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Teratogenic Studies with Hiconcil (Amoxicillin) on SWR/J Mice

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ABSTRACT. Normal adult inbred SWR/J mice were used in the investigation of the possible teratogenic and other effects of Hiconcil. Pregnant females were treated intraperitoneally (ip) daily from day 7 through day 12 of pregnancy. Hiconcil at a dose of 500 or 650 mg/kg body weight resulted in teratogenic effects in foetuses of treated mothers including the development of abnormal hindlimbs, heads and tails. Moreover, some abnormally small foetuses were also obtained from treated mothers given these doses of the drug. However, the drug appeared safe to treated dams at all dose levels and throughout gestation.

Hiconcil (Amoxicillin) is a semisynthetic broad spectrum antibiotic that is effective against many Gram-positive and Gram-negative bacteria infecting man, such as those causing skin infections, gonorrhoea, urinary and respiratory tract infections and otitis media (Sutherland *et al.* 1972, Alergant 1973, Burns and Devitt 1974, Pankey 1974, Nolan and White 1978, Schwartz *et al.* 1981, Savard-Fenton *et al.* 1982, Avener *et al.* 1983, Stahl *et al.* 1984, Masterton *et al.* 1985, Gerstner *et al.* 1989 and Anon 1990). It has also been used in the treatment of various experimental infections in mice (Boon and Beale 1987, Beale *et al.* 1988, Gisby and Beale 1988, Gelber 1991 and Gisby *et al.* 1991).

The use of antibiotics is sometimes obligatory during pregnancy although there may be risks associated with the use of chemotherapeutic agents during this critical time of development. Hence, the safety of such drugs to both mother and the

developing offspring is of paramount importance and should be ascertained well before use. Since the study of Abou-Tarboush (1994) has indicated that the prenatal administration of Hiconcil at a dose of 500 or 650 mg/kg body weight resulted in both teratogenic and toxic effects on foetuses of treated CBA mothers, an attempt was made in the present study to evaluate the effects of various doses of Hiconcil both on pregnant SWR/J mice and on their foetuses.

Materials and Methods

The mice used were adult inbred SWR/J mice bred in the Department of Zoology Animal Facility of King Saud University for several generations. All laboratory mice in the Facility were housed in plastic boxes that were maintained in an environmentally - controlled room with a light/dark cycle of 14/10 hrs. Each box housed 3-5 nulliparous females, together with a single male.

The detection of vaginal plugs in mated females signalled the commencement of pregnancy, and the day the plug was observed was designated as day 0 of gestation. Starting from day 7 through day 12 of gestation, the females were injected once daily intraperitoneally (ip) with 300, 500, or 650 mg/kg of Hiconcil (Mead Johnson and Company, Evansville, Indiana, U.S.A.) in sterile physiological saline, which correspond to the normal dosage levels used in humans. Control mice were injected with only the corresponding volumes of sterile physiological saline. The mice were killed by cervical dislocation on day 17 of gestation and the number of live foetuses and resorptions was noted. Each foetus was then examined macroscopically both externally and internally for gross developmental defects. Ten foetuses (from five different females) from each group were subsequently cleared and stained for skeletal examinations according to the modification of the method of McLeod (1980) by Abou-Tarboush (1987).

Data were statistically analysed using the Student t-test and a 2x2 contingency table (X^2) for the actual numbers obtained (Sokal and Rohlf 1981).

Results

Table 1 reveals that there were no significant differences between Hiconcil-treated and control groups in mean number of implantation sites, mean live litter size, the proportion of resorptions or overall mean live foetal body weight at any dose level used. However, a low incidence of abnormalities was observed in eight foetuses obtained from dams treated at 500 and 650 mg/kg (Table 1). The defects included abnormal hindlimbs, tails and heads in four of the foetuses (Figs. 1-3). Two

types of tail abnormalities were observed, a shortening of tail length, as well as a U-shaped bending of it. Moreover, backward bending of hindlimbs was also observed. However, the abnormalities of the head were of an unclassified nature. The mean live body weight of these defective foetuses was significantly ($P < 0.05$) lower than that of controls (Table 2). The other four foetuses were abnormally small (Figs. 1, 3) and their mean live body weight was very much less than that of controls. The difference in mean live body weight between these foetuses and that of the controls was highly significant at $P < 0.01$ (Table 2). Skeletal abnormalities were not seen in any group at any dose level.

