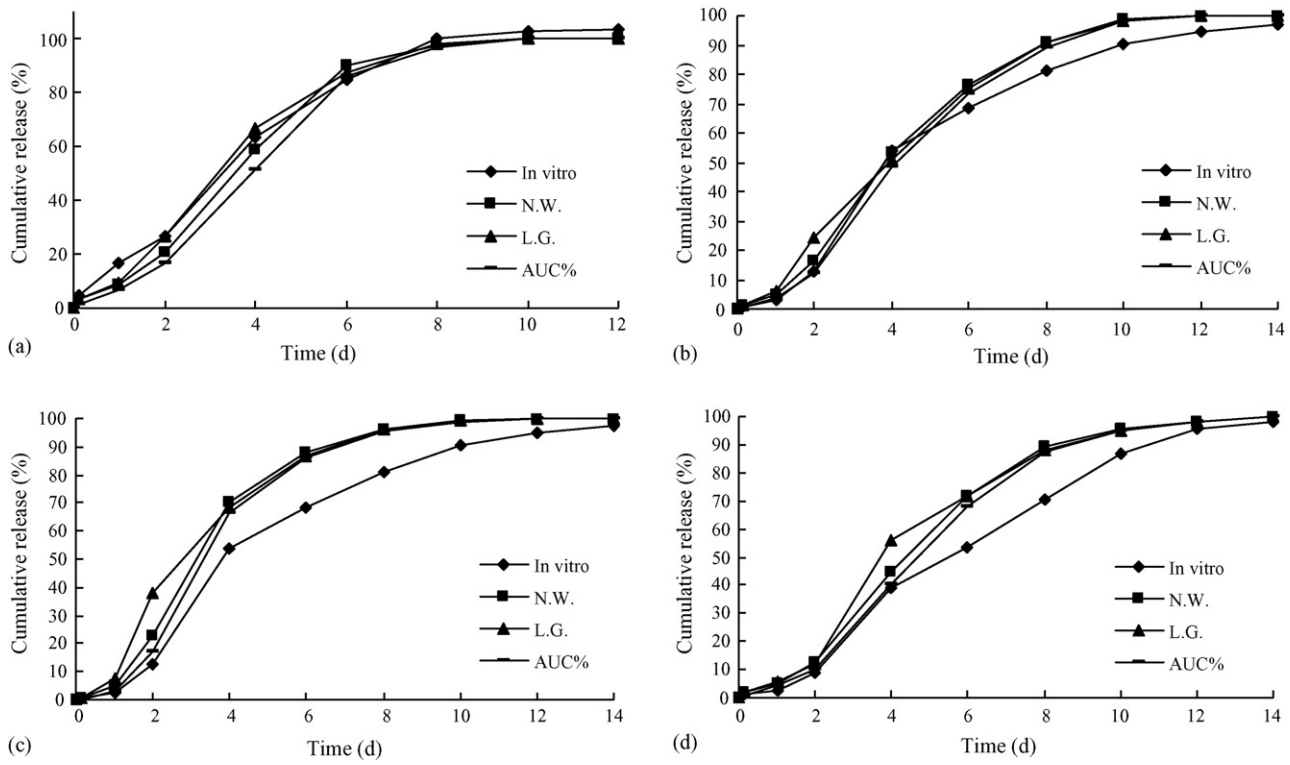


Fig. 4. *In vitro* releases in PBS at 37 °C and *in vivo* cumulative releases using the Wagner–Nelson and Loo–Riegelman methods and percent AUC of huperzine A in dogs from PLGA microspheres (a) A, i.m., (b) E, i.m., (c) E, s.c. and (d) F, i.m.

