TERATOGENICITY OF CAFFEINE IN MICE RELATED TO ITS MODE OF ADMINISTRATION

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In teratological experiments with drugs, the injection by ways of parenteral routes is often used for the advantage of securing certain effects, especially exact dosage-response relation. It has been presumed that the teratogenic action of a drug varies with the mode of parenteral administration, even if the same total daily dosage is employed and this may be due to the variations in the nature of metabolites of a drug and their concentration or duration in the embryonic tissues. There is little information in this area at hand. Isaacson and Chaudhry (1) reported that the incidence of cortisone-induced cleft palate in mouse embryos is higher when administration of 4 small doses at 6-hour interval is given rather than a single dose of the total amount.

The teratogenicity of caffeine in mice was established and some of its mechanisms were studied by our group (2-4). The rapid metabolic rate and no apparent accumulation in the body of this compound established in man (5) can be presumably applied to other mammals. So the administration of caffeine could be an advantageous tool for analytical studies of teratogenicity in experimental mammals. This study was undertaken to establish a relationship between teratogenic activity and some modes of administration by using this compound.

METHODS

The ICR-JCL mice at the weaning age supplied by the Central Laboratory for Experimental Animals (Meguro, Tokyo) were raised for 6 weeks at our Laboratory (Okazaki). During this period as well as throughout the experiments mentioned below, they were kept in an air-conditioned room at $22^{\circ}\pm 1^{\circ}\text{C}$ and $55\pm 5\%$ of humidity and given the compressed pellets for mice and rats (OA-2) manufactured by Japan CLEA Co. Ltd. (Meguro, Tokyo) and fresh tap water *ad libitum*.

Nulliparous females of 9-13 weeks of age were mated all night (17 hours) with adult males. Copulation was checked by the presence of a vaginal plug and the day when the plug was found, was designated as day zero of gestation. One hundred and sixty-five pregnant mice were divided into eight groups of about equal numbers on day 12 of gestation and they were treated as follows:

Group 1a: 1% aqueous solution of caffeine (J.P.) was injected once at 9:30 a.m.

at a dosage of 200 mg/kg into the nape subcutaneously.

Group IIa: The same solution was injected once at 9:30 a.m. at the same dosage intraperitoneally.

Group IIIa: The same solution was injected twice at 9:30 a.m. and 11:30 a.m. (at

TABLE 1. Effect of prenatal administration of caffeine (1% aqueous solution) given on gestational day 12 upon mouse offspring observed at term.

Group	No. of mothers treated	Dosage at single injection (mg/kg)	Total implants (per litter)	Dead or resorbed (%)	No. of mothers with abnormal fetuses (%)	Live fetuses		
						No. of live fetuses (per litter)	Average body weight $\pm$ Sx (g)	No. of abnormal (% out of total)
Ia	20	200	287 (14.4)	39* (13.6)	14* (70.0)	248 (12.4)	1.28* 0.06	51* (20.6)
IIa	20	200	273 (13.7)	22* (8.1)	14 (70.0)	251 (12.6)	1.27* 0.007	46*++++ (18.3)
IIIa	23	100	308 (13.4)	43++ (14.0)	11* (47.8)	265 (11.5)	1.32* 0.007+++	18* (6.8)
IVa	22	100	307 (14.0)	45+++ (14.7)	11* (50.0)	262 (11.9)	1.27* 0.006	16* (6.1)
Ib	21	0	262 (12.5)	18 (6.9)	1 (4.8)	244 (11.6)	1.36 0.007	1 (0.4)
IIb	19	0	235 (12.4)	17 (7.2)	3 (15.8)	218 (11.5)	1.36 0.008	3 (1.4)
IIIb	20	0	261 (13.1)	23 (8.8)	1 (5.0)	238 (11.9)	1.36 0.006	1 (0.4)
IVb	20	0	268 (13.4)	18 (6.7)	1 (5.0)	250 (12.5)	1.33 0.007	1 (0.4)

Ia : administered once subcutaneously ; IIa : administered once intraperitoneally ; IIIa : administered twice intraperitoneally at an interval of 2 hours ; IVa : administered twice intraperitoneally at an interval of 4 hours ; Ib, IIb, IIIb and IVb : respective control groups for Ia, IIa, IIIa and IVa.

* : significantly different from those of respective control groups ($P < 0.01$)

+ : significantly different from that of group Ia ($P < 0.05$)

++ : significantly different from that of group IIa ($P < 0.05$ respectively)

+++ : significantly different from that of groups IIa and IVa ($P < 0.01$)

++++ : significantly different from that of groups IIIa and IVa ($P < 0.01$)

2-hour interval) at a dosage of 100 mg/kg respectively intraperitoneally.

Group IVa: The same solution was injected twice at 9:30 a.m. and 1:30 p.m. (at 4-hour interval) at a dosage of 100 mg/kg respectively intraperitoneally.

Group Ib, IIb, IIIb and IVb: The same volume of saline was injected in the same way with the above mentioned groups as the respective control groups.

