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Effects of Indomethacin on Dams and Foetuses of C57 BL/6J Mice

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Abstract. Normal adult inbred C57 BL/6J mice were used to investigate the teratogenic and other possible toxic effects of indomethacin on both dams and their foetuses. The dams have been treated with various doses of the drug once each day from day 7 to day 12 of pregnancy. The prenatal administration of the drug at 2, 3, 4 or 6 mg/kg body weight has significantly increased foetal mortality rate and decreased the mean number of live foetuses. The highest dose used (6 mg/kg body weight) has also significantly decreased the live foetal body weight. None of the doses used has induced any foetal malformations but the highest doses of the drug (4 and 6 mg/kg body weight) used were toxic on the dams.

Introduction

Indomethacin (Id) is an effective non-steroid anti-inflammatory agent with marked analgesic properties [1; 2]. It is a potent inhibitor of prostaglandin biosynthesis [1; 3-5] and increases embryonic death and resorption in rabbits [6-10], mice [11] and rats [12; 13]. If given in late pregnancy, it reduces uterine motility and prolongs parturition resulting in high foetal mortality in rats [14]. However, the teratogenic effects of the drug have not been well investigated. Hence, an attempt is being made in the present study to investigate teratogenic and other possible toxic effects of Id given to pregnant C57 BL/6J mice in various doses at days 7-12 of gestation.

Materials and Methods

Normal adult inbred male and female C57 BL/6J mice, maintained within a closed colony, were used. They were housed in plastic boxes in an environmentally controlled room with a temperature of 22±1°C, a relative humidity of 45± 5% and

a light/dark cycle of 14/10 hrs. Food (commercial mouse chow available in Saudi Arabia) and water (in drinking bottles) were offered *ad libitum* throughout the study period. In each box, 3-5 nulliparous females were caged together with a single male.

The commencement of pregnancy was determined by the detection of vaginal plugs in mated females, and the day the plug was observed was counted as day 0 of gestation. On day 7 through day 12 of pregnancy, the females were injected once daily intraperitoneally (ip) with 1, 2, 3, 4 or 6 mg/kg Indomethacin (Merck Sharp and Dohme Limited, Hoddesdon, Hertfordshire, England) dissolved in vegetable oil. Control mice were injected with the corresponding volumes of the vehicle alone. On day 17 of pregnancy, the mice were killed by cervical dislocation and the number of live foetuses and resorptions was noted. Each foetus was then examined macroscopically, both externally and internally for gross developmental abnormalities. Fifteen foetuses from each group were then cleared and stained according to a modificaiton of the method of [15] for skeletal examinations.

The data were statistically analysed using a student's t-test and a 2X2 contingency table (X^2) for the actual numbers obtained [16].

Results

The effects of Indomethacin on pregnant mice and their foetuses are shown in Tables 1 and 2. The treatment with Id at doses of 4 and 6 mg/kg body weight has significantly ($P<0.05$ and $P<0.01$, respectively) increased female mortality with deaths on days 9-16 of gestation (Table 1). On the other hand, the mean number of live foetuses has significantly ($P<0.05$ at dose level of 3 mg/kg body weight, $P<0.01$ at other dose levels) and progressively decreased at the dose level of 3 mg/kg body weight through 6 mg/kg body weight (Table 2). Moreover, the rate of foetal resorptions has also significantly ($P<0.05$ at dose level of 2 mg/kg body weight, $P<0.01$ at other dose levels) and progressively increased at dose level of 2 mg/kg body weight through 6 mg/kg body weight, while the mean live foetal body weight was only significantly ($P<0.05$) decreased at the highest dose level used (6 mg/kg body weight, Table 2). Nevertheless, no gross developmental abnormality has been observed in any of the foetuses at any of the dose levels of the drug used.

Discussion

The present study has clearly demonstrated the toxic, deleterious and growth suppressing effects of Id on the treated mice and their foetuses. Toxic effects of the drug on foetuses had already been reported [6-14; 17; 18], but toxic effects of the drug on treated dams is only reported for the second time in the present study [18].

