International Journal of Pharmaceutics 510 (2016) 296–303

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

In vitro and in vivo investigation of the gastrointestinal behavior of simvastatin

Sophie Geboers[a], Jef Stappaerts[a], Jan Tack[b], Pieter Annaert[a], Patrick Augustijns[a,*]

[a] Drug Delivery and Disposition, KU Leuven, Department of Pharmaceutical and Pharmacological Sciences, Belgium
[b] University Hospitals Leuven, Department of Gastroenterology, Belgium

ARTICLE INFO

Article history:
Received 11 April 2016
Received in revised form 1 June 2016
Accepted 19 June 2016
Available online 20 June 2016

Keywords:
Simvastatin
Simvastatin acid
Gastrointestinal behavior
Human gastrointestinal fluids
Clinical trial
Prodrug

ABSTRACT

Simvastatin (SV) is marketed as a lactone ester prodrug which is hydrolyzed to the active simvastatin hydroxyacid (SVA). SV is characterized by a low solubility and undergoes extensive first-pass metabolism. In this study, the influence of the upper gastrointestinal environment on the intraluminal behavior of simvastatin was investigated by a series of in vitro experiments. Dissolution, stability and two-stage dissolution tests were performed using simulated and human gastrointestinal fluids.

The dissolution studies revealed a relatively slow dissolution of SV as well as conversion of SV to SVA. The hydrolysis of SV was further examined and stability studies indicated a faster conversion in gastric fluids than in intestinal fluids. These isolated phenomena were then confirmed by the more integrative two-stage dissolution studies.

To estimate the predictive value of the in vitro tests, an additional in vivo study was performed in which the gastrointestinal concentration-time profiles also revealed a slow dissolution of SV and faster degradation of SV to SVA in the stomach than in the intestinal tract. However, the plasma concentrations of SV and SVA did not directly correlate with the observed gastrointestinal concentrations, suggesting that gut wall and hepatic metabolism have a greater impact on systemic exposure of SV than the intraluminal interconversion between SV and SVA.

1. Introduction

Nowadays, most drug candidates suffer from low aqueous solubility or low intestinal permeability, resulting in poor oral bioavailability. (Lipinski et al., 2001) The development of prodrugs exhibiting improved solubility and/or permeability has been successful as a strategy to counter these challenges. (Jarkko et al., 2008) It has been demonstrated, however, that premature intraluminal hydrolysis mediated by hydrolyzing enzymes present in the intestinal fluids may significantly alter the intestinal absorption of a prodrug. (Brouwers et al., 2007); (Stappaerts et al., 2015) It has for instance been shown that intraluminal degradation of the ester prodrug tenofovir disoproxil fumarate takes place in vivo, illustrating that the esterases present in the intestinal fluids may undermine the intended enhanced permeability. In another recently described study, the hydrolyzing capacity of intestinal fluids was revealed to be an effective trigger causing abiraterone supersaturation upon administration of the ester prodrug abiraterone acetate; esterase-mediated hydrolysis was shown to be beneficial for the intestinal absorption of abiraterone. (Stappaerts et al., 2015) It is clear that the intraluminal behavior of ester prodrugs can be diverse and may have significant repercussions on intestinal drug absorption. In vitro stability testing in biorelevant media containing hydrolyzing enzymes is clearly an important step in assessing the feasibility of a prodrug approach.

Statins are indispensable in the primary and secondary prevention of cardiovascular diseases worldwide. (Scandinavian Simvastatin Survival Study Group, 1994) This class of drugs competitively inhibits the rate-limiting step of cholesterol biosynthesis, mediated by 3-hydroxy-3-methylglutaryl-coenzym A reductase, decreasing cholesterol neogenesis. (Vickers et al., 1990b) Statins are on the market as either the active hydroxyacid form (atorvastatin, fluvastatin, rosuvastatin and pravastatin) or as lactone (a cyclic ester) prodrug (simvastatin (SV) and lovastatin). (Li et al., 2011); (Lennernäs and Fager, 1997) SV is hydrolyzed to the active metabolite simvastatin hydroxyacid (SVA) by esterases,

* Corresponding author at: Drug Delivery and Disposition, KU Leuven, Department of Pharmaceutical and Pharmacological Sciences, Gasthuisberg O&N 2, Herestraat 49, Box 921, 3000 Leuven, Belgium.
E-mail address: patrick.augustijns@pharm.kuleuven.be (P. Augustijns).

http://dx.doi.org/10.1016/j.ijpharm.2016.06.048
0378-5173/

paraoxonases and by non-enzymatic hydrolysis (Fig. 1). (Pedersen and Tobert, 2004); (Prueksaritanont, 2002)

Simvastatin has a low oral bioavailability of less than 5%, which may be attributed to low intestinal uptake and extensive first-pass metabolism. (Kato, 2008) Solubilized simvastatin, on the other hand, is well absorbed from the gastrointestinal tract. (Mauro, 1993) This was confirmed by a high apical to basolateral transport across Caco-2 cell layers for both SV and SVA. (Li et al., 2011) Hepatic uptake of SV occurs through a combination of passive and active transport mediated by the liver-specific isoforms of the Organic Anion Transporting Polypeptide (OATP) family, i.e. OATP 1B1/1B3. (Thompson, 2013) In the liver, SV is metabolized via various pathways including acid/lactone interconversion. Both SV and SVA are substrates for CYP3A4. (Fig. 1) (Vickers et al., 1990b); (Pedersen and Tobert, 2004); (Prueksaritanont, 2002); (Bottorff and Hansten, 2000); (Cheng et al., 1994) Biliary excretion is found to be the major route of elimination of the SV metabolites. (Vickers et al., 1990a)

In view of our recent findings on the intraluminal stability of several ester prodrugs, the aim of this study was to gain more insight into the intraluminal behavior of the cyclic ester SV. To reach this goal, several in vitro experiments were designed involving the use of biorelevant media such as simulated and human gastric and intestinal fluids. In addition, more complex in vitro models were used including two compartmental set-ups to further increase the in vivo similarity. In addition, a clinical study was performed to investigate (1) for the first time the in vivo intraluminal behavior of SV and (2) the predictive value of the performed in vitro tests.

