Biomaterials 31 (2010) 6031–6038

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Tyrosine-based rivastigmine-loaded organogels in the treatment of Alzheimer's disease

Guillaume Bastiat [a], François Plourde [a], Aude Motulsky [a], Alexandra Furtos [b], Yvan Dumont [c], Rémi Quirion [c], Gregor Fuhrmann [a,d], Jean-Christophe Leroux [a,d,*]

[a] Faculty of Pharmacy, University of Montreal, Montreal, QC, Canada
[b] Department of Chemistry, University of Montreal, Montreal, QC, Canada
[c] Douglas Institute, McGill University, Montreal, QC, Canada
[d] Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zürich, Switzerland

ARTICLE INFO

Article history:
Received 25 February 2010
Accepted 5 April 2010
Available online 15 May 2010

Keywords:
Organogel
Rivastigmine
Alzheimer's disease
Pharmacokinetic
Biocompatibility
AChE inhibition

ABSTRACT

Organogels can be prepared by immobilizing an organic phase into a three-dimensional network coming from the self-assembly of a low molecular weight gelator molecule. In this work, an injectable subcutaneous organogel system based on safflower oil and a modified-tyrosine organogelator was evaluated *in vivo* for the delivery of rivastigmine, an acetylcholinesterase (AChE) inhibitor used in the treatment of Alzheimer's disease. Different implant formulations were injected and the plasmatic drug concentration was assayed for up to 35 days. In parallel, the inhibition of AChE in different brain sections and the biocompatibility of the implants were monitored. The pharmacokinetic profiles were found to be influenced by the gel composition, injected dose and volume of the implant. The sustained delivery of rivastigmine was accompanied by a significant prolonged inhibition of AChE in the hippocampus, a brain structure involved in memory. The implant induced only a minimal to mild chronic inflammation and fibrosis, which was comparable to poly(D,L-lactide-co-glycolide) *in situ*-forming implants. These findings suggest that tyrosine-based organogels could represent an alternative approach to current formulations for the sustained delivery of cholinesterase inhibitors.

1. Introduction

An organogel is a non-glassy thermoreversible semi-solid system composed of an organic liquid phase entrapped in a three-dimensionally cross-linked network. The liquid phase can be an organic solvent or an oil, whereas the structuring network is formed by self-assembled low molecular weight or polymeric organogelator molecules. Organogels have been investigated in fields as diverse as molecular photonics [1], art conservation [2] and food industry [3]. In the area of pharmaceutical sciences, organogels have only received a marked interest in recent years [4–6]. They have been tested with more or less success for the administration of inflammatory/analgesic drugs [7–9], cardiovascular drugs [4], antipsychotics [10] as well as nucleic acids [11] and peptides [12] by the transdermal, rectal, oral and buccal routes. In drug delivery, organogels are generally prepared using biocompatible and safe gelating molecules such as lecithin [8,13–15], sorbitan monostearate (SMS) [4,12,16] and amino acid derivatives [17]. A promising avenue for organogels lies in their use as depot formulations following parenteral extravascular injection. Compared to polymeric hydrogels, organogels have the ability to better retain low molecular weight polar compounds in their matrix [18]. Also, as opposed to implants based on lactic acid copolymers [19], their inner structure does not acidify upon degradation, which could be an advantage for the formulation of acid-sensitive pharmacological agents. Injectable organogels based on SMS or glyceryl esters of fatty acids have been successfully employed as vaccine adjuvants [16] and depot formulations for contraceptive steroids [20].

Over the past 6 years, we have been studying the ability of hydrophobized amino acids such as L-alanine [21] and L-tyrosine [22] to self-assemble in vegetable oils and form semi-solid/solid systems at body temperature. In order to partially inhibit gelation at room temperature and allow the injection of the formulation, a small amount of *N*-methyl pyrrolidone (NMP), a biocompatible and water-soluble hydrophilic organic solvent [23], is added to the

* Corresponding author. Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zürich, Switzerland. Tel.: +41 (0) 44 633 7310; fax: +41 (0) 44 633 1314.
E-mail address: jleroux@ethz.ch (J.-C. Leroux).

