EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES 37 (2009) 43–52

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Fenofibrate-loaded PLGA microparticles: Effects on ischemic stroke

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ARTICLE INFO

Article history:
Received 7 October 2008
Received in revised form
9 December 2008
Accepted 27 December 2008
Published on line 9 January 2009

Keywords:
Local delivery
Brain
PLGA
Microparticle
Controlled release

ABSTRACT

Many drugs are not able to cross the Blood Brain Barrier (BBB) and, thus, cannot reach a target site within the Central Nervous System (CNS). Local controlled drug delivery can help to overcome this restriction. However, this is a highly challenging approach and only one product is yet available on the market: Gliadel, which is used to reduce the risk of local tumor recurrence upon resection of malignant glioma. The aim of this study was to evaluate the potential of local controlled drug delivery to the CNS to reduce the consequences of ischemic stroke. Fenofibrate as well as its active metabolite fenofibric acid were encapsulated within PLGA microparticles. Importantly, fenofibrate-loaded microparticles effectively reduced the consequences of ischemic stroke in Wistar rats: the total, cortical and striatal infarct volumes decreased from 257 to 197, 193 to 139, and 64 to 58 mm^3, respectively. Interestingly, fenofibric acid-loaded microparticles did not show significant *in vivo* efficacy, which might be attributable to a potentially limited distribution pattern within the brain and/or limited cell uptake. Thus, local controlled drug delivery to the CNS also has a significant potential for the treatment/prevention of other types of diseases than cancer. Furthermore, this approach can help to provide proof of concept *in vivo* in the early drug discovery phase, if the drug candidate cannot cross the BBB.

1. Introduction

The treatment of diseases of the Central Nervous System (CNS) is highly challenging because of the Blood Brain Barrier (BBB) (Abott and Romero, 1996; Wang et al., 2002). Generally, only low molecular weight, lipid-soluble molecules and a few peptides and nutrients can cross this barrier to a significant extent, by either passive diffusion or using specific transport mechanisms (Grieg, 1987). For most drugs it is hardly possible to achieve therapeutic levels within the brain tissue upon intravenous, intramuscular, subcutaneous or oral administration. Furthermore, highly potent drugs (e.g., used for the treatment of cancer) that may be necessary to be delivered to the CNS, often cause serious toxic side effects when administered systemically.

One possibility to overcome these restrictions is to directly administer the drug into the brain tissue (intracranially) (Wang et al., 2002; Siepmann et al., 2006). To reduce the required administration frequency (and associated considerable risk of CNS infections), time-controlled drug delivery systems with

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doi:10.1016/j.ejps.2008.12.016

release periods of several weeks or months can be highly suitable for this type of applications (Reinhard et al., 1991; Brem et al., 1995). The use of *biodegradable* matrix formers is particularly beneficial, because the removal of empty remnants can, thus, be avoided (Brem et al., 1993). However, the underlying mass transport processes governing drug release from the dosage forms and subsequent drug transport within the living brain tissue are complex and yet not fully understood (Siepmann et al., 2006). The first (and so far only) commercially available product which is based on the principle of local controlled drug delivery to the brain is Gliadel (Valtonen et al., 1997; Brem and Gabikian, 2001). It is a disc-shaped wafer, consisting of BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea; carmustine] as the drug (loading: 3.85%) and poly[bis(p-carboxyphenoxy)] propane–sebacic acid (PCPP:SA) as the biodegradable polymer. Gliadel has been developed in the early/mid nineties by the group of Henry Brem. In addition to brain cancer, neurological disorders have been in the focus of past and ongoing research on local controlled CNS delivery (Benoit et al., 2000; Menei et al., 2005). However, it can be expected that this type of advanced drug delivery also offer major advantages for other types of diseases. Furthermore, the use of local controlled drug delivery to the brain can also be expected to be highly suitable during the early drug discovery process: even if a novel, promising compound is not able to cross the BBB it can, thus, be made available at the target site during pre-clinical proof of concept studies. If the drug candidate shows to be efficient, it might either be chemically modified in order to be able to cross the BBB on its own, being delivered using an advanced drug targeting system (Beduneau et al., 2007), or be administered directly into the brain tissue using a local controlled delivery system as final dosage form.

