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Reproductive Toxicology 21 (2006) 94–103
Reproductive Toxicology
www.elsevier.com/locate/reprotox

Monoisoamyl dimercaptosuccinic acid induced changes in pregnant female rats during late gestation and lactation

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Received 8 January 2005; received in revised form 17 May 2005; accepted 24 May 2005
Available online 22 July 2005

Abstract

Monoisoamyl dimercaptosuccinic acid (MiADMSA), a vicinal thiol chelating agent and an analogue of a conventional metal chelating agent, *meso*-2,3-dimercaptosuccinic acid (DMSA) has recently been gaining recognition to be more effective chelating agent than DMSA in mobilizing lead, mercury and arsenic. However, very little information is available on the toxicological properties of this chelator. In the present study, MiADMSA was administered to pregnant female rats from day 14 of gestation to day 21 of lactation at different doses through oral (p.o.) and intraperitoneal (i.p.) routes to examine the toxicity in the pups and dams. Results suggested that MiADMSA had no effect on period of gestation, litter-size, sex ratio, and viability and lactation. No skeletal defects were observed following the administration of the chelator. However, MiADMSA administration produced few signs of oxidative stress in dams particularly at the higher doses (100 and 200 mg/kg) as evident from increased thiobarbituric acid reactive substances (TBARS) in RBCs and decrease in the δ-aminolevulinic acid dehydratase (ALAD) activity. Administration of MiADMSA also caused some alterations in the essential metal concentration in the soft tissues especially tissue copper loss in lactating mothers and pups, which would be of some concern. Apart from copper, changes were also observed in the tissue zinc concentrations in mothers and pups following MiADMSA administration. The study thus suggests that the chelator is relatively safe during late gestation and it does not cause any major alteration in the mothers and the developing pups. However, detailed studies with MiADMSA, post-toxic metal exposure in pregnant animals may provide useful information.

Keywords: Developmental toxicity; Maternal toxicity; MiADMSA; DMSA; Chelation therapy; Pregnancy; Rats

1. Introduction

Conventional chelating agents that are used clinically are known to have serious side effects following administration during pregnancy in animals [1]. Clinical use of chelating agents like ethylenediamine tetra acetic acid (EDTA) and British anti-lewisite (BAL) has been mainly restricted due to their large number of side effects reported in experimental animals, particularly when administered during pregnancy/lactation [1–8].

Meso-2,3-dimercaptosuccinic acid (DMSA) has been used successfully for the treatment of arsenic, lead and mercury [9–12]. These analogues of BAL have shown promise compared to BAL, for less toxicity, greater water solubility and no lipid solubility, available orally and more effective [13,14]. Toxicity studies in rats have shown DMSA to have a higher safety ratio than BAL [14–20].

Recently, a number of mono and diesters of DMSA especially the higher esters were synthesized and tested for metal intoxication especially against lead [21,22], cadmium [23], mercury [24–26] and gallium arsenide [27,28]. Among these monoesters, MiADMSA (monoisoamyl DMSA), a C_5 branched alkyl monoester has got the ability to gain intracellular access to various endogenous ligands [29], thus has an added advantage over its parent (DMSA). It was reported that subcutaneous injections of MiADMSA at 23.8, 47.6 and 95 mg/kg/day given for four consecutive days following a single teratogenic dose of sodium arsenite or methyl mer-

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doi:10.1016/j.reprotox.2005.05.008

cury chloride showed protective effects on the embryo/fetal toxicity of these compounds [19,25]. However, only one study reported the effect of MiADMSA when injected i.p. to pregnant mice on day 5 through day 15 of gestation, maternal toxicity was noted at the 95 and 190 mg/kg/day. Embryo/fetal toxicity, consisting of a significant increase in the number of late resorptions as well as in the percentage of postimplantation loss, reduced fetal body weight and increase in the incidence of skeletal defects, were noted at 190 mg/kg/day [30]. The effect of MiADMSA on trace element metabolism during gestation has not been specifically examined [1]. Domingo [1] also suggested the promising role of MiADMSA in metal-induced toxicity but raised concern about the absence of toxicological/pharmacological data of this drug during human pregnancy and hence administration of this potent chelator cannot be recommended during human pregnancy. We have recently reported the data about the toxicological safety of MiADMSA in male and female rats as well as its comparison with other metal chelators [31–34] and found that the chelator does not produce any adverse effects on the tissue except for a mild hepatotoxicity and copper loss.

