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M. Angeles Bosque · José L. Domingo · Jacinto Corbella

Assessment of the developmental toxicity of deferoxamine in mice

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Abstract Deferoxamine (DFO), an efficient chelating agent available for the treatment of iron and aluminium overload, was evaluated for developmental toxicity in Swiss mice. Intraperitoneal injections of DFO were given to pregnant animals at 0, 44, 88, 176, and 352 mg/kg per day on gestational days 6 through 15. Maternal clinical status was monitored daily during and after treatment. Fetal parameters, including external, visceral, and skeletal malformations and variations, were assessed. Mice were killed on day 18. No maternal mortality was observed, but dams exhibited reduced body weight gain during treatment at 88, 176, and 352 mg/kg per day. Body weight at termination, corrected body weight, and food consumption were reduced in all groups. In contrast, the only significant treatment-related embryo/fetal effect was a decrease in the number of live fetuses per litter at 352 mg/kg per day. The no-observable-adverse-effect level (NOAEL) for maternal toxicity of DFO was <44 mg/kg per day, whereas the NOAEL for developmental toxicity was 176 mg/kg per day. In summary, intraperitoneal administration of DFO to mice during organogenesis produced developmental toxicity in the presence of maternal toxicity. Because of the remarkable maternal toxicity of DFO, extreme caution in the use of this drug is recommended during pregnancy.

Key words: Maternal toxicity
Developmental toxicity · Deferoxamine · Mice

M. Angeles Bosque · José L. Domingo (✉)
Laboratory of Toxicology and Biochemistry,
School of Medicine, "Rovira I Virgili" University,
San Lorenzo 21, E-43201 Reus, Spain

M. Angeles Bosque · Jacinto Corbella
Department of Toxicology, School of Medicine,
University of Barcelona, Casanova 143,
E-08036 Barcelona, Spain

Introduction

The trihydroxamic acid deferoxamine (desferrioxamine, DFO), is a hexadentate chelator which has been successfully used for the treatment of acute iron poisoning and chronic iron overload (Sephton-Smith 1962; Barry et al. 1974; Hoffbrand and Wonke 1989). Although the best route of administration is unclear, because DFO is not well absorbed from the gastrointestinal tract, for optimum activity it must be given parenterally.

In recent years, DFO has also been used to treat aluminium overload in patients with chronic renal failure (Ackrill and Day 1985; Molitoris et al. 1988). DFO has also been reported to benefit anemia in chronic hemodialysis patients through improvement of erythropoiesis (Tielmans et al. 1985), as well as aluminium-related osteomalacia (Malluche et al. 1984).

Despite the widespread use of DFO, therapy with this drug has been associated with a number of toxic side-effects including increased susceptibility to acute infections (Boelaert et al. 1994), pulmonary complications (Tenenbein et al. 1992), ocular toxicity (Cases et al. 1990), auditory neurotoxicity (Gallant et al. 1987; Cases et al. 1990), as well as other serious toxic side effects such as thrombocytopenia, nausea and vomiting, and renal toxicity (Swartz 1985; Domingo 1989; Porter and Huehns 1989). However, there is a remarkable lack of information about experimental studies on the embryotoxicity and teratogenicity of DFO.

Because the treatment of pregnant women with chelating agents is controversial, the use of DFO during pregnancy would be of special concern. Although the manufacturer's package insert states that DFO should not be given in pregnancy unless the expected benefits outweigh the risks, in a recent review on the consequences of an iron overdose and its therapy with DFO in pregnancy, it was concluded that in acute iron poisoning, treatment with DFO should not be withheld

merely because of the pregnancy (McElhatton et al. 1991). In order to extend the knowledge on the maternal and developmental toxicity of DFO, the current study was conducted to assess the potential embryo/fetal toxicity of this drug in pregnant mice.

Materials and methods

Deferoxamine (DFO) was purchased from Ciba-Geigy (Barcelona, Spain). DFO was dissolved in 0.9% saline and solution concentrations were adjusted so that a 30-g mouse would receive 0.15 ml. Virgin male and female Swiss mice (weighing 27–31 g) were obtained from Interfauna Ibérica (Barcelona, Spain). After a 7-day quarantine period, female mice were mated with males (2:1) overnight and examined the following morning for copulatory plugs. The morning on which a vaginal plug was found was designated as gestational day (gd) 0. Animals were housed in a thermostatically maintained room at 22 ± 2°C and 50 ± 10% relative humidity, illuminated with a 12 h/day light cycle regulated by a time switch. Food (Panlab rodent chow, Barcelona) and tap water were available ad libitum throughout the study.

