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Controlled Release Tacrine Delivery System for the Treatment of Alzheimer's Disease

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Alzheimer's disease is a neurodegenerative condition that affects ~5 million people and is the fourth leading cause of death in America. Tacrine is one of the three drugs approved by the FDA for the treatment of Alzheimer's disease. However, the drug has a short biologic half-life of 2–3 hr and gastrointestinal, cholinergic, and hepatic adverse reactions that are associated with high doses of the drug. The aim of our study was to formulate a controlled release delivery system of tacrine that could be used to minimize the side effects associated with the drug. Microparticles of tacrine were formulated using poly(D,L-lactide-co-glycolide) (PLG). PLG and tacrine were dissolved in mixed organic solvents and added to a polyvinyl alcohol solution that was stirred at a constant rate. The organic solvent was evaporated overnight and the formed microparticles were collected by filtration, dried, and sieve-sized. The effects of such formulation variables, as molecular weight of polymer, stir speed during preparation, and drug loading on encapsulation efficiency (EEF), and *in vitro* release profiles of tacrine were investigated. An increase in the molecular weight of polymer from 8,000 to 59,000 and 155,000 resulted in ~10-fold increase in EEF, but the rate of release decreased with increasing molecular weight. Stir speed during preparation had an effect on the EEF but not on the rate of release. Drug loading did not have a significant effect on the EEF but had an effect on the rate of tacrine release. The results suggest that tacrine could be delivered at controlled levels for weeks for the treatment of Alzheimer's disease.

Keywords Alzheimer's Disease, Bioerodible, Controlled Release, Microspheres, Tacrine

Alzheimer's disease (AD) is a neurodegenerative condition that afflicts ~5 million people and is the fourth leading cause of death in America (Iverson et al. 1993). The Alzheimer's Association estimates that the number of people suffering from Alzheimer's disease could reach 14 million by the middle of 2001 unless a cure or prevention is found (Alzheimer's Association 2000). In its simplest model, AD is a condition of memory loss due to progressive degeneration of the brain's acetylcholine neuron system (Cowley 1989). Manifestations of AD also extend beyond memory loss and include personality changes, neuromuscular changes, seizures, and occasional psychotic features (Cooper 1991). Alzheimer's disease is the most common cause of dementia in the United States. The incidence of the disease has risen steadily over the past decade, especially in elderly patients (Cooper 1991). The Alzheimer's Association estimates the disease's direct health expenditures at $100 billion a year (Alzheimer's Association 2000).

Exact causes of Alzheimer's disease are not completely understood and still remain a mystery. A definitive diagnosis of this disease is usually made during autopsy if the histologic examination of the brain tissue reveals characteristic neurofilbrillary tangles and neuritic plagues. Today, new diagnostic tools make it possible to obtain a diagnosis of probable Alzheimer's with an accuracy of ~90% (Alzheimer's Association 2000).

At present, there is no cure for Alzheimer's disease (Dinsmore 1999; Sramek and Cutler 1999; Qizilbash et al. 2000; Simonson 2000). Symptomatic treatment helps maintain patients' activities daily and alleviates symptoms brought on by the disease, such as depression, insomnia, and agitation (Baldinger and Schroeder 1995). Therapeutic treatments are aimed at stopping or reversing the disease process. However, these regimens have been primarily experimental and have had limited clinical efficacy. Investigational agents have included metabolic enhancers, vasodilators, nootropics, psychostimulants, acetylcholine precursors, and acetylcholine agonists (Gamzu et al. 1989; Harris et al. 1989; Beary 1990; Alzheimer's Association

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2000). To date, there are three Food and Drug Administration (FDA) approved drugs for the treatment of Alzheimer's disease—tacrine, donepezil and rivastigmine—and several others in clinical trials (Alzheimer's Association 2000). Tacrine, an acetylcholinesterase inhibitor, was approved by the FDA in 1993 for treatment of Alzheimer's disease. Tacrine is indicated for patients with mild to moderate Alzheimer's disease, and its clinical effects have been limited due to associated cholinergic, hepatic, and gastrointestinal adverse reactions (Abramowicz 1993; Qizilbash et al. 2000). One recent study showed that gastrointestinal side effects, such as diarrhea, anorexia, dyspepsia, and abdominal pain, and raised serum liver enzymes were the major reason for withdrawal (Qizilbash et al. 2000).

Controlled drug delivery systems offer an advantage over the conventional approach because the polymer that is applied in the design of such systems controls the rate at which the drug is released. Microencapsulated tacrine could be delivered systemically for a prolonged period of time at controlled lower concentrations to reduce the hepatic adverse reactions and bypass the gastrointestinal route. Encapsulation of water-soluble drugs by the use of nonaqueous systems such as oil-in-oil emulsion technique generally results in high encapsulation efficiency (O'Donnell and McGinity 1998; Marinina et al. 2000). However, processing the microparticles could be quite tedious if vegetable and mineral oils are involved. Our paper describes the formulation and characterization of a controlled release tacrine delivery system prepared by a simple oil-in-water emulsion technique with a high encapsulation efficiency, intended for the treatment of Alzheimer's disease.