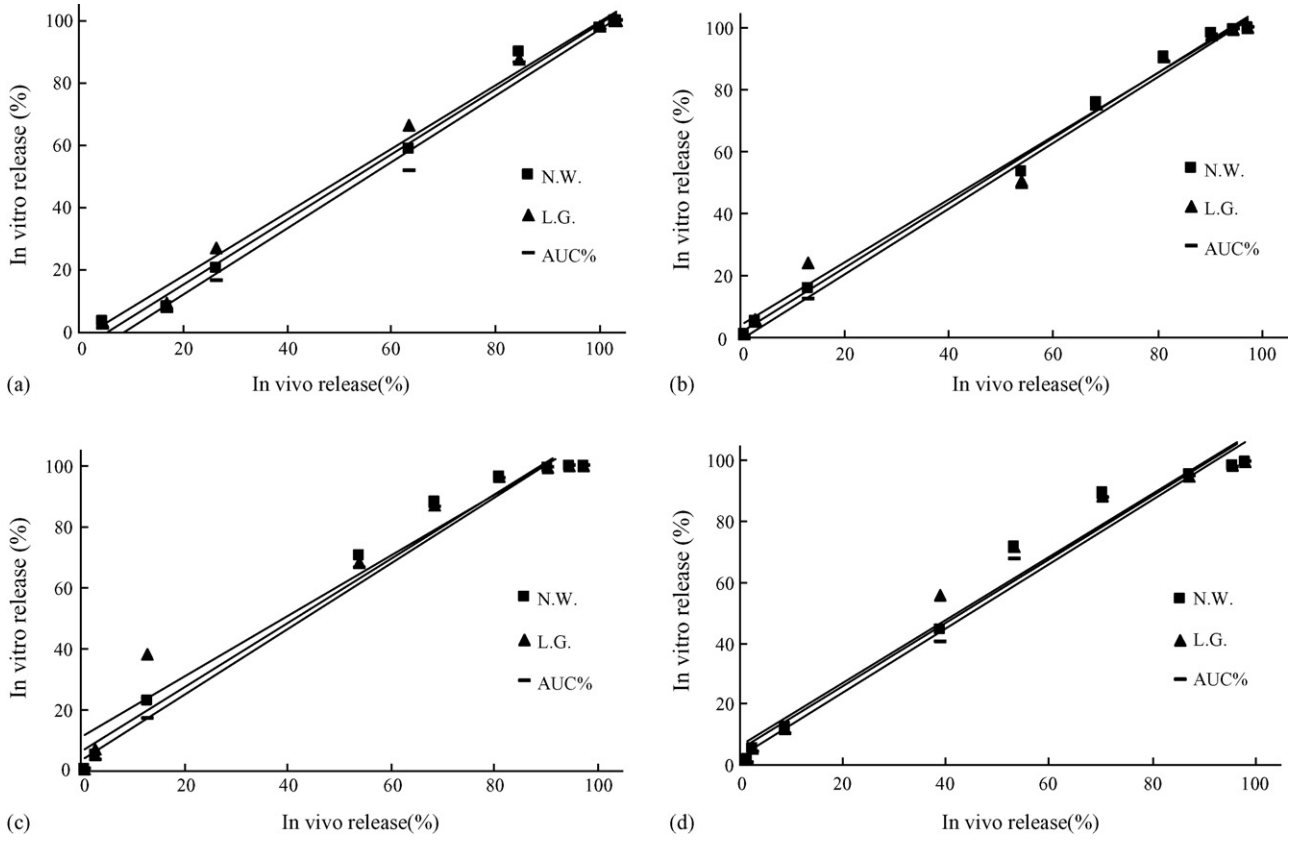


Fig. 5. IVIVC model linear regression plots of cumulative absorption and percent AUC vs. percent dissolution of huperzine A from PLGA microspheres (a) A, i.m., (b) E, i.m., (c) E, s.c. and (d) F, i.m.

Table 2
The values of correlation coefficient ($R^2$), slope and intercept obtained in the IVIVC model using linear regression analysis

| Formulation | *In vivo* absorption model | $R^2$ | Slope | Intercept |
|---|---|---|---|---|
| A (i.m.) | WN[a] | 0.992 | 1.039 | −5.436 |
| | LR[b] | 0.993 | 1.013 | −2.116 |
| | AUC%[c] | 0.992 | 1.06 | −9.244 |
| E (i.m.) | WN | 0.990 | 1.054 | 1.428 |
| | LR | 0.988 | 1.02 | 3.824 |
| | AUC% | 0.990 | 1.066 | −1.111 |
| E (s.c.) | WN | 0.976 | 1.052 | 6.158 |
| | LR | 0.959 | 0.99 | 11.02 |
| | AUC% | 0.984 | 1.078 | 2.954 |
| F (i.m.) | WN | 0.974 | 1.043 | 4.985 |
| | LR | 0.968 | 1.029 | 6.661 |
| | AUC% | 0.98 | 1.056 | 2.525 |

[a] Wagner–Nelson method.
[b] Loo–Riegelman method.
[c] $(AUC_{0-t}/AUC_{0-\infty}) \times 100$.

