Short Communication

AN EXAMINATION OF THE INTERPLAY BETWEEN ENTEROCYTE-BASED METABOLISM AND LYMPHATIC DRUG TRANSPORT IN THE RAT

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ABSTRACT:
The current study has examined whether drugs that are transported to the systemic circulation via the intestinal lymph (and therefore associate with lipoproteins within the enterocyte) are accessible to enterocyte-based metabolic processes. The impact of changes to the mass of lipid present within the enterocyte-based lymph lipid precursor pool (LLPP) on the extent of enterocyte-based drug metabolism has also been addressed. Low (5 mg oleic acid/h) or high (20 mg oleic acid/5.2 mg lyso-phosphatidylcholine/h) lipid dose formulations containing halofantrine (which is lympathically transported and metabolized) or dichlorodiphenyltrichloroethane (DDT) (which is lympathically transported and relatively metabolically inert) and radiolabeled oleic acid were infused into the duodenum of lymph duct-cannulated rats. After 5 h, drug and radiolabeled oleic acid were removed from the infusions, allowing calculation of the first order turnover rate constants describing drug and oleic acid transport from the LLPP into lymph from the washout profiles. In one group of animals, bolus doses of ketoconazole were also administered to inhibit cytochrome P450-based metabolism. The rate constant describing halofantrine transport from the LLPP into the lymph was lower than that of oleic acid, whereas these differences were abolished in the presence of ketoconazole. DDT and oleic acid exhibited similar turnover rate constants. The data therefore suggest that enterocyte-based metabolism removes halofantrine from the LLPP before transport into the lymph. Furthermore, enhancing the lymphatic transport of halofantrine by coadministration of larger quantities of lipid reduced the difference between the turnover rate constant for halofantrine and oleic acid and seemed to reduce the extent of enterocyte-based metabolism.

After oral delivery, drugs may be transported from the intestine to the systemic circulation via the intestinal lymph or the portal blood (Porter and Charman, 2001a). Drugs that are significantly transported via the intestinal lymphatic system usually do so in association with the lipid-rich lipoprotein fraction of lymph (Sieber et al., 1974; Porter and Charman, 2001a). For this reason, only highly lipophilic drugs (typically log P > 5, triglyceride solubility > 50 mg/g) (Charman and Stella, 1986a), which can associate with developing lipoproteins during transport through the enterocyte, are appreciably transported via the intestinal lymphatic system.

Relatively little has been published detailing the processes that dictate the association of lipophilic drugs with enterocyte-based lipoproteins; however, a recent study from our laboratory (Trevaskis et al., 2006) has shown that the size and dynamics of the LLPP seems to dictate the rate and extent of lymphatic transport of a highly lipophilic drug (halofantrine). The LLPP consists of multiple droplets of lipid situated in the smooth endoplasmic reticulum (SER) and Golgi of the enterocyte. The lipids in the LLPP are destined for assembly into lipoproteins, which are subsequently transported from the enterocyte to the systemic circulation via the intestinal lymphatic system. The LLPP in the SER and Golgi is distinct from the lipid droplets that are located in the enterocyte cytoplasm. These cytoplasmatic lipids are primarily transported to the systemic circulation via the portal vein (Tipton et al., 1989; Nevin et al., 1995).

Interestingly, the previous study from our laboratory showed that, whereas drug transport into the lymph was, in large part, a function of the mass of lipid in the LLPP available to solubilize drug (where fatty acid was used as a marker for lipid), the first order rate constant describing halofantrine transport from the LLPP into the lymph was significantly lower than the equivalent rate constant describing fatty acid transport (Trevaskis et al., 2006). Because the fractional turnover of fatty acid from the LLPP was more rapid than that of drug, the data suggested that halofantrine was removed from the LLPP, not only by transfer into the lymph, but also by an additional mechanism. The hypothesis underlying the current study was that the differences in halofantrine and lipid turnover may be accounted for by loss of halofantrine from the LLPP as a result of enterocyte-based metabolism.

