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Solubility and stability of dalcetrapib in vehicles and biological media

Günter Gross*, Joseph Tardio, Olaf Kuhlmann

F. Hoffmann-La Roche Ltd, Basel, Switzerland

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ABSTRACT

Dalcetrapib solubility was determined in aqueous and in non-aqueous vehicles and in biorelevant media. In a pure aqueous environment the solubility was low but could be increased by addition of surfactants or complexing agents. This was also reflected in the solubility seen in simulated gastrointestinal (GI) fluids, with almost no solubility in simulated gastric fluid, but reasonable solubilisation in simulated intestinal fluids containing lecithin and bile salt. Additionally, the stability of dalcetrapib was determined in simulated GI fluids with and without pancreatic lipase. In solutions without lipase, dalcetrapib was slowly hydrolysed, but in the presence of lipase the hydrolysis rate was significantly faster depending on pH and enzyme activity. In biological fluids, dissolved dalcetrapib appeared to behave similarly being rapidly hydrolysed in human intestinal fluids with a half-life below 20 s with no degradation observed in human gastric fluids at low pH. The results provide supportive evidence that absorption is higher under fed conditions and indicate lipase inhibitors might interfere with oral absorption of dalcetrapib.

1. Introduction

Dalcetrapib, a thioester compound, modulates plasma cholesteryl ester transfer protein activity to increase high-density lipoprotein cholesterol and promote reverse cholesterol transport, thus potentially reducing cardiovascular risk (Schwartz et al., 2009).[1] Dalcetrapib undergoes rapid hydrolysis by non-specific esterases *in vivo* to generate the pharmacologically active free thiol, which was found to be the main component in human plasma (Derks et al., 2010). Systemically, the free thiol exists in oxidation–reduction equilibrium with the disulfide dimer (Fig. 1; Heinig et al., 2012).

Carboxylesterase enzymes (CES1 and CES2), a family of serine hydrolases highly expressed in tissues including the liver (Yang et al., 2009), stomach and intestines, are known to play a significant role in xenobiotic thioester hydrolysis in a number of species including human and cynomolgus monkey (Williams et al., 2011). Dalcetrapib is subject to significant hydrolysis in the gut, followed by further metabolism, glucuronidation and conjugation of the thiol prior to reaching the liver. For example, following initial passage of dalcetrapib through the gut in cynomolgus monkeys, only 15% of drug-related material in the plasma of the portal vein was observed to be dalcetrapib thiol, the remainder being inactive metabolites, mainly *S*-glucuronide metabolite and a small amount of *S*-methyl metabolite (Kuhlmann and Heinig, 2011). This level of pre-systemic metabolism contributes to observed low bioavailability after oral administration.

To better understand the behaviour of dalcetrapib *in vivo*, further knowledge on the solubility and stability of dalcetrapib in biorelevant media is needed. The solubility and stability of dalcetrapib in aqueous vehicles and different organic solvents, biorelevant media, i.e., Fed State Simulated Intestinal Fluid (FeSSIF), Fasted State Simulated Intestinal Fluid (FaSSIF), and Simulated Gastric Fluid (SGF), and in biological media, i.e., saliva and both fasted and fed (stimulated) human gastrointestinal (GI) fluids was therefore investigated.

2. Materials and methods

2.1. Materials

Study drug and glycocholic acid were provided by F. Hoffmann-La Roche Ltd, Basel, Switzerland. Pancreatin was from Merck Chemicals (Darmstadt, Germany), lipase from hog pancreas was from Fluka (Sigma–Aldrich Steinfurt, Germany), soybean lecithin from Lipoid AG (Steinhausen, Switzerland) and all other chemicals used were from Merck Chemicals (Sigma–Aldrich, Steinfurt, Germany), or Roche (F. Hoffmann-La Roche Ltd, Basel, Switzerland).

2.1.1. Biorelevant media

Simulated GI fluids were prepared according to the composition given by Galia et al. (1998).

* Corresponding author at: F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124, CH-4070, Basel, Switzerland. Tel.: +41 61 6886113; fax: +41 61 6888689.
E-mail address: guenter.gross@roche.com (G. Gross).

[1] The phase III trial dal-OUTCOMES was terminated in May 2012 due to the absence of beneficial cardiovascular effects.

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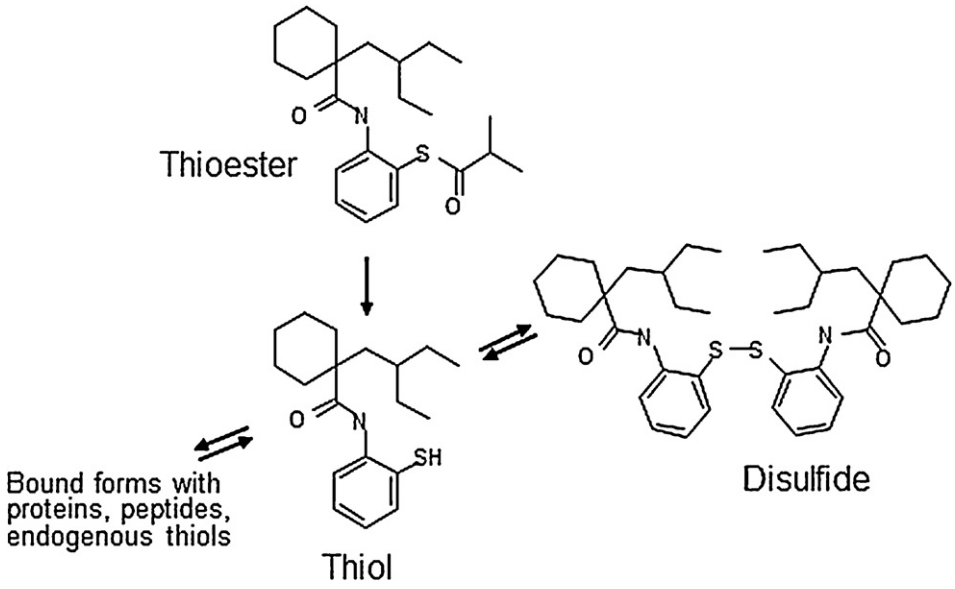