MATERIALS AND METHODS

Materials

Poly(D,L-lactide-co-glycolide) (PLG) (50/50) of different molecular weight grades were supplied by Birmingham Polymers (Birmingham, AL, USA). Polyviny1 alcohol (PVA) (M.W. 30,000–70,000) and tacrine were obtained from Sigma Chemical Company (St. Louis, MO, USA). Methylene chloride and methanol were supplied by Fisher Scientific (Norcross, GA, USA). Distilled de-ionized water was used. All materials were used as supplied.

Preparation of Microspheres

Microspheres were prepared by the emulsion solvent evaporation procedure. PVA was dissolved in water to prepare 1% w/v solution. Then 500 mg of PLG and 300 mg of tacrine were dissolved in a mixed organic solvent of methylene chloride and methanol. Afterward, the drug-polymer solution was added to 25 mL of PVA solution that was being stirred at 500 rpm with a Lightnin Mixer (General Signal, NY, USA). The emulsion was stirred overnight to remove the organic solvents. The microparticles were collected by filtration, washed with water, and dried in air for 2 days. All products were sieve-sized using a combination of U.S. standard sieve numbers 40, 60, 120, and 400. Fractions collected between 40/60 (250–425 μm), 60/120 (125–250 μm), and 120/400 (37–125 μm) were used for further studies. The effects of polymer molecular weight, stir speed during preparation, and drug loading on the encapsulation efficiency, and kinetics of tacrine release were investigated. The experiments were conducted in triplicates.

Morphology of Microspheres

The surface morphology, shape and size of tacrine microspheres, was obtained using an Electroscan Model E-3 scanning electron microscope. The environmental scanning electron microscope allowed samples to be examined in their natural state without modification or preparation.

Determination of Tacrine Encapsulation Efficiency (EEF)

Fully 10 mg of tacrine microspheres were dispersed in 10 mL of methylene chloride that dissolved the polymer but not the drug. Tacrine was extracted five times with a total of 100 ml of water and analyzed spectrophotometrically. The amount of drug in the microspheres was determined using an UV-1201 spectrophotometer (Schimadzu Scientific Instruments, MD, USA) at 323 nm. The EEF was determined as the ratio of the amount analyzed to the initial amount of the drug added during preparation.

In Vitro Release of Tacrine from Microspheres

The equivalent of 10 mg of microspheres or free drug was used in all studies. The microspheres or the free drug was placed in 900 mL of distilled deionized water in dissolution beakers (USP Apparatus 2), and stirred at 100 rpm using a VK 7000 dissolution testing station (VanKel Technology Group, NC, USA). The VK 7000 was interfaced with the ADS 2000 sampling station and an UV-1201 spectrophotometer. The dissolution system was programmed to collect samples automatically through full flow filters at specified time intervals from the various beakers and circulated through the spectrophotometer for automatic recording of absorbances on a computer. Each sample was automatically returned to its original beaker after each measurement. The volume of the dissolution medium in each beaker was maintained at 900 mL throughout the dissolution studies. Absorbances of dissolved tacrine were measured at 323 nm and concentrations of the drug were calculated from a standard curve. Release data for three batches of microspheres were combined in one plot.

In another release study conducted for more than 1 month in an incubator, microspheres were suspended in 200 ml of pH 7.4 phosphate buffer in closed containers and agitated at 50 rpm. Samples of dissolved tacrine were removed at specified time intervals for spectrophotometric analysis. Fresh buffer was added each time to replace withdrawn samples to maintain the volume at 200 ml.

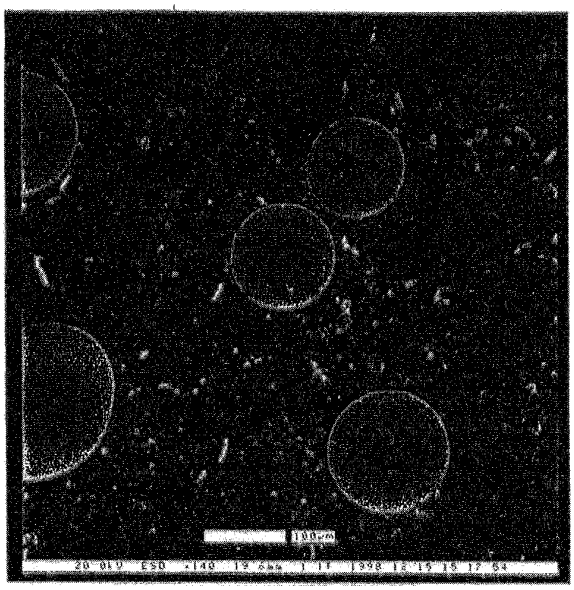

FIG. 1. Scanning electron micrograph shows the external appearance of tacrine microspheres prepared at 500 rpm with poly(D,L-lactide-co-glycolide) of molecular weight 155,000.