The primary mechanism of halofantrine metabolism is via N-dealkylation to desbutyl-haloafantrine (Halliday et al., 1995), and this is thought to occur in both the enterocyte and the liver. Previous studies have demonstrated that the plasma ratio of desbutyl-haloafantrine to haloafantrine is significantly higher after oral as opposed to i.v. administration and that the metabolism of haloafantrine after oral administration can be reduced by coadministration with the cyto-

ABBREVIATIONS: LLPP, lymph lipid precursor pool; SER, smooth endoplasmic reticulum; LPC, l-α-lyso-phosphatidylcholine; DDT, dichlorodiphenyltrichloroethane; (dX/dt)ss, steady-state rate of total fatty acid transport into lymph; KX, first order rate constant describing fatty acid transport from the LLPP into the lymph; XLP, mass of lipid in the LLPP; (dD/dt)ss, steady-state rate of drug transport into lymph; KD, first order rate constant describing drug transport from the LLPP into the lymph; DLP, mass of drug in the LLPP; HPLC, high performance liquid chromatography; BP, benzo(a)pyrene.
chrome p450 3A inhibitor, ketoconazole (Porter and Charman, 2001b). Coadministration of halofantrine with food also results in a reduction in the desbutyl-halofantrine to halofantrine plasma ratios (Humberstone et al., 1996). The latter aspect is thought to reflect increased lymphatic transport of halofantrine postprandially, resulting in a smaller proportion of the dose being absorbed via the portal blood (and therefore available for first pass hepatic metabolism) and/or abstraction of halofantrine into developing lymph lipoproteins in the enterocyte, effectively protecting halofantrine from enterocyte-based metabolism.

The current study has therefore investigated whether lymphatically transported drugs such as halofantrine are available for metabolism within the enterocyte and whether this might explain our previous observations of a reduced turnover rate for halofantrine (when compared with fatty acid) into the lymph (Trevaskis et al., 2006). This was examined by comparing the turnover rate constants describing drug and lipid transport from the LLPP into the lymph using either halofantrine in both the presence and absence of ketoconazole (to inhibit cytochrome p450-mediated metabolism) or DDT [a lipophylic, lymphatically transported compound that is not metabolized during transport through the enterocyte (Sieber et al., 1974; Charman and Stella, 1986b)]. The potential impact of increasing the size of the intracellular LLPP and, therefore, the extent of lymphatic lipid and drug transport, on enterocyte-based drug metabolism was also examined.

Materials and Methods
Preparation of Halofantrine Formulations. Lipid formulations contained 1 μCi of [14C]oleic acid, 100 μg of halofantrine or DDT, and 5 mg of oleic acid or 20 mg of oleic acid/5.2 mg of lyso-phosphatidylcholine (LPC) dispersed in 2.8 ml of 5 mM taurocholate bile salt in phosphate buffer, pH 6.9. These formulations were administered at a rate of 2.8 ml/h, and prepared and checked for stability as described previously (Trevaskis et al., 2005). A moderate lipid dose of 5 to 20 mg/h (equivalent to 1–5 g/kg to a human on a mg/kg basis) was chosen for the formulations. LPC was also added to the higher lipid dose formulation since previous studies have shown that intraduodenal administration of LPC expands the LLPP (Trevaskis et al., 2006). In this way, addition of LPC to the formulation facilitated assessment of the effect of increasing the size of the LLPP on enterocyte-based drug metabolism. The addition of LPC to the high lipid dose formulation did not markedly alter the physical properties of the lipid dispersion when compared with the low lipid group (5 mg of oleic acid), and previous examination of identical formulations revealed mean particle diameters of 205 nm and 273 nm for the low and high lipid dose formulations, respectively (Trevaskis et al., 2006).