Table 1. Effects of various doses of Hiconcil given daily to pregnant SWR/J mice on their foetuses taken on day 17 of pregnancy

Hiconcil dose (mg/kg)	No. of females	Mean no. of implantation sites (± S.E.)	Mean no. of live foetuses (± S.E.)	Resorption (%)	Mean live foetal body wt. (g) (± S.E.)	Abnormalities observed*
Control	30	9.27 ± 0.44	9.00 ± 0.45	8(2.88)	0.95 ± 0.02	None
300	30	9.23 ± 0.34	9.07 ± 0.33	5(1.81)	0.91 ± 0.02	None
500	30	8.90 ± 0.43	8.63 ± 0.44	8(3.00)	0.93 ± 0.02	1 H,L
650	30	9.73 ± 0.44	9.13 ± 0.50	18(6.16)	0.91 ± 0.02	2L, T; 1H

*H = Abnormal Head L = Abnormal Hindlimbs T = Abnormal Tail

Table 2. Variations in mean live foetal body weight of abrnomal foetuses taken from Hiconcil treated SWR/J and control female mice on day 17 of gestation

Mean live foetal body wt. (g) of the control (± S.E.)	Mean live foetal body wt. (g) of defective foetuses (± S.E.)	Mean live foetal body wt. (g) of abnormally small foetuses (± S.E.)
0.95 ± 0.02	0.82 ± 0.06*	0.65 ± 0.02**

*Differences in body weight between treated and controls are statistically significant at $P < 0.05$.
**Differences in body weight between treated and controls are statistically significant at $P < 0.01$.

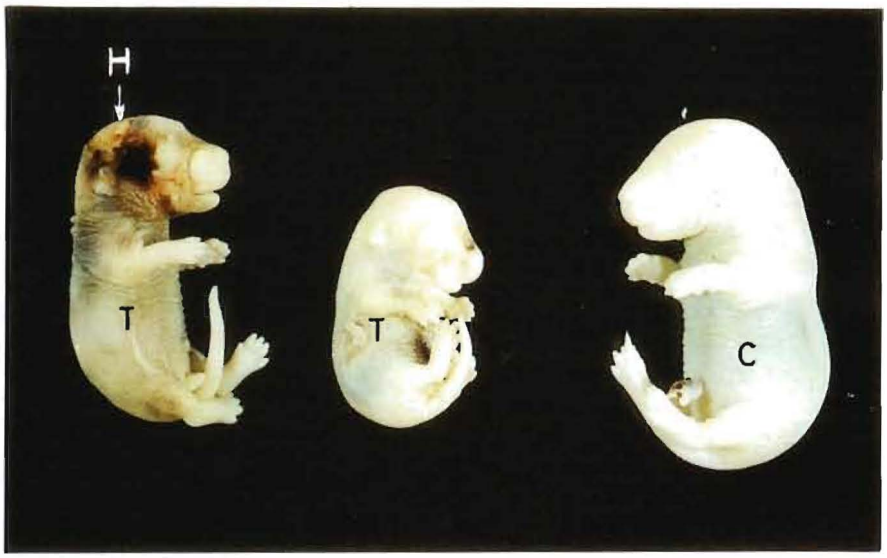

Fig. 1. Foetuses obtained on day 17 of pregnancy from treated dams (500 mg/kg Hiconcil) and from controls. (H = abnromal head)

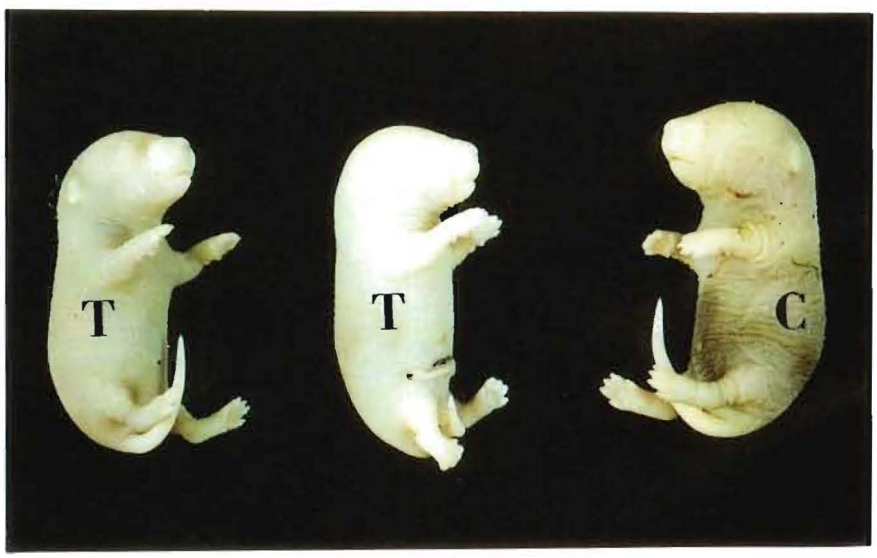

Fig. 2. Foetuses obtained on day 17 of pregnancy from treated dams (650 mg/kg Hiconcil) and from controls. C= control T = treated