0142-9612/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2010.04.009

oil/organogelators/drug mixture. Gel formation occurs after the subcutaneous (s.c.) injection of the formulation upon diffusion of the gelation inhibitor NMP into the surrounding tissues. The implants based on *N*-stearoyl L-alanine methyl ester (SAM) (in safflower oil) were found to be well tolerated in rats [24] and effective to deliver some drugs in a prolonged manner. The incorporation of leuprolide, an inhibitor of testosterone secretion, into these implants resulted in an efficient chemical castration of male rats for up to 50 days [25]. More recently, the same SAM organogels were assessed for the parenteral sustained delivery of rivastigmine, an AChE inhibitor prescribed to patients suffering from mild-to-moderate Alzheimer's disease [26]. Rivastigmine is currently marketed as oral solution, capsules or transdermal patch requiring daily dosing [27]. It is therefore of potential interest to develop a sustained-release formulation that would only require a few injections per year. *In vivo*, the SAM gels were found to release the rivastigmine during only 10 days [25]. Moreover, the analytical assay involved the use of tritium-labelled rivastigmine and therefore the plasma concentration could have been partly biased by radiolabeled metabolites or degradation products.

Recently, we discovered that organogels prepared with L-tyrosine derivatives yielded implants with better mechanical properties, higher gel–sol transition temperatures and lower burst release *in vitro* [22]. In the present study, the sustained-release properties of such implants following s.c. injections were investigated for the first time in rats. The effects of gel composition (L-alanine *vs.* L-tyrosine), dose (15 *vs.* 25 mg kg^{-1}) and implant volume (300 *vs.* 500 μL) on rivastigmine plasma concentrations were evaluated by LC/MS/MS. In addition, the AChE activity of the formulation in different sections of the brain as well as the biocompatibility of the rivastigmine-loaded implants were assessed in rats (Fig. 1).

2. Materials and methods

2.1. Materials

SAM and *N*-behenoyl L-tyrosine methyl ester (BTM) were synthesized as previously described [22]. NMP and 7-(β hydroxyethyl) theophyllin were purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada) and used as supplied. Superrefined safflower oil (fatty acid composition of the triglycerides: 72.0% linoleic acid (C18:2), 16.6% oleic acid (C18:1), 7.4% palmitic acid (C16:0), 2.5% stearic acid (C18:0), and 1.5% *v/v* others) was kindly provided by Croda Inc. (Toronto, ON, Canada). Rivastigmine hydrogen tartrate was purchased from LGM Pharmaceuticals Inc. (Boca Raton, FL). Eligard® (22.5 mg, 3 months) was from Sanofi Aventis (Laval, QC, Canada).

2.2. Formulation preparation

BTM, SAM and rivastigmine were sterilized by γ-irradiation at 25 kGy using a ^{60}Co source (Nordion Inc., Laval, QC, Canada). Stability after sterilization of the organogelators and drug was confirmed by ^{1}H NMR. The chemical shifts (ppm) of the organogelator in $CDCl_3$ obtained with a Bruker ARX-400 spectrometer (400 MHz, Bruker, Milton, ON, Canada) before and after γ-irradiation did not change: *BTM*: 0.9 (triplet t, 3H), 1.2 (multiplet m, 16H), 1.6 (quintuplet q, 2H), 2.2 (t, 2H), 3.0 (m, 2H), 3.7 (singlet s, 3H), 4.9 (doublet of triplets dt, 1H), 5.5 (s, 1H), 5.8 (doublet d, 1H), 6.7–7.0 (m, 4H); *SAM*: 0.9 (t, 3H), 1.2 (m, 32H), 1.4 (d, 3H), 1.6 (q, 2H), 2.2 (t, 2H), 3.7 (s, 3H), 4.6 (q, 1H), 6.0 (d, 1H). Safflower oil and NMP were sterilized on 0.2-μm polytetrafluoroethylene filters. All formulations were prepared under aseptic conditions. Organogelator (5% *w/w*) and safflower oil were weighed into a polyethylene flask, mixed and heated to 90 °C. Once the organogelator was dissolved, the temperature was decreased to 80 °C and rivastigmine powder was physically dispersed in the oily solution by magnetic stirring. NMP (3% *w/w* of oil) was then added and syringes (20G1-gauge needle) were immediately filled with the hot dispersion (400 or 600 μL) and placed on ice for 30 min. Control formulations included an oil/NMP/rivastigmine solution devoid of organogelator (pharmacokinetic study, PK), a saline solution of rivastigmine (0.045% *w/v*) (enzymatic titration study, ET) and gel formulation devoid of rivastigmine (biocompatibility study, BC). Table 1 lists the composition of each formulation and controls tested *in vivo*.