Surgery, Formulation Infusion, and Lymph Collection. All surgical and experimental procedures were approved by the local institutional Animal Ethics Committee and were modified slightly from those described previously (Trevaskis et al., 2005). Male Sprague-Dawley rats (280–320 g) were fasted overnight with free access to water, and anesthesia was induced and maintained using a combination of fentanyl, fluanisone, and midazolam (Trevaskis et al., 2005). The trachea, mesenteric lymph duct, and duodenum were cannulated using a combination of fentanyl, fluanisone, and midazolam (Trevaskis et al., 2005). After completion of the surgery, a continuous intraduodenal infusion of 2.8 ml/h normal saline was initiated and the animals were stabilized for 0.5 h before infusion of the experimental lipid formulations. Experimental formulations were then continued infused into the duodenum at 2.8 ml/h and contained hourly doses of 100 μg of halofantrine or 100 of μg DDT, 1 μCi of [14C]oleic acid, and either 1) 5 mg of oleic acid dispersed in 5 mM bile salt solution or 2) 20 mg oleic acid/5.2 mg LPC dispersed in 5 mM bile salt solution. In another group, rats were preloaded with 50 mg/kg ketocozole (dispersed in 1 ml of 40% v/v propylene glycol, 10% v/v ethanol, and 50% v/v Milli-Q water; Millipore, Milford, MA), at −24 h and −12 h (by oral gavage) and at −0.5 h (via the intraduodenal cannula) before initiation of the intraduodenal infusion of a formulation containing hourly doses of 100 μg of halofantrine, 1 μCi of [14C]oleic acid, and 5 mg of oleic acid dispersed in 5 mM bile salt solution.

The animals were administered a further dose of 50 mg/kg ketocozole via the intraduodenal cannula at 5 h after initiation of the intraduodenal infusion. The protocol by which ketoconazole was administered in the current paper was chosen after an initial study was conducted using a similar method, but in which 5 mg/h ketocozole was infused together with halofantrine throughout the experiment and ketoconazole was not predosed before the experiment. In these initial studies, infusing halofantrine with ketoconazole (i.e., no ketoconazole predosing) did not result in a change to the first order rate constant describing halofantrine transport into the lymph (data not shown). The inhibition of enterocyte-based metabolism of halofantrine by ketoconazole therefore appears to be dependent on the dose and timing of administration of halofantrine and ketoconazole.

The lipid formulations were administered continuously for 5 h to obtain steady-state rates of fatty acid and drug transport into the lymph as described previously (Trevaskis et al., 2006). After 5 h, the formulations were replaced with new formulations of the same composition but excluding the [14C]oleic acid radiolabel and drug. Lymph samples were then collected for another 5 h into tared tubes which were changed every 0.5 h. Removal of the [14C]oleic acid label and drug from the infusion after 5 h allowed quantification of the washout kinetics of fatty acid and drug into the lymph and calculation of the mass of fatty acid and drug in the LLPP at steady state, as described previously (and briefly summarized below) (Trevaskis et al., 2006). The data reported for halofantrine infusion in the absence of ketoconazole are reproduced from a previous publication (Trevaskis et al., 2006) for comparative purposes.

Analysis of Samples. Lymph concentrations of halofantrine were determined as described previously (Trevaskis et al., 2005). DDT was extracted from lymph using the same method as that described for halofantrine (Trevaskis et al., 2005). Using this method, recovery of DDT spiked into blank lymph (at concentrations of 8, 20, and 80 μg/ml) was >95% (n = 5 analyses at each concentration). DDT concentrations were measured by HPLC. The HPLC system consisted of a Waters 590 programmable HPLC pump, Waters 717 autosampler, Waters 486 Tunable absorbance detector (Waters, Milford, MA) and Shimadzu C-R5A Chromatopac integrator (Shimadzu, Kyoto, Japan). The detector was set at a wavelength of 238 nm. After extraction from lymph, 50-μl samples were injected onto a reverse phase ODS Beckman Ultrasphere column (25 cm × 4.6 mm; Beckman Coulter, Fullerton, CA) with a pore size of 5 μm, fitted with a Brownlee RP-18 precolumn (7 μm 15 × 3 mm; Alltech Associates, Baulkman Hills, Australia). The mobile phase consisted of 90% methanol and 10% Milli-Q water, and the flow rate was 1 ml/min. The run time was 9 min, and DDT eluted at 5.9 min. The DDT HPLC method was validated by assuring that precision and accuracy were acceptable (±10%) for replicate (n = 3) standard curves (concentrations 100, 200, 500, 1000, and 2500 ng/ml DDT in acetomitrile) run on three separate days.

