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ORIGINAL ARTICLE

Pharmacokinetics of the adrenocorticolytic compounds 3-methylsulphonyl-DDE and *o,p′*-DDD (mitotane) in Minipigs

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Abstract The pharmacokinetics of the adrenocorticolytic drug candidate 3-Methylsulphonyl-DDE (3-$MeSO_2$-DDE) and the anticancer drug *o,p′*-DDD (mitotane) were studied in Göttingen minipigs. The animals were given 3-$MeSO_2$-DDE or *o,p′*-DDD as single oral doses (30 mg/kg). Concentrations in plasma and subcutaneous fat were measured by gas chromatography at different time points during 180 days. Maximal plasma concentrations appeared within 24 h for both compounds, but were about 2 times higher for 3-$MeSO_2$-DDE. *o,p′*-DDD plasma concentrations declined rapidly to low levels during 4 days. 3-$MeSO_2$-DDE also decreased rapidly, but remained at high concentrations throughout the study. In fat, 3-$MeSO_2$-DDE reached about 25-fold higher levels than *o,p′*-DDD at 30 days, and both substances were eliminated slowly from this tissue. 3-$MeSO_2$-DDE liver concentrations were about 18-fold higher than those in plasma at 180 days. In contrast, *o,p′*-DDD liver and plasma levels were about equal at 180 days. *o,p′*-DDD had roughly 45 times larger CL/F than 3-$MeSO_2$-DDE, confirming that the elimination of this compound was more rapid. Both compounds were characterised by their localisation and retention in fat tissue, and the individual size of the fat stores clearly determined the plasma concentrations. It is concluded that although 3-$MeSO_2$-DDE is an interesting candidate for therapeutic use due to its potential characteristics to specifically target adrenocortical tumour cells the slow elimination of the compound might make it challenging to design appropriate dosage regimes.

Keywords Pharmacokinetics · *o,p′*-DDD · Mitotane · 3-$MeSO_2$-DDE · Adrenal cortex · Minipig

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Introduction

The DDT-metabolite 3-$MeSO_2$-DDE (2-(4-chloro-3-methylsulphonylphenyl)-2-(4-chlorophenyl)-1,1-dichloroethene) was originally discovered in blubber of Baltic grey seal in 1976 [19]. 3-$MeSO_2$-DDE has been identified as a highly potent and tissue-specific adrenal toxicant that induces a targeted cell death in the glucocorticoid-forming zona fasciculata in mice [30]. The high adrenal toxicity is mediated by cytochrome P45011B1 (CYP11B1), a mitochondrial CYP form, catalysing formation of a reactive 3-$MeSO_2$-DDE metabolite that binds covalently in cells expressing CYP11B1 in mice [23, 30, 31]. The endogenous function of CYP11B1 is to catalyse the formation of adrenal glucocorticoids, and a disrupted corticosterone formation is observed both in 3-$MeSO_2$-DDE -exposed adult and foetal mice [24]. As shown in a subsequent study on human adrenocortical mitochondrial fractions, 3-$MeSO_2$-DDE appears

to be activated and covalently bound also to the human adrenal cortex in vitro [25]. The previous results were verified when 3-MeSO$_2$-DDE was found to bind covalently in zona fasciculata and reticularis, using a human adrenal tissue-slice culture procedure [29]. This was due to that the human adrenal cortex expresses CYP11B1 both in zona fasciculata and reticularis. 3-MeSO$_2$-DDE also inhibits the catalytic CYP11B1 activity in the human adrenocortical H295R cell line [21]. Based on these results, 3-MeSO$_2$-DDE has been proposed as a lead compound for an improved pharmacotherapy for adrenocortical hypersecretion and tumour growth [29]. Due to the high and specific accumulation in the adrenal cortex in vivo, 3-MeSO$_2$-DDE labelled with ^{11}C or ^{17}F is also a proposed imaging agent for diagnosis of adrenocortical disorders by means of positron emission tomography (PET) [5].