For drug content analysis a 100-μL sample from each syringe was dissolved in 1 mL of NMP, mixed with 9 mL of phosphate-buffered saline (PBS) (104 mM

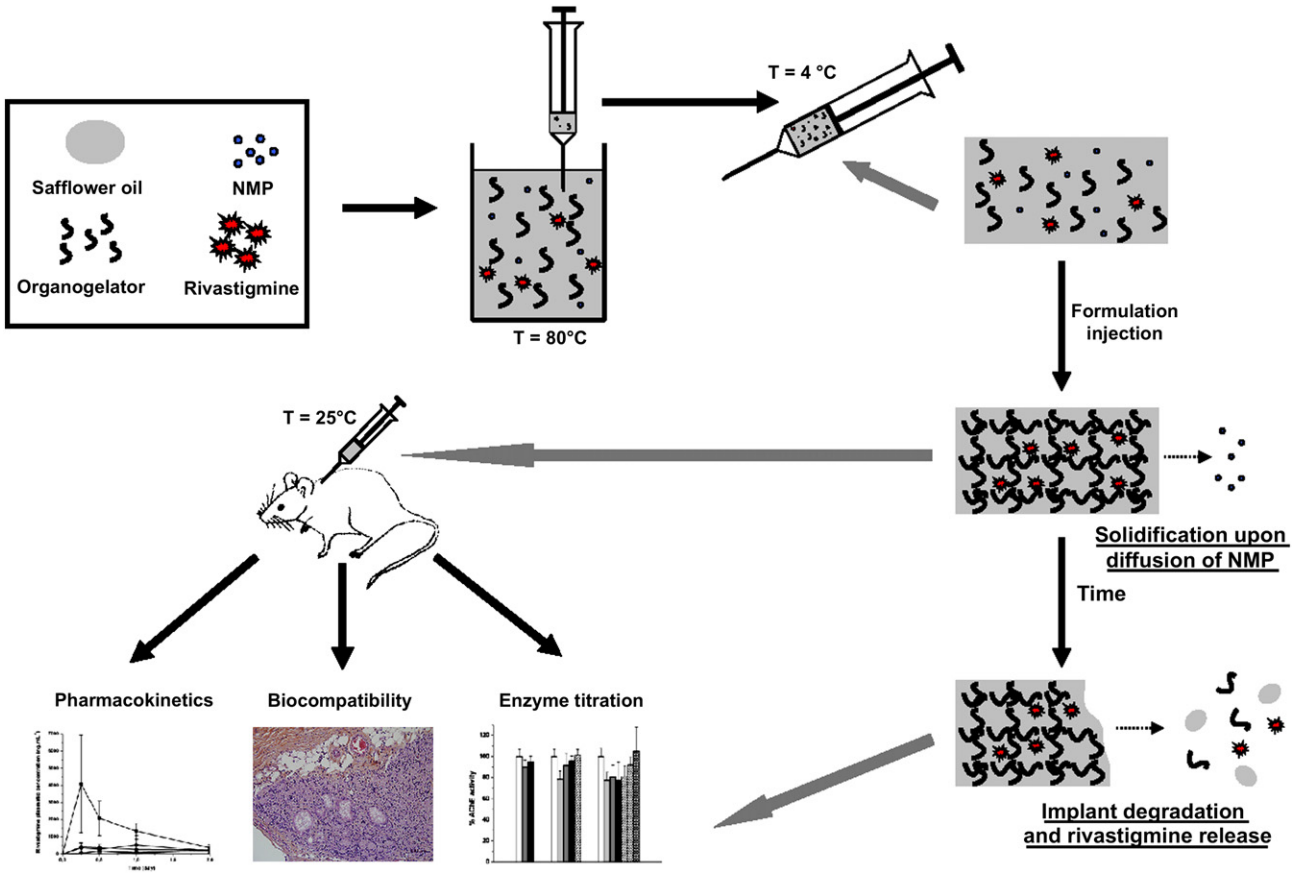

Fig. 1. Schematic representation of the experimental design: implant preparation, formation and biodegradation, and *in vivo* experiments.