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Oleic acid increases intestinal absorption of the BCRP/ABCG2 substrate, mitoxantrone, in mice

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HIGHLIGHTS

- Oleic acid at relevant doses for humans, increased absorption of the BCRP substrate mitoxantrone, in mice.
- Oleic acid increased the levels of mitoxantrone in plasma, brain, kidney and liver compared to control groups.
- Increased gene expression in the intestine from mice exposed to oleic acid was found, although not statistically significant.
- The BCRP inhibitor Ko143 caused increased absorption of mitoxantrone in liver and a tendency of increased absorption in plasma and blood.

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ABSTRACT

The efflux transporter breast cancer resistance protein (BCRP/ABCG2) decrease intestinal absorption of many food toxicants. Oleic acid increases absorption of the specific BCRP substrate mitoxantrone (MXR), and also BCRP gene expression in human intestinal Caco-2 cells, suggesting that oleic acid affect the BCRP function. Here, we investigated the effect of oleic acid on intestinal absorption of MXR in mice. Mice were orally dosed with 2.4 g oleic acid/kg b.w. and 1 mg MXR/kg b.w., and sacrificed 30, 60, 90 or 120 min after exposure, or were exposed to 0.6, 2.4 or 4.8 g oleic acid/kg b.w. and 1 mg MXR/kg b.w., and sacrificed 90 min after exposure. Mice were also treated with Ko143 together with MXR and sacrificed after 60 min, as a positive control of BCRP-mediated effects on MXR absorption. Absorption of MXR increased after exposure to oleic acid at all doses, and also after exposure to Ko143. Intestinal BCRP gene expression tended to increase 120 min after oleic acid exposure. Our results in mice demonstrate that oleic acid decreases BCRP-mediated efflux, causing increased intestinal MXR absorption in mice. These findings may have implications in humans, concomitantly exposed to oleic acid and food contaminants that, similarly as MXR, are substrates of BCRP.

1. Introduction

Humans are exposed to toxic substances in food in combination with a mixture of nutrients such as fat, proteins, carbohydrates and vitamins. Fat rich meals are known to increase absorption of certain drugs used in human and veterinary medicine (Awadzi et al., 1994; Custodio et al., 2008). Fatty acids, which are released during gastro-intestinal digestion of fat, are surface active and may cause increased intestinal absorption of normally poorly absorbed water-soluble substances by opening the barrier of tight junctions in the paracellular absorption pathway (Aspenstrom-Fagerlund et al., 2007, 2009; Lindmark et al., 1998). Another barrier for intestinal absorption is efflux transporters in the apical cell membranes of the intestinal epithelial cells, which extrude a wide range of substances back to the intestinal lumen after they have been taken up from the intestinal lumen into the intestinal cells. Fatty acids may interfere with the transcellular absorption pathway for substances, by impairment of the function of apical efflux transporters in the intestinal epithelium. As a result an increased intestinal absorption of certain hydrophilic substances may occur (Aspenstrom-Fagerlund et al., 2012).

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One important efflux transporter for many food toxicants is the breast cancer resistance protein (BCRP also termed ABCG2) which belongs to the ATP-binding cassette (ABC) transporter family (Doyle and Ross, 2003). BCRP has a broad substrate specificity and mediates efflux of numerous substances, including food toxicants like benzo(a)pyrene, bisphenol A (BPA) and several mycotoxins (Ebert et al., 2007; van Herwaarden et al., 2006), as well as substances/nutrients important for normal health, like vitamins (van Herwaarden et al., 2007) and hormones (Gram et al., 2009). BCRP is commonly expressed in tissues involved in drug and xenobiotic absorption (small and large intestinal epithelia), distribution (the blood–brain and placental barriers) and elimination (kidney, liver) (Gutmann et al., 2005; Maliepaard et al., 2001; Tanaka et al., 2005). BCRP is also expressed in the apical membranes of different polarized cell models used for transport studies, *e.g.* intestinal Caco-2 cells. Recently we showed that oleic acid increases the absorption of mitoxantrone (MXR), and also caused an increased gene expression of BCRP in Caco-2 cells (Aspenstrom-Fagerlund et al., 2012). MXR is a well-known model substance for BCRP transport (Doyle and Ross, 2003). Oleic acid is the most common fatty acid in the Swedish diet (National Food Agency, 2012) and was therefore selected for studies of fatty acid-induced effects on the absorption of MXR. Certain inhibitors of BCRP, *e.g.*, Ko143, can be used to verify that BCRP is functional in the intestinal epithelium of mice or in cell models like Caco-2 cell monolayers (Allen et al., 2002).

The aim of the present study was to investigate if oleic acid causes an increased absorption of MXR *in vivo* by impairment of Bcrp mediated efflux in mice.