Five groups of plug-positive female mice were given intraperitoneal doses of DFO (0, 44, 88, 176, and 352 mg/kg per day) on gd 6–15. In a previous study, the intraperitoneal LD_{50} of DFO in mice was estimated to be 1760 mg/kg (Domingo et al. 1990). Control mice received intraperitoneal injections of 0.9% saline on gd 6–15. Animals were observed daily during and after treatment for overt signs of toxicity. Body weight gain and food consumption were computed for the pretreatment, treatment, and post-treatment periods from daily records of these parameters. Following termination on gd 18, the maternal body, liver, kidneys, and intact uterus were weighed. Uterine contents were evaluated for the number of implantation sites, and live, dead and resorbed fetuses. Each live fetus was weighed and examined for sex and external morphological abnormalities.

Subsequently, fetuses were preserved intact in Bouin's solution and processed for the evaluation of visceral anomalies using the free-hand razor blade sectioning method of Wilson (1965), or fixed in 70% ethanol, macerated with potassium hydroxide and stained with alizarin red S (Staples and Schnell 1968). Results of the quantitative continuous variables (e.g., maternal body weight, food consumption, organ weights, etc.) were compared using one-way analysis of variance (ANOVA). When ANOVA revealed a significant effect, Student's t test or Mann-Whitney U-test were used to determine which dose groups differed significantly from the control group. Statistical analyses of morphological defects were performed on both an individual fetus and a litter basis. Frequency data for a given parameter were analyzed by the chi-square method. If significance was observed Fisher's exact test was then used. The level of significance for all tests was $p < 0.05$.

Results

Although all plug-positive mice survived to scheduled termination, early deliveries were observed in the 88 (31.8%), 176 (22.2%), and 352 (29.4%) mg DFO/kg per day groups, which would be a substantial effect of the drug. Those litters were discarded and were not included in the data here presented. Maternal toxicity was indicated by a significant reduction in food consumption and in maternal body weight gain. Food consumption was significantly reduced at 44, 88, and 176 mg/kg per day on gd 6–15, 15–18, and 0–18, whereas at 352 mg/kg per day food consumption was reduced on gd 15–18 and 0–18, but not during the treatment period (gd 6–15) (Table 1).

Body weight gain was significantly depressed during the treatment period (gd 6–15) and during the total

Table 1 Maternal weight gain and food consumption in mice injected intraperitoneally with DFO on gestational days 6–15[a]

Gestation days	Dose (mg/kg per day) 0	44	88	176	352
No. of plug-positive females	17	18	22	18	17
Body weight gain (g)					
0–6 (pretreatment period)	4.80 ± 1.03	5.83 ± 2.33	4.73 ± 1.16	5.21 ± 1.19	4.67 ± 1.07
6–9	1.92 ± 1.01	1.75 ± 2.61	2.00 ± 1.01	1.93 ± 1.49	1.67 ± 1.07
6–12	8.58 ± 2.12	7.00 ± 3.49	6.27 ± 3.37	7.64 ± 2.02	7.58 ± 1.62
6–15 (treatment period)	18.49 ± 2.27	16.42 ± 2.91	13.47 ± 5.12[c]	13.64 ± 4.05[c]	14.36 ± 2.11[d]
15–18 (posttreatment period)	11.11 ± 1.37	10.25 ± 2.77	8.12 ± 4.70	8.90 ± 2.81	10.71 ± 5.31
0–18 (total study period)	34.40 ± 3.06	32.50 ± 7.14	26.32 ± 10.53[b]	27.75 ± 7.31[b]	29.74 ± 4.28[b]
Food consumption (g)					
0–6 (pretreatment period)	36.41 ± 2.52	35.32 ± 4.40	33.03 ± 5.69	35.29 ± 2.76	34.14 ± 3.66
6–9	17.09 ± 3.65	14.87 ± 2.20	16.03 ± 2.43	16.55 ± 0.72	17.87 ± 2.41
6–12	36.29 ± 4.38	33.05 ± 2.31	32.59 ± 3.20[b]	33.60 ± 1.23	35.27 ± 3.85
6–15 (treatment period)	58.75 ± 6.16	50.52 ± 4.79[c]	50.92 ± 6.49[c]	51.61 ± 2.50[d]	54.81 ± 6.89
15–18 (posttreatment period)	25.41 ± 1.98	22.66 ± 1.19[d]	16.45 ± 2.86[d]	16.50 ± 5.63[d]	15.87 ± 1.55[d]
0–18 (total study period)	120.57 ± 8.49	108.50 ± 5.88[d]	100.40 ± 9.98[d]	103.40 ± 6.68[d]	104.82 ± 6.37[d]

[a] Values indicate mean ± SD
[b] Significantly different from control group, $p < 0.05$
[c] Significantly different from control group, $p < 0.01$
[d] Significantly different from control group, $p < 0.001$

