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Interindividual differences in *o,p′*-DDD enantiomer kinetics examined in Göttingen minipigs

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ABSTRACT

Five minipigs were given a single oral dose of a racemic mixture of *o,p′*-DDD (30 mg kg^{-1} b.w., EF = 0.49). Blood plasma and subcutaneous adipose tissue were collected for analysis, at different time-points over 180 d. At the end of the experiment also liver, kidney and brain tissue were collected. Low concentrations of *o,p′*-DDD still remained after 180 d in plasma (mean 0.5 ± 0.3 ng g^{-1} f.w.) and in adipose tissue (mean 40 ± 40 ng g^{-1} f.w.). The mean concentrations in liver and kidney were 500 ± 300 pg g^{-1} f.w. and 90 ± 50 pg g^{-1} f.w., respectively. The enantiomers of *o,p′*-DDD were isolated by HPLC and the absolute configuration of the enantiomers were determined by X-ray crystallography and polarimetry as *R-(+)-o,p′*-DDD and *S-(−)-o,p′*-DDD. The enantiomer fractions (EFs) of *o,p′*-DDD were determined in plasma, adipose tissue and kidney using GC/ECD equipped with a chiral column. The EFs of *o,p′*-DDD in the individual minipigs showed large variability, ranging from 0.2 to 0.6 after 24 h in plasma and from 0.2 to 0.7 after 90 d in adipose tissue. Hence in two of the minipigs, the *S-(−)-o,p′*-DDD enantiomer was dominating while the other enantiomer, *R-(+)-o,p′*-DDD was dominating in three minipigs. We propose that a yet not identified factor related to polymorphism, regulating the metabolism and/or elimination of the enantiomeric *o,p′*-DDD, is responsible for the differences in enantiomeric retention of the compound in the minipigs.

1. Introduction

The persistent chlorinated hydrocarbon DDT has been the most widely used insecticide in the world. Despite being banned in industrialized countries for several decades, DDT and its persistent metabolites are still present in relatively high concentrations in human blood serum, milk, and placental tissue (Zumbado et al., 2005; Carreno et al., 2007; Lopez-Espinosa et al., 2007; Tanabe and Kunisue, 2007). Due to the high persistency and excellent insecticidal properties, DDT is still used for combating malaria in certain parts of the world. The health risks posed by DDT and its biologically active metabolites in humans and wildlife are therefore still an important issue. Taking advantage of the insecticidal properties, DDT has also been used as a pharmaceutical drug to treat certain parasite infestations such as scabies in humans and animals. Technical DDT contains about 79% *p,p′*-DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane), 15% *o,p′*-DDT (1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane) and the rest is other impurities (Buser and Müller, 1995). In the environment, *o,p′*-DDT is degraded to *o,p′*-DDE (1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethene) and *o,p′*-DDD (1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane). Both *o,p′*-DDT and its metabolite *o,p′*-DDD are chiral compounds.

The pharmacokinetics of chiral compounds may be different among enantiomers as discussed in a review by Wong (2006). Pharmacokinetic processes like absorption, distribution, metabolism and excretion can be highly enantioselective because individual enantiomers do interact differentially with drug-metabolizing enzymes, carrier proteins and ATP-dependent transporters. Passive processes such as diffusion of lipid-soluble substances across cell membranes and accumulation in adipose tissue, do not generally involve macromolecular interactions and stereochemistry does not seem important (Rozman and Klaassen, 2001). Not only the pharmacokinetics but also the pharmacodynamics may show differences between enantiomers, each enantiomer having a specific biological effect or each enantiomer having the same effect but with different potency. Degradation of enantiomers may proceed along different routes; one where two enantioselective enzymes exist, each converting only one enantiomer; another, where both enantiomers are converted by one enzyme but at different rates or enantioselective conversion of one enantiomer by one enzyme and isomerization of the other enantiomer by an isomerase (Müller and Kohler, 2004).

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Chiral chemicals used for technical purposes and as pesticides are generally released into the environment as racemic mixtures (Williams, 1996). The relative abundances of enantiomers can however be changed by microbiological degradation or by enantioselective absorption, metabolism and bioaccumulation. The relative content of enantiomers is in general either reported as enantiomeric ratio, ER (Faller et al., 1991) or enantiomeric fraction, EF (Harner et al., 2000). Chirality of persistent organic pollutants in biota has become of increasing interest (Kallenborn and Hühnerfuss, 2001) but there are still few studies evaluating the enantiomeric composition of *o,p′*-DDD.

Shen and co-workers (2006) found that the enantiomers of *o,p′*-DDD in human placenta deviated significantly from 1:1 ratio at low concentrations, suggesting a more rapid elimination of the first eluting enantiomer than of the second. Another study recently reported EFs of *o,p′*-DDD in the atmosphere suggesting a preferential depletion of one enantiomer over the other (Venier and Hites, 2007). Konwick and co-workers observed no significant difference in EF of *o,p′*-DDD in fish fed with a racemic mixture of chiral organochlorines including *o,p′*-DDD, indicating non-enantioselective biotransformation (Konwick et al., 2006). However, the concentrations of *o,p′*-DDD in biota are in general too low to allow enantiospecific analysis, hence hampering conclusions to be made on this matter.

o,p′-DDD given as a racemic mixture seems to be a metabolism-activated toxicant in the adrenal cortex in several species including human (Lindhe et al., 2002), dog (Nelson and Woodard, 1949), mink (Jönsson et al., 1993) and bird (Jönsson et al., 1994). To our knowledge very little is known about the biological activity of each enantiomer. It has been shown that the (−)-*o,p′*-DDT enantiomer is an active estrogenic compound whereas the hER activity of the (+)*o,p′*-DDT is negligible (Hoekstra et al., 2001). Given its toxicity to the adrenal cortex, *o,p′*-DDD (Mitotane) has for 40 years been used for treatment of adrenocortical carcinoma (ACC) (Bergenstal et al., 1960) and Cushing's syndrome (Benecke et al., 1991). The efficacy and potency is however low, and *o,p′*-DDD treatment is frequently associated with severe side effects (Allolio and Fassnacht, 2006).

