3-METHYLSULPHONYL-DDE: A POTENT TOXICANT FOLLOWING METABOLIC ACTIVATION IN THE ADRENAL *ZONA FASCICULATA* IN MICE

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ABSTRACT

The DDT-metabolite 3-methylsulphonyl-DDE is a highly potent toxicant in the adrenal *zona fasciculata* in adult and neonatal mice. The metabolite is activated by adrenal cytochrome P-450 to a reactive intermediate that binds irreversibly to protein. The activating enzyme appears to reside in the mitochondria, the prime subcellular target. A depletion of the mitochondrial glutathione pool in the *zona fasciculata* is suggested to contribute to the high toxicity observed.

KEY WORDS

adrenal toxicity, 3-methylsulphonyl-DDE, cytochrome P-450, irreversible binding

INTRODUCTION

Methylsulphone-containing metabolites of PCB and DDT are present in adipose tissue of Baltic grey seal (1), a seal population suffering from a syndrome characterized by gross morphological and histological lesions in a variety of tissues (2). A high incidence of adrenocortical hyperplasia has been observed among seals of different ages. Although no conclusive information about the etiology of the syndrom is available, a causal relationship to the high bodyburden of persistent halogenated hydrocarbons in these animals has been proposed (2).

In connection with studies on methylsulphone metabolites formed from PCB, hexachlorobenzene or DDT we observed that injection of the ^{14}C-labelled DDT-metabolite 3-methylsulphonyl-DDE ($MeSO_2$-DDE) resulted in a high accumulation of radioactivity in the adrenal cortex of mice (3). Autoradiography of solvent-extracted tissue-sections (4) showed that a high concentration of an irreversibly bound residue was confined to the *zona fasciculata*. Based on these observations we performed histopathology and found that $MeSO_2$-DDE was a potent toxicant in the adrenal *zona fasciculata* (5). In this report our studies on the adrenal toxicity of $MeSO_2$-DDE are briefly reviewed.

MATERIALS AND METHODS

$MeSO_2$-(^{14}C)DDE and unlabelled $MeSO_2$-DDE were prepared as described by Bergman and Wachtmeister (6). The spec. act. of the labelled compound was 13.4 mCi/mmol and the radiochemical purity 98%. Nonpregnant, pregnant and lactating C57Bl mice were given single injections of the labelled or unlabelled substance. For the autoradiographic experiments $MeSO_2$-(^{14}C)DDE was injected iv (4-5 mg/kg). For histopathological and ultrastructural studies $MeSO_2$-DDE was injected ip (3-50 mg/kg). For studies on excretion in milk, $MeSO_2$- (^{14}C)DDE was injected ip (1.5 or 25 mg/kg).

For binding studies *in vitro*, homogenates from female adrenal cortici were prepared (generally 300xg supernatants). The homogenates were used in incubation mixtures containing $MeSO_2$-(^{14}C)DDE and a NADPH-generating system. After incubation, the irreversible binding of radioactivity to protein was determined after exhaustive extraction according to Baker and van Dyke (7).

RESULTS

Morphological changes in the adult adrenal cortex. Histopathology showed that $MeSO_2$-DDE was a potent adrenocorticolytic agent that induced extensive necrosis in the adrenal cortex one day after exposure to single doses down to 25 mg/kg. At 12.5 mg/kg degenerative changes were evident at the light microscopic level of resolution. The lesion was confined to the *zona fasciculata*, leaving the *zona glomerulosa* and *reticularis* (X-zone) intact. The affected cells became pycnotic, showed signs of extensive cytoplasmic vacuolation and finally were resorbed. Four days after treatment the *zona fasciculata* virtually had disappeared, leaving a ditch of debris and necrotic residues. At later time-points (45 days) the *zona fasciculata* seemed to regenerate.

To define the subcellular origin of the lesion, adrenals were subjected to electron microscopy following ip treatment of mice with $MeSO_2$-DDE. The results revealed early mitochondrial swelling, disappearence of mitochondrial cristae followed by reduced numbers of mitochondria in the *zona fasciculata*. Already six hours after application of the lowest dose examined (3 mg/kg), degenerative mitochondrial changes were obvious.

