EMBRYOTOXIC AND TERATOGENIC EFFECT OF DISODIUM GLYCYRRHETINIC ACID HEMIPHTHALATE IN MICE

H. Marzban

Department of Anatomy, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract - Disodium glycyrrhetinic acid hemiphthalate (GAP) was evaluated for developmental toxicity in pregnant NMRI mice. GAP was administered intraperitoneally on days 8, 9, 10, of gestation at doses of 0, 25, 50 and 75 mg/kg/day. On gestation day of 18, after operational delivery, the fetuses were examined for soft tissue, external and skeletal defects.

Increased absorptions, dead fetuses, and reduce fetal body weight per litter were observed at doses of 50 and 75 mg/kg/day. In addition by using different doses of GAP at different critical gestation times, developmental delay was observed as reduction in ossification centers and vertebral number in caudal region. It seems that GAP at doses of (50 and 75 mg/kg/day) has a embryotoxic and teratogenic potential in mice.

Acta Medica Iranica 36 (1): 59 - 63 ;1998

Key words: Disodium glycyrrhetinic acid hemiphthalate, mice, embryotoxicity, teratogenicity

INTRODUCTION

Glycyrrhizin, a saponin obtained from the root of liqurice (Glycyrrhiza glabra L.) is a conjugate of glycyrrhetinic acid (3β- hydroxy - 11 - oxoalean-12-en-30-oic acid), with two molecules of glucouronic acid. It is known to be effective as an anti-inflammatory, antiallergenic, antihepatitis agent (1) and shows steroid like action (2). Among glycyrrhetinic acid derivatives the sodium salt of glycyrrhetinic acid dihemiphthalate has been shown to suppress the edema induced by histamine, bradykinin, and platelet activating factor (1). Our previous investigations showed that glycyrrhiza derivatives provided protection against gastric ulceration induced by non-steroidal anti-inflammatory drugs (3, 4, 5, 6).There is no obvious data onembryotoxicity or teratogenicity of GAP in animals and human beings.

With respect to the fact that GAP is going to find a place in treatment of human disease, it seems necessary to find its embryofetal effects. Consequently, we were interested to investigate effects of GAP during gestation period in mice.

MATERIALS AND METHODS

Animals

Sixty female virgin NMRI mice (weighing 25 - 30g) were purchased from institute of Razi. They were maintained on a standard laboratory chow and tap water provided ad libitum, in rooms at a temperature of 22 ± 0.5°c and on 12 : 12hr light: dark cycle. After 1 week of acclimatization, the animals were mated (male: female ratio of 1:3) and the mating success was monitored by vaginal smear on the following morning. If spermatozoa found, the mating was considered successful (and the day was designed day 1 of pregnancy). The pregnant females were caged separately and allocated to four groups.

Materials

GAP was obtained from Daroupakhsh pharmaceutical research center, Tehran-Iran, prepared as described previously (7).

Treatment Schedule

Doses of GAP were selected according to its LD_{50} (7). Group 1 received a proportionate volume of physiological saline intraperitoneally (IP). Group 2 was injected IP with a single dose

of 25 mg/kg body weight of GAP on days 8, 9 and 10 of gestation.

Group 3 was injected IP with a single dose of 50 mg/kg body weight of GAP on days 8, 9 and 10 of gestation.

Group 4 was injected IP with a single dose of 75 mg/kg body weight of GAP on days 8, 9 and 10 of gestation. All animals were killed by cervical dislocate on day 18 of gestation and fetuses were delivered by hysterectomy.

Embryotoxicity and Teratogenesis Parameters

The ovaries were excised and the number of corpora lutea were counted. The following parameters were recorded: the number of implantations, resorptions and dead fetuses in each uterin horn, the number, weight, crown - rump length and biparietal diameter of live fetuses, and the presence of any external malformation. The "embryotoxicity score" (ETS) was evaluated for each litter as follows:

$$ETS = \frac{\text{No. of resorptions + No. of dead fetuses}}{\text{Total No. of implants 0}} \times 100$$

The fetuses from each litter were divided randomly into two groups of almost equal size.

One group was fixed in 95% ethanol and stained with alizarin red'S (8) to evaluate: the variations of bone structures and the degree of skeletal ossification according to parameters chosen as indicators (namely: number of ossified caudal vertebrae, middle phalanxes of hand or foot , and tarsus). Another group was fixed in Bouin's fluid to be examined later for soft tissue abnormalities according to Wilson's section method (9).

Data Management and Statistical Analysis

Data for most of parameters were collected both on a "litter" basis and on a "total fetuses" basis. Statistical analysis was performed using either parametrical or non - parametircal tests, namely ANOVA and Kruskal - wallis tests were used respectively. Significancy was assumed at $p<0.05$.

RESULTS

A number of gestation parameters in the 50 and 75 mg/kg/day groups differed significantly from those of control. These include increased number of resorptions and dead fetuses in animals treated with GAP. Mortality rate and resorption sites in control and experimental groups were 1.35%, and 6% respectively.

Examination of live fetuses for external malformation did not show significant difference in any groups (Table 1).

