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Development, characterization and application of *in situ* gel systems for intranasal delivery of tacrine

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ABSTRACT

The present study aimed to develop an *in situ* gel formulation for intranasal delivery of tacrine (THA), an anti-Alzheimer's drug. Thermosensitive polymer Pluronic F-127 was used to prepare THA *in situ* gels. Sol–gel transition temperature ($T_{sol-gel}$), rheological properties, *in vitro* release, and *in vivo* nasal mucociliary transport time were optimized. The pharmacokinetics and brain dispositions of *in situ* gel were compared with that from THA oral solution in rats. The *in situ* gel demonstrated a liquid state with Newtonian fluid behavior under 20 °C, while it exhibited as non-flowing gel with pseudoplastic fluid behavior beyond its $T_{sol-gel}$ of 28.5 °C. Based on nasal mucociliary transport time, the *in situ* gel significantly prolonged its retention in nasal cavity compared to solution form. Moreover, the *in situ* gel achieved 2–3 fold higher peak plasma concentration (C_{max}) and area under the curve (AUC) of THA in plasma and brain tissue, but lowered C_{max} and AUC of the THA metabolites compared to that of oral solution. The enhanced nasal residence time, improved bioavailability, increased brain uptake of parent drug and decreased exposure of metabolites suggested that the *in situ* gel could be an effective intranasal formulation for THA.

1. Introduction

Tacrine (THA), a reversible and non-selective acetylcholinesterase inhibitor, was the first oral prescription medication approved by FDA in 1993 to improve the symptoms of mild to moderate Alzheimer's disease (AD) (Summers, 2006). Due to its flat configuration and medium lipophicility with a Log *P* of 2.71 (Summers, 2000), THA has a good intestinal permeability with an apparent permeability coefficient of 2.5×10^{-5} cm/s across rat jejunum (Jasti et al., 2009). Its clinical use is, however, hampered by low oral bioavailability (17–24% in human) due to extensive first-pass metabolism (Hartvig et al., 1990; Madden et al., 1995) and dose-dependent hepatotoxicity (O'Brien et al., 1991; Qizilbash et al., 1998). The hepatotoxicity of THA is probably resulted from its reactive metabolites (Patocka et al., 2008). Therefore, it is worth exploring alternative routes of administration to avoid its first-pass metabolism as well as to enhance the bioavailability and brain targeting effect of THA (Fig. 1).

Intranasal delivery is a non-invasive and convenient method that could provide efficient systemic delivery for certain therapeutic compounds (Dhuria et al., 2010). The nasal route might also avoid the first-pass metabolism if the nasal drug could be retained and absorbed in the nasal cavity, thereby reducing the biotransformation of the parent drug to metabolites (Wong and Zuo, 2013; Wong et al., 2012; Wong and Zuo, 2010). Jogani et al. developed a nasal mucoadhesive microemulsion of [^{99m}Tc]-THA and, according to total radioactivity measurement, it exhibited a 3-fold higher brain disposition than that after intravenous administration of [^{99m}Tc]-THA solution (Jogani et al., 2008). This indicated that the nasal route could be an alternative way for THA administration. The limitation of radioactivity measurement is that the parent drug could not be differentiated from its metabolites. Therefore, the exact systemic and brain dispositions of THA and its metabolites after intranasal THA administration remain to be established.

It was noticed that the nasal absorption of liquid dosage form is often limited by its short residence time due to quick clearance from nasal cavity (Illum, 2003). Several strategies, such as adding absorption enhancers (Illum et al., 1994) and using nasal gel or powder dosage forms (Illum et al., 2002; Wang et al., 2013), have

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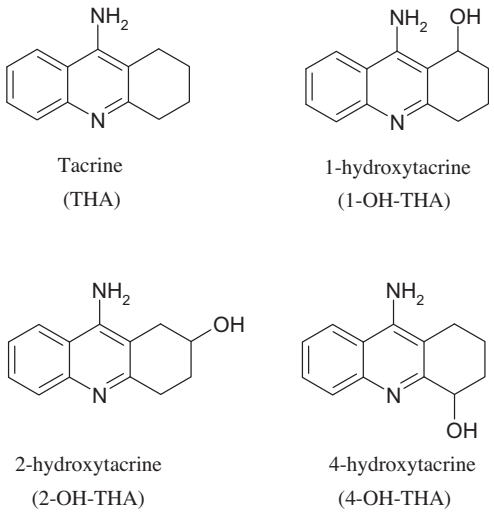

Fig. 1. Chemical structure of tacrine (THA), 1-hydroxytacrine (1-OH-THA), 2-hydroxytacrine (2-OH-THA) and 4-hydroxytacrine (4-OH-THA).

