COMPARISON OF TERATOGENIC ACTION OF SUBSTANCES RELATED TO PURINE METABOLISM IN MOUSE EMBRYOS

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Teratogenic and embryocidal actions of adenine in the mouse were demonstrated (1-2). Since adenine is an important purine metabolite in animals of all kinds as well as in plants, it was decided to investigate other purine metabolites and a xanthine oxidase inhibitor as to whether or not similar actions on developing mouse embryos could be demonstrated.

METHODS

Weanling mice of ICR-JCL strain purchased from Japan CLEA Co. (Takatsuki, Osaka) were raised for about 8 weeks at N.M.B. Research Laboratories. During this period as well as throughout the experiments, they were kept in an air-conditioned room at $22 \pm 1^\circ\text{C}$ and $55\% \pm 5$ of humidity, fed the solid mice chow (CA-1, Japan CLEA Co.) and allowed fresh water ad libitum. Nulliparous females of 10-13 weeks of age were mated overnight (17 hr) with adult males of the same strain. Copulation was established the next morning by the presence of a vaginal plug and that day was designated as day 1 of presumed gestation.

Inosin-5'-monophosphate disodium*(IMP) was intraperitoneally injected at a dose of 250, 500 or 1000 mg/kg (groups IMP-250, IMP-500 and IMP-1000) by weight after having been dissolved in saline solution. Hypoxanthine*(HX) at a dose of 600, 800 or 1000 mg/kg (groups HX-600, HX-800 and HX-1000), guanosine*(GR) at 750 or 1000 mg/kg (groups GR-750 and GR-1000) and 4-hydroxypyrazolopyrimidine*(HPP), a xanthine oxidase inhibitor at a dose of 50 or 100 mg/kg (groups HPP-50 and HPP-100) were intraperitoneally injected after having been suspended in 0.5% CMC vehicle. All treatments were made at 9 AM on day 10 or 13 of gestation. The volume of the solutions injected was calculated to be 10 ml/kg of body weight. Control animals were not treated. Females were sacrificed on day 19 of gestation. The number of implantation sites and signs of death such as fetal resorption or maceration were checked in situ. Then, the live fetuses were removed from the uterus, weighed and examined for external deformities including those in the oral cavity. They were fixed in 95% ethyl alcohol, cleared and stained with alizarin red S for examination of skeletal malformations.

* The compounds were obtained through Katayama Chemical Company, (Osaka, Japan).

RESULTS

Injection on day 10: Results of fetal examination are summarized in Table 1. Significantly high frequency of dead offspring was found in groups IMP-1000, HX-800, HX-1000, GR-1000 and HPP-100. Body weight of live fetuses was significantly decreased in most groups and lowest in group HPP-100. Significantly high rate of fetuses with external malformations was shown only in groups HPP-50 and HPP-100. A few insignificant cases of malformation were observed in groups where these three metabolites had been administered. Skeletal observation revealed significantly high frequencies of fetuses with malformations in most metabolite-treated groups but not in the HPP-treated groups.

Types of external and skeletal malformations are listed in Table 2. Several cases of limb or digital defects in the forelimbs were observed in groups treated with the metabolites. Most of them were located in the left side. In groups treated with HPP, main defects were cleft palate and harelip. Types of skeletal malformation in groups treated with the metabolites were fusion, deformation and absence of vertebral and rib bones. These malformations were frequently found to be in the lower thoracic to lumbar region.

Injection on day 13: Results are summarized in Table 3. Fetal mortality significantly increased under treatment of metabolites and HPP except for group HPP-50. Fetal body weight significantly decreased in all groups. External malformations were found in most groups, but only in group GR-1000 was the frequency significantly higher than the control.

TABLE 1. Effects on mouse embryos of a single maternal ip injection of inosine-5'-monophosphate disodium (IMP), hypoxanthine (HX), guanosine (GR), and 4-hydroxypyrazolopyrimidine (HPP) on gestation day 10.