Table 1. Parameters of embryotoxicity for mice given GAP on days 8, 9, and 10 of pregnancy and killed on day 18

Parameters	group1	group 2	group3	group4
mean of crown - rump length±0.01	24.45	24.33	22.66*	22.53*
mean of biparietal diameter±0.01	6.28	6.26	5.15*	5.20*
mean of weight±0.001	1.514	1.515	1.181*	1.176*
grossly malformation fetuses (%)	0.22	0.25	0.22	0.18

The value for the prevalence of affected fetuses marked with an asterisk differ significantly from those of control group 1 ($P<0.01$). * : cm below costal margin

Results of the skeletal examinations are summarized in (Table 2). Examination of live fetuses for skeletal malformations showed significant effects in experimental groups at 50 and 75 mg/kg/day compared to the control group. The reduction in the number of caudal vertebrae, ossification centers in middle phalanxes of hand and foot, and tarsus were characteristics of growth retarded fetuses of experimental groups (Figs. 1a, b, c, d) ($p<0.01$).

Evaluation of histologic sectioning, showed that cartilage calcification decreased in experimental groups. In other words, cartilage cells growth grade was 4 and 5 in control group but 3 and 4 in experimental groups ($p<0.03$).

No significant differences were found in the renal, cerebral, internal hemorrhages and other anomalies of soft tissue.

Fig. 1.a

Fig. 1.c

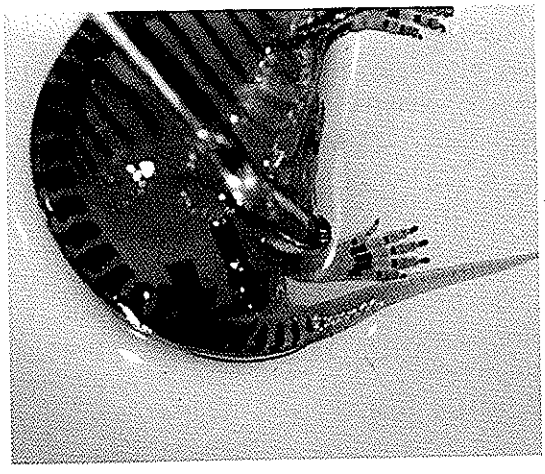

Fig. 1.b

Fig. 1.d

Fig. 1. Alizarin red - S stained skeletons of fetuses of day 18 of gestation. (a, b) dose: 50 mg/kg/day, 8th day. (c, d) dose: 75 mg/kg/day, 8th day. (a, c) control groups, ossification centers in tarsus, metatarsus, middle phalanx, and caudal vertebrae have normal growth. (b, d) experimental groups (GAP groups). Ossificaion centers in tarsus, metatarsus, middle phalanx, and caudal vertebrae have poor development.

Table 2. Ossification parameters in fetuses of mice given GAP on days 8, 9 and 10 of pregnancy and killed on day 18

Parameters	group 1	group 2	group 3	group4
No. of caudal vertebrae	14.66	14.82	10.71*	10.85*
NO. middle phalanx of hand	3.22	3.25	1.88*	1.82*
of Tarsus	1.55	1.50	0.28*	0.28*
ossified middle phalanx of foot	2.88	2.92	0.14*	0.16*

The values for the prevalence of affected fetuses marked with an asterisk differ significantly from those of control group 1 ($P<0.01$). * : cm below costal margin

DISCUSSION

Administration of GAP to pregnant mice at 50 and 75 mg/kg/day of 8, 9 and 10 of gestation period caused an increase in the number of resorption sites and dead fetuses per litter. Teratogenicity was also observed at 50 and 75 mg/kg/day as evidenced by significant decrease in the number of caudal vertebrae ossification centers in middle phalanxes of hand and foot, and tarsus. Also, fetotoxicity (decrease in fetal body weight), decrease in crown-rump lenght, and biparietal diameter were observed in the 50 and 75 mg/kg/day groups. The embryo - fetotoxic effects of a teratogenic compound may be the result of a direct contact of the parent compound and/or its metabolites with the embryonic or fetal tissue and/or the indirect result of maternal toxicity (10, 11). It is not known whether GAP itself or its metabolites lead to its teratogenic effects. Also there is not enough evidence to confirm for transport of GAP or its metabolite(s) from placenta.

In recent years, a number of authors have demonstrated that one of the derivatives of glycyrrhetinic acid has stabilizing effect on cellular, lysosomal and endoplasmic in phospholipid and cholestrol and phosphatidil-choline. This effect can lead to an increase in phospholipid and cholestrol of membrane (12). Also there is evidence that some of derivatives of glycyrrhetinic acid affect vascular permeability and inhibit cell membrane transduction (13). Therefore, it could be concluded that GAP before entering to fetuses, contacts to syncytium trophoblast then stabilizes syncytium trophoblast membrane, increases cholestrol and phospholipid level, and inhibitory effect on vascular (membrane) permeability lead of fetus growth delay. Meanwhile, there is coincidence between growth delay and changes in chondrocyte differentiation. These changes may be due to effects of drug on fetus cell replication and chondrocyte development (14). So, it is not surprising that GAP administration during gestation period induce an inhibitory effect on ossification centers of skeleton.

A dose related increase in embrotoxicity and minor anomalies with oral administration of glycyrrhizic acid to the rat has been found earlier but it was not found in this study. There is also no major external or internal anomalies which is supported by previous workes (15). In conclusion, the present study demonstrated that the IP administration of GAP to mice results in growth retardation. Further examinations by electron microscopy imaging are proposed to explore more details of embryotoxicity and teratogenicity potential of GAP at cellular level.

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