Table 1. Effects of Indomethacin on treated C57 BL/6J dams

Id dose (mg/kg)	No. of dams	Mortality rate (%)	Days of death
Control	20	0.0	–
1	15	0.0	–
2	15	0.0	
3	15	13.33	D13, D15
4	20	30.00*	D11-D16
6	20	55.00**	D9-D16

* Differences are statistically significant from the controls at $P<0.05$.

**Differences are statistically significant from the controls at $P<0.01$.

D – Day of pregnancy

Table 2. Effects of Indomethacin on foetuses of C57BL/6J dams examined on day 17 of pregnancy

Id dose (mg/kg)	No. of dams	No. of implantation sites	No. of foetuses (Mean+SE)	No. of live foetuses (Mean±SE)	Resorption (%)	Live foetal body wt. in gms (Mean±SE)	Abns. observed
Control	20	146	7.30±0.39	6.95±0.41	7(4.79)	0.77±0.04	None
1	15	112	7.47±0.55	7.27±0.59	3(2.68)	0.83±0.03	"
2	15	114	7.60±0.34	6.67±0.54	14(12.28)*	0.74±0.02	"
3	15	101	6.73±0.52	5.47±0.78*	19(18.81)**	0.82±0.03	"
4	20	150	7.50±0.40	4.60±0.83**	58(38.67)**	0.81±0.04	"
6	20	151	7.55±0.29	2.85±0.78**	94(62.25)**	0.66±0.05*	"

* Differences are statistically significant from the controls at $P<0.05$.

**Differences are statistically significant from the controls at $P<0.01$.

The toxic effects of the drug on treated dams might be due to a particular sensitivity of mice strains to the drug which is known to cause some degree of gastrointestinal, renal and hepatotoxicity [1; 2; 19-22]. Moreover, in accordance with the dose rate, the drug is known to cause denaturation or sloughing of gastric mucosa with changes in its permeability together with a reduction in acid output and increase in the production of histamine or pentagastrin [19; 20]. On the other hand, the inhibition of prostaglandins biosynthesis brought about by the drug may well modify renal function to the extent of pathology [2] and could also, at least in part, explains the observed reduction of foetal body weight. Such reduction could also be due to the inhibitory effects of the drug on cell proliferation [23-26] and/or to the known anorexogenic effects of the drug. However, foetal gross developmental defects have not been observed in the present study at all dose levels used which is in agreement with the results of [18].

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تأثيرات عقار الإندوميثاسين على أمهات وأجنة فئران التجارب من سلالة C57 BL/6J

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ملخص البحث. استعملت في هذه الدراسة فئران صادقة التوالد طبيعية ناضجة جنسيًا من سلالة C57BL/6J لدراسة العيوب الخلقية والتأثيرات السامة الأخرى المحتملة لعقار الإندوميثاسين على الأجنّة التي عولجت أمهاتها بجرعات مختلفة من هذا العقار يوميًا منذ اليوم السابع إلى اليوم الثاني عشر من بداية الحمل. ولقد أوضحت الدراسة أن حقن الأمهات بالجرعات ٢، ٣، ٤ أو ٦مجم/ كجم من وزن الجسم قد أدى إلى زيادة ذات دلالة معنوية في معدّلات موت الأجنّة وانخفاض ذي دلالة معنوية في معدّلات عدد الأجنّة الحيّة. هذا بالإضافة إلى انخفاض ذي دلالة معنوية في معدّلات أوزان الأجنّة الحيّة عند الجرعة ٦ مجم/ كجم من وزن الجسم مقارنة بالمجموعة الضابطة. كما أوضحت الدراسة بأن هذا العقار ليس له خاصية استحداث عيوب خلقية في أجنّة الأمهات المعالجة إلا أن له تأثيراً مميتًا (سامًا) على تلك الأمهات عند حقنه بالجرعات ٤ و ٦مجم/ كجم من وزن الجسم وهذا يتفق مع نتائج الدراسة السابقة التي استخدمت فيها السلالة SWR/J من فئران التجارب (١٨).