Group (mg/kg)	No. of mothers treated	Total implants	Dead offspring %	Live fetuses % : out of total live			
				Mean body wt	with external malfor- mation %	with subcu- taneous hema- toma %	with skeletal* malfor- mation %
IMP-250	16	207	10.1	1.35*	0	0	12.4*
IMP-500	17	230	9.1	1.33*	0	0	15.3*
IMP-1000	18 ¹	248	16.5*	1.35*	1.0	0	14.0*
HX-600	16	215	8.8	1.37*	0.5	0	0.5
HX-800	14	198	13.3**	1.40	1.2	0	0.6
HX-1000	18	257	13.2**	1.30*	3.1	0	11.8*
GR-750	19	237	7.2	1.32*	2.3	0	7.7*
GR-1000	19 ²	248	26.2*	1.34*	0.5	0	14.2*
HPP-50	20	272	10.3	1.29*	4.9*	0	1.2
HPP-100	14 ³	186	41.4*	1.09*	10.1*	0	2.8
Control	18	232	6.5	1.40	0.5	0	0

1 : Three mothers found dead next morning after injection.

2 : One mother only with resorptions excluded from autopsy data.

3 : Four mothers only with resorptions excluded from autopsy data.

4 : Those observed externally were excluded.

* : Significant at $P < 0.01$

** : Significant at $P < 0.05$

TABLE 2. Classified fetal malformations of mouse fetuses caused by a single maternal ip injection of IMP, HX, GR and HPP on gestation day 10.

Group (mg/kg)	No. of live fetuses	Classified no. of fetuses				
		Abnor- malities	External malfor- mations	Types ¹	Skeletal malfor- mations	Locations ² or types
IMP-250	186	23*	0	—	23*	TR 11 LR 14
IMP-500	209	32*	0	—	32*	TR 12 SR 2 LR 17 CR 3
IMP-1000	207	30*	2	CP 1 DD 1	29*	TR 16 SR 1 LR 20
HX-600	196	2	1	CP 1	1	LR 1
HX-800	172	2	2	AT 1 CP 1	1	LR 1 SR 1
HX-1000	223	27*	7	AM 2 GE 3 CP 1 HL 2 DD 2 HM 3 EC 1 PF 2	26*	TR 16 SR 2 LR 17 CR 1
GR-750	220	20*	5	BT 1 HM 1 CP 2 LD 1 DD 1	17*	TR 10 LR 8 CR 1
GR-1000	183	26*	1	HM 1	26*	TR 8 SR 4 LR 20 CR 2
HPP-50	244	13*	12*	BT 2 HL 4 CF 1 OE 1 CP 8	3	WR 3
HPP-100	109	11*	11*	CF 2 GE 1 CP 11 HL 4 DD 33	3	LR 1 BL 2
Control	217	1	1	CP 1	0	—

* : Significant at $P < 0.01$

1 : Code for type, AM : Amelia, AT : Absent tail, BT : Bent tail,

CF : Club foot, CP : Cleft palate, DD : Digital defect,

EC : Exencephalus, GE : Generalized edema, HL : Harelip,

HM : Hemimelia, LD : Limb defect, OE : Open eyelid,

PF : Encephalocele.

2 : Code for location or type, TR : Thoracic region, LR : Lumbar region,

SR : Sacral region, CR : Caudal region, BL : Bent long bones,

WR : Waved ribs.

Subcutaneous hematoma was induced by the treatment of HX and GR, but not IMP and HPP. A significantly high frequency of skeletal defects was seen only in groups treated with HPP. External and skeletal malformations are listed in Table 4. Frequencies of fetuses with some abnormality were significant in groups HX-1000 and GR-1000. Main types of external malformation in groups treated with HX or GR were digital defects and cleft palate, while in groups treated with HPP, only cleft palate was seen. Skeletal malformations in groups treated with HPP were mostly waved ribs and bent