The aim of the present study was to evaluate the potential of local controlled drug delivery to the brain to reduce the consequences of ischemic stroke. A stroke is caused by the interruption of the blood supply to the brain, usually because a blood vessel bursts (hemorrhagic stroke) or is blocked by a clot (ischemic stroke). This cuts off the supply of oxygen and nutrients, causing damage to the brain tissue. The importance of the consequences of a stroke essentially depends on which part of the brain is injured and how severely it is affected. A very severe stroke can cause sudden death. Every year 15 million people in the world suffer from a stroke, 5 million of which die and another 5 million are left permanently disabled (WHO) (Mackay and Mensah, 2004). This makes a stroke the first leading cause of disability and the third leading cause of death in industrialized countries. Until now the treatment is limited to the prevention of cerebrovascular risk factors and to the modulation of the coagulation cascade during the acute phase. Obviously, the existing treatments are insufficient (Sacco et al., 2007; Young et al., 2007). The abrupt lack of oxygen and glucose to neuronal tissues during a stroke results in a series of pathological cascades finally leading to neuronal death. A strategy which protects neurons and reduces their vulnerability against the consequences of ischemic injury and the metabolic consequences should, thus, be promising. Recently, PPAR (Peroxisome Proliferator Activated Receptors) activators have been proposed as neuroprotective drugs against ischemic stroke due to their effects on oxidative stress and inflammation (Bordet et al., 2006; Heneka and Landreth, 2007). Fenofibrate is a PPARα activator and successfully used for the treatment of hypertriglyceridemia. Its active metabolite is fenofibric acid (Streel et al., 2000). In the present study, both drugs were encapsulated within poly(lactic-co-glycolic acid) (PLGA) microparticles. The latter offer the major advantage to be: (i) biodegradable (Visscher et al., 1985; Anderson and Shive, 1997), (ii) biocompatible with brain tissue (irrespective of the drug carrier shape and implantation site, the CNS tissue response to intracranial PLGA drug carrier administration is a moderate and non-specific inflammatory reaction due to the mechanical trauma) (Fournier et al., 2003; Menei et al., 2004), (iii) able to control the resulting release rate of a drug during a few days up to several months (Freiberg and Zhu, 2004; Berkland et al., 2007), and (iv) administrable via stereotaxy into desired CNS regions.

The major aim of this study was to evaluate the potential of local controlled drug delivery to the brain and also for other types of CNS diseases than cancer and neurological disorders. The investigated devices are not necessarily intended to serve as final dosage forms, but can also be of great help during the early drug discovery process allowing for a proof of concept *in vivo* even in the case of substances that are not able to cross the BBB.

2. Materials and methods

2.1. Materials

Poly(D,L lactic-co-glycolic acid) (PLGA; Resomer RG 502H; PLGA 50:50; containing 25% D-lactic units, 25% L-lactic units and 50% glycolic units; Boehringer Ingelheim, Ingelheim, Germany), fenofibric acid (Tyger Scientific, Ewing, USA), fenofibrate (Sigma–Aldrich, Steinheim, Germany), acetonitrile, ethyl acetate and dichloromethane (VWR, Fontenoy-sous-Bois, France), polyvinyl alcohol (Mowiol 4–88; Kuraray Specialities Europe, Frankfurt, Germany), sodium carboxymethylcellulose (NaCMC 7HCF), mannitol and Tween 80 (Cooper, Melun, France).

2.2. Microparticle preparation

2.2.1. Fenofibrate-loaded PLGA microparticles

Fenofibrate-loaded PLGA microparticles were prepared using an oil-in-water (O/W) solvent extraction/evaporation technique: 350 mg of drug and 1 g of the polymer were dissolved in 9 g of dichloromethane. This organic phase was dispersed within 2.5 L of an outer aqueous polyvinyl alcohol solution (0.25%, w/w) under stirring with a three-blade propeller for 30 min (2000 rpm). The formed microparticles were hardened upon addition of 2.5 L further outer aqueous phase and 4 h gentle stirring (700 rpm). The particles were separated by filtration and subsequently freeze-dried to minimize the residual solvents' content. Drug-free microparticles were prepared accordingly without fenofibrate. Larger particles were separated by sieving (average pore size of the sieve: 50 μm; sieve, Retsch, Haan, Germany).