Lymph triglyceride and phospholipid concentrations were determined using commercial enzymatic colorimetric methods running on a Cobas Mira lipid analyzer (Khoo et al., 2001). Calculation of total fatty acid transport into lymph (endogenous plus exogenous) assumed that each mole of lymph triglyceride and phospholipid comprised 3 and 2 mol of fatty acid, respectively.

Data Analysis and Calculations. Removal of [14C]oleic acid and drug from the intraduodenal lipid infusion after 5 h allowed quantification of their washout kinetics into lymph. We have previously shown under similar conditions that the decline in concentration of [14C]oleic acid and drug in total fatty acid in lymph (which is assumed to be the same as the decline in concentration of [14C]oleic acid and drug in total fatty acid in the LLPP) is controlled by a first order rate process (Trevaskis et al., 2006). A semilog plot of ln (concentration of [14C]oleic acid or drug in total fatty acid in the lymph) versus time (hours) after removal of [14C]oleic acid or drug from the intraduodenal lipid infusion was therefore linear, with a gradient equal to the first order rate constants describing fatty acid (Kf) or drug (Kd) transport from the LLPP into the lymph.

Because the steady-state rate of lipid and drug transport from the LLPP into the lymph followed first order kinetics, the transport rate of total lipid (endogenous plus exogenous fatty acid) (dX/dt) and drug (dD/dt) from the LLPP into the lymph per unit time at steady state was a function of the mass of lipid (Xf) or drug (Df) in the LLPP at steady state and the rate constant describing lipid (Kf (h⁻¹)) and drug (Kd (h⁻¹)) turnover from the LLPP into the lymph, respectively, where
concentration of Hf or DDT in lipid (measured as total fatty acid) (rats (K_{drug (\text{in vivo}))) dispersed in 5 mM bile salt solution. Data are also shown for halofantrine (Hf) and a dose of 50 mg/kg ketoconazole (KC) at -24, -12, -0.5, and 5 h before or after initiation of formulation administration, 3) 100 \mu g of DDT, to mesenteric lymph duct-cannulated, anesthetized rats (n = 4, mean \pm S.E.M.). B, concentration of Hf or DDT in lipid (measured as total fatty acid) (\mu g/\mu mol) in the LLPP (black bars) or lymph (white bars) after continuous intraduodenal infusion of formulations containing hourly doses of 5 mg of oleic acid dispersed in 5 mM bile salt solution and either 1) 100 \mu g of halofantrine (Hf), 2) 100 \mu g of Hf and a dose of 50 mg/kg ketoconazole (KC) at -24, -12, -0.5, and 5 h before or after initiation of formulation administration, or 3) 100 \mu g of DDT or hourly doses of 20 mg oleic acid/5.2 mg LPC, and 4) 100 \mu g Hf or 5) 100 \mu g DDT, to mesenteric lymph duct-cannulated, anesthetized rats (n = 4, mean \pm S.E.M.). The data obtained for Hf administered in the absence of KC are reproduced from a previous publication for comparison (Trevaskis et al., 2006).

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\left( \frac{dX_t}{dt} \right)_{\text{ss}} = K_{X}X_{t,0} \quad \text{and} \quad \left( \frac{dD}{dt} \right)_{\text{ss}} = K_{0,D}D_{t,0}
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\(X_{t,0}\) and \(D_{t,0}\) were therefore calculated using the values of \(K_X\) and \(K_D\) obtained from the washout profiles and the measured rate of fatty acid (\(dX_t/dt\)) and drug (\(dD_t/dt\)) transport into lymph obtained during the 4- to 5-h period after initiation of lipid infusion (and attainment of steady-state lipid and drug transport in lymph). The concentration of drug in the LLPP and lymph was subsequently calculated from the mass ratio of drug (micrograms) to total fatty acid (micromoles) in the LLPP or lymph.