2. Materials and methods

2.1. Materials

Radioactive ^{3}H MXR (specific activity 10 Ci/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, USA). MXR dihydrochloride (purity >99%), oleic acid (protonated form, purity 98–99%), Ko143, hydroxyl propyl methyl cellulose, Tween 80, HEPES and dimethylsulfoxid (DMSO, Riedel-de Haën) were purchased from Sigma–Aldrich Sweden AB (Stockholm, Sweden). Glucose was purchased by BDH Limited, (VWR International Ltd.) Poole (England). The experimental medium Hank's balanced salt solution (HBSS) with $CaCl_2$ and $MgCl_2$ was purchased from Invitrogen/Gibco/Life Technologies Ltd. (Paisley, UK). RNA later was purchased from Qiagen® Nordic (Solna, Sweden). Isoflurane was purchased from Baxter Medical AB (Kista, Sweden). Biolute-S (tissue solubilizer) was purchased from Zinsser Analytic, GmbH (Frankfurt, Germany). Ultima Gold (scintillation liquid) was purchased from Sigma–Aldrich Sweden AB (Stockholm, Sweden). EDTA was purchased from MP Biomedicals (Solon, USA). Heparin was from Leo Pharma (Ballerup, Denmark). All other chemicals were purchased from Merck (Darmstadt, Germany). For isolation of total RNA from the mouse small intestine the NucleoSpin® RNAII kit with DNaseI from Macherey–Nagel GmbH&Co, KG (Düren, Germany) was used. QuantiTect one-tube RT-PCR kit with SYBR Green was obtained from Qiagen® Nordic (Solna, Sweden) and was used for the qRT-PCR analysis. All chemicals used in the experiments were of analytical grade.

2.2. Mice

FVB/NCrl male mice (8 weeks) were purchased from Charles River (Sulzfeld, Germany) via Scanbur AB (Sollentuna, Sweden). Mice were randomly assigned to different cages (n = 5–6) and acclimatized for one week in a temperature- and humidity-controlled room with a 12 h light/dark cycle. Tap water and food pellets were given *ad libitum* during one week before the experiment. The food pellets were purchased from Special Diets Services (Rat and Mouse No. 1 Maintenance Autoclavable, Essex, UK), and contained 7.34 energy (E)-% fat (0.77% oleic acid, 18% protein and 75% carbohydrates). Mice were fasted overnight with free access to water before the experiments. At the time of the experiment the mice weighed 20–25 g and were 9 weeks old. The experiment has been reviewed and approved by the Uppsala Ethical Committee on Animal Experiments, application C334/9, Uppsala, Sweden.

2.3. Experimental methods

2.3.1. Absorption experiments

Each mouse was administered MXR in a dose of 2 μmol MXR/kg b.w., corresponding to 1 mg MXR/kg b.w. spiked with 3 μCi ^{3}H MXR/mouse as tracer. Protonated free oleic acid in HBSS was emulsified by sonication, using a water bath shaker, immediately before used in the experiments. To avoid heating of the oleic acid emulsions they were put in the bath for a very short time (just a few seconds) twice. Finally, MXR and ^{3}H-MXR in HBSS was added to the experimental emulsion, immediately before treatment of mice through gavage. The BCRP inhibitor Ko143 was used as a positive control of BCRP-mediated effects on MXR absorption in mice. Since KO143 is poorly soluble in water, a micellar suspension was prepared as described previously (Allen et al., 2002). Shortly, a stock solution of 5 mg Ko143 and 200 μl DMSO was prepared, of which 200 μl was mixed with 800 μl 0.2 M HCl. The final Ko143 formulation was obtained by mixing the Ko143 solution with 1.5 ml of hydroxyl propyl methyl cellulose (10 mg/ml) in Tween 80 (5%) and finally diluted with 0.5 ml glucose (5%). Two formulations were prepared, one containing Ko143 and one without Ko143. Each mice treated with Ko143 received a dose of 10 mg Ko143/kg b.w.

Firstly a time-dependency study (experiment 1) and secondly a dose-dependency study (experiment 2) were carried out where mice were treated with MXR and oleic acid at different time points and different doses, respectively. Thirdly, a study using Ko143 as a positive control for the effects of BCRP inhibition on MXR absorption was carried out (experiment 3).

2.3.1.1. Experiment 1. Mice, (4 groups, n = 5–6), were dosed orally by gavage with an oleic acid emulsion (2.4 g oleic acid/kg b.w.) and MXR. Each oleic acid-exposed group had its own control group, only receiving MXR in HBSS. The dose was divided into three administrations with a 30 min interval in-between during 1 h (0, 30 and 60 min). Groups of mice were sacrificed 30, 60, 90 and 120 min after the last administration and MXR levels in various tissues were measured as described below.

2.3.1.2. Experiment 2. Three groups of mice (n = 6) were dosed orally with MXR in oleic acid by gavage, and a fourth group (n = 6) with MXR in HBSS (control group), as described in the time-dependency study above. The total dose of oleic acid for each mouse was 0.6, 2.4 or 4.8 g/kg b.w. Mice were sacrificed 90 min after the last administration and MXR levels in various tissues were measured as described below.

2.3.1.3. Experiment 3. Two groups of mice (n = 6) were treated orally by gavage with MXR in formulations with or without Ko143. One control group was pre-treated by gavage with the formulation without Ko143, 30 min prior to administration of MXR in Ko143-free formulation three times during one hour with a 30 min interval in-between (0, 30 and 60 min). Another group of mice was pre-treated with Ko143 formulation by gavage 30 min before treatment with Ko143 formulation containing MXR three times