Fig. 1. Structures of dalcetrapib, dimer and thiol.

2.1.2. Collection of biological media

Fresh saliva samples were collected from two subjects (Roche, Basel). Samples of human GI fluids were collected at the Roche Clinical Pharmacology Unit (Strasbourg) under the study protocol WP21518. Informed consent was given for the collection of saliva and other human fluids.

Collection of GI fluid was performed *via* a nasogastric or nasojejunal tube both following an overnight fast and under stimulated conditions. Stimulated GI fluid was collected according to directions pre-specified in the study protocol; 20 min prior to beginning, stimulated collection volunteers were provided with chewing-gum and requested to chew for 15 min and to replace sticks of gum approximately every 2 min to maintain taste. During stimulation, volunteers were requested not to swallow saliva; this was spat out regularly or aspirated *via* a dental suction catheter.

For some experiments, fasted gastric fluid samples were pooled (subjects 3001, 3002, 3003 and 3004) and the pH of the pooled sample adjusted to 4.5. This was also performed for stimulated gastric fluid (subjects 4001, 4002, 4003 and 4004) and the pH of the pooled sample also adjusted to 4.5. Also, pooling of fasted intestinal fluid (subjects 1001, 1002, 1005 and 1006) was performed; final pH 6.1, and of stimulated intestinal fluid (subjects 2003, 2005 and 2006); final pH 6.6. The combined fluids were centrifuged for 5 min at 700 × *g* to remove coarse matter, and aliquots of 0.5 or 1.0 mL were frozen at −20 °C.

2.2. Solubility and stability

2.2.1. Solubility of dalcetrapib in aqueous vehicles and in solvents

2.2.1.1. Preparation of buffer solutions. Phosphate buffered saline was prepared by dissolving 3.2 g NaCl and 2.0 g sodium dihydrogen phosphate dehydrate ($NaH_2PO_4 \cdot 2H_2O$) to a final volume of 500 mL water adjusted to the desired pH with 1 N HCl or 1 N NaOH.

2.2.1.2. Assessment of solubility. Dalcetrapib was added in excess to the solvents (listed in Table 1) or the aqueous vehicles (listed in Table 2) in closed vials. The suspension was stirred at room temperature for 24 h, filtered using a 0.45 μm filter, adequately diluted and subjected to analysis by high-performance liquid chromatography (HPLC).

Table 1
Solubility of dalcetrapib in solvents and pharmaceutically relevant vehicles at 25 °C.

Vehicle	Solubility (mg/mL)
Ethanol	146
2-Propanol	>100
Glycofurol 75	>100
Polyethyleneglycol 400	16.8
Propyleneglycol	2.8
Glycerin	0.5
Pharmasolve (N-methyl pyrrolidone)	>500

2.2.2. Solubility of dalcetrapib in biorelevant media

2.2.2.1. Preparation of stock solutions. FeSSIF: 10-fold concentrated FeSSIF stock solution was prepared as follows: 300 mg soybean lecithin was added to 1 mL ethanol and the lecithin brought into solution by agitation. Eight hundred and six milligrams sodium taurocholate was added and dissolved. The ethanol was evaporated in a rotary evaporator at 45 °C initially at 100 mbar for ∼15 min. The resulting film was further dried at 45 °C at 10 mbar for a further 2 h. 10-fold FeSSIF concentrate was prepared by dissolving the residue in 9 mL of water. FeSSIF concentrate was then diluted 10-fold in buffer solution containing NaCl and acetic acid to provide final concentrations of 202 mM NaCl and 144 mM acetic acid with a pH adjusted to 5.0 with 1 N NaOH. Final concentrations were 3.75 mM soybean lecithin (3.0 mg/mL) and 15 mM sodium taurocholate (8.06 mg/mL).

Table 2
Solubility of dalcetrapib in aqueous vehicles at 25 °C.

Aqueous vehicle	Solubility μg/mL
Buffer solutions (pH 3–7)	∼0.02
Water–ethanol (50:50 v/v)	135
Water–2-propanol (50:50 v/v)	1156
Water–ethanol (80:20 v/v)	0.65
Water–2-propanol (80:20 v/v)	0.49
30% w/v sulfobutylether-β-cyclodextrin in physiologic NaCl solution	22.4
30% w/v hydroxypropyl-β-cyclodextrin in physiologic NaCl solution	73.1
Mixed micelles solution (200 mM sodium glycocholate, 200 mM lecithin)	2800