

Maternal and Fetal Toxicity of Mefenamic Acid In SWR/J Mice

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Abstract: Normal adult inbred SWR/J mice were used to investigate the teratogenic, and other possible toxic effects of mefenamic acid (Ponstan) on both dams and their fetuses. The dams were treated orally with various dose levels of the drug once each day from day 7 to day 12 of pregnancy. Treatment at 25, 75 or 150 mg/kg body weight of the drug had no toxic effect on fetuses and their dams. However, treatment at 300, 450 or 600 mg/kg body weight significantly ($P<0.01$) increased females' mortality. Moreover, treatment at 450 or 600 mg/kg significantly ($P<0.01$) decreased the mean body weight gain of the treated females on day 17 of their pregnancies or at the day of their death. On the other hand, the mean number of live fetuses significantly ($P<0.01$) and progressively decreased at the dose levels of 300, 450 or 600 mg/kg of the drug. Furthermore, the percentage of resorptions was also significantly ($P<0.01$) and progressively increased at 300, 450 or 600 mg/kg, while the mean live fetal body weight was only significantly ($P<0.05$) decreased at the dose levels of 450 or 600 mg/kg of the drug. However, apart from abnormal hindlimbs in only one fetus (300 mg/kg), no gross developmental defects were observed in any of the fetuses at any of the dose levels of mefenamic acid used.

Key Words: Mefenamic acid, teratogenicity, fetomaternal toxicity, SWR/J mice.

Introduction

Mefenamic acid (Ponstan) is a non-steroidal anti-inflammatory agent with analgesic properties and a demonstrable antipyretic effect (Flower *et al.*, 1980; Smolinske *et al.*, 1990; Brigges *et al.*, 1994). As an analgesic, it has been used to relieve pain of rheumatic conditions, soft-

tissue injuries, other painful musculoskeletal conditions and dysmenorrhea (Flower *et al.*, 1980). As an anti-inflammatory agent, it has mainly been tested in short-term treatment trials of osteoarthritis and rheumatoid arthritis (Flower *et al.*, 1980). Despite its effectiveness, it frequently causes side effects, mainly in the gastrointestinal

system, including dyspepsia and diarrhea associated with steatorrhea (Chadwick and Hossenbous, 1976; Flower *et al.*, 1980). There have also been reports of bleeding ulcers associated with the drug (Wolfe *et al.*, 1976; Smolinske *et al.*, 1990). Other less frequently observed side effects include transient abnormalities of hepatic and renal functions, central nervous system effects and skin rashes (Flower *et al.*, 1980). Moreover, a potentially serious side effect of the drug is a hemolytic anemia, possibly of an autoimmune type in isolated cases (Jackson *et al.*, 1970; Kennedy and Robertson, 1971).

Mefenamic acid, similar to other aspirin-like drugs, is a potent inhibitor of prostaglandin biosynthesis and there is an increasing literature on the potentially deleterious effects of such drugs on human pregnancy (Moncada *et al.*, 1975; Moncada and Vane, 1979; Rudolph, 1981; Truter *et al.*, 1986; Menahem, 1991a, b).

However, to the best of my knowledge, no experimental study has been reported on the teratogenic effect of mefenamic acid in mice and rats with the sole exception of the study of Montenegro and Palomino (1990) who have reported cleft palate induced by mefenamic acid, in conjunction with four other prostaglandin inhibitors in offspring of treated female mice.

In Saudi Arabia, however, mefenamic acid is commonly used through prescriptions, and in high doses without prescription (Abou-Tarboush, 1996). Hence, the purpose of this study was to

investigate the possible toxic effects of the drug on pregnant SWR/J mice, and of its possible teratogenic and other embryotoxic effects on the offspring of such females at various dose levels administered on days 7 to 12 of gestation (The period corresponding to the duration of organogenesis in mice).

Materials and Methods

Inbred normal SWR/J male and female mice, 10-12 weeks old and weighting 28.5-33.7 g, were used throughout the study. Animals were kept and bred in an environmentally controlled room with a temperature of $22\pm 1^{\circ}\text{C}$, a relative humidity of $45\pm 5\%$ and a light/dark cycle of 10/14 h. Mouse food (commercially available in Saudi Arabia) and water were offered *ad libitum*.

In each box, 4-5 nulliparous females were caged together with a single male. The females were examined each morning for the presence of a vaginal plug; the day the plug was detected was considered as day 0 of gestation and the pregnant females were placed in separate cages. A total of 105 pregnant females were used, and were divided into 7 groups (I to VII), 15 females in each. On days 7-12 of pregnancy, the females of groups II-VII were treated orally once daily with 25, 75, 150, 300, 450, or 600 mg/kg of Ponstan (Parke, Davis and Company, Pontypool, U.K.) dissolved in

Table 1. Effect of various dose levels of mefenamic acid (Ponstan) on SWR/J female mice.