Metabolic activation of $MeSO_2$-DDE in vitro. A tissuespecific metabolic activation of $MeSO_2$-(^{14}C)DDE in the adrenal *zona fasciculata* was indicated by the high irreversible binding observed by autoradiography in this zone. To determine and examine such an activation, $MeSO_2$-(^{14}C)DDE was incubated with adrenal homogenates as described (5). The results demonstrated a time- and concentration-dependent irreversible binding of radioactivity to protein (apparent K_m = 2.1 µM; V_{max} = 104 pmoles/mg protein/30 min) and a formation of watersoluble radioactive metabolite(s). The irreversible protein binding and the formation of watersoluble metabolite were inhibited by the cytochrome P-450 inhibitors metyrapone (K_i = 1 µM) and carbon monoxide. Further, the binding was inhibited by addition of reduced glutathione to the incubation medium, whereas in this case the formation of the watersoluble labelled metabolite(s) increased. As determined by experiments with various subcellular fractions, the activating enzyme activity appeared to reside in the mitochondrial fraction.

Transplacental and transmammary toxicity. When $MeSO_2$-(^{14}C)DDE was injected into pregnant mice, autoradiography revealed a high and specific accumulation of irreversibly bound radioactivity in the late gestational fetal adrenal cortex. Likewise, there was a high

binding in the adrenal *zona fasciculata* in suckling mice following ip injection of $MeSO_2$-(^{14}C)DDE to the dam. As in adult animals, $MeSO_2$-DDE was found by histopathology to be toxic to the fetal and postnatal adrenal cortex. When the transmammary transport of $MeSO_2$-(^{14}C)DDE was examined, higher concentrations of radioactivity were present in the postnatal than in the maternal liver and adrenal, both at a low (1.5 mg/kg) or a high (25 mg/kg) dose of $MeSO_2$-(^{14}C)DDE to the mother.

DISCUSSION

The present studies have shown that the DDT-metabolite $MeSO_2$-DDE is highly toxic to the adrenal cortex in mice. Notably, ultrastructural changes in the mitochondria were evident after a single dose of 3 mg/kg, the lowest dose examined. Following ip administration $MeSO_2$-DDE became irreversibly bound in its target tissue, the glucocorticosteroid producing *zona fasciculata*.. This is in contrast to PCB-methyl sulphones which are reversibly bound in their target cells in the lung and kidney (3).

Studies *in vitro* showed that the adrenals posess a high ability to activate $MeSO_2$-DDE to a reactive, tissuebinding metabolite. The results indicated that cytochrome P-450 was involved in the metabolic activation of $MeSO_2$-DDE. Since the *zona fasciculata* was the only tissue in the body that contained significant amounts of bound $MeSO_2$-DDE *in vivo*, the activating form of cytochrome P-450 would be expected to be expressed predominantly or specificly in this tissue. Based on these considerations and the observation that the activating enzyme activity appeared to reside in the mitochondrial fraction, we propose that a mitochondrial cytochrome P-450 catalyses the activation of $MeSO_2$-DDE. The enzyme may be identical with the11ß-hydroxylase, the cytochrome P-450 which converts deoxycorticosterone to the glucocorticosteroid corticosterone in the mouse adrenal cortex. This contention is further supported by the observed high inhibitory activity of metyrapone, a potent inhibitor of 11ß-hydroxylase, and by the recent finding that corticosterone blood levels are decreased in mice exposed to $MeSO_2$-DDE (Jönsson *et al.* unpublished). Notably, deoxycorticosterone was also an inhibitor of the irreversible binding of $MeSO_2$-(^{14}C)DDE to adrenal protein in vitro (K_i = 3 µM).

The experiments *in vitro* further suggest that metabolically activated $MeSO_2$-DDE is conjugated with glutathione. Considering the results discussed above, we propose that a mitochondrial activation of $MeSO_2$-DDE results in depletion of the mitochondrial glutathione pool. Such a mechanism could contribute to the strikingly high toxicity of $MeSO_2$-DDE in the adrenal *zona fasciculata*.

In conclusion, $MeSO_2$-DDE is a highly potent adrenal toxicant at ecotoxicologically relevant doses. The observation that exposure via mothers milk resulted in higher concentrations of radioactivity in the postnatal liver and adrenals than in the corresponding maternal organs indicates an efficient elimination of $MeSO_2$-DDE via milk. This route of exposure may be particularly important in seals since the suckling neonatal seal will increase its body weight 4-5 times in the first three weeks of life. Notably, $MeSO_2$-DDE has been identified also in human breast milk (8).

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