In 1960, the first successful treatment with *o,p′*-DDD (mitotane) of a patient with adrenocortical carcinoma (ACC) was reported [2]. *o,p′*-DDD is currently the main drug of choice in treatment of ACC as well as overproduction of glucocorticoids due to a pituitary tumour, Cushing's disease. The drug-induced cell death in the adrenal cortex occurs after a CYP-catalysed hydroxylation and a subsequent spontaneous dehydrochlorination at the side-chain β-carbon of *o,p′*-DDD. The resulting reactive acyl chloride, binds covalently to primarily mitochondrial proteins [7, 32]. Steroidogenesis also seems to be directly affected since *o,p′*-DDD has been reported to inhibit steroidogenic enzymes, e.g. CYP11A1 (cholesterol side-chain cleaving enzyme) [12, 13]. Reports and evaluations of the medical treatment with *o,p′*-DDD show that there are several severe side effects, such as gastrointestinal irritation and CNS toxicity (80 and 40% of patients, respectively) [9], due to the narrow therapeutic window of the drug. Also, the therapeutic efficacy on ACC cells seems to be inadequate in a majority of patients. A retrospective study of published reports on ACC patients treated between 1972 and 1992 concluded that only 35% of tumours responded to treatment with *o,p′*-DDD [40]. Since ACC is a rare disease, with an incidence of 1–2 per million/year [10, 11], little research has been focused on developing new therapeutic alternatives. There is a strong need for a more specific and effective therapeutic agent with few side effects.

In order to contribute information for an improved chemotherapy of ACC, the adrenocorticolytic characteristics of 3-MeSO$_2$-DDE and *o,p′*-DDD are currently being examined in vivo and in vitro. As part of this work, the present study was carried out, primarily to investigate the long-term pharmacokinetics of the lead compound 3-MeSO$_2$-DDE following a single oral dose in minipigs. We also compared 3-MeSO$_2$-DDE with the current therapeutic alternative, *o,p′*-DDD. The minipig, often used in pharmacological and toxicological studies, was chosen as an animal model, because of its body composition, and since it is easy for handling.

The pharmacokinetic information for 3-MeSO$_2$-DDE and *o,p′*-DDD is deficient. The distribution of 3-MeSO$_2$-DDE after an i.v. injection has been studied in mice using autoradiography [30]. Trace levels of 3-MeSO$_2$-DDE have been reported to be present in blood serum and adipose tissue in human subjects as well as in wild seals and polar bears [8, 22, 28, 38, 39]. The presence of 3-MeSO$_2$-DDE in these species could be due both to direct intake via food and metabolic formation in the body from *p,p′*-DDE, also a highly persistent DDT-metabolite ubiquitously present in human and wildlife adipose tissue [19, 38]. According to the European Public Assessment Report (EPAR) from European Medicines Agency (EMEA) [9], most information about human pharmacokinetics of *o,p′*-DDD comes from a single study published in 1961, based on 18 cancer patients [34]. Since *o,p′*-DDD is used pharmacologically in dogs as well, the oral absorption from various vehicles has been determined in some dog breeds [37]. *o,p′*-DDD is biotransformed to hydroxylated metabolites and a carboxylic acid, *o,p′*-DDA, but also the lipophilic *o,p′*-DDE and a methylthio-containing metabolite have been reported [1, 16].

The results reveal that 3-MeSO$_2$-DDE is persistent in minipigs and could be traced during the whole study period in high levels both in plasma and fat. In contrast to *o,p′*-DDD, there seemed to be a lack of rapid elimination pathways for 3-MeSO$_2$-DDE, and this caused large differences in the kinetic behaviour of the two substances.

Materials and methods

Chemicals

3-MeSO$_2$-DDE was synthesised by Synthelec AB, Lund, Sweden, using procedures developed by Bergman and Wachtmeister [3]. *o*,p′-DDD (puriss, 99%) was obtained from Aldrich Chemical Company Inc., Milwaukee, USA.

