European Journal of Pharmaceutics and Biopharmaceutics 79 (2011) 285–293

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Development and physico-chemical characterization of a liposomal formulation of istaroxime

Paola Luciani, Maréva Fevre, Jean-Christophe Leroux *

Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, Zurich, Switzerland

ARTICLE INFO

Article history:
Received 29 December 2010
Accepted in revised form 21 April 2011
Available online 29 April 2011

Keywords:
Istaroxime
Liposome
Controlled release
Congestive heart failure treatment
pH gradient
Poly(ethylene glycol)–660-hydroxystearate

ABSTRACT

Istaroxime, an investigational new drug that targets defective Ca^{2+} cycling without compromising cardiac efficiency, may represent a promising and safe treatment of both acute and chronic heart failure. Even though the compound demonstrated good tolerability in a phase I/II safety study, symptoms related to the gastro-intestinal tract and pain at the injection site were reported as the most frequent side effects. The aim of this study was to encapsulate istaroxime in a drug delivery system (DDS) that could minimize the pain perceived upon administration. The DDS was designed to be quickly destabilized in plasma, in order to minimize alteration of the pharmacokinetic profile of istaroxime. To meet those requirements, a balance between the encapsulation efficiency and the release rate was sought. Transmembrane pH-gradient liposomes formulated with different phosphatidylcholines were investigated as vehicles for an efficient active drug loading. Poly(ethylene glycol)–660-hydroxystearate (PEG–HS) was chosen as excipient to modulate the bilayer fluidity and the release properties of the liposomes. A fast and efficient encapsulation was obtained by modulating the drug-to-lipid ratio, the amount of PEG–HS, and the incubation temperature. High encapsulation efficiency was achieved by incubating the drug with liposomal dispersions at room temperature for 10 min. Almost complete release was obtained in physiological conditions in less than 10 min, suggesting a model formulation potentially useful for drugs presenting similar features and side effects.

1. Introduction

Heart failure (HF) afflicts over 5 million Americans and 15 million Europeans and Mediterraneans [1,2]. It represents a serious public health burden, associated with major morbidity and mortality. It has been described as the "emerging" epidemic for the 21st century: among all adults aged 40 years, 1 in 5 will develop heart failure at some point in his/her lifetime [3]. Since the introduction of digoxin into clinical practice, inotropic agents have played a pivotal role in HF treatment. However, their proarrhythmic potential has led to a radical reassessment of their use for this indication [4]. Agents with innovative mechanisms of action are currently being tested, and further improvements are ongoing [5,6].

Istaroxime (Fig. 1) is a novel Na^+/K^+-ATPase inhibitor that has been shown to have the unique property of increasing sarcoplasmic reticular (SR) calcium adenosine triphosphatase isoform 2a (SERCA2a) activity [7,8]. By inhibiting Na^+/K^+-ATPase activity, a cytosolic calcium accumulation is induced (inotropism) while, due to SERCA2a stimulation, subsequent SR calcium storage is increased, preventing Ca^{2+} intoxication and facilitating myocardial relaxation (lusitropism) [9]. By exerting both inotropic and lusitropic activities, istaroxime targets defective Ca^{2+} cycling without compromising cardiac efficiency, representing an effective and safe treatment of both acute and chronic HF [10]. Animal models have shown that istaroxime promotes both muscle contraction and relaxation and prevents the cytosolic calcium overload and arrhythmia associated with digoxin [9]. The compound demonstrated good tolerability in a phase I/II safety study. However, unexpected symptoms related to the gastro-intestinal (GI) tract and pain at the injection site were reported as most frequent side effects [11,12]. Pain upon injection represents a serious drawback for parenteral administration. Such a problem could be solved by reducing the interaction of the drug with the surrounding tissues at the injection site through encapsulation in suitable formulations [13].

In choosing an appropriate drug delivery system for istaroxime, several aspects should be considered: (a) a final dosage form that

Abbreviations: DPPC, 1,2-myristoyl-sn-glycero-3-phosphocholine; DMPC, 1,2-palmitoyl-sn-glycero-3-phosphocholine; DOPC, 1,2-oleoyl-sn-glycero-3-phosphocholine; EPC, egg phosphocholine; PEG–HS, poly(ethyleneglycol)–660-hydroxystearate; HPTS, 1-hydroxypyrene-3,6,8-trisulfonic acid; DPX, p-xylenebis(pyridinium bromide; FBS, fetal bovine serum; Ch-BODIPY, cholesteryl 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

* Corresponding author. Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zurich, Wolfgang-Pauli-Str. 10, HCI H301, CH-8093 Zurich, Switzerland. Tel.: +41 44 633 7310; fax: +41 44 633 1314.
E-mail address: jean-christophe.leroux@pharma.ethz.ch (J.-C. Leroux).

0939-6411/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2011.04.013

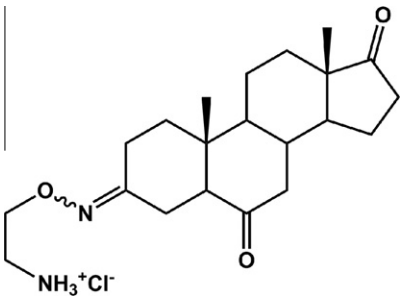

Fig. 1. Chemical structure of istaroxime.

can be easily stored and reconstituted; (b) the presence of a biocompatible barrier able to interfere with the pain perceived at the injection site; and (c) rapid destabilization of liposomes after injection to minimize alteration of the drug pharmacokinetic profile. Liposomes appeared to be an ideal choice to overcome the drawbacks presented by the current administration route of istaroxime, providing versatility in terms of formulation composition and drug-loading procedures [13]. The chemical properties of istaroxime played a crucial role in selecting the most appropriate loading procedure. Istaroxime is an amphipathic weak base (pK_a 9.25) that could, in principle, be remotely loaded within large unilamellar vesicles (LUVs) exhibiting a transmembrane pH gradient [14,15]. The good stability of the drug in its dry form and its relative chemical lability in aqueous media led to the choice of adopting an active *in situ* loading procedure [16]. The drug would therefore be stored as a powder, reconstituted, and loaded into the liposomes just prior to the intravenous infusion. Drug release should occur rapidly after injection to avoid a drastic change in the pharmacokinetic profile of istaroxime.

