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Effects of Indomethacin on Dams and Foetuses of CBA Mice*

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ABSTRACT. Normal adult inbred CBA mice were used to investigate the teratogenic and other possible toxic effects of indomethacin on both dams and their foetuses. The dams were treated with various doses of the drug once each day from day 7 to day 12 of pregnancy. Treatments at 3, 4 or 6 mg/kg body weights significantly increased the foetal mortality rate and decreased the mean number of live foetuses. At a dose of 4 mg/kg body weight, the live foetal body weight was also significantly decreased. None of the doses used induced any foetal malformations, but higher ones (4 and 6 mg/kg body weight) were toxic to the dams.

Indomethacin (Id) is an effective non-steroid anti-inflammatory agent with marked analgesic properties (Flower 1974 and Nickander *et al.* 1979). It is a potent inhibitor of prostaglandin biosynthesis (Vane 1971, Tomlinson *et al.* 1972, Flower 1974 and Jansakul *et al.* 1990) and increases embryonic death and resorption in rabbits (El-Banna *et al.* 1976, Hoffman 1978, El-Banna 1980, 1983 and 1986), mice (Lau *et al.* 1973, Abou-Tarboush and Massoud 1993 and Abou-Tarboush 1995) and rats (Gavin *et al.* 1974 and Tanaka *et al.* 1991). If given in late pregnancy to rats, it reduces uterine motility and prolongs parturition, resulting in high foetal mortality (Aiken 1972).

The drug is recommended for the treatment of the active stages of rheumatoid arthritis, osteoarthritis, degenerative hip joint disease, gout, ankylosing spondylitis,

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bursitis and tendonitis. Moreover, it may be useful in relieving low-back pain and the pain following dental or bone surgery, as well as a short-term supportive measure to reduce fever.

Similar to previous studies on other strains of mice (Abou-Tarboush and Massoud 1993 and Abou-Tarboush 1995), the purpose of this study was to investigate teratogenic and other toxic effects of Id on pregnant CBA strain of mice at various dose levels administered on days 7-12 of gestation.

Materials and Methods

Normal adult inbred male and female CBA 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 10/14 hrs. Food (commercial mouse chow) 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 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 modification of the method of McLeod (1980) for skeletal examinations.

The data were statistically analysed using a student's t-test and a 2 x 2 contingency table (X^2) for the actual numbers obtained (Sokal and Rohlf 1981).

Results

Treatment with Id at doses of 4 and 6 mg/kg body weight significantly ($P < 0.05$ and $P < 0.01$, respectively) increased female mortality with deaths on days 9-16 of gestation (Table 1). The mean number of live foetuses significantly and progressively decreased at dose levels of 4 and 6 mg/kg body weight ($P < 0.05$ and P

< 0.01, respectively, Table 2). The rate of foetal resorptions had also significantly (P < 0.01) and progressively increased at dose levels of 3 mg/kg body weight through 6 mg/kg body weight, while the mean live foetal body weight was only significantly (P < 0.01) decreased at a dose level of 4 mg/kg body weight (Table 2). Nevertheless, no gross developmental abnormalities were observed in any of the foetuses at any of the dose levels used.

Table 1. Effects of Indomethacin on treated CBA dams

Id dose (mg/kg)	No. of dams used	No. of deaths of dams	Mortality rate (%)	Days of death
Control	20	–	0.0	–
1	10	–	0.0	–
2	10	–	0.0	–
3	15	2	13.33	D13, D14
4	20	5	25.00*	D10-D14
6	20	10	50.00*	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 CBA dams examined on day 17 of pregnancy

Dose used (mg/kg)	No. of dams used	No. of implantation sites	No. of foetuses (Means ± SE)	No. of live foetuses (Means ± SE)	No. of resorptions (%)	Live foetal body wt. in gms (Mean ± SE)	Abns. observed
Control	20	130	6.50 ± 0.36	6.10 ± 0.38	8(6.15)	0.75 ± 0.02	None
1	10	72	7.20 ± 0.39	6.50 ± 0.50	7(9.72)	0.72 ± 0.04	None
2	10	63	6.30 ± 0.40	5.60 ± 0.22	7(11.11)	0.72 ± 0.04	None
3	15	101	6.73 ± 0.30	5.53 ± 0.68	18(17.82)**	0.71 ± 0.03	None
4	20	143	7.15 ± 0.30	4.70 ± 0.69*	49(34.27)**	0.66 ± 0.03**	None
6	20	142	7.10 ± 0.36	2.95 ± 0.80**	83(58.45)**	0.71 ± 0.05	None

*Differences are statistically significant from the controls at $P < 0.05$.
** Differences are statistically significant from the controls at $P < 0.01$.

