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Short communication

Fetal cartilage malformation by intravenous administration of indium trichloride to pregnant rats

Mikio Nakajima [a], Hiroki Takahashi [a], Ken Nakazawa [b], Makoto Usami [b,*]

[a] *Pharmaceuticals R and D Division, Asahi Kasei Pharma Corporation, Japan*
[b] *Division of Pharmacology, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya, Tokyo 158-8501, Japan*

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Abstract

The effects of indium on bone and cartilage development in rat fetuses were examined. Pregnant Sprague Dawley (SD) rats were treated with indium trichloride (0.1, 0.2, or 0.3 mg/kg) by single intravenous administration on Day 10 of gestation, and their fetuses were examined on Day 21. Half of each litter was prepared for skeletal examinations using a skeletal double-staining technique to allow evaluation of cartilage as well as bone. Dose-related increased incidences of external and skeletal fetal malformations occurred at doses of 0.2 mg/kg or more. The incidences of cartilage malformations in the vertebrae, ribs, and forepaw phalanges were significantly increased at 0.3 mg/kg. Malformations of the axial bone were accompanied by cartilage malformations. It was concluded from these results that indium produced cartilage malformations, that were considered to be the underlying cause for the majority of fetal skeletal malformations observed in rats in this study.

Keywords: Cartilage; Bone; Skeleton; Fetus; Malformation; Indium trichloride; Sprague Dawley rat; Teratogenicity

1. Introduction

Indium, a metal commonly used for semiconductors in industry and for scintigraphy in medicine, is teratogenic in rats. Single intravenous administration of indium trichloride (0.4 mg/kg) to pregnant rats on Days 8, 9, or 10 of gestation caused external, skeletal, and visceral malformations in the fetuses [1,2]. Brachyury, anury, kinked tail, anal atresia, and oligodactyly were observed as external malformations. Undesdended testis and dilatation of renal pelvis were observed as visceral malformations. Skeletal malformations were observed in the vertebrae, ribs, and sternebrae.

Indium may also affect cartilage development in the fetuses since the axial skeleton is formed by replacement of the cartilage by bone [3]. Potential cartilage malformations produced by indium, however, have not been reported, since fetal specimens in previous studies were examined following staining only for ossified bones using alizarin red S. Therefore in the present study, we extended the examination of indium teratogenicity in rats by using a double-staining method, which allowed an assessment of both fetal bone and cartilage development in rat fetuses.

2. Materials and methods

2.1. Animals and administration of indium

Pregnant Sprague Dawley (SD) rats (Crl: CD (SD), 9–11 week's old, weighing 240–280 g, Charles River laboratories Japan, Inc., Kanagawa, Japan) at Days 5 or 6 of gestation (sperm positive vaginal smear, Day 0) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). They were housed as previously described [1]. Indium trichloride ($InCl_3 \cdot 4H_2O$, Wako pure chemical industry Ltd., Osaka, Japan) dissolved in physiological saline was injected into a tail vein of the pregnant rats on Day 10 of gestation. Pregnant rats in the control group were given physiological saline at 1 ml/kg in the same manner. Forty rats were divided into four groups (10 per group) and of which 8 (control), 10 (0.1 mg/kg), 9 (0.2 mg/kg), and 10 (0.3 mg/kg) rats were confirmed as pregnant at term.

2.2. Observation and examination

Clinical observation of the animals was made daily. Body weight was measured on Days 6, 8, 10, 12, 14, 16, 18, and 21 of gestation. Body weight gain was

* Corresponding author. Tel.: +81 3 3700 1141x342; fax: +81 3 3707 6950.
E-mail address: usami@nihs.go.jp (M. Usami).

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calculated for each interval between the body weight measurements. The pregnant rats were sacrificed on Day 21 of gestation by exsanguination under ether anesthesia. The gravid uterine horns were removed and the numbers of dead implants and live fetuses were counted. Live fetuses were sexed, weighed individually, and examined for external malformations. The placentas were weighed individually. All live fetuses were examined for potential skeletal malformations after standard KOH clearing and staining. Half of the fetuses from each litter was double stained with alizarin red S and alcian blue 8GS for assessment of bone and cartilage [4]. The remaining half of the fetuses from each litter was stained only with alizarin red S [5] as in a previous study [1]. No visceral examination of the fetuses was made in the present study.

2.3. Statistical analysis

A pregnant animal or a litter was used as a sample unit, and statistical significance of differences between the control and indium groups was examined at 5 and 1% probability levels. Fisher's exact test was used for categorical data. One-way analysis of variance was used for parametric data with homogeneous variance among the groups as determined by Bartlett test. Kruskal–Wallis H test was used for parametric data without homogenous variance and for nonparametric data. When the results of these parametric or nonparametric analysis of variance were significant at a 5% probability level, comparisons were made between the control and indium groups by parametric or nonparametric Dunnett's test using SAS Version 6.12 incorporated in a toxicology study-supporting system TOXstaff21 (CTC laboratory systems corp., Tokyo, Japan).