$P < 0.001$ for the Wagner–Nelson method; $R^2 = 0.959$–$0.993$, $P < 0.001$ for the Loo–Riegelman method) and percent $AUC_{0-\infty}$ ($R^2 = 0.98$–$0.992$, $P < 0.001$) after i.m. and s.c. administrations of the huperzine A loaded microspheres in dogs. Similar results were also obtained in mice, which will be reported elsewhere.

## 4. Discussion

To obtain a microsphere formulation with the appropriate sustained release profile, encapsulation efficiency and particle size for the treatment of Alzheimer's disease, PLGA polymers of 502 and 503H and their mixture were used to encapsulate huperzine A. Based on the above data, formulation E, which had an appropriate property, was potential for the treatment of Alzheimer's disease and was applied in the pharmacokinetic study.

To investigate the effect of administration route on huperzine A absorption, s.c. injection was chosen as an alternative parenteral route other than i.m. injection of microspheres. After formulation E was administrated to dogs via either s.c. or i.m. injection, though similar AUCs were found, the differences in the plasma concentration–time profiles and *in vivo* cumulative release profiles were significant.

From the profiles, it was clear that the release of huperzine A from the microspheres after s.c. administration was faster than that after i.m. administration. Many factors can affect drug absorption after i.m. or s.c. injection, such as molecular size and hydrophilicity of drug, blood supply at injection site and so on (Zuidema et al., 1988). It has been reported that blood flow rate in musculature was higher than that in hypodermia (Liang, 2004), and as a result, the i.m. injection of hydrophilic drug often had a higher $C_{max}$ and a shorter $T_{max}$ than that of s.c. injection (Vaag et al., 1990; Tassi et al., 1994; Zuidema et al., 1994; Ranheim et al., 2002).

Huperzine A is a small molecule alkaloid with a solubility of about 0.89 mg/mL in water. It was expected that the absorption rate of huperzine A in musculature should be faster than that in hypodermia, but an unusual result was obtained from the experiments: $C_{max}$ after s.c. injection of the huperzine A loaded microspheres was higher than that after i.m. injection. Huperzine A can penetrate easily the biologic membrane (Tang and Han, 1999). Therefore, the rate-limited release of huperzine A from the microspheres in the injection sites would be a dominant factor resulting in the pharmacokinetic differences. Drug release from biodegradable delivery systems of PLGA polymer occurs by a combined mechanism of drug diffusion and polymer degradation or erosion (Furr and Hutchinson, 1992; Shah et al., 1992). It had been concluded that the site of administration had an effect on the degradation rate of PLGA microspheres, and the microspheres at the s.c. site degraded faster than that at the i.m. site (Sandor et al., 2002). PLGA microspheres administrated i.m. had a lower level of cellular infiltration than that of the microspheres administrated in the s.c. site (Sandor et al., 2002). Action of macrophages can help to contribute a surface erosion effect to the normally bulk-eroding polymer. Alternatively, macrophages can produce an acidic microenvironment in the vicinity of the polymer to be digested. This might create an acid-catalyzed degradation effect with the oligomers produced being quickly phagocytosed (Huffman and Casey, 1985; Gogolewski et al., 1993). These indicated that the mass loss for PLGA microspheres administrated i.m. was mainly due to the hydrolytic degradation and the decrease in molecular weight, which was similar to the typical *in vitro* situation, whereas for the microspheres administrated s.c., due to more enzymatic, macrophages and other cellular activity within the surrounding tissue, increased erosion existed (Sandor et al., 2002).

For formulation A, due to the lower molecular weight of PLGA, the *in vivo* release of huperzine A was faster than that of formulation E, which agreed with their *in vitro* releases. Larger mean particle size of formulation F resulted in a slower *in vivo* release of huperzine A than that of formulation E with smaller mean particle size, which was also similar to the results of the *in vitro* dissolution assays.

Although an IVIVC can be defined with a minimum of two formulations with different release rates, three or more formulations with different release rates are recommended (FDA, 1997). In this study, formulation E and other two formulations A and F with relative higher encapsulation efficiency and different release rates were used to establish the IVIVC of huperzine A loaded microspheres in dogs. Huperzine A can penetrate easily the biologic membrane, which resulted in a rapid absorption of huperzine A *in vivo*. For a rapid absorption process of drug, it is appropriate to use the Wagner–Nelson procedure or the Loo–Riegelman method for obtaining an absorption profile (Hwang et al., 1993), because the difference between the cumulative amount released and the cumulative amount absorbed, i.e. the amount of drug released from the dosage form but not yet absorbed, could be negligible. The Loo–Riegelman method is used usually in the calculation of the cumulative absorption of the drug which is fitted to a two-compartment model. Though the Wagner–Nelson method is mainly applied to the pharmacokinetic study of the drug fitted to a one-compartment model, due to its simplicity, this method is also used for the drugs fitted to a two-compartment model (Schliecker et al., 2004). In this

study, a similar IVIVC indicated that for the IVIVC analysis of huperzine A loaded microspheres, these two methods could be used alternatively.

