LONG-TERM KINETICS OF 3-MESO$_2$-DDE FOLLOWING A SINGLE ORAL DOSE IN MINIPIGS

Veronica Hermansson[1], Tatiana Cantillana[2], Karolina Törneke[3], Karl Ljungvall[4], Ulf Magnusson[4], Åke Bergman[2] and Ingvar Brandt[1]

[1]Department of Environmental Toxicology, Uppsala University, Norbyvägen 18A, SE-752 36 Uppsala, Sweden
[2] Department of Environmental Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden
[3]Swedish Medical Products Agency, Box 26, SE-751 03 Uppsala, Sweden
[4]Department of Clinical Sciences, Swedish University of Agricultural Sciences, Centre for Reproductive Biology in Uppsala, Box 7054, SE-750 07 Uppsala, Sweden

Introduction

3-Methylsulphonyl-2,2-bis(4-chlorophenyl)-1,1-dichloroethene (MeSO$_2$-DDE) is a persistent environmental pollutant formed during the metabolism of the insecticide *p,p'*-DDT (1,1,1,-trichloro-2,2-bis(4-chlorophenyl)ethane). Originally, MeSO$_2$-DDE was identified in adipose tissue from Baltic grey seal[1], and can now be found in human tissues[2] and breast milk[3], fat tissue of fish-eating species like polar bears[4], minks[5] and cormorants[6]. MeSO$_2$-DDE is activated by CYP11B1[7] to a reactive intermediate, which binds irreversibly in the adrenal zona fasciculata in mice. Adrenocortical cell death and disruption of corticosterone formation are subsequently developed[8]. Subsequent results indicate that MeSO$_2$-DDE may be toxic to the human adrenal cortex as well[9]. *o,p'*-DDD (mitotane) has, since the 1960's, been used to treat patients suffering from adrenocortical carcinoma (ACC) and Cushing's syndrome[10]. Treatment with *o,p'*-DDD has been reported to be insufficient in many cases, and is often associated with severe side effects[11]. There is consequently a need for therapeutic alternatives. Based on the zone-specific metabolic activation and toxicity in glucocorticoidproducing adrenal tissue, MeSO$_2$-DDE is being examined as a lead compound for an improved pharmacotherapy of ACC. As part of these studies, we are presently comparing the pharmacokinetic profiles of *o,p'*-DDD and MeSO$_2$-DDE in minipigs. Since patients with functional adrenocortical tumours and Cushing's syndrome often show characteristic accumulations of fat, the minipig is a suitable test animal because of its extensive body fat storages. A short preliminary account for the long-term pharmacokinetics of MeSO$_2$-DDE is given below. Preliminary data for *o,p'*-DDD is given in a report by Cantillana et al presented at this symposium[12].

Materials and Methods

Chemical

3-MeSO$_2$-DDE, 99 % pure, was synthesized by Synthelec AB, Ideon, Lund, using procedures developed by Bergman and Wachtmeister[13].

Animals and samples

Five female minipigs were obtained from Ellegaard Göttingen minipigs ApS, Dalmose, Denmark. Upon arrival they were 6-7 months old and weighed 15.4-17.6 kg. All animals were housed in the same pen, with free access to water. Twice a day they were fed with a standard minipig low calory pelleted diet. The pen had concrete floor, wooden or metal walls and was bedded with straw. For acclimatization, the animals were kept under these conditions for three weeks before treatment. The pigs were fasted the same morning as the administration was implemented. As a single dose, MeSO$_2$-DDE (15 mg/kg body weight), dissolved in corn oil, was given by gastric intubation. The dose was chosen to approximately correspond to 1 g *o,p'*-DDD, i.e. half a common daily dose given to ACC patients. 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 of the test substance. At day 180, blood samples were drawn from the heart following anaesthesia with an i.p. injection of 20 ml pentobarbital 100 mg/ml (Apoteket AB, Sweden). 30, 60, 90, 120 and 180 days after administration, the pigs were weighed and subcutaneous fat samples from the chin were collected with a biopsy punch, after local anaesthesia with mepivacain (Carbocain® 2%). The blood samples were collected in 5 ml EDTA-tubes and centrifuged for 10 minutes at 3000 rpm

(Eppendorf centrifuge). The plasma was transferred to Eppendorf tubes and stored in -20ºC until analysis. The fat samples were immediately frozen and stored at -70ºC. After 180 days, the anaesthetized pigs were killed by an intracardial injection of Pentobarbital 100 mg/ml (Apoteket AB, Sweden). All procedures were approved by the animal experimentation committee in Uppsala.

