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The conflict between *in vitro* release studies in human biorelevant media and the *in vivo* exposure in rats of the lipophilic compound fenofibrate

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ABSTRACT

The performance of four different lipid-based (Tween 80–Captex 200P, Tween 80–Capmul MCM, Tween 80–Caprol 3GO and Tween 80–soybean oil) and one commercially available micronized formulation (Lipanthyl Micronized®) of the lipophilic compound fenofibrate was compared *in vitro* in various biorelevant media and *in vivo* in rats. In simulated gastric fluid without pepsin (SGF_{sp}) and fasted state simulated intestinal fluid (FaSSIF), only Tween 80–Captex 200P system resulted in a stable fenofibrate concentration, but no supersaturation was obtained. The other three lipid based systems created fenofibrate supersaturation; however they did not maintain it. In fed state simulated intestinal fluid (FeSSIF), all lipid-based formulations resulted in complete dissolution of fenofibrate during the experiment, which represented a supersaturated state for Tween 80–Capmul MCM and Tween 80–Caprol 3GO systems. In both FaSSIF and FeSSIF, all lipid-based formulations yielded a higher fenofibrate concentration than the micronized formulation. Contrary to the *in vitro* results, no significant difference in the *in vivo* performance was observed among the four tested lipid-based formulations both in the fasted and the fed states. The *in vivo* performance of all lipid-based formulations was better than that of Lipanthyl Micronized®, in the fasted as well as in the fed state. The fact that for the lipid based systems the *in vitro* differences in pharmaceutical performance were not translated into *in vivo* differences can be attributed to the continuous excretion of bile in the gastrointestinal tract of rats, causing enhanced solubilizing capacity for lipophilic drugs. This study clearly points to the conflicting situation that might arise during the preclinical phase of the development of lipid based formulations of lipophilic drugs as the performance of such systems is very often evaluated by both *in vitro* release studies in human biorelevant media as well as *in vivo* studies in rats. Care must be taken to select a relevant animal model.

1. Introduction

Oral administration has attractive advantages for drug delivery including the ease of application and high patient compliance, and is the preferred route for chronic drug therapy (Shen and Mitragotri, 2002; Barakat, 2010). However, drug solubility and its dissolution are among many factors determining drug bioavailability after oral administration (Rolan and Molnar, 2006). Low solubility is the reason why BCS (Biopharmaceutics Classification System) class II drugs often show poor and variable oral bioavailability (Grove et al., 2007). Several strategies dealing with the formulation problems of poorly water soluble compounds have been developed and described in literature. Lipid-based drug delivery systems, including oil solutions, oil suspensions, emulsions and self-(micro)emulsifying drug delivery systems (SMEDDS), constitute one of the possible approaches to improve drug bioavailability (Pouton, 2000). There are different advantages using lipid-based drug delivery systems such as the drug already being in solution, allowing elimination of the dissolution step (Porter et al., 2004), and the benefits observed in the presence of food as even small doses of lipid have been shown to stimulate a post-prandial response (Khoo et al., 2003). Significant interest has been paid to lipid-based drug delivery systems after the commercial success of Sandimmune Neoral® (cyclosporine A), Fortovase® (saquinavir) and Norvir® (ritonavir) (Grove et al., 2006), as well as the proven increase in bioavailability of different compounds such as ontazolast (Hauss et al., 1998), halofantrine (Khoo et al., 1998), danazol (Porter et al., 2004) and seocalcitol (Grove et al., 2006, 2007) when

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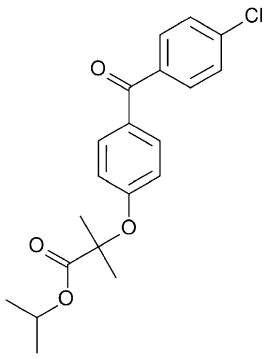

Fig. 1. Structural formula of fenofibrate.

administered in lipid-based delivery systems compared to a solid dosage form.

Various studies have been carried out in order to compare the ability of medium chain lipids versus long chain triglycerides with respect to increasing drug bioavailability. Medium chain lipids have higher fluidity, better solubilizing properties and provide a better chemical stability for the drug substance owing to the purity of the lipid and the lack of double bonds compared with long chain triglycerides (Shah et al., 1994; Grove et al., 2006). However, the latter is likely to enhance the lymphatic transport of a lipophilic drug substance, which prevents first-pass metabolism (Caliph et al., 2000; Porter et al., 2007). In some cases, such as vitamin D3 (Holmberg et al., 1990), halofantrine (Caliph et al., 2000) and danazol (Porter et al., 2004), drug bioavailability has been shown to be significantly higher after co-administration with long chain triglycerides compared with medium chain triglycerides. In contrast, the intestinal absorption of progesterone and griseofulvine was higher when administered with medium chain lipids when compared to long chain lipids (Porter et al., 2008). On the other hand, no significant differences in bioavailability were found for seocalcitol (Grove et al., 2006) and dexamethasone (MacGregor et al., 1997), irrespective of the chain length of the lipid employed. One of the remaining difficulties in formulation development during the preclinical stage is to select the optimal type of tests to evaluate the performance with respect to *in vivo* exposure. A lot of excellent research has been done to develop biorelevant (to humans) media to test *in vitro* drug dissolution or drug release (Jantratid et al., 2008; Vertzoni et al., 2004). Most often, *in vitro* dissolution tests are performed in biorelevant media and consecutively (or concurrently), based on the outcome of the biorelevant dissolution studies animal tests are executed.

