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Inhibition of cholesterol transport in an intestine cell model by pine-derived phytosterols

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ABSTRACT

We have quantified the inhibition of intestinal cholesterol transport by pine-derived phytosterols using an HT29-MTX intestine cell model that forms a mucus layer similar to that in the intestine. An artificial intestinal fluid consisting of digested fat, bile salt, cholesterol, and phytosterols was formulated in order to mimic the conditions in the intestine. The apparent permeability coefficient (P_{app}) of the positive control, i.e., 0.1 mM of cholesterol solubilized in the artificial intestine fluid, was found to be 0.33 ($\pm$ 0.17) $\times$ 10^{-6} cm/s. When 0.1 mM β-sitosterol was solubilized alongside, P_{app} was effectively zero, corresponding to a total inhibition of cholesterol transport. A similar strong inhibition was found when commercial pine-derived phytosterols, PinVita™ FSP DuPont, were co-solubilized with cholesterol in the dietary model micelles, leading to P_{app} = 0.06 ($\pm$ 0.06) $\times$ 10^{-6} cm/s, i.e., 5.5 times lower than the cholesterol positive control. Additionally, the effect of potential oral administration formulations generated by the pine-derived phytosterols was also characterized. The formulations were produced as a liquid formulation of the cholesterol-containing artificial intestine fluid. Six liquid formulations were tested of which four displayed a P_{app} in the range of 0–0.09 $\times$ 10^{-6} cm/s. The remaining two formulations did not show any inhibition effect on cholesterol transport and even enhanced cholesterol transport. It was furthermore observed that the phytosterols were found in the collected intestine cells but not transported to the basolateral region in the intestinal cell model system.

1. Introduction

Cholesterol is a lipid that plays a pivotal role in many biochemical and biophysical processes. Cholesterol is the precursor of various steroid hormones, bile salts, and vitamin D_3, and is essential for maintaining the proper rigidity of the plasma membrane (Rozner and Garti, 2006). However, excess amount of cholesterol in the blood vessels is the main reason for provoking cardiovascular disease (CVD), coronary heart diseases (CHD) and increased mortality rate (Martin et al., 1986; Weingartner et al., 2011). Cholesterols are supplied to the body through the food (exogenous) or synthesized in liver (endogenous). The exogenous cholesterol is delivered to the apical side of the intestines after food digestion by means of bile salts and lipases. Continuously, the degraded lipid components of the intestines (monoglycerides, fatty acids, and cholesterols) are transformed from hydrophobic material into a colloidal structure, such as micelles, with aid of the bile salts (Carey et al., 1983). Ashworth and Lawrence showed that the 3–25 nm diameter of monoglyceride-oleic acid of digested lipid micelles were optimized to be absorbed into enterocytes (Ashworth and Lawrence, 1966). When the micelles enter into the enterocytes, cholesterols, monoglycerides, and fatty acids are re-esterified as cholesteryl esters, triglycerides, and phospholipids, which are main components of secreted particles (chylomicrons), and then transferred to the basolateral region (Carey et al., 1983; Chang et al., 2009), as illustrated in Fig. 1.

Phytosterols and plant-derived compounds are known for their ability to lower cholesterol levels in the blood stream (Brauner et al., 2012; Jones et al., 1997; Ostlund, 2004; Ostlund et al., 2002; Raederstorff et al., 2003). Phytosterols are lipids with a very similar chemical structure to cholesterol, they are more hydrophobic, and humans cannot synthesize them. Therefore, the phytosterols are available orally from vegetables, plant oils, plant extracts, or plant origin supplements (Ostlund, 2007). The mechanism behind