Liposomal membrane permeability can be adapted by varying the phospholipid composition and by incorporation of additives, such as surfactants. Phosphatidylcholines (PC) with different chain lengths and levels of saturation were chosen as main components of the bilayer. In order to keep the formulation as simple as possible, permeability of the bilayer was modulated by the addition of a well-tolerated surfactant, poly(ethylene glycol)–660-hydroxystearate (PEG–HS). This excipient was chosen for its low toxicity profile, its good tolerance upon parenteral administration, and its compatibility with the lipid bilayer. Parameters such as drug-to-lipid ratio, PEG–HS concentration, and incubation temperature were varied, and their effect on encapsulation efficiency and release kinetics were investigated.

2. Materials and methods

2.1. Chemicals

Istaroxime was provided by Debiopharm S.A. (Gland, Switzerland). 1,2-myristoyl-*sn*-glycero-3-phosphocholine (DMPC),1,2-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-oleoyl-*sn*-glycero-3-phosphocholine (DOPC), and egg phosphocholine (EPC) were a gift from Lipoid (Ludwigshafen, Germany). Poly(ethylene glycol)–660-hydroxystearate (PEG–HS) was kindly donated by BASF (Friedrichshafen, Germany). 1-hydroxypyrene-3,6,8-trisulfonic acid (HPTS), *p*-xylenebis(pyridinium bromide) (DPX), Gibco fetal bovine serum (FBS), and cholesteryl 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-dodecanoate (Ch-BODIPY) were obtained from Invitrogen (Eugene, OR). All the other chemicals were purchased from Sigma Aldrich (St. Louis, MO). The solvents used in the serum extraction protocol were HPLC grade. Water was distilled and deionized in a Barnstead NANOpure Diamond Ultrapure Water Systems (Thermo Scientific, Rockford, IL). The osmolalities of the solutions used were measured with a Wescor 5500 vapor pressure osmometer (Wescor, Logan, UT) and eventually adjusted with NaCl to obtain isotonicity.

2.2. Liposome preparation

Lipid vesicles containing the selected phosphatidylcholines (DOPC, EPC, DMPC, or DPPC, unless otherwise stated) mixed with 0, 5, 10, or 15 mol% PEG–HS were prepared according to the film hydration extrusion method [17]. Briefly, stock solutions of lipids in $CHCl_3$ and a stock solution of PEG–HS in ethanol were mixed in order to obtain the chosen molar percentage of surfactant. Organic solvents were evaporated under a stream of nitrogen until dry. Traces of solvent were further removed by keeping the lipid films under vacuum overnight. The swelling buffer and the procedures followed to obtain LUV or multilamellar vesicles (MLV) are described in more detail in the Supplementary Information. Unless otherwise stated, the total lipid concentration was 5 mM.

2.3. Dynamic light scattering (DLS)

The mean diameter and size distribution of liposomes was measured by DLS at a scattering angle of 165° using a Delsa Nano S instrument (Beckman Coulter, Krefeld, Germany) equipped with a 658-nm laser diode and a temperature controller. The intensity size distributions of the liposomes were typically unimodal, and therefore, the autocorrelation functions were analyzed according to the cumulant method.

2.4. Differential scanning calorimetry (DSC)

DSC measurements were performed using a DSC Q200 (TA Instruments, New Castle, DE) and the data analyzed with Q Series software. Temperature and enthalpy calibration was performed using indium as reference. About 10 μL of liposome solution was transferred to an aluminum pan and hermetically sealed. The reference pan was filled with the appropriate buffer solution. The weight of each DSC pan was verified before and after the temperature scan to check for eventual water leakage. The scan rate was 2 °C/min. After an initial isothermal period of 5 min, the MLV were scanned between the temperature ranges reported in Supplementary Table S1.

2.5. Fluorescence spectroscopy

2.5.1. Steady-state fluorescence polarization

The liposome dispersions (EPC, DOPC, DMPC, or DPPC) were diluted into quartz cuvettes (final concentration 0.1 mM), and 1,6-diphenyl-1,3,5-hexatriene (DPH) from a THF stock solution was added (probe/lipid molar ratio 1:300). The dispersions were stirred and incubated at a temperature above T_m until fluorescence intensity reached a plateau. The eventual presence of free DPH in solution was checked by recording the fluorescence after addition of a small excess of liposomal suspension [18]. The change in polarization as a function of temperature and surfactant concentration was measured by means of a Cary Eclipse Fluorescence spectrophotometer (Varian Inc., Palo Alto, CA) equipped with manual polarizers and a Peltier circulating water bath to strictly control the temperature (λ_{ex} = 360 nm and λ_{em} = 430 nm). After each temperature change or surfactant addition, the samples were allowed to equilibrate for 5 min. The steady-state fluorescence anisotropy was calculated according to Eq. (1):

$$r = \frac{(I_{VV} - I_{VVblank} IG) \times (I_{VH} - I_{VHblank})}{(I_{VV} - I_{VVblank} 2G) \times (I_{VH} - I_{VHblank})} \quad (1)$$