Animals

The Göttingen minipig is a crossbreed between the Minnesota minipig, the Vietnamese potbelly swine and the German Landrace [4]. Ten females Göttingen minipigs, of specific pathogen-free quality (as specified by the supplier), were obtained from Ellegaard, Dalmose, Denmark. Upon arrival, the animals were 6–7 months old and weighed 15.4–19.8 kg (mean 16.7 kg). They were randomly divided into two groups, which were housed in separate pens. The pigs were fed with a standard low calorie diet (Maintenance diet for minipigs, Special Diet Services, Witham, England)

and given free access to water. The pens had concrete floor, wooden or metal walls and were bedded with straw. For acclimatisation, the animals were kept under these conditions for 3 weeks before treatment. They showed no signs of infection during this adaptive period to a microbiologically conventional environment. The pigs were fasted the same morning as the administration was implemented. A single dose of 3-$MeSO_2$-DDE or *o,p′*-DDD (30 mg/kg body weight) dissolved in corn oil was given by gastric intubation. This dose corresponds to approximately half a daily dose of *o,p′*-DDD given to ACC patients and was expected to give reasonable levels for detection. The doses were given as pure substance in corn oil, since earlier studies demonstrate that the bioavailability of *o,p′*-DDD is improved when given in oil emulsion compared to tablets [33, 37]. All described procedures were approved by the Local Ethics Committee for Research on Animals (C101/4).

Samples

Plasma

Blood samples were drawn from vena jugularis before administration and 0.5, 1, 3, 8, 24, 48 h, 4, 10, 30, 60, 90, 120 days after administration. In addition, blood samples were drawn from the heart after anaesthesia with an intraperitoneal injection of pentobarbital (2 g, 100 mg/ml, Apoteket AB, Sweden) at study termination by day 180. The blood samples were collected in 5-ml EDTA-tubes and centrifuged for 10 min at 3,000 rpm (Eppendorf centrifuge). The plasma was transferred to Eppendorf tubes and stored in −20°C until analysis.

Adipose tissue

At 30, 60, 90, 120 and 180 days after administration, the pigs were weighed and subcutaneous fat samples from the chin were collected using a biopsy punch, after local anaesthesia with mepivacain (Carbocain® 2%, AstraZeneca AB). The fat samples were frozen and stored at −70°C.

Other tissues

At 180 days, after blood sampling, the pigs were killed by an intracardial injection of pentobarbital. Samples from the adrenals, liver and abdominal fat, were collected, immediately frozen and stored in −70°C. Small pieces of adrenals and liver were fixed in 4% phosphate-buffered formaldehyde (pH 7.4) for histological evaluation. For eight of the pigs, there were no signs of disease during the investigative period. However, two pigs (one pig from each treatment group) became ill with an unidentified disease and were killed 1 and 4 days before the end of the experiment. In spite of the circumstances, plasma, subcutaneous and abdominal fat, adrenal and liver were collected from these pigs. The condition of these pigs was considered not to affect the outcome of the experiment since their plasma values and histopathology of organs did not differ from the other individuals.

Sample analysis

Instrument

Gas chromatography with electron capture detection (GC-ECD) was performed on a Varian 3400 GC equipped with a Varian 8100 auto sampler and a split/splitless injector operated in splitless mode. A non-polar column containing CP-SIL 8CB (25 m × 0.15 mm × 0.12 μm); Chrompack, (EA Middelburg, The Netherlands) was used with hydrogen as carrier gas and nitrogen as make-up gas. The GC temperature program was 80°C (1 min) and 20°C/min to 300°C, which was maintained for 10 min. The injector temperature was 260°C and the detector temperature was 360°C. The chromatographic data were recorded and processed by Elds Pro (Chromatography Data Systems AB, Stockholm, Sweden).

Material

All the solvents used were of pro analysis quality. Silica gel (0.063–0.200 mm) purchased from Merck KgaA (Darmstadt, Germany), was activated by heating overnight at 280°C. For the silica gel columns empty polypropylene reservoirs 1.5 ml, Extract-Clean, (Alltech, Deerfield IL 60015–1899, US) with male luer outlets were used with loose frits (20 μm) at the bottom. A 12-port vacuum manifold (Alltech) was used to process multiple columns simultaneously. All glassware was heated at 300°C overnight prior to use.