The Wagner–Nelson and Loo–Riegelman methods as well as numerical deconvolution are recommended by FDA to calculate the absorption profile (FDA, 1997). When these procedures were used to acquire the IVIVC, pharmacokinetic parameters from drug immediate release formulation is necessary. Percent AUC has been used in IVIVC analysis of microsphere formulation without using parameters from immediate release formulation (Woo et al., 2004). In this study, though no parameters were lacked, to further estimate IVIVC by percent $AUC_{0-\infty}$, it was tried to use percent AUC to establish IVIVC of the sustained release formulations of huperzine A and a successful IVIVC was obtained.

Because the *in vivo* degradation of PLGA was faster than that *in vitro* due to the foreign body response (Spenlehauer et al., 1989; Tracy et al., 1999), when the release of drug was mainly controlled by the degradation of the polymer matrix, the *in vitro* releases of PLGA-based release systems in PBS were slower than those *in vivo* (Soriano et al., 1996; Machida et al., 2000; Jiang et al., 2003). While for diffusion-controlled sustained-release systems, the release of drug depended more on the permeability of the polymeric matrix and was contributed less by the degradation of the polymer. In this case, a proper IVIVC can be found (Van Dijkhuizen-Radersma et al., 2004). Since huperzine A is a small molecule alkaloid, its release from the microspheres was contributed mainly by the dissolution and diffusion of huperzine A in the swollen microspheres. As a result, though there was a longer erosion period of the PLGA used (4–6 weeks), the diffusion-controlled release of huperzine A from the swollen PLGA matrix was sustained within two weeks. Also, huperzine A was absorbed rapidly, which resulted that the *in vivo* absorption profile was a good approximation of the release profile of huperzine A from microspheres. Therefore, the good IVIVC of huperzine A loaded PLGA microspheres was obtained and could be explained by the diffusion-controlled sustained-release of huperzine A from PLGA microspheres combined with a rapid *in vivo* absorption of huperzine A.

Via the same route of i.m. administration, the values of $R^2$ of the formulation E were higher than these of formulation F. Formulation F had a larger particle size than that of formulation E, which slowed the diffusion of huperzine A from microspheres and increased the effect of PLGA degradation on the release of huperzine A. Based on the faster *in vivo* degradation of PLGA matrix, the larger particle size of formulation F increased the difference between the *in vitro* and *in vivo* releases. All of the values of $R^2$ of formulation A were higher than these of formulation E. An IVIVC after i.m. injection of huperzine A loaded PLGA (lactide/glycolide ratio, 75/25; $M_n$, 15 000) microspheres to rats (Fu et al., 2005) was also reported with a $R^2$ of 0.98, which was lower than these in this study. The reason should be similar, a higher molecular weight and ratio of lactide/glycolide of the PLGA slowed the diffusion of huperzine A and increased the effect of polymer degradation on the release of huperzine A. In the case of the formulation E, when it was i.m. injected, the values of $R^2$ were higher than that when it was s.c. injected. This agreed with that described above; the physiological factors in hypodermia accelerated the degradation of PLGA matrix, which led to a decreased $R^2$ after the s.c. administration.

## 5. Conclusion

Huperzine A loaded PLGA microspheres were successfully prepared using an O/W method. With the increase of the PLGA molecular weight and the particle size, the *in vitro* and *in vivo* release periods of huperzine A were prolonged. The release of huperzine A from microspheres following s.c. injection was faster than that following i.m. injection due to an accelerated degradation of PLGA in hypodermia. Since huperzine A was diffusion-controlled released from the polymeric matrix and was absorbed *in vivo* rapidly, a good linear regression relationship was observed between the percent *in vitro* dissolution in PBS at 37 °C and the percent absorption or percent AUC. Larger particle size slowed the diffusion of huperzine A from microspheres and increased the effect of PLGA degradation on the release of huperzine A, which led to the lower values of $R^2$ than these of the smaller microspheres. Because physiological factors affected the degradation of PLGA matrix in hypodermia more than that in musculature, the values of $R^2$ after i.m. administration were higher than these after s.c. administration.

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