**Statistical Analysis.** Statistically significant differences were determined by analysis of variance followed by Tukey’s test for multiple comparisons at a significance level of \(\alpha = 0.05\). All statistical analysis was performed using SPSS for Windows version 11.5.0. (SPSS Inc., Chicago, IL).

**Results and Discussion**

Figure 1A shows the first order rate constants describing fatty acid (\(K_X\)) and drug (\(K_D\)) turnover from the LLPP into the lymph at steady state after administration of formulations containing 100 \mu g/h halofantrine or DDT and 5 mg/h oleic acid dispersed in 5 mM bile salt solution. Data are also shown for halofantrine after coadministration with ketoconazole. As reported previously (Trevaskis et al., 2006), the first order turnover rate constant describing halofantrine transport from the LLPP into the lymph was significantly lower than that of fatty acid (\(P < 0.05\)). The difference between the first order rate constants describing lipid and halofantrine transport from the LLPP into the lymph was attenuated, however, by administration of halofantrine with the cytochrome p450 3A inhibitor, ketoconazole. The turnover rate constants describing lymphatic transport of lipid and the nonmetabolizable substrate DDT were also not significantly different. The data are therefore consistent with the hypothesis that the difference between the first order rate constants describing fatty acid and halofantrine transport from the LLPP into the lymph resulted from halofantrine removal from the LLPP by metabolism.

Further support for this suggestion is provided by the data in Fig. 1B, which shows the concentration of drug (halofantrine or DDT) present in the LLPP or lymph lipids after administration of low (5 mg oleic acid/d) or high (20 mg oleic acid/5.2 mg LPC per h) lipid dose formulations containing halofantrine (with or without ketoconazole) or DDT. After administration of halofantrine in the absence of ketoconazole (with either a low or high lipid load), the concentration of halofantrine in LLPP lipids was significantly greater than the equivalent concentration of halofantrine in the lymph. In contrast, the concentrations of halofantrine in LLPP and lymph lipids were not significantly different after administration of halofantrine with ketoconazole (\(P < 0.05\)). The concentration of DDT in the LLPP and lymph lipids was also not significantly different (\(P < 0.05\)).

The effect of increasing the lipid dose (from 5 mg oleic acid to 20 mg oleic acid/5.2 mg LPC) on the mass of fatty acid in the LLPP, the first order turnover rate constants describing fatty acid (\(K_X\)) and drug (\(K_D\)) transport from the LLPP into the lymph, and the fractional difference between the turnover rate constants for fatty acid and drug are presented in Table 1. The LLPP expanded from 23 to 140 \mu mol of total fatty acid and \(K_X\) decreased after administration of halofantrine or DDT with the higher rather than the lower lipid dose. The first order rate constant for DDT transport from the LLPP into the lymph also decreased in line with the data observed for fatty acid transport, whereas the first order rate constants for halofantrine were significantly less than those for fatty acid and DDT. Interestingly, the fractional difference between the first order turnover rate constant for fatty acid and halofantrine transport from the LLPP into the lymph was attenuated after administration of the higher lipid dose, suggesting that the rate of halofantrine metabolism decreased when the LLPP was expanded. This is consistent with previous in vitro studies, which have suggested that the larger fat droplets that are present within the enterocyte after a fatty meal protect benzo(a)pyrene (BP) from metabolism by reducing transfer of BP from fat droplets in the SER membrane to microsomal enzymes responsible for BP metabolism (Van Veld et al., 1987).