Plasma

The extraction of the blood samples was carried out as described elsewhere [14]. Due to the small sample amount the solvent volumes had to be reduced to half. Plasma (5 g for 180 days samples and 0.2 g for all other samples) was transferred to a screw-capped test tube and the internal standards (3-$MeSO_2$-CB141 and CB189) were added. The samples were denatured with hydrochloric acid (6 M, 0.5 ml) and 2-propanol (3 ml). The denatured plasma was extracted twice with hexane:methyl-tert-butyl ether (1:1, 3 ml) and the organic phase was partitioned into a 1% potassium chloride-solution by gentle mixing. After centrifugation the organic phase was transferred to a test tube and

the solvent evaporated. To separate phenolic compounds from the neutrals the extract was dissolved in hexane (4 ml) and partitioned with a potassium hydroxide-solution (0.5 M in 50% ethanol, 2 ml). The organic phase was transferred to another test tube and represented the neutral fraction. The solution was re-extracted with hexane (2 ml). The alkaline solution was then acidified with hydrochloric acid (2 M, 1 ml) and the phenolic compounds were extracted with hexane:metyl-tert-butyl ether (9:1, 3 ml). The remaining lipids were removed by an impregnated multilayer silica gel column. The column consisted of two 1.5 ml polypropylene tubes connected with an adapter with a loose frit on the bottom. The column was packed from bottom to top with 0.1 g activated silica gel, 0.4 g of 0.1 M potassium hydroxide/silica gel (1:2, w/w) and 0.8 g of 90% sulphuric acid/silica gel (1:2, w/w). The column was placed in a vacuum manifold. 3-$MeSO_2$-DDE was eluted with dichloromethane (18 ml) and *o,p′*-DDD with hexane:dichloromethane (1:1, 8 ml).

Adipose tissue

The method used for extraction of adipose tissue has been described elsewhere [20]. Due to the small sample amount (1 g) 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, 2 ml). The solvent was evaporated and the samples were dissolved with hexane and spiked with 3-$MeSO_2$-CB141 and CB189. Separation of neutral and phenolic compounds was performed as described above. A first lipid reduction was performed by anhydrous dimethyl sulphoxide partitioning for the 3-$MeSO_2$-DDE samples while the *o,p′*-DDD samples were treated with concentrated sulphuric acid.

Both methods have been described elsewhere [14, 15]. Further lipid removal was performed with a multilayer column as for the plasma samples described earlier.

Liver

The liver samples (1 g) were homogenised according to a method previously reported [18], but the solvent volumes were reduced to 10% of the original method and the extraction was performed in test tubes. The samples were homogenised in 2-propanol:diethyl ether (5:2, 5 ml) and extracted with 2-propanol (2 ml) and hexane:diethyl ether (9:1, 3 ml). The lipid reduction was performed as mentioned above. After adjustment of sample volume all samples were analysed and quantified by GC-ECD.

Data analysis

Pharmacokinetic parameters were determined by non-compartmental analysis performed for each individual, using the software program WinNonlin version 5.0.1. (Pharsight Corporation, CA, USA). The area under curve (AUC) was determined using the trapezoidal rule. The slope of the terminal phase, lambda z (λ_z), was calculated by regression analysis using data points from 10 to 180 days with the following exclusions: 1. For all individuals given 3-$MeSO_2$-DDE, the 60-days plasma concentration values were excluded from the calculation of λ_z, due to a temporary weight loss of the pigs, caused by too restricted feeding.

2. The 180-days' plasma concentration values for all individuals given *o,p′*-DDD were excluded from the λ_z calculations. The *o,p′*-DDD concentrations in these samples were close to the detection limit and the measured values were possibly influenced by the higher sample volume extracted at this time-point.

Calculations of the terminal half-lives ($t½$) were based on λ_z. Since the bioavailabilities (F) of the two substances were not known, the volume of distribution of the terminal phase (V_z) and clearance (CL) are presented V_z/F and CL/F, respectively.

Histology

To exclude any persistent overt toxicity in the livers and adrenals that could disturb the overall kinetics, pieces from these organs, were fixed in phosphate-buffered formaldehyde (pH 7.4), embedded in methacrylate, sectioned (2 µm) and stained with hematoxylin/eosin for light microscopic examination.

Results

No detectable amounts of 3-$MeSO_2$-DDE and *o,p′*-DDD were present in the plasma samples collected before administration. No pig showed any signs of reaction that could be assumed to be treatment-related following administration of either test compound. The histology of the adrenals and livers were normal in all pigs, with no difference between the groups. These observations indicate that any effect on morphology that possibly might have arisen from the treatment was not permanent.