2. Materials and methods

2.1. Chemicals

Simvastatin and simvastatin acid were both obtained from Sigma-Aldrich (St. Louis, MO) as well as rosuvastatin (RSV), monobasic potassium phosphate monohydrate ($KH_2PO_4 \cdot H_2O$), bis-4-nitrophenylphosphate and pancreatin from porcine pancreas (powder, suitable for cell culture, 4 x USP specifications). Dimethylsulfoxide (DMSO) and tetrabutylammonium sulfate were obtained from Acros-Organics (Geel, Belgium). Acetic acid was purchased from Chem-lab (Zedelgem, Belgium). Acetonitrile was purchased from Fisher Scientific (Leicestershire, UK). Simulated intestinal fluid (SIF) powder was purchased from Biorelevant (Croydon, UK). Methanol and sodium acetate trihydrate were purchased from VWR International (Leuven, Belgium). Water was purified with a Maxima system (Elga Ltd., High Wycombe Bucks, UK). For the measurements of the pH, a Portamess 911 pH-meter (Knick GmbH & Company, Berlin, Germany) was used. All stock solutions were prepared in DMSO.

2.2. Stabilization mixture

Precautions were taken to guarantee stability of the samples before the analysis. It is known that the stability of simvastatin decreases with increasing temperature whereas sufficient stability has been reported in a pH range of 3–6. (Álvarez-Lueje et al., 2005); (Di and Kerns, 2009) All samples were immediately diluted 1/100 in a stabilization mixture (pH 3.5), consisting of MeOH:0.02 N HCl (50:50) containing 400 μM of the esterase inhibitor bis-4-nitrophenylphosphate. The stability of the ester prodrug was confirmed in this stabilization mixture.

2.3. Media

Fasted state simulated intestinal fluid (FaSSIF) and fasted state simulated gastric fluid (FaSSGF) were made according to the manufacturer's preparation protocol (Biorelevant®, Croydon, UK). FaSSIF was prepared by dissolving SIF powder (2.24 mg/mL) in a phosphate buffer (pH 6.5). To provide the simulated fluids with hydrolyzing capacity, FaSSIF was supplemented with pancreatin (10 mg/mL) as described by Borde et al. (Borde et al., 2012) After vortex mixing, this suspension was centrifuged (2880*g*) and the supernatant was used for the stability study. FaSSGF was prepared by dissolving SIF powder (0.06 mg/mL) in an HCl/NaCl solution (pH 1.6). For the two-stage dissolution experiment double

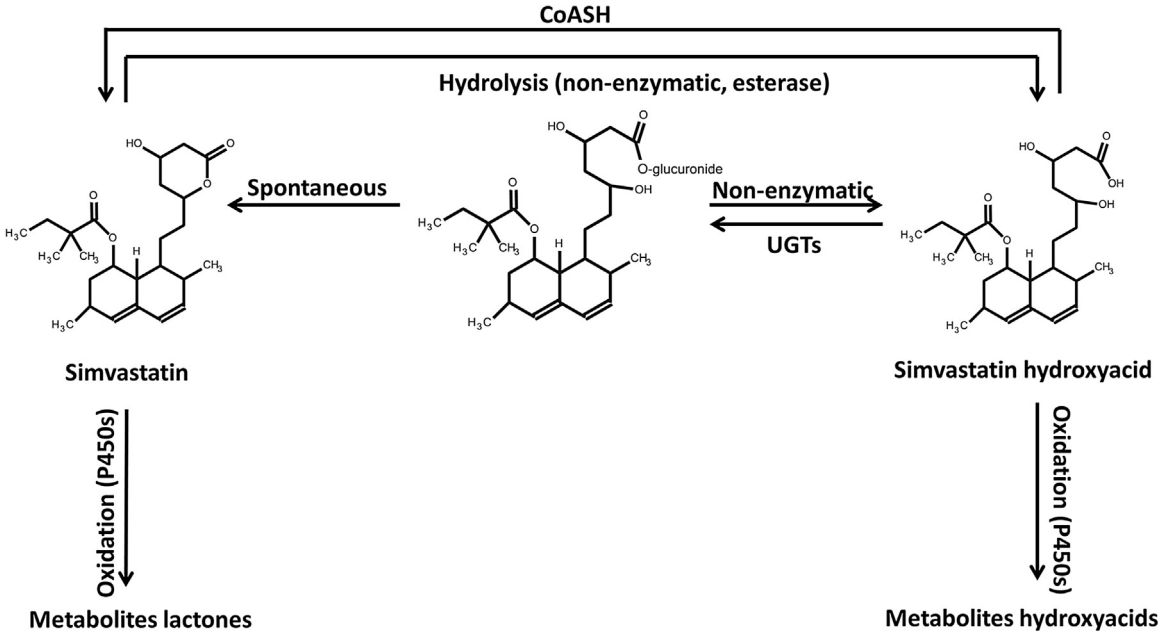

Fig. 1. Metabolism of simvastatin in man.
Source: Adapted from Pedersen and Tobert (2004) and Prueksaritanont (2002).