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Human biofluid concentrations of mono(2-ethylhexyl)phthalate extrapolated from pharmacokinetics in chimeric mice with humanized liver administered with di(2-ethylhexyl)phthalate and physiologically based pharmacokinetic modeling

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ABSTRACT

Di(2-ethylhexyl)phthalate (DEHP) is a reproductive toxicant in male rodents. The aim of the current study was to extrapolate the pharmacokinetics and toxicokinetics of mono(2-ethylhexyl)phthalate (MEHP, a primary metabolite of DEHP) in humans by using data from oral administration of DEHP to chimeric mice transplanted with human hepatocytes. MEHP and its glucuronide were detected in plasma from control mice and chimeric mice after single oral doses of 250 mg DEHP/kg body weight. Biphasic plasma concentration–time curves of MEHP and its glucuronide were seen only in control mice. MEHP and its glucuronide were extensively excreted in urine within 24 h in mice with humanized liver. In contrast, fecal excretion levels of MEHP glucuronide were high in control mice compared with those with humanized liver. Adjusted animal biomonitoring equivalents from chimeric mice studies were scaled to human biomonitoring equivalents using known species allometric scaling factors and *in vitro* metabolic clearance data with a simple physiologically based pharmacokinetic (PBPK) model. Estimated urine MEHP concentrations in humans were consistent with reported concentrations. This research illustrates how chimeric mice transplanted with human hepatocytes in combination with a simple PBPK model can assist evaluations of pharmacokinetics or toxicokinetics of the primary or secondary metabolites of DEHP.

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1. Introduction

Recent biomonitoring techniques for determining various manmade and naturally occurring chemicals have become valuable tools for quantitatively evaluating human exposure from environmental and/or incidental sources. The phthalate di(2-ethylhexyl)phthalate (DEHP, Fig. 1) is used in the production of polyvinyl chloride as a plasticizer and exhibits low toxicity from both acute and chronic exposures (Koch et al., 2006). Primary and secondary phthalate monoester metabolites of DEHP (Fig. 1) have been detected in human urine (Herr et al., 2009). DEHP is reportedly rapidly hydrolyzed to mono(2-ethylhexyl)phthalate (MEHP) in microsomal/cytosolic fractions of selected human organs (Choi et al., 2012). Studies have shown that absorbed monoester metabolites are usually oxidized in human bodies and excreted in urine, largely as glucuronide conjugates (Albro et al., 1982). Although no information is available on the chronic, reproductive, developmental, or carcinogenic effects of DEHP in humans, animal studies have reported that oral exposure has resulted in developmental, reproductive, or carcinogenic effects in rats and mice (Kluwe et al., 1983). A study by the United State National Toxicology Program showed that DEHP administered orally increased the incidence of liver tumors in rats and mice: DEHP has been classified as a Group B2, probable human carcinogen (Rusyn and Corton, 2012), as its hazard summary shows (US EPA, 1998). Comparisons of metabolic profiles of MEHP, a primary metabolite of DEHP, between rats, marmosets, and/or humans have been recently reported (Rhodes et al., 1986; Kurata et al., 2012a,b) without significant toxicological effects on animals in the range of 100–2000 mg DEHP/kg body weight. In a German study conducted in order to assess infant exposure to DEHP in infants (Volkel et al., 2014), the 95-percentile daily intake values of DEHP calculated from biomonitoring data have been 0.0054 mg/kg for infants and 0.0233 mg/kg for their mothers. There has been important understanding that a wide range of assumed linearity from the non-toxicological levels in animal toxicokinetic experiments to actual human daily exposure doses of chemicals is generally accepted.

A lot of work has been reported on the *in vitro–in vivo* extrapolations of hepatic clearance, volume of distribution, and on the estimation of unbound microsomal fraction (Poulin and Haddad, 2013). In the present study, the pharmacokinetics of DEHP in chimeric mice transplanted with human hepatocytes were investigated. Our observations showed that transplanted human hepatocytes were able to effect the excretion of primary and secondary metabolites of DEHP into urine in chimeric mice. A simplified physiologically based pharmacokinetic (PBPK) model was able to estimate human plasma and urine concentrations of MEHP after ingestion of DEHP and was capable of both forward and reverse dosimetry.

2. Materials and methods

2.1. Chemicals, animals, and enzyme preparations

DEHP, MEHP, and β-glucuronidase (2000 units/mg protein, *Ampullaria* source) were purchased from Wako Pure Chemicals (Osaka, Japan). Uridine diphosphate glucuronic acid (UDPGA) was obtained from Sigma–Aldrich (St. Louis, MO, USA) and pooled human liver microsomes (H150) from Corning (Woburn, MA, USA). Liver microsomes from mice were prepared as described previously (Tsukada et al., 2013). Recently developed TK-NOG mice (Hasegawa et al., 2011; Yamazaki et al., 2012; Higuchi et al., 2014) are treated to express a herpes simplex virus type 1 thymidine kinase within the livers of severely immunodeficient NOG (non-obese diabetic/severe combined immunodeficiency/interleukin-2 receptor gamma chain-deficient) mice. TK-NOG mice were induced by a non-toxic dose of ganciclovir and then human liver cells were transplanted without the need for ongoing drug treatment. Control mice (TK-NOG mice with no transplanted human hepatocytes) and humanized TK-NOG mice (~20–30 g body weight) (Hasegawa et al., 2011) were used in this study. In the chimeric mice, more than 70% of liver cells were estimated to have been replaced with human hepatocytes, as judged by measurements of human albumin concentrations in plasma (Hasegawa et al., 2011; Yamazaki et al., 2012). Hereafter, the terms "mouse" or "mice" refer to control TK-NOG mice. The use of animals for this study was approved by the Ethics Committees of the Central Institute for Experimental Animals and Showa Pharmaceutical University. Other reagents used in this study were obtained from sources described previously or were of the highest quality commercially available (Suemizu et al., 2014).

2.2. In vitro and in vivo metabolic studies of DEHP and MEHP

Elimination rates of MEHP for liver microsomes from control mice, humanized mice, and humans were measured using a liquid chromatography (LC) system. Briefly, a typical incubation mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), an NADPH-generating system, 10 mM $MgCl_2$, 3 mM UDPGA, liver microsomes (0.10 mg protein/mL) with pretreatment with 50 μg/mL alamethicin, and DEHP or MEHP (100 μM) in a final volume of 0.25 mL. Incubations were carried out at 37 °C for 15–30 min. Reactions were terminated by adding 0.5 mL of acetonitrile. Supernatant samples (50 μL)

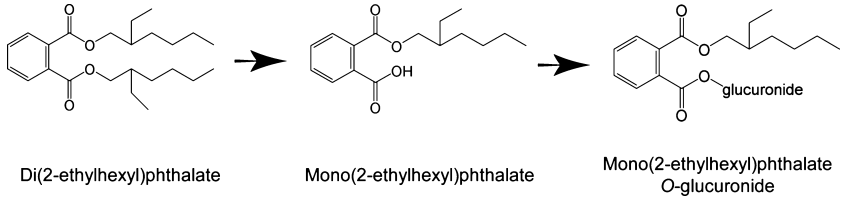

Fig. 1 – Metabolic pathway of di(2-ethylhexyl)phthalate to mono(2-ethylhexyl)phthalate and its glucuronide.