Previously, we have reported that oral administration of halofantrine to dogs with a fatty meal or ketoconazole reduces the plasma concentration ratio of desbutyl-halofantrine to halofantrine (Humberstone et al., 1996). Administration of halofantrine with a fatty meal was suggested to avoid halofantrine metabolism either in the enterocyte, by altering the intracellular compartmentalization of halofantrine and, therefore, access to metabolic enzymes, or in the liver by promoting lymphatic transport since the lymphatic system empties directly into the system circulation and effectively bypasses the liver. The current study suggests that halofantrine is available for enteroocyte-based metabolism when trafficked into the LLPP but that metabolism is reduced when the LLPP is expanded by the coadministration of lipid (Table 1). Enhancing the lymphatic transport of
halofantrine via coadministration with lipid therefore reduces halofantrine metabolism by reducing both enterocyte-based and hepatic first pass metabolism.

Figure 2 suggests a simple model to describe the interplay between enterocyte-based metabolism and lymphatic drug transport at steady state based on the current and previous studies (Trevaskis et al., 2006). Figure 2A shows the kinetic processes that dictate the rate of fatty acid transport from the LLPP into the lymph at steady state. A constant mass of fatty acid (X_{LLPP}) is present in the LLPP at steady state, and fatty acid turnover from the LLPP into the lymph may be described by a first order rate constant, K_X. The rate of lipid transport into the lymph at steady state (dX_{LYMP}/dt)_{ss} is therefore described by the equation (dX_{LYMP}/dt)_{ss} = K_X \cdot X_{LLPP}. After administration of a higher lipid dose, X_{LLPP} increases and K_X decreases; however, the overall effect is an increase in the rate of lipid transport into lymph (Trevaskis et al., 2006).

Figure 2B summarizes the kinetic processes that dictate the rate of drug transport into the lymph at steady state. A constant mass of drug (D_{LLPP}) is present in the LLPP at steady state but, in contrast to lipid, drugs may be removed from the LLPP by two first order processes. Drugs that are not subject to metabolism (e.g., DDT) are transported from the LLPP into the lymph in conjunction with lipid (and with the same proportional turnover kinetics) such that the rate of lymphatic transport of such drugs is described by the equation (dD_{LYMP}/dt)_{ss} = K_X \cdot X_{LLPP}. In contrast, drugs that are subject to metabolism (e.g., halofantrine) are removed from the LLPP at a first order rate by metabolism (with constant K_m) and also by turnover from the LLPP into the lymph in conjunction with lipid. The overall effect is that the drug turnover from the LLPP into the lymph is described by an apparent first order rate constant, K_D, where K_D = K_X - K_m, and where (dD_{LYMP}/dt)_{ss} is described by the equation dD_{LYMP}/dt = K_D \cdot D_{LLPP} = (K_X - K_m) \cdot D_{LLPP}.

Conclusion

In this study, the relationship between enterocyte-based metabolism and lymphatic drug transport was examined via determination of the first order rate constants describing fatty acid and drug transport from the LLPP into the lymph. The first order rate constant describing halofantrine transport from the LLPP into the lymph was significantly less than the equivalent rate constant for fatty acid; however, this difference was attenuated by administration of halofantrine with the cytochrome p450 inhibitor, ketoconazole. Furthermore, the first order rate constants describing the turnover of the nonmetabolizable probe, DDT, and fatty acid from the LLPP into the lymph were similar. The difference between the first order rate constants for halofantrine and fatty acid therefore appears to be due to removal of halofantrine from the LLPP by metabolism. The influence of the quantity of lipid in the enterocyte-based intracellular LLPP on enterocyte-based metabolism was subsequently examined by comparing the difference between the turnover rate constant for halofantrine and fatty acid transport from the LLPP into the lymph after administration of halofantrine with a higher lipid dose. Increasing the size of the intracellular LLPP (and, as a result, the extent of lymphatic drug transport) by coadministration with larger quantities of lipid appeared to reduce the extent of metabolism. Coadministration of lymphatically transported drugs with food or lipid-based formulations that stimulate intestinal lymphatic transport may therefore increase oral bioavailability both by avoidance of hepatic first pass metabolism and also by local avoidance of metabolism in the enterocyte.

References


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