Ponstan dose (mg/kg)	No. of females used	% of females mortality	Days of death & No.	(Mean±SE) Body weight gain in g at	
				D7	D17 or days of death
Control	15	00.00	-	3.37±0.26	15.11±0.80
25	15	00.00	-	2.83±0.25	17.33±0.89
75	15	00.00	-	3.35±0.12	15.08±1.72
150	15	00.00	-	2.91±0.15	16.80±1.26
300	15	26.67*	D11(2); D12(1); D14(1)	3.12±0.26	12.04±2.53
450	15	80.00**	D9(1); D10(3); D11(1); D13(2); D15(3)	3.52±0.38	-1.31±2.56**
600	15	93.33**	D9(1); D10(2); D11(1); D12(2); D13(5); D14(1); D15 (2)	2.94±0.14	-2.61±1.81**

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

** Differences are statistically significant from the control group at $P<0.01$ Day7-D17=day of gestation.

sterile normal saline. Control mice (group I) were similarly treated with the corresponding volumes of the vehicle alone, and the animals were kept under daily observations. On day 17 of pregnancy, the mice were killed by cervical dislocation and the numbers of live fetuses and resorptions were noted. Each fetus was then examined macroscopically, both externally and internally for gross developmental abnormalities. Ten to fifteen fetuses 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 2x2 contingency table (X^2) for the actual numbers obtained (Sokal and Rohlf, 1981).

Results

The treatment with Ponstan at the dose levels of 300, 450 or 600 mg/kg body weight significantly ($P<0.01$) increased females' mortality with deaths on days 9-15 of mortality with deaths on days 9-15 of gestation (Table 1). The highest (7.78%)

Table 2. The numbers and percentages of mefenamic acid-treated SWR/J female mice that have died at various days of gestation.

Ponstan dose (mg/kg)	Days of death						
	D9	D10	D11	D12	D13	D14	D15
Control							
25.75 or 150	-	-	-	-	-	-	-
300	-	-	2	1	-	1	-
450	1	3	1	-	2	-	5
600	1	2	1	2	5	1	2
Total	2	5	4	3	7	2	7
% mortality of treated dams.	2.22	5.56	4.44	3.33	7.78	2.22	7.78

D=day of gestation.

and the lowest (2.22%) female mortality occurred on days 13 and 15, and 9 and 14, respectively (Table 2). Moreover, treatment with the drug at the dose levels of 450 or 600 mg/kg significantly ($P<0.01$) decreased the mean body weight gain, at day 17 or day of death, of the treated females (Table 1). On the other hand, mean numbers of live fetuses were significantly ($P<0.01$) and progressively decreased at the dose levels of 300, 450 or 600 mg/kg of the drug (Table 3). Furthermore, the percentage of resorptions was also significantly ($P<0.01$) and progressively increased at the same dose levels (Table 3), while the mean live fetal body weight was only significantly ($P<0.05$) decreased at the dose levels of 450 or 600 mg/kg of Ponstan. However, no gross developmental abnormality was observed in any of the fetuses at any of the dose levels of the drug used, except that one fetus with abnormal hindlimbs was observed in the treated group at the dose

level of 300 mg/kg (Table 3).

Discussion

The present study has clearly demonstrated the toxic and deleterious effects of high doses of mefenamic acid (Ponstan) on treated pregnant SWR/J mice and on their fetuses. However, no developmental abnormalities of any type, with the sole exception of a single fetus with abnormal hindlimbs in the group treated at the dose level of 300 mg/kg of the drug, were observed in the fetuses of treated females at any of the dose levels used.

The toxic and potentially deleterious effects of this drug, and of other prostaglandin inhibitors on treated mouse dams and on their fetuses might be due to the reported detrimental side effects that they cause in experimental animals and man. Such effects are well documented (Lewis and Schulman, 1973; Collins and Turner, 1975; Turner and Collins, 1975;

Table 3. Effects of various dose Levels of mefenamic acid (Ponstan) on fetuses of treated SWR/J dams examined On day 17 of pregnancy.