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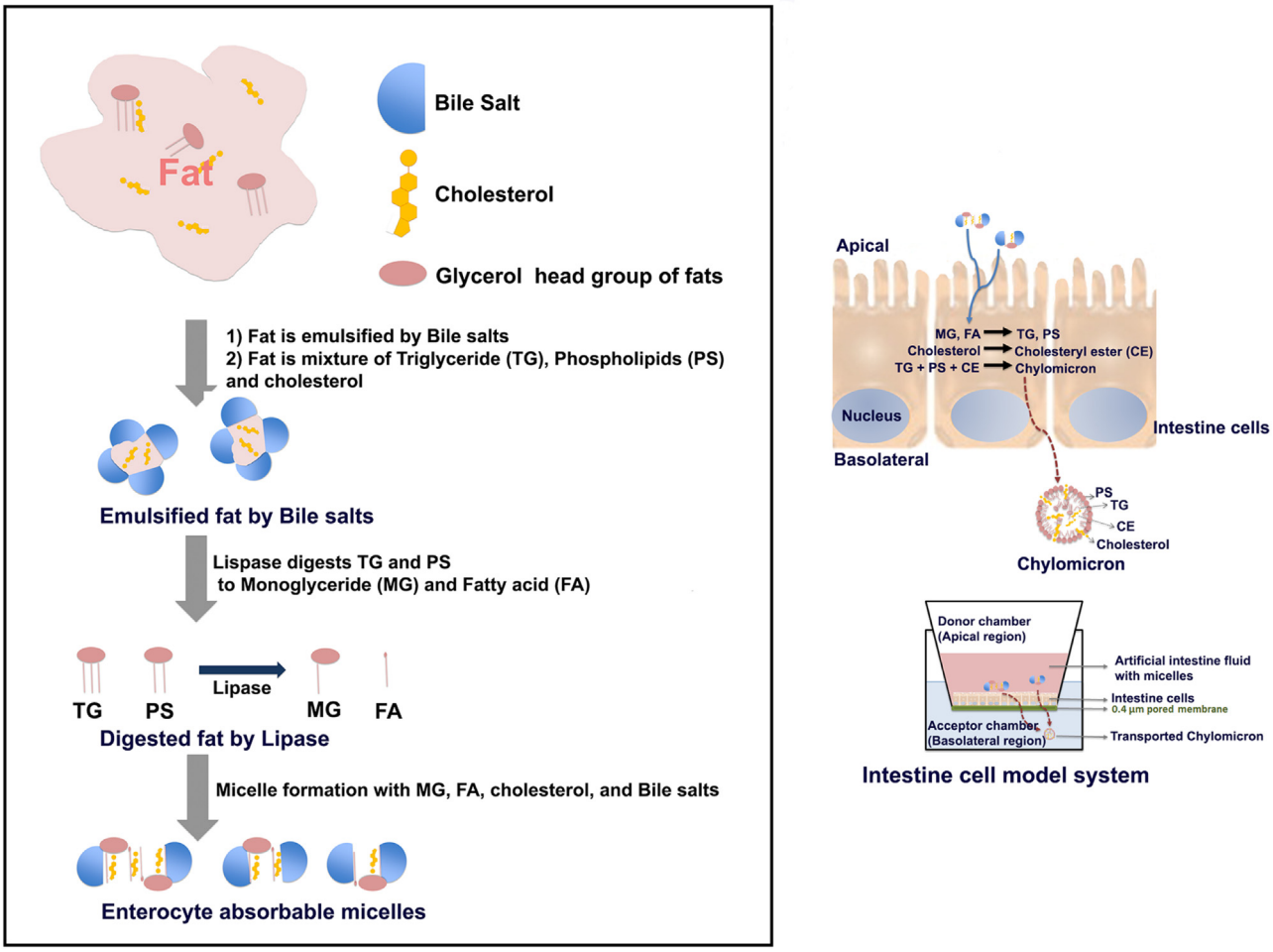

Fig. 1. The schemes of the intestinal cell model system for cholesterol transport. Triglycerides, cholesterol ester, and phospholipids are digested by bile salts and lipases in the intestine lumen, and the digested monoglycerides, fatty acids, and cholesterol-containing micelles enter into intestine cells and are transported to the basolateral region in the form of chylomicrons. Fat digestion and absorbable micelle formation in the intestine lumen (left). The digested fat including cholesterol is transported from the apical region to the basolateral region by forming chylomicrons (upper right). The experimental set-up for the intestinal cell model system (lower right). The set-up of the intestinal cell model system for cholesterol transport is also shown (lower right). **BA**: bile salt, **MG**: monoglyceride, **FA**: fatty acid, **TG**: triglyceride, **PS**: phospholipid, **C**: cholesterol, **CE**: cholesteryl ester.

inhibition of cholesterol uptake by phytosterols is still not well characterized. It was previously reported that the phytosterols can inhibit cholesterol uptake through creating bio-unavailable crystals with cholesterol in the stomach and intestines (Christiansen et al., 2003; Rozner and Garti, 2006), limiting the cholesterol solubility in the dietary micelles, blocking metabolic effects of cholesterol in intestinal cells (Brauner et al., 2012; Trautwein et al., 2003). Still, the effect of phytosterols against cholesterol absorption in the serum appears well established in practice, and consequently phytosterol supplements are used for cholesterol lowering efficacy (Brauner et al., 2012; De Smet et al., 2012). In order to optimize such supplementation there is a need for a deeper and quantitative understanding of the inhibition mechanism.

The intestine cell model system is a very useful tool to investigate drug absorption, nutrient transport, nanoparticle transport, and sterol transport in vitro (Ehrhardt and Kim, 2008; Hilgendorf et al., 2000; Langerholc et al., 2011; Rubas et al., 1993; Sun and Pang, 2008). Previously, cholesterol transport was tested in an intestinal model system using Caco-2 cells (Field et al., 1997; Palmgren et al., 2005; Petruzzelli et al., 2009). Caco-2 cells have been widely applied to study drug absorption *in vitro* and to predict drug bioavailability (Rubas et al., 1993; Sun and Pang, 2008). In recent work, HT29-MTX cells were preferred over Caco-2 cell model for studies of lipophilic molecular transport, since the HT29-MTX cell line forms a mucus layer on the surface of the apical region of the cells (Behrens et al., 2001). Because this model system is closer to the natural intestine tissue structure as it consist of mixed population of absorptive cells and mucus-producing cells (Lesuffleur et al., 1990, 1991). Finally, from the intestine cell model measurements, it is possible to calculate the apparent permeability coefficient (hereafter, P_{app}) to be compared with that obtained in the Caco-2 cell model system (Rubas et al., 1993). The P_{app} values of hydrophobic compound transport found when using HT29-MTX cells are lower than the values pertaining to Caco-2 cells due to a physical barrier of the mucus layer formed by HT29-MTX cells (Behrens et al., 2002). P_{app} is the relevant assessment value of drug delivery and compares to the in vivo effect indirectly (Rubas et al., 1993).

The aim of the present paper is to quantify the transport of cholesterol in the intestine cell model using a customized artificial intestinal fluid with lipophilic micelles composed of digested fats. Simultaneously, the inhibition of cholesterol transport by phytosterols (β-sitosterol and the commercial pine-derived phytosterol) was characterized by determination of P_{app}. In the current study, we have used the non-modified sterols in order not to influence the