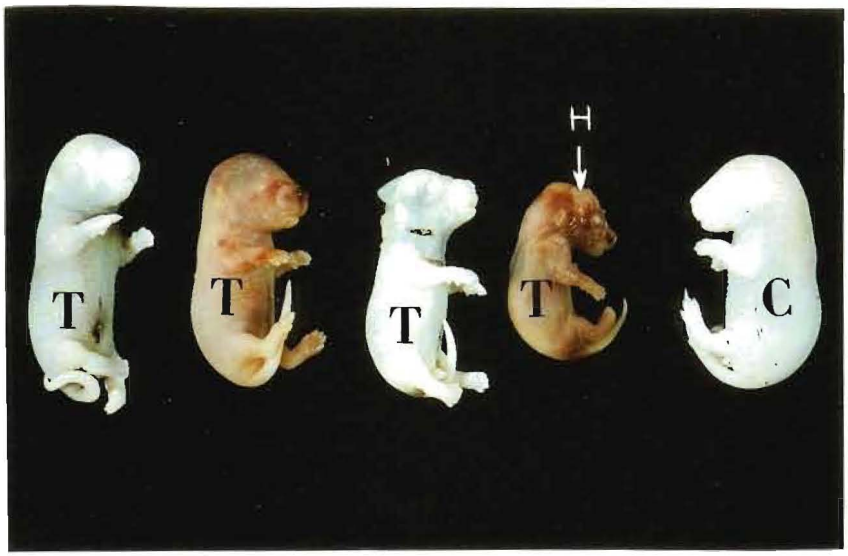

Fig. 3. Foetuses obtained on day 17 of pregnancy from treated dams (500 mg/kg Hiconcil) and from controls. (H = abnromal head) C = control T = treated

Discussion

As seen in Abou-Tarboush (1994) in CBA mice, teratogenic effects of Hiconcil were observed on developing SWR/J mice in the present study only in mouse embryos whose mothers had been treated with 500 and 650 mg/kg body weight of the drug during organogensis. However, SWR/J mouse embryos seem more resistant to the toxic and the growth-suppressing effects of Hiconcil than CBA counterparts. The proportion of resorptions was significant ($P < 0.05$) at 500 and 650 mg/kg of Hiconcil in the CBA strain (Abou-Tarboush 1994), but was not significant at any dose level used in the SWR/J strain. Furthermore, the mean live body weight of the defective foetuses was about 50% that of the control in the CBA strain (Abou-Tarboush 1994), but was approximately 80% compared to the control in the SWR/J strain.

Such results cast doubt on the view that antibiotics (natural or synthetic) are completely safe throughout pregnancy in humans (Weistein 1975, Weistein 1979, Berkowitz *et al.* 1981, Schwartz 1981 and Briggs *et al.* 1986). In fact, Masterton *et al.* (1985) and Gerstner *et al.* (1989) have reported that Hiconcil is effective and safe even at a single-dose of 3g or a 4-day course of 3 doses of 750 mg when given over a mean duration of pregnancy of 25 weeks in humans. Differences in the human

studies and the present studies could well be due to the time of drug administration or to the species genetic differences or to both. Moreover, the treated mouse dams did not show any ill effects at any dose level of the drug used during any time of gestation in terms of general activities and signs of disease.

Hence, this study suggests that Hiconcil may produce teratogenic and toxic effects on foetuses whose mothers are treated with high doses of this antibiotic during organogenesis. This suggests that, if possible, application of this drug should be avoided in the first 3-7 weeks of pregnancy in humans, which is equivalent to the time teratogenesis occurs in the current mouse model.

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دراسة تشويهية لعقار الهايكونسيل (الأموكسيسيلين) على فئران التجارب من السلالة SWR/J

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استعملت في هذه الدراسة حيوانات طبيعية يافعة من فئرات التجارب التي تنتمي للسلالة النقية المعروفة بإسم **SWR/J** لدراسة احتمال حدوث تشوهات خلقية نتيجة لإستعمال عقار الهايكونسيل (أموكسيسيلين) على الأجنة التي حقنت أمهاتها بالجرعات ٣٠٠ ، ٥٠٠ ، أو ٦٥٠ مغم/ كغم من وزن الجسم عند الحقن وخلال الفترة من اليوم السابع إلى اليوم الثاني عشر من الحمل . ولقد كان الحقن مرة واحدة يومياً . هذا وقد أوضحت نتائج هذه الدراسة أن حقن هذا العقار للأمهات خلال الفترة المشار إليها وبالجرعة ٥٠٠ أو ٦٥٠ مغم/ كغم من وزن الجسم له تأثيراً مشوهاً على الأجنة المتحصل عليها من الأمهات المعالجة . إلا أنه ليس لهذا العقار أية تأثيرات ضارة على الأمهات المعالجة بأي من الجرعات المستخدمة في أي وقت خلال فترة الحمل .

من الواضح من نتائج هذه الدراسة ونتائج الدراسة السابقة أن لعقار الهايكونسيل تأثيراً مشوهاً على الأجنة المتحصل عليها من أمهات عولجت بجرعات عالية من هذا العقار خلال فترة تكون الأعضاء ، لذا ينصح بتجنب استخدام هذا العقار من قبل النساء خلال الأسابيع ٣-٧ الأولى من فترة الحمل ، إن أمكن ذلك .