3. Results

No obvious toxicological signs and necropsy findings were observed in the pregnant rats in any experimental groups. There were no significant differences in the body weight of pregnant rats between the control and indium treatment groups, although reduced body weight gains were observed after the administration of indium on Days 14, 18, and 21 of gestation at 0.3 mg/kg and on Day 14 at 0.2 mg/kg probably due to the increased mortality of implants and the decreased fetal weights (Fig. 1). Table 1 shows fetal growth on Day 21 of gestation in pregnant rats treated with indium. There were no significant differences at 0.1 and 0.2 mg/kg. Mortality of implants was significantly increased at 0.3 mg/kg. There were dose-related decreases in fetal weight with remarkable significant decreases at 0.3 mg/kg in both male and female fetuses. Placental weight was significantly decreased at 0.3 mg/kg in female fetuses.

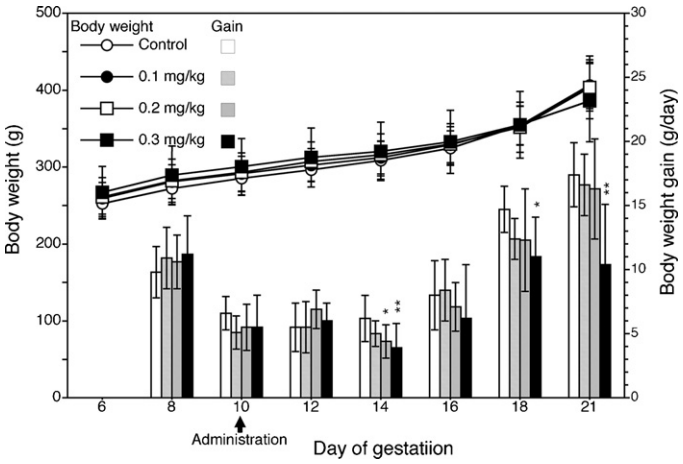

Fig. 1. Body weight and body weight gain of pregnant rats treated with indium trichloride on Day 10 of gestation. Vertical bars represent S.D. Asterisks indicate significant differences compared with the control group (*, $p < 0.05$, **, $p < 0.01$).

External malformations observed in the fetuses are shown in Table 2. There were significant increases in the incidence of external malformations at 0.2 and 0.3 mg/kg. Malformations of caudal part, that is, brachyury, anury, kinked tail, anal atresia, and of digits were observed at high incidences. The incidence of kinked tail at 0.2 mg/kg was higher than that at 0.3 mg/kg in contrast to the increased incidences of anury and brachyury at 0.3 mg/kg, suggesting that the kinked tail was a moderate manifestation of tail malformations by indium.

Skeletal abnormalities observed in alizarin red S-stained fetal specimens are shown in Table 3. There were significant increases in the incidence of skeletal malformations at 0.2 and 0.3 mg/kg. It was noted that malformations of the axial skeleton, that is, the vertebrae, ribs, and sternebrae were observed at high incidences. Malformations of the forelimb were also observed at high incidences at 0.3 mg/kg.

Skeletal abnormalities observed in double-stained fetal specimens are shown in Table 4. The incidences of cartilage malformations in the vertebrae, ribs, and forepaw phalanges were significantly increased at 0.3 mg/kg. Severe cartilage mal-

Table 1
Fetal growth in pregnant rats treated with indium trichloride

	Dose (mg/kg)			
	0 (Control)	0.1	0.2	0.3
No. of pregnant rats	8	10	9	10
No. of implants[a]	13.3 ± 1.3	13.6 ± 2.1	13.6 ± 1.9	14.6 ± 2.8
Mortality of implants (%)	1.0	4.5	3.3	31.3**
No. of live fetuses[a]	13.1 ± 1.5	13.0 ± 2.3	13.1 ± 1.8	10.1 ± 4.4
Sex ratio (M/M + F)	0.49	0.52	0.46	0.48
Fetal weight (g)[a]				
Male	5.73 ± 0.43	5.46 ± 0.44	5.33 ± 0.32	3.76 ± 1.16**
Female	5.48 ± 0.28	5.21 ± 0.48	5.05 ± 0.27	3.47 ± 0.95**
Placental weight (g)[a]				
Male	0.45 ± 0.05	0.45 ± 0.04	0.45 ± 0.04	0.38 ± 0.13
Female	0.45 ± 0.06	0.45 ± 0.06	0.44 ± 0.07	0.35 ± 0.11*

[a] Means ± S.D. are shown. Asterisks indicate significant differences compared with the control group (*, $p < 0.05$; **, $p < 0.01$).