Plasma

The plasma concentration-time profiles for 3-$MeSO_2$-DDE and *o,p′*-DDD during the first 10 days after administration are given in Fig. 1a. Maximal plasma concentration was about two times higher for 3-$MeSO_2$-DDE and T_{max} was calculated to 8.0–24.0 h compared to 8.0 h for all pigs exposed to *o,p′*-DDD. In both groups, the maximal plasma concentrations showed individual differences, indicated by large standard deviations during the first 24 h. Plasma

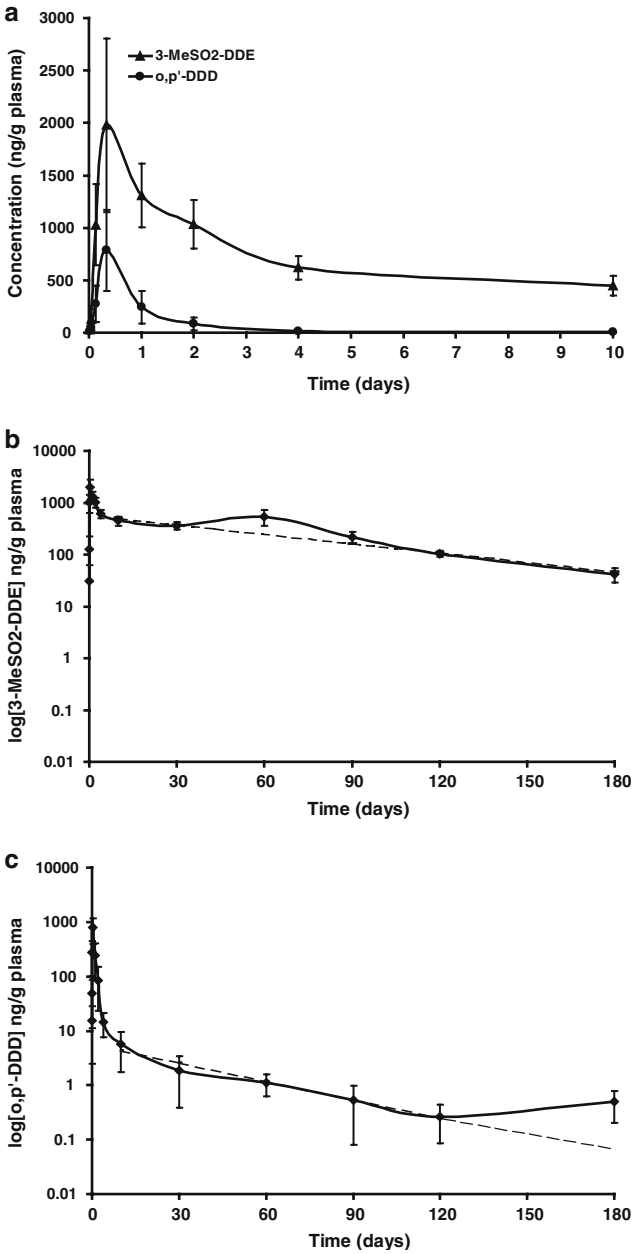

Fig. 1 **a** Plasma concentrations of 3-$MeSO_2$-DDE and *o,p′*-DDD in minipigs plotted against time (days) the first 10 days after administration. Each data point represents the mean value with standard deviation bars. Log plasma concentrations of 3-$MeSO_2$-DDE (**b**) and *o,p′*-DDD (**c**) in minipigs plotted against time (days). Each data point represents the mean value with *standard deviation bars*. Note the logarithmic *y*-axis. In both **b** and **c** the lambda z (λ_z) slope is marked with a *broken line*

concentrations for the two substances during the whole experimental period are given in Fig. 1b and c.

Both *o,p′*-DDD and 3-$MeSO_2$-DDE were rapidly distributed. Plasma concentrations then rapidly declined for *o,p′*-DDD, presumably due to both elimination and distribution, and only low concentrations remained after 4 days. 3-$MeSO_2$-DDE also decreased rapidly after the first peak. Unexpectedly, a second concentration peak was reached at 60 days after administration. Most likely, this second peak was due to a temporary weight loss in four out of five pigs in this group at this time point, with a subsequent redistribution from fat tissue into the blood. The same phenomenon was observed in one of the pigs given *o,p′*-DDD, which was the only pig losing weight in this group.