RESULTS AND DISCUSSION

The preparation conditions involving a volume fraction of 22% and a PVA concentration of 1% w/v were found to produce microspheres of desirable characteristics. The scanning electron micrograph of the external appearance of tacrine microspheres prepared at 500 rpm shows that the microparticles are spherical and have smooth surfaces (Figure 1). The EEF of tacrine in the microspheres depended on the molecular weight, stir speed during preparation, and particle size distribution of the microspheres, but it was independent of the drug loading. However, the release profile was found to depend on the molecular weight of polymer and drug loading, but independent of the stir speed used during preparation.

The EEF of microspheres prepared with PLG of different molecular weight grades are provided in Table 1. An increase in the molecular weight of polymer from 8,000 to 59,000 and 155,000 resulted in ~10-fold increase in EEF for microspheres of size range 250–425 μm and 125–250 μm, respectively. One of the factors that determine the rate of polymer precipitation is its molecular weight, which is related to the intrinsic viscosity of the polymer. At a high polymer intrinsic viscosity, the microencapsulation process yielded a high EEF. These observations are consistent with what has been reported for microparticles prepared with PLG and poly(epsilon-caprolactone) polymers (Cleland et al. 1997; Youan et al. 1999; Yang et al. 1999). This could be attributed to the extraction of methanol from the solvent mixture of the microdroplet into the aqueous phase coupled with solvent evaporation and exposure of the microdroplet to the aqueous phase. These processes allowed microspheres prepared with the higher molecular weight polymer to harden more quickly. Thus, there is significant slowing down of loss of tacrine into the external aqueous phase. Consequently, the EEF of microspheres of size range 250–425 μm prepared with PLG of molecular weight 155,000 and 8,000 were 85% and 7%, respectively. Similarly, the release profiles of tacrine from the microspheres indicate that the higher the molecular weight of the polymer used for the preparation, the lower the rate of drug release (Figure 2). About 5%, 25%, and 70% of the drug was released in 24 hr from microspheres prepared with PLG 155,000, 59,000, and 8,000, respectively.

TABLE 1
Encapsulation efficiency of microspheres prepared with PLG of different molecular weight grades

Molecular weight of polymer	EEF (%)	
	250–425 μm	125–250 μm
155,000	85.0 (1.1)	33.0 (1.1)
59,000	62.5 (2.5)	47.5 (1.5)
8,000	7.4 (0.5)	4.4 (0.3)

Standard error of the mean shown in parenthesis.

Since our objective was to formulate a controlled release dosage of tacrine that would release the drug for weeks in treating Alzheimer's disease, we decided to use PLG of molecular weight 155,000 for further studies. The effect of stir speed during preparation on the EEF of tacrine microspheres is shown in Table 2. Increasing the stir speed during preparation from 400 or 500 rpm to 800 rpm resulted in a 100% decrease in EEF for microspheres of 250–425 μm. This could be attributed to a significant loss of the drug from the microdroplets into the aqueous phase at a higher stir speed. Another significant aspect

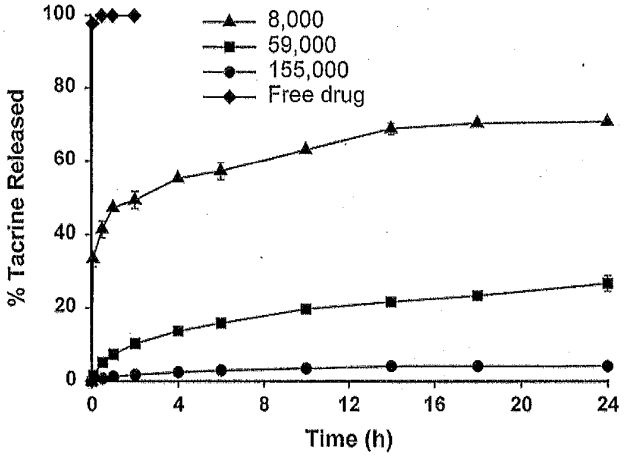

FIG. 2. The effect of molecular weight of polymer during preparation at a constant PVA concentration of 1% w/v and a volume fraction of 22% on the release profiles of tacrine microspheres. (▲) 8,000, (■) 59,000, (●) 155,000. The dissolution of the free drug is shown for comparison (◆).

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