been applied to prolong the contact time of drugs with the nasal mucosa or enhance drug permeability across the nasal mucosa. In our preliminary study, addition of chitosan hydrochloride or various types of cyclodextrins did not show significant improvement of nasal permeability of THA across Calu-3 cell monolayer (data not shown), which might be due to its inherent high nasal permeability (P_{app} on Calu-3 cell: 1.1×10^{-5} cm/s (Qian et al., 2010)) with transcellular diffusion as its major membrane transport pathway. Therefore, we speculated that composition with only absorption enhancer in THA solution might not be able to provide significant enhancement in its nasal bioavailability. Illum et al. developed a mucoadhesive microsphere powder of morphine and found a 5–7 fold higher nasal bioavailability in sheep in comparison to its nasal solution, which could be, at least partially, attributed to the prolonged contacting time between the drug and nasal epithelium (Illum et al., 2002). Nasal gels have also been employed to enhance drug delivery efficiency by reducing swallowing and anterior leakage of formulation. Several products such as vitamin B12 nasal gel have been marketed (Suzuki et al., 2006). However, both nasal powder and ordinary gel encounter several problems including difficulty in accurate dosing, nasal mucosa irritation and a gritty feeling in nose (Behl et al., 1998). Recently, the nasal *in situ* gel appears very promising since it exists as fluid before nasal administration and thus could be easily spray from nebulizer device with an accurate dose, while it forms gel film in the nasal cavity with effective contact to nasal epithelium (Watts and Smith, 2009).

In general, *in situ* gelation can be achieved by using thermosensitive polymers which forms gel on instillation by sensing nasal temperature. Pluronic F-127, with excellent thermosensitive gelling properties at physiological temperature, low toxicity and irritation, excellent water solubility, good drug release characteristics and compatibility with other chemicals (Anderson et al., 2001; Jeong et al., 2002; Li and Guan, 2011), has been extensively investigated for developing *in situ* gel systems for nasal, transdermal, rectal and ocular applications (Escobar-Chavez et al., 2006).

The aim of the present study was to develop a THA nasal *in situ* gel using Pluronic F-127 as gelling agent. The optimized THA *in situ* gel with favorable gelation temperature, *in vitro* release and rheological properties would be selected to perform *in vivo* evaluations in rats. Systemic pharmacokinetics and brain dispositions of THA as well as its metabolites after intranasal administration of the THA *in situ* gel would be compared to that obtained from the conventional oral route.

2. Materials and methods

2.1. Materials

THA hydrochloride was purchased from Enzo Life Sciences Inc. (Farmingdale, NY, USA). 1-Hydroxytacrine (1-OH-THA), 2-hydroxytacrine (2-OH-THA) and 4-hydroxytacrine (4-OH-THA) were kindly gifted from Pfizer Inc. (Groton, CT, USA). Pluronic F-68, Pluronic F-127, polyethylene glycol 8000 (PEG 8000) and indigo carmine were obtained from Sigma (St. Louis, MO, USA). Chitosan hydrochloride with average molecular weight of ~200 kDa and deacetylation degree of 83% was purchased from Zhejiang Golden-Shell Biomedical Co. Ltd. (Zhejiang, China). Calcium chloride and potassium chloride were purchased from BDH (Poole, England). All other reagents were of at least analytical grade and were used without further purification. Distilled and deionized water was used for the preparation of solutions.

2.2. Preparation of THA in situ gels

The thermosensitive *in situ* gel systems were prepared by cold method described by Schmolka (Schmolka, 1972). Briefly, THA, Pluronic F-68, chitosan and PEG 8000 were stirred in distilled water at room temperature until all of them completely dissolved. The mixture, together with the container, was then put into ice bath followed by addition of Pluronic F-127 into the mixture. The final transparent solution was stored at 4 °C for further evaluations.

2.3. Physicochemical characterizations of the in situ gels

2.3.1. Sol–gel transition temperature measurement

The sol–gel transition temperature ($T_{sol-gel}$) of the prepared *in situ* gel formulations was determined as previously described by Gillert et al. (Gilbert et al., 1987). Briefly, 2 ml of the prepared formulation was transferred into a test tube (10 ml) with a diameter of 1.0 cm and sealed with a parafilm. The tube was kept in a circulation water bath at 8 °C, and the temperature of water bath was increased at an increment of 2–3 °C in the beginning (from 8 °C to 18 °C) and then at 0.2–0.5 °C until gelation. After each setting of the water bath temperature, 10 min was allowed for equilibration. The test tube was then taken out and placed horizontally to observe the state of the sample, and gelation was said to occur when the meniscus would no longer move upon.

2.3.2. In vitro drug release from the in situ gels

In vitro release studies of THA *in situ* gel formulations were performed in 500 ml of simulated nasal electrolyte solution (containing 1.29 mg/ml KCl, 7.45 mg/ml NaCl and 0.32 mg/ml $CaCl_2 \cdot 2H_2O$, pH adjusted to 5.7 by HCl) (Callens et al., 2003) at 35 ± 0.5 °C using the USP II method. A dialysis bag (Spectra/Por® membrane, MWCO: 12,000–14,000, Spectrum Laboratories Inc., CA, USA) containing 1 g of *in situ* gel formulation was attached onto the paddle by thread and the rotating speed was set at 50 rpm. Samples (2 ml) were collected through 0.22 μm syringe filters at 5, 10, 30, 45, 60, 90, 120, 180, 240, 360, and 600 min. The same volume of fresh medium was replaced after each sampling. The *in vitro* release experiments were run in five replicates for each formulation. The release of THA from its solution formulation (dissolving THA in normal saline) was also tested to serve as the control.

THA concentrations in the samples were determined by HPLC/UV method. The HPLC/UV system consists of Waters 600 controller (pump), Waters 717 auto sample and Waters 996 Photodiode Array detector. Data collection was performed using a

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