Analysis
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 (25m x 0.15mm x 0.12um); 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 (1min), 20˚C/min to 300˚C which was held 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.

The extraction and cleanup of the blood samples was carried out as earlier described[14], with a slight modification. Plasma (0.2-5g) was transferred to a screw-capped test tube and the internal standard (3-$MeSO_2$-CB141) was added. The samples were denatured with HCl and 2-propanol. The denatured plasma was extracted twice with hexane/methyl-tert-butyl ether and the organic phase was partitioned into a KCl-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. To separate the phenolic compounds from the neutrals the extract was dissolved in hexane and was partitioned with a KOH-solution. The organic phase was transferred to another test tube and represents the neutral fraction. The lipids were removed by a silica/sulfuric acid gel column. The compounds were eluted with dichloromethane (15 ml). After adjustment of sample volume the compounds were analyzed and quantified by GC-ECD. The method used for extraction of the fat samples has been described eralier[15], but due to the small sample amount the method has been scaled down. The samples were mixed with hexane:acetone 2:5 and extracted twice with hexane:methyl-tert-butyl ether. The lipid amount was determined as described above. The samples were dissolved with hexane and spiked with 3-$MeSO_2$-CB141 before lipid removal. A first lipid reduction was performed by anhydrous dimethyl sulfoxide partitioning as described earlier[16]. Further lipid removal was performed with a silica/ sulphuric acid column. The compounds were analyzed and quantified by GC-ECD.

Results and Discussion
No trace of $MeSO_2$-DDE could be detected in the plasma samples collected before administration of the test

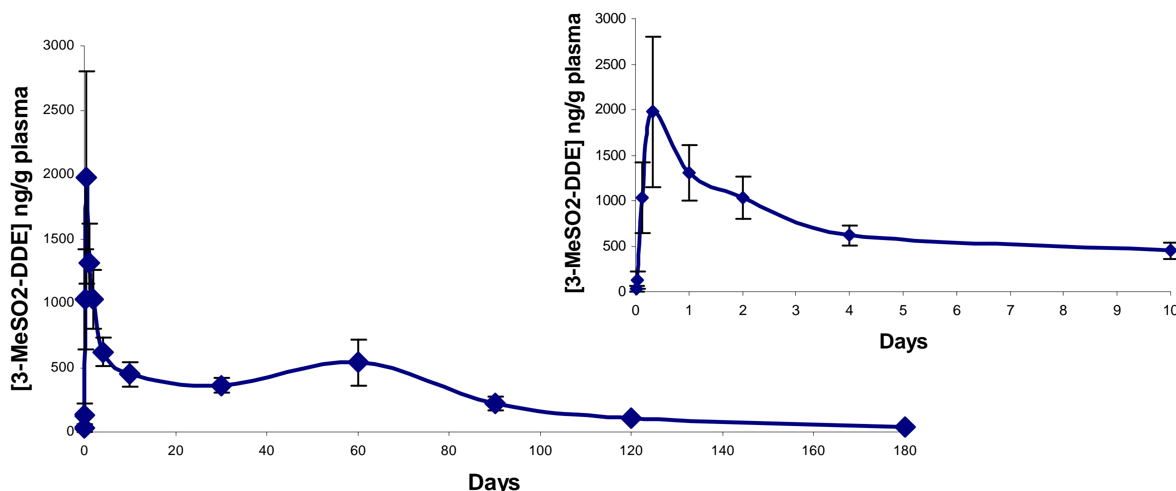

Figure 1. Plasma concentrations of 3-$MeSO_2$-DDE plotted against time (days) (A). Each data point represents a

mean value from the five pigs, with standard deviation bars. The smaller graph (B) is a magnification of the curve during the first 10 days.

compound. After treatment, no pig showed any sign of treatment-related effect. The plasma concentration curve for $MeSO_2$-DDE during the whole investigative period is shown in figure 1A. A more detailed illustration of the kinetics during the first 10 days is given in figure 1B. Plasma concentrations increased rapidly during the first hours after administration and peaked within 24 hours in all individuals. After the concentration peak, the curve declined rapidly for the next 3-9 days. The plasma levels then decreased very slowly during the remaining months. As demonstrated in figure 1A, the concentrations unexpectedly increased in almost all animals at 60 days after dosing. This is discussed below.

As shown in figure 2, there was a strong accumulation and retention of $MeSO_2$-DDE in subcutaneous fat, which reached a 230-fold higher concentration than blood plasma at day 30. At day 180, the concentration in subcutaneous fat was almost 300-fold higher that that in plasma.