Table 2 Maternal body and organ weights at termination in mice injected intraperitoneally with DFO on gestational days 6–15[a]

	Dose (mg/kg per day)				
	0	44	88	176	352
No. of plug-positive females	17	18	22	18	17
Body weight at termination (g)	66.64 ± 4.68	59.08 ± 7.39[d]	53.75 ± 11.36[d]	55.10 ± 6.54[e]	57.86 ± 4.59[e]
Gravid uterine weight (g)	24.30 ± 2.44	20.96 ± 5.36	19.02 ± 7.58	17.87 ± 7.01[c]	20.62 ± 4.58[c]
Corrected body weight (g)	42.34 ± 2.96	38.12 ± 3.38[d]	34.73 ± 3.94[e]	37.23 ± 3.50[d]	37.24 ± 3.08[d]
Corrected body weight change (g)	10.06 ± 1.39	10.12 ± 2.92	6.73 ± 3.34[d]	10.13 ± 2.26	9.95 ± 1.80
Liver weight (g)	3.36 ± 0.38	2.88 ± 0.36[c]	2.86 ± 0.42[c]	3.07 ± 0.43	3.22 ± 0.54
Relative liver weight (%)[b]	7.94 ± 0.65	7.56 ± 0.65	8.23 ± 0.54	8.24 ± 0.77	8.64 ± 1.55
Kidney weight (g)	0.56 ± 0.05	0.53 ± 0.16	0.46 ± 0.07[d]	0.50 ± 0.06	0.47 ± 0.06[d]
Relative kidney weight (%)[b]	1.32 ± 0.06	1.39 ± 0.48	1.32 ± 0.17	1.34 ± 0.12	1.26 ± 0.23

[a] Values indicate mean ± SD
[b] Calculated as percentage of corrected body weight
[c] Significantly different from control group, $p < 0.05$
[d] Significantly different from control group, $p < 0.01$
[e] Significantly different from control group, $p < 0.001$

Table 3 Development toxicity in pregnant Swiss mice following intraperitoneal maternal exposure to DFO on gestational days 6–15[a]

	Dose (mg/kg per day)				
	0	44	88	176	352
No. of plug-positive females	17	18	22	18	17
No. implants/litter	14.54 ± 1.92	12.33 ± 3.39	11.75 ± 3.50	12.60 ± 4.12	12.43 ± 3.31
No. live fetuses/litter	13.27 ± 1.35	11.17 ± 3.21	10.00 ± 5.86	10.40 ± 4.79	10.86 ± 3.72[b]
No. non-viable implants/litter					
early resorptions	0.82 ± 1.08	0.67 ± 0.77	0.12 ± 0.35	1.50 ± 1.90	1.00 ± 1.53
late resorptions	0.45 ± 0.93	0.32 ± 0.49	0.37 ± 0.74	0.60 ± 0.69	0.00 ± 0.00
dead fetuses	0.00 ± 0.00	0.17 ± 0.39	1.26 ± 2.55	0.10 ± 0.32	0.57 ± 1.13
% postimplantation loss	8.72 ± 8.03	9.40 ± 7.72	14.84 ± 18.95	17.42 ± 16.11	12.62 ± 13.15
Sex ratio (M/F)	0.87 ± 0.39	1.10 ± 0.44	0.91 ± 0.85	0.91 ± 0.48	1.26 ± 1.15
Fetal weight (g)	1.31 ± 0.08	1.37 ± 0.98	1.21 ± 0.12	1.29 ± 0.17	1.30 ± 0.15

[a] Values indicate mean ± SD
[b] Significantly different from control group, $p < 0.05$

gestational period (gd 0–18) at 88, 176, and 352 mg/kg per day (Table 1). In addition, although not dose-related, maternal body weight on gd 18 and corrected body weight (body weight at termination minus gravid uterine weight) were significantly decreased in all the DFO-treated groups (Table 2). Gravid uterine weight was only reduced at 176 and 352 mg/kg per day, whereas corrected maternal body weight change (corrected body weight minus body weight on gd 0) was significantly affected by treatment at 88 mg/kg per day. Maternal absolute liver weight was decreased relative to controls at 44 and 88 mg/kg per day, whereas absolute kidney weights were depressed at 88 and 352 mg/kg per day. In contrast, maternal relative and kidney weights were not affected by DFO administration (Table 2).

Table 3 shows the gestational parameters in mice treated intraperitoneally with DFO during organogenesis. Although a reduction in the number of live fetuses per litter was observed in all treated groups, the level of significance was only reached at 352 mg/kg per day. There were no effects of treatment on the number of total or nonviable implantations per litter, or on the percentage postimplantation loss. Sex ratio and average fetal body weight were also unaffected by treatment.