The goal of this work was to investigate the relevance of combining *in vitro* release studies in human biorelevant media with *in vivo* studies in rats for the lipophilic model compound fenofibrate formulated in four different lipid based drug delivery systems (containing medium or long chain lipids) and one commercially available micronized formulation (Lipanthyl Micronized®). This combination of *in vitro* testing with animal experiments is often done in pharmaceutical industry during the early formulation development phase and is based on the philosophy "to test as much as possible with minimal efforts and costs". Often, the physiological suitability of the animal model selected is not clear and rats are frequently used in an early stage of development as they are cheap and easy to handle. Moreover, rats have been used as animal model in various published studies of lipid-based formulations (Grove et al., 2006; Yin et al., 2009). The ability of the lipid-based delivery systems and the micronized formulation to maintain fenofibrate supersaturation was studied in various biorelevant media including those simulating the fasted as well as the fed state.

Fenofibrate (Fig. 1) is a neutral, lipophilic drug ($\log P = 5.2$) (Munoz et al., 1994), which is poorly soluble in water (aqueous solubility < 0.5 mg/l) (Vogt et al., 2008); it has a high permeability and hence is considered as a Class II drug according to the BCS (Granero et al., 2005). Fenofibrate is a lipid-lowering agent, which is mainly used to reduce cholesterol levels in patients at risk of cardiovascular disease (Wysocki et al., 2004).

2. Materials and methods

2.1. Chemicals

Fenofibrate was purchased from Indis (Aartselaar, Belgium) and fenofibric acid from ABCR (Karlsruhe, Germany). Carbamazepine was bought from PharmInnova (Waregem, Belgium). Captex 200P (propylene glycol mono- and dicaprylate and mono- and dicaprate), Capmul MCM (glyceryl mono- and dicaprate) and Caprol 3GO (polyglycerol-3 oleate) were kindly provided by Abitec Corp. (Janesville, WI, USA). Soybean oil was purchased from Sigma–Aldrich Chemie (Steinheim, Germany). Tween 80 was purchased from Alfa Aesar (Karlsruhe, Germany).

Sodium taurocholate was bought from ICN Biomedicals (Eschwege, Germany), lecithin from Nattermann Phospholipid (Köln, Germany), and chloroform from Chemlab (Zedelgem, Belgium). $NaH_2PO_4 \cdot H_2O$, NaCl and 0.1 M HCl were purchased from Fisher Scientific (Tournai, Belgium).

2.2. Methods

2.2.1. Preparation of lipid-based formulations

Four formulations (Tween 80–Captex 200P, 3–1; Tween 80–Capmul MCM, 5–1; Tween 80–Caprol 3GO, 5–1; and Tween 80–soybean oil, 7–1; w/w) were prepared by mixing Tween 80 and each oil at 50–60 °C. Fenofibrate was then dissolved into the mixture of surfactant and oil by constant stirring and kept at 50–60 °C until a clear solution was obtained. The final concentration of fenofibrate in the lipid-based system was 5%. All mixtures remained clear at room temperature. The commercially available micronized formulation, Lipanthyl Micronized® (dose strength 67 mg) was obtained from Solvay Pharma (Brussels, Belgium).

2.2.2. Droplet size measurement

The droplet size of the formulations was determined at 0.5% (w/v) concentration of the formulation in water by photon correlation spectroscopy using a CGS-3 spectrometer (Malvern Instruments, Worcestershire, UK) equipped with a goniometry, auniphase 22 mV He–Ne laser operating at 632.8 nm, an avalanche photodiode detector and an ALV-5000/EPP multi-angle tau correlator. Light scattering was monitored at 90°.

2.2.3. Preparation of release media

Fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) were prepared according to the formula described in Vertzoni et al. (2004), and simulated gastric fluid without pepsin (SGF_{sp}; USP). The following chemicals were used for the preparation of the biorelevant media: sodium acetate (VWR, Brussels, Belgium), acetic acid (Chemlab, Zedelgem, Belgium), sodium taurocholate (practical grade) (ICN Biomedicals, Eschwege, Germany), lecithin (Phospholipon 90G,Nattermann Phospholipid, Köln, Germany), $NaH_2PO_4 \cdot H_2O$, NaCl and 1 M HCl (Fisher Scientific, Tournai, Belgium) and chloroform (Chemlab, Zedelgem, Belgium).

2.2.4. Solubility measurements

The solubility of fenofibrate was assessed in various aqueous media by the shake-flask method: an excess amount (approximately 2 mg) of fenofibrate was dispersed in 1.5 ml of medium containing placebo lipid-based formulations and shaken for 24 h at