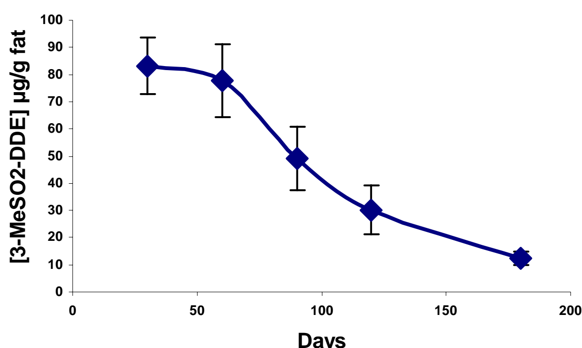

Figure 2. The fat concentrations of $MeSO_2$-DDE plotted against time (days). Each data point represents the mean value from the five pigs, with standard deviation bars.

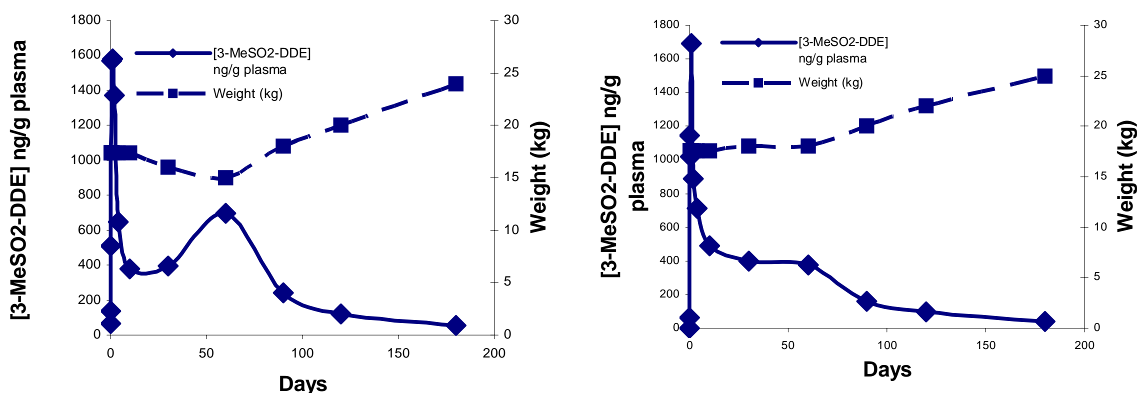

Figure 3. Illustration of how the body weight of the minipigs affected the plasma concentrations of $MeSO_2$-DDE. The left graph (A) represents an individual pig, which lost weight for 60 days after administration. The right graph (B) represents a pig that retained its body weight during the first 60 days. Both pigs gained weight during day 60 to 180. In pig A the plasma level almost doubled from day 10 to day 60.

As illustrated in figure 3A-B, it seems that the weight of the pigs, and probably the fat stores, affected the plasma concentrations of $MeSO_2$-DDE. All but one pig had lost weight at two months after administration. The diet was

therefore increased, resulting in a subsequent weight gain. This observation should be relevant also for human subjects, e. g. for an ACC patient with Cushing's syndrome, in which a loss of adipose tissue would result in a redistribution of the drug to blood plasma, and subsequently to more sensitive tissues such as the adrenal cortex and tumour tissue expressing CYP11B1. Such a pharmacokinetic profile would, however, make the plasma drug concentration difficult to control under conditions where body fat is lost. Provided a favourable effect/side-effect ratio of the drug, the long plasma half-life could also provide an advantage in that maintenance dosing needs to be less frequent.

As mentioned above, $MeSO_2$-DDE is present in wildlife, and accumulates in fat tissue of various species. In this context, this study gives an insight in the kinetic behaviour of $MeSO_2$-DDE in wild animals subject to decreased body fat stores due to hibernation or starvation. It seems obvious that, during periods of body fat degradation, lipophilic pollutants such as $MeSO_2$-DDE will be redistributed from fat, via the blood, to sensitive target tissues such as the adrenal cortex. The present observations could therefore be useful not only in the study of $MeSO_2$-DDE as a drug candidate lead compound, but also for the understanding of risks posed by lipophilic pollutants in environmentally exposed animals in general.

In conclusion, this is the first time that the kinetics of $MeSO_2$-DDE has been studied in a large mammal. Our preliminary data show that the compound is absorbed from the gastro-intestinal tract and reaches maximal plasma concentrations within a few hours after administration. Although the concentrations in both plasma and fat decreased over time, considerable amounts were retained after 180 days. This is a preliminary report, and adequate calculations need to be performed in order to more conclusively define the pharmacokinetic characteristics for $MeSO_2$-DDE in minipigs.

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