Discussion

The present study has clearly demonstrated the toxic and deleterious effects of Id on treated CBA mice and their foetuses. Similar effects of the drug have been reported for other mammals and other mouse strains (Aiken 1972, Lau *et al.* 1973, Gavin *et al.* 1974, El-Banna *et al.* 1976, Csaba *et al.* 1978, Hoffman 1978, El-Banna 1980, 1983, 1986, Tanaka *et al.* 1991, Abou-Tarboush and Massoud 1993 and Abou-Tarboush 1995).

The toxic effects of the drug on treated dams might be due to a particular sensitivity of the mouse strains to the drug which is known to cause some degree of gastrointestinal, renal and hepato-toxicity (Flower 1974, Arrigoni-Martelli 1977, Shriver *et al.* 1977, Nickander *et al.* 1979, Guglietta *et al.* 1990 and Wallace *et al.* 1991). 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 increases in the production of pharmacologically active substances such as histamine or pentagastrin (Arrigoni-Martelli 1977 and Shriver *et al.* 1977). On the other hand, the inhibition of prostaglandin biosynthesis brought about by the drug may well modify renal function to the extent of pathology (Nickander *et al.* 1979) and could also, at least in part, explain the observed reduction of foetal body weight. Such a reduction could also be due to the inhibitory effects of the drug on cell proliferation (Kuwayama *et al.* 1990, Wlodarski 1990, Alino *et al.* 1991 and Gelin *et al.* 1991) and/or to the known anorexogenic effects of the drug. Furthermore, it could also be concluded that the reduction in some of the free amino acids in uterine fluid caused by Id treatment could lead to a reduction in the growth rates of the embryos and consequently to increases in embryonic deaths and resorptions (El-Banna *et al.* 1993). Similar to the observations on other mouse strains (Abou-Tarboush and Massoud 1993 and Abou-Tarboush 1995), foetal gross developmental defects were not observed in this mouse strain at any dose level of the drug used. Hence, it may be concluded from the present and other studies (Abou-Tarboush and Massoud 1993 and Abou-Tarboush 1995) that Id is toxic to pregnant mice and their foetuses, but it is not teratogenic.

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تأثيرات عقار الإندوميثاسين على أمهات وأجنة فئران التجارب من سلالة CBA

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استعملت في هذه الدراسة فئران صادقة التوالد طبيعية ناضجة جنسياً من سلالة **CBA** لدراسة العيوب الخلقية والتأثيرات السامة الأخرى المحتملة لعقار الإندوميثاسين على الأجنة التي عولجت أمهاتها بجرعات مختلفة من هذا العقار يومياً منذ اليوم السابع إلى اليوم الثاني عشر من بداية الحمل . ولقد أوضحت الدراسة أن حقن الأمهات بالجرعات ٣ ,٤ أو ٦ مجم/ كجم من وزن الجسم قد أدى إلى زيادة ذات دلالة معنوية في معدلات موت الأجنة وانخفاض ذي دلالة معنوية في معدلات عدد الأجنة الحية . هذا بالإضافة إلى انخفاض ذي دلالة معنوية في معدلات أوزان الأجنة الحية عند الجرعة ٤ مجم/ كجم من وزن الجسم مقارنة بالمجموعة الضابطة . كما أوضحت الدراسة بأن هذا العقار ليس له خاصية استحداث عيوب خلقية في أجنة الأمهات المعالجة إلا أن له تأثيراً مميتاً (ساماً) على تلك الأمهات عند حقنه بالجرعات ٤ و ٦ مجم/ كجم من وزن الجسم .