All the mothers were sacrificed on day 18 of gestation. The implantation sites and maceration of the fetuses were inspected *in situ* and all living fetuses were removed, weighed and examined for external deformities including those in the oral cavity.

RESULTS AND DISCUSSION

Most animals of group Ia and IIa showed transient dyspnea and slight convulsions several minutes after the caffeine injection, while those of group IIIa and IVa exhibited transient dyspnea and depressed activity after the injection. All the mothers survived until term.

The main effects on the offspring observed on day 18 of gestation are shown in Table 1.

The incidence of dead or resorbed fetuses in the caffeine treated groups is higher than that of each of the respective control group. Number of the mothers with the abnormal fetuses in the caffeine treated groups is more than that of respective control group. It was also shown that the incidence of the fetuses with abnormalities in each of the caffeine treated groups is significantly higher than that of the respective control groups.

If the group Ia is compared with the group IIa, lethal effect is severer in the group

TABLE 2. Classification of abnormal fetuses according to type of defects.

Group	No. of live fetuses	No. of fetuses with abnormalities	Incidence of fetuses with each abnormality (% out of live)				
			Cleft palate	Micrognathia	Defect of digits	Hematoma	Clubfoot
Ia	248	51	48* (19.4)	15* (6.0)	1 (0.4)	15* (6.0)	3 (1.2)
IIa	251	46	41** (16.3)	11** (4.4)	—	11*** (4.4)	3 (1.2)
IIIa	265	18	16* (6.0)	1 (0.4)	1 (0.4)	5* (1.9)	2 (7.5)
IVa	262	16	5 (1.9)	—	—	—	11* (4.2)
Ib	244	1	—	—	—	—	1 (0.4)
IIb	218	3	—	—	—	—	1 (1.4)
IIIb	238	1	1 (0.4)	—	—	—	—
IVb	250	1	1 (0.4)	—	—	—	—

* Significantly different from those of respective control groups ($P < 0.01$)

+ Significantly different from those of group IIIa and IVa ($P < 0.01$)

+ Significantly different from that of group IVa ($P < 0.01$)

Ia than in the group IIa. However, no significant difference of the incidence of mothers with abnormal fetuses as well as that of the malformed fetuses is shown between the group Ia and IIa.

Next, the comparison among the three intraperitoneally injected groups, IIa, IIIa and IVa, revealed the findings that the lethality is higher in the groups IIIa and IVa than in the group IIa, while the incidence of the fetuses with abnormalities higher in the group IIa than in the groups IIIa and IVa. Also it was found that there is no appreciable difference of the effects between the group IIIa and IVa.

The fetuses with abnormalities are classified according to types of defects in Table 2.

All treated groups showed the high incidence of cleft palate and the occasional occurrence of clubfoot. The hematoma was accompanied in most cases by some adjacent skeletal defects. The incidence of cleft palate in the group IIa is significantly higher than that of the groups IIIa and IVa. The same tendency is shown in the incidence of micrognathia. Subcutaneous hematoma was found around the mouth and at the digits in the three treated groups.

The above mentioned findings indicate some significant facts on the dependence of embryotoxic effects of caffeine upon the mode of its administration. That is, the subcutaneous injection of its single dose induces a severer lethal effect on the embryos than the intraperitoneal injection of the same dose, but no difference in the teratogenic effect is shown between these two routes; by the intraperitoneal route, administration of two divided small doses at 2- or 4-hour intervals produces severer lethality, and lower teratogenicity than that of the single total dose. There is no doubt that the rate of absorption following subcutaneous injection of caffeine is lower than intraperitoneal injection. It may be possible that administration of a single dose leads to the higher concentration and the shorter duration of caffeine or its metabolic products in the blood as well as the embryonic tissues than administration of the divided small two doses at the interval. Although with what drug metabolism in mothers and embryos such variations in embryotoxicity are linked awaits further studies, the above results may be accounted for by the speculation that the lethality of caffeine on the mouse embryos is related to the duration of exposure of the embryos to the compound or its metabolic by-products while the teratogenicity is rather attributable to the sufficiently high concentration. At any rate, dissociation of both lethality and teratogenicity to the embryos revealed by varying the mode of administration might suggest some difference in the pathway involved in causing both effects.

SUMMARY

Caffeine was administered to the ICR-JCL pregnant mice on day 12 of gestation at the dosage of 200 mg/kg causing transient maternal toxic symptom by four different methods: once subcutaneously, once intraperitoneally, twice at 2-hour interval at the half dosage intraperitoneally and twice at 4-hour interval at the half dosage intraperitoneally. The fetuses were examined at term externally. Main findings are as follows:

1. Lethal effect of a single subcutaneous injection is severer than that of a single intraperitoneal injection.

2. A single intraperitoneal injection causes higher teratogenic effects as well as causation of subcutaneous hematoma in the jaws and digits, but lower lethality than the two times administration of half dose at 2- or 4-hour interval. Such differences in teratogenic effects are conspicuous in the facial defects such as cleft palate and micrognathia.

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