Ponstan dose (mg/kg)	No. of females used	No. of implantation sites	No. of fetuses (Mean±SE)	No. of live fetuses (Mean±SE)	No. of resorptions (%)	Live fetal body weight in g (Mean±SE)	Abnormalities observed
Control	15	178	11.87±0.36	11.13±0.36	11 (6.18)	0.92±0.02	None
25	15	169	11.27±0.41	10.93±0.42	5(3.96)	0.93±0.02	None
75	15	163	10.87±0.36	10.07±0.70	12 (7.36)	0.96±0.02	None
150	15	187	12.47±0.72	12.00±0.79	7 (3.74)	0.88±0.02	None
300	15	176	11.73±0.27	8.27±0.73*	52 (29.55)**	0.88±0.02	1 fetus with abn. Hindlimbs
450	15	177	11.80±0.47	2.40±1.29**	141 (79.66)**	0.84±0.03*	None
600	15	180	12.00±0.49	0.80±0.80**	168 (93.33)**	0.80±0.01*	None

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

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

Moncada and Vane, 1979; Flower *et al.*, 1980; Rudolph, 1981; Smolinske *et al.*, 1990; Menahem, 1991a, b) and include gastrointestinal effects, diarrhea, bleeding ulcers, transient abnormalities of the hepatic and renal functions, central nervous system effects, uncoupling of oxidative phosphorylation and the ability of these drugs to inhibit or interfere with a variety of enzymes and cellular systems. Moreover, duct constriction and even duct closure could be induced by such prostaglandin inhibitors leading to an increase in perinatal morbidity and mortality (Truter *et al.*, 1986; Moise *et al.*, 1988; Menahem, 1991a, b). Furthermore, severe cases of closure fetal duct has been reported as possible causes of hydrops fetalis and of intrauterine fetal death (Mogilner *et al.*, 1982; Truter *et al.*, 1986). On the other hand, the inhibition of prostaglandin biosynthesis that might have resulted by the high doses of the drug, could have well resulted in the modification of both hepatic and renal functions (Nickander *et al.*, 1979) which could, at least in part, explain the observed reduction in fetal body weight. Such a reduction could also be due to the known anorexogenic effect of the drug on the treated females (Flower *et al.*, 1980; Abou-Tarboush, 1996).

The present observation of virtual absence of fetal gross defects is in line with similar effects of another prostaglandin inhibitor (Indomethacin) on pregnant mice (Abou-Tarboush and Massoud, 1993; Abou-Tarboush, 1995, 1996).

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السمية الأمومية والجنينية لحمض الميفيناميك في فئران السلالة SWR/J

فيصل محمد أبو طربوش

قسم علم الحيوان - كلية العلوم - جامعة الملك سعود - ص.ب (٢٤٥٥) الرياض ١١٤٥١ - المملكة العربية السعودية .

استعملت في هذه الدراسة فئران مختبرية طبيعية ناضجة جنسياً من السلالة SWR/J لدراسة التأثيرات السامة والمشوهة المحتملة لعقار حمض الميفيناميك (البونستان) على الأجنة التي عُولمت أمهاتها بجرعات مختلفة من هذا العقار يومياً منذ اليوم السابع وحتى اليوم الثاني عشر من بداية الحمل وعلى الأمهات المعاملة نفسها . ولقد أوضحت نتائج هذه الدراسة أن إعطاء الأمهات هذا العقار عن طريق الفم وبالجرعات ٢٥ أو ٧٥ أو ١٥٠ مجم / كجم من وزن الجسم لم يكن له تأثير سام على الأجنة أو على أمهاتها ، إلا أن الجرعات ٣٠٠ أو ٤٥٠ أو ٦٠٠ قد أدت إلى زيادة نسبة موت الأمهات المعاملة بطريقة ذات دلالة معنوية ($p < 0.01$) ، كما أدت هذه المعاملة إلى انخفاض متوسطات الزيادة في أوزان أجسام الأمهات ، عند اليوم السابع عشر من فترة الحمل (أو عند يوم موتها) ، وبطريقة ذات دلالة معنوية ($p < 0.01$) عند الجرعتين ٤٥٠ و ٦٠٠ مجم / كجم . كما أوضحت نتائج هذه الدراسة أن هناك انخفاضاً في متوسطات عدد الأجنة الأحياء المتحصل عليها من الأمهات المعاملة وبطريقة ذات دلالة معنوية ($p < 0.01$) عند الجرعات ٣٠٠ و ٤٥٠ و ٦٠٠ مجم / كجم . هذا بالإضافة إلى أن المعاملة بهذا العقار قد أدت ، أيضاً ، إلى زيادة نسبة الأجنة الممتصة المتحصل عليها ، وبطريقة ذات دلالة معنوية عند نفس الجرعات ، بينما انخفضت متوسطات أوزان أجسام الأجنة الأحياء المتحصل عليها وبطريقة ذات دلالة معنوية ($p < 0.05$) عند الجرعتين ٤٥٠ و ٦٠٠ مجم / كجم فقط . ولقد أوضحت نتائج هذه الدراسة ، أيضاً ، أن هذا العقار ليس له خاصية استحداث عيوب خلقية في أجنة الأمهات المعاملة عند كل الجرعات المستخدمة ويستثنى من ذلك الحصول على جنين واحد فقط معاب الأرجل الخلفية من مجموعة الأمهات المعاملة بالجرعة ٣٠٠ مجم / كجم .