It is therefore necessary to evaluate the reproductive, embryotoxic and teratogenic potential of MiADMSA before its approval of administration during pregnancy. The following study was performed to assess the effect of the chelator (MiADMSA): (i) on late gestation, parturition, lactation, and postnatal viability when administered through two routes (p.o. and i.p.) at different dose concentrations and were compared with the parent drug, DMSA, (ii) to assess the trace metal status especially of zinc, copper, magnesium, iron and calcium in dams and pups and (iii) to assess the effects on some sensitive biochemical variables in blood of lactating dams.

2. Material and methods

2.1. Chemicals and reagents

Monoisoamyl DMSA (MiADMSA) was synthesized in Synthetic Chemistry division of our establishment by the controlled esterification of DMSA with the corresponding alcohol in acidic medium [35]. The products were purified and characterized using spectral and analytical methods before animal experimentation. All the samples were stored, refrigerated in a dessicator to avoid oxidation and thermal decomposition. DMSA and δ-aminolevulinic acid (ALA) were purchased from Sigma Chemical Co., (St. Louis, MO, USA). All other analytical laboratory chemicals and reagents were purchased from E. Merck (Germany), Sigma (USA), Fluka (Germany), or BDH Chemicals (India).

2.2. Animals and treatment

Female Wistar rats weighing 120–150 g were housed in stainless steel cages in an air-conditioned room. Rats were allowed standard rat chow diet (Lipton's India Ltd., metal contents of diet, in ppm dry weight Zn 45, Cu 10, Mn 55, Fe 70, Co 5) and water ad libitum for the duration of the experiment. Following acclimatization female rats and their sexually mature male rats weighing approximately 150 g, were caged for breeding in a 2:1 ratio. The day on which vaginal plugs were observed and confirmed by microscopic examination of sperms in the vaginal smear was denoted as day 0 of gestation. Each pregnant rat was kept individually in a separate cage and the cages were kept in a room with alternating light and dark cycle of 12 h each. Animal Use Ethical Committee of DRDE approved all the protocols for the experimentation. Forty-five mated females were divided into nine groups but each female was individually caged. The groups were divided and treated as below

- Group 1: Control
- Group 2: MiADMSA, 50 mg/kg, p.o. administration
- Group 3: MiADMSA, 100 mg/kg, p.o. administration
- Group 4: MiADMSA, 200 mg/kg, p.o. administration
- Group 5: DMSA, 200 mg/kg, p.o. administration
- Group 6: MiADMSA, 25 mg/kg, i.p. administration
- Group 7: MiADMSA, 50 mg/kg, i.p. administration
- Group 8: MiADMSA, 100 mg/kg, i.p. administration
- Group 9: DMSA, 100 mg/kg, i.p. administration

The two chelators (MiADMSA and DMSA) were administered daily from day 14 of gestation to day 21 of lactation. Both MiADMSA and DMSA were dissolved in sodium bicarbonate solution. All the antidote solutions were prepared immediately before use. The injection volume amounted to 4 ml/kg-body weight and administered in the morning. Each female was assessed daily twice for condition (live, dead, moribund) and for general signs of toxicity. Body weights were recorded on day 0, 6, 13 and 18 of gestation, and on postnatal days 0, 4, 14 and 21. Beginning day 20 of gestation, all dams were checked for signs of delivery. The following parameters were assessed at birth: duration of pregnancy, number of live, dead or moribund pups, sex and body weights of live born pups. Each pup was weighed again on postnatal days 4, 14, 18 and 21. The following indices were then calculated:

$$n\text{-day viability index} = \#\,\text{pups viable at lactation day } n / \#\,\text{viable pups born}$$

where n is the day of lactation (i.e. day 4, 14, 18 or 21).

Developmental milestones of pinna detachment, incisor eruption and eye opening were also monitored.

At weaning (day 21 of lactation), all dams and weanlings were euthanized under light ether anesthesia for autopsy. Blood was collected in heparinized vials. The liver, kidney, brain and spleen were removed, washed free of extraneous materials and weighed and the ratio of organ weight/body weight was then calculated. A few biochemical parameters were also performed in blood to assess sign of toxicity in dams.