No external or visceral malformations and variations were noted. Treatment-related skeletal defects included reduced ossification of parietal bone, metacarpals, and metatarsals (Table 4). The number of fetuses with reduced ossification was significantly increased at 88, 176, and 352 mg/kg per day. However, when the statistical analysis of these data was carried out using the litter as the unit of comparison, no significant differences between DFO-treated groups and the control group could be observed.

Table 4 Skeletal anomalies in offspring of Swiss mice following intraperitoneal maternal exposure to DFO on gestational days 6 15

	Dose (mg/kg per day)				
	0	44	88	176	352
No. fetuses examined/litters	119/17	134/12	92/15	64/14	63/12
Reduced ossification					
Parietal bone	6/4	17/8	34[b]/9	18[b]/7	19[b]/8
Metacarpals	3/2	2/2	4/4	4/3	3/3
Metatarsals	8/6	15/6	14/10	10/6	12[a]/7
Total skeletal variations	10/9	21/9	39[b]/11	18[b]/7	24[b]/9

[a] Significantly different from control group, $p < 0.05$
[b] Significantly different from control group, $p < 0.001$

Discussion

Although there is a paucity of information concerning the maternal and embryo/fetal toxicity of DFO, the results of this study confirm and extend some data of previous investigations in pregnant rats, rabbits and mice (Lauro et al. 1968; Thomas and Skalicka 1980; Blanc et al. 1984). Only minor developmental effects (retardation of bone ossification, vertebral aplasia, bifurcation, and fusion of ribs) were seen following DFO exposure.

In the present study, pregnant mice were given DFO intraperitoneally at doses of 44, 88, 176, and 352 mg/kg per day throughout organogenesis. Similar amounts to the lowest two doses of DFO (44 and 88 mg/kg) have been administered to pregnant women for treatment of iron overload, with no evidence for major adverse fetal effects in association with DFO (Thomas and Skalicka 1980; Martin 1983; Blanc et al. 1984; Olenmark et al. 1987). Intraperitoneal exposure of pregnant mice to DFO on gd 6–15 resulted in maternal toxicity at 44, 88, 176, and 352 mg/kg per day, as evidenced by significantly lowered food consumption, maternal body weight at termination and corrected body weight. In animal developmental toxicology studies, maternal toxicity has been deemed to occur when administration of a test substance to a pregnant animal during any portion of gestation results in at least one of the following: death, significant reduction in maternal body weight, clinical signs of toxicity, deleterious effects on behavior, appearance, organ weight, organ function, or incidence of gross or microscopic lessions (Khera 1984, 1985; Black and Marks 1986).

In contrast, there was no evidence of embryo/fetal toxicity at 44, 88, and 176 mg/kg per day, whereas the number of live fetuses per litter was significantly reduced at the highest dose of DFO compared with the control group. However, the incidence of fetuses with skeletal variations was significantly increased in the 88, 176, and 352 mg/kg per day groups, although the number of litters with skeletal defects at these doses did not differ significantly from those in the control group. Consequently, developmental toxicity of DFO was only present at 352 mg/kg per day, a dose which also produced significant maternal toxicity. An association between maternal and embryo/fetal toxicity was reported when a number of teratogenicity studies in rodent species – including diverse mouse strains – were extensively reviewed (Khera 1984, 1985).

On the other hand, it has been reported that since DFO is negligibly absorbed across the gastrointestinal tract, and is a charged and relatively large molecule, it should not be expected to cross the placenta (Blanc et al. 1984; Tenenbein 1990). Thus, although in the current study no pharmacokinetic data were obtained, the developmental toxicity observed here may be the direct consequence of maternal toxicity.

In recent reviews on the consequences of iron overdose and its treatment with DFO during gestation (Olenmark et al. 1987; McElhatton et al. 1991), it was concluded that as iron overdose in human pregnancy can be fatal, and because no major adverse fetal effects have been associated with DFO treatment during gestation, the use of this drug in such situations might be appropriate although the risk of spontaneous abortion should not be excluded.

In the present study, the no-observable-adverse-effect level (NOAEL) for developmental toxicity of DFO was 176 mg/kg per day. The lowest-observable-adverse-effect level (LOAEL) for maternal toxicity was 44 mg/kg per day, whereas the NOAEL for maternal toxicity would be lower than the lowest dose of DFO administered. This fact would indicate that DFO might be toxic in pregnant women under current conditions of clinical use. Consequently, extreme caution in the administration of DFO is suggested, if therapy with this chelator is decided in iron or aluminium overdoses during pregnancy. Further studies testing the effects of DFO in pregnant animals which were previously overloaded with iron or aluminium might also be of concern.

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