The objective of the present study was to identify the enantiomer pattern of *o,p′*-DDD in plasma and tissues following oral administration of the racemic mixture to Göttingen minipigs. We also aimed to isolate the *o,p′*-DDD enantiomers and confirm their absolute configuration and chiroptical rotation. The EF of *o,p′*-DDD in Lysodren tablets (HRA Pharma, Paris; Bristol Meyer Squibb, New York), the registered preparation for ACC in the EU and USA, was also determined.

2. Materials and methods

2.1. Test chemical and material

o,p′-DDD, purity 99%, was obtained from Aldrich Chemical Company Inc. (Milwaukee, USA). All solvents used were of analytical grade. Silica gel (0.063–0.200 mm) was from Merck KgaA (Darmstadt, Germany) and was activated by heating overnight at 280 °C before use. Empty polypropylene reservoirs (1.5 mL), from Extra-Clean (Alltech, Deerfield IL, US) with male Luer outlets and with loose polyethylene frits (20 μm) at the bottom were used for the silica gel columns. A 12-port vacuum manifold (Alltech) was used to process multiple columns simultaneously. All glassware was heated at 300 °C overnight prior to use.

2.2. Animals and treatment

Five female minipigs, 6–7 months old (15–20 kg), were obtained from Ellegaard (Dalmose, Denmark). The pigs were fed with a standard low calorie diet (Maintenance diet for minipigs) from Special Diet Services (Witham, England) and given free access to water. The pigs were housed in a pen with concrete floor, wooden or metal walls and the pen was bedded with straw. For acclimatisation the animals were kept under these conditions for three weeks before treatment. The pigs were given a single oral dose (30 mg *o,p′*-DDD kg^{-1} body weight dissolved in corn oil) by gastric intubations. This dose corresponds to approximately half a daily dose *o,p′*-DDD given to adrenocortical carcinoma patients. All described procedures were approved by the Local Ethics Committee for Research on Animals (C101/4).

2.3. Samples

Blood samples were drawn into heparin, from *vena jugularis* before administration, at 0.5, 1, 3, 8, 24, 48 h and 4, 10, 30, 60, 90, 120 d after administration. At day 180 blood samples were drawn from the heart after intraperitoneal pentobarbital anaesthesia and the pigs were then killed by an intracardial injection of pentobarbital. The blood samples were centrifuged and the collected plasma was stored in −20 °C until analysis. Thirty, 60, 90 and 120 d after administration the pigs were weighed and subcutaneous fat samples from the chin were collected with a biopsy punch after a local anaesthesia. The fat samples and tissues (liver, kidney and brain samples) taken at day 180 were frozen and stored at −70 °C until analysis. The minipigs showed no sign of being unwell during the experiment.

2.4. Sample preparation

The extraction and the clean up of the blood samples were carried out as described elsewhere (Hovander et al., 2000), but due to the small amount of plasma the method had to be slightly modified. Plasma (0.2 g) was transferred to a screw cap tube and the internal standard was added. The samples were denatured with 6 M hydrochloric acid (0.5 mL) and 2-propanol (3 mL). The denatured plasma was extracted twice by adding hexane:methyl-*tert*-butyl ether (1:1, 3 mL) and inverting the extraction tubes for 5 min. The organic phase was partitioned into a 1% potassium chloride solution by gentle mixing. After centrifugation the organic phase was transferred to a pre-weighed test tube and the solvent was evaporated. The lipid content was determined gravimetrically and was estimated to be approximately 0.2%. To separate the phenolic compounds from the neutrals the extract was dissolved in hexane and was partitioned with 0.5 M potassium hydroxide in 50% ethanol solution (2 mL). The lipids were removed by a multilayer column containing activated silica gel (0.1 g), 0.1 M potassium hydroxide:silica gel (1:2, 0.4 g) and 90% (w/w) sulfuric acid:silica gel (1:2, 0.8 g) as described elsewhere (Hovander et al., 2006). The analytes were eluted with hexane:dichloromethane (1:1) to obtain the *o,p′*-DDD fraction.

The method used for extraction of the fat samples has been described elsewhere (Jensen et al., 1983) but due to the small sample amount (100–200 mg) the method was scaled down. The samples were mixed with hexane:acetone (2:5, 4 mL) and extracted twice with hexane:methyl-*tert*-butyl ether (9:1, 4 mL). The lipid amount was determined gravimetrically and was calculated to about 60%. The samples were dissolved with hexane and spiked with CB-189 before lipid removal. The phenolic compounds were separated as described above. A first lipid reduction from the neutral fraction was performed by sulfuric acid treatment. Further lipid removal was performed with a column of silica gel:sulfuric acid (2:1, w/w, 1 g) and the analytes were eluted with hexane.

Liver, kidney and brain samples were homogenized according to the method described by Jensen et al. (2003). About 5 g sample was weighed into a 50 mL centrifuge tube and homogenized with