The kinetic parameters calculated for the two substances in plasma are summarised in Table 1. The area under the plasma concentration–time curve (AUC_{last}) for 3-$MeSO_2$-DDE confirms that large amounts of this substance, compared to *o,p′*-DDD, remained in the body long-term. For example, at 3 months after administration of 3-$MeSO_2$-DDE, the remaining plasma concentration was still about 17% of the observed maximal plasma concentration, compared to about 0.2% for *o,p′*-DDD. Even if the true values of volume of distribution could not be estimated, some information could be gained from V_z/F, as there was a distinct difference between V_z/F for the two compounds. Especially for *o,p′*-DDD, V_z/F was very large (median 20.3×10^3 L), showing that this substance preferably resides in other compartments than plasma.

The elimination from plasma was more rapid for *o,p′*-DDD than for 3-$MeSO_2$-DDE, indicated by the much larger CL/F for *o,p′*-DDD. This was also reflected by long terminal *t½*, which was ∼50 and ∼28 days for 3-$MeSO_2$-DDE and *o,p′*-DDD, respectively. Since the value of *t½* in this case does not consider the whole time course, we also calculated the mean residence time (MRT), which might be a more suitable parameter in this case. The MRT was estimated to ∼56 days for 3-$MeSO_2$-DDE and ∼12 days for *o,p′*-DDD.

Subcutaneous fat

The concentrations in subcutaneous fat samples, expressed on a fat weight basis, are shown in Fig. 2a–b. 3-$MeSO_2$-DDE reached about 25-fold higher fat levels than *o,p′*-DDD at 30 days. For both substances the *t½* in subcutaneous fat was approximately equivalent to that in plasma, that is, about 52 and 22 days for 3-$MeSO_2$-DDE and *o,p′*-DDD, respectively. Table 2 shows selected parameters describing the kinetics in subcutaneous fat.

The long half-lives in fat are also reflected in the AUC:s, which were very large, particularly for 3-$MeSO_2$-DDE. Despite that both substances are strongly lipophilic, the partition between fat and plasma was different. At day 30, the ratio subcutaneous fat/plasma was about 230 for 3-$MeSO_2$-DDE, compared to about 3,400 for *o,p′*-DDD. For both substances, the levels in the abdominal fat samples collected at 180 days were about equal to the levels in the 180 days subcutaneous fat samples.

Table 1 Kinetic parameters calculated for 3-$MeSO_2$-DDE and *o,p′*-DDD in plasma after a single oral dose to minipigs. Data are given as median values (range)

Parameter	3-$MeSO_2$-DDE	*o,p′*-DDD
C_{max} (ng/g plasma)	1690 (1581–2922)	834 (199–1212)
T_{max} (h)	8.0 (8.0–24.0)	8.0
AUC_{last} 180 days (h × ng/ml)	1.2×10^6 (9.3×10^5–1.4×10^6)	2.7×10^4 (8.2×10^3–3.4×10^4)
t½ (days)	49.9 (37.3–61.3)	28.1 (20.9–37.6)
MRT (days)	56.1 (50.4–56.6)	12.1 (5.1–18.3)
V_z/F (L)	0.8×10^3 (0.5×10^3–1.2×10^3)	20.3×10^3 (17.2×10^3–87.3×10^3)
CL/*F* (L/h)	0.5 (0.4–0.6)	22.4 (17.6–67.1)

C_{max} Maximum plasma concentration, T_{max} time at Cmax, AUC_{last} area under plasma concentration versus time curve, *t½* terminal plasma half-life, *MRT* mean residence time, V_z/F volume of distribution and CL/*F* clearance (*F* bioavailability)

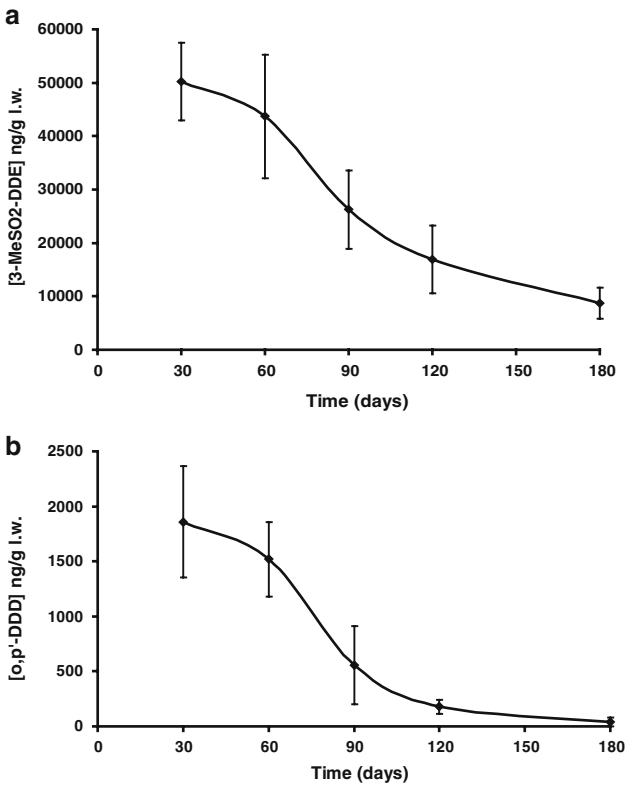

Fig. 2 The fat concentrations of 3-$MeSO_2$-DDE (**a**) and *o,p′*-DDD (**b**) in minipigs plotted against time (days). Each data point represents the mean value, with *standard deviation bars*. Note different scales on the *y*-axis. *lw* lipid weight

Liver

Compared to *o,p′*-DDD, high levels of 3-$MeSO_2$-DDE remained in the liver at 180 days after administration. The median concentration of 3-$MeSO_2$-DDE was 986.5 (234.4–1329.8) ng/g fresh weight (fw) and only 0.5 (0.2–0.9) ng/g fw for *o,p′*-DDD. At 180 days, the concentrations of 3-$MeSO_2$-DDE were 11.4–29.4 times higher in the liver than in plasma. *o,p′*-DDD, on the other hand, was found in about the same levels in both liver and plasma (ratio liver plasma 0.3–2.5).

Discussion

Both 3-$MeSO_2$-DDE and *o,p′*-DDD are intended for treatment of ACC, a condition frequently associated with increased cortisol secretion, weight gain and characteristic obesity. This could certainly have consequences to the distribution of highly fat-soluble compounds. The present study was therefore carried out to characterise and compare the pharmacokinetic behaviour of 3-$MeSO_2$-DDE and *o,p′*-DDD in a species with large body fat stores, the Göttingen minipig. Earlier data from humans show that both substances are accumulated in body fat [2, 8, 38].

The plasma concentration of 3-$MeSO_2$-DDE peaked within 24 h, reaching a maximum level of about 1.7 μg/g plasma, which was about two times higher than for *o,p′*-DDD. $AUC_{Day1–4}$ for 3-$MeSO_2$-DDE was almost six times larger than for *o,p′*-DDD (calculations not shown). Shortly after reaching peak plasma levels, concentration of *o,p′*-DDD rapidly declined, most likely due to elimination and distribution, particularly to adipose tissue. Human subjects, given a single oral dose (2 g) of *o,p′*-DDD in oil emulsion, reached a C_{max} of 3–4 μg/ml ($n = 6$) within the first 5 h after administration [33]. When correcting for the lower dose given to the pigs, these human data are similar to those of the present study, where *o,p′*-DDD reached a median maximal concentration of 0.83 μg/g plasma (T_{max} = 8.0 h). After the first steep slope, only low levels remained in plasma, but more was found in fat tissue. The very high fat/plasma concentration ratio for *o,p′*-DDD at 30 days, exceeding the ratio for 3-$MeSO_2$-DDE about 15-fold, reflects the high lipophilicity of this substance. Also 3-$MeSO_2$-DDE declined rapidly after the first peak but plasma concentrations remained considerably higher. Both test compounds had large V_z/F values, but especially *o,p′*-DDD showed surprisingly large values for this parameter supporting the indication of higher lipophilicity and thus accumulation of non-hydrophilic compartments for this compound compared to 3-$MeSO_2$-DDE. However, affinity to plasma proteins such as albumin and steroid carrier proteins are important for the

Table 2 Kinetic parameters for 3-$MeSO_2$-DDE and *o,p′*-DDD in subcutaneous fat of minipigs. Data are given as median values (range)

Parameter	3-$MeSO_2$-DDE	*o,p′*-DDD
AUC_{last} 180 days (h × ng/g lw)	1.8×10^8 (1.7×10^8–2.2×10^8)	4.9×10^6 (4.3×10^6–7.5×10^6)
t½ (days)	51.7 (48.9–75.6)	22.3 (18.3–29.2)

AUC Area under fat concentration versus time curve, *t½* terminal fat half-life, *lw* lipid weight

magnitude of the V_z and the degree of plasma protein binding is not known for any of the compounds.

3-$MeSO_2$-DDE has been reported to be present at five times higher concentrations in liver than in fat in humans and seals, calculated on a lipid weight basis [8, 27, 38]. As shown in the present study, 3-$MeSO_2$-DDE was retained in high levels in the liver of minipigs, exceeding the plasma levels about 18 times (median ratio 18.1). This high retention could presumably be due to protein binding, because aryl methyl sulphones, such as certain $MeSO_2$-PCBs are known to associate with ligand-binding proteins in different organs of experimental animals [6, 26]. So far, such specific protein binding of $MeSO_2$-PCBs has not been correlated with adverse toxic effects. In contrast to 3-$MeSO_2$-DDE, *o,p′*-DDD was not retained in liver .

In general, the terminal *t½* is influenced by the sensitivity of the analysis method and the duration of the study, and it might be considerably longer than the elimination *t½*. Therefore, MRT may be a more appropriate parameter to discuss as it is calculated from the complete plasma versus time profile. For *o,p′*-DDD, the calculated MRT was about 12 days compared to the terminal *t½* which was about 28 days. The concentrations of 3-$MeSO_2$-DDE were notably far above the detection limit, and in contrast to *o,p′*-DDD the terminal *t½* was similar to MRT. Thus, the elimination *t½*, although not calculated from the present data, is likely to differ more than what is implied by the *t½* calculations presented.

During the course of the study, the body weight of the pigs increased from 16.7 ± 1.3 kg to 24.0 ± 2.9 kg. The influence of the weight gain on the plasma values could not be corrected for, since the exact body composition of each individual (the relation between muscle and fat mass) was unknown. The dose regimen for *o,p′*-DDD treatment is generally not standardised, and the plasma levels need to be monitored to avoid side effects caused by overdosing. Since *o,p′*-DDD retains in fat, fat tissue will act as a drug reservoir from which the compound could be released in high concentrations in case of rapid loss of fat tissue. As the therapeutic window of this drug is likely to be narrow this could result in toxicity in an individual that has reached the therapeutic plasma concentration before the weight loss. The same effect should be expected also for 3-$MeSO_2$-DDE and other structurally related drugs. This scenario was unintentionally illustrated when four out of five pigs treated with 3-$MeSO_2$-DDE temporarily lost weight around 60 days after administration. This weight loss resulted in markedly increased 3-$MeSO_2$-DDE plasma concentrations at this time point (∼1.5 times higher compared to the concentration 30 days earlier). A similar redistribution to plasma was observed in one *o,p′*-DDD-exposed pig that also lost weight.

Metabolic pathways for *o,p′*-DDD are present in humans, as well as in other animal species like rats, rabbits and guinea pigs [17, 35, 36]. The higher CL/*F* for *o,p′*-DDD confirms that these pathways were present also in minipigs. In contrast, CL/*F* for 3-$MeSO_2$-DDE was more than 60 times lower, implying a very slow metabolic degradation of this substance in minipigs. In mice, no significant excretion of 3-$MeSO_2$-DDE in bile has been observed, although there seemed to be a discharge of this substance directly into the large intestinal contents [30].

In conclusion, the present study shows that 3-$MeSO_2$-DDE and *o,p′*-DDD have different pharmacokinetic profiles when administered as single oral doses to minipigs. Both compounds were accumulated in fat tissues, and resided in the body for an extended time, but 3-$MeSO_2$-DDE was eliminated slower than *o,p′*-DDD although the fat/plasma ratio was considerably lower. Although 3-$MeSO_2$-DDE is an interesting candidate for therapeutic use, due to its potential characteristics to specifically target adrenocortical tumour cells, the slow elimination of the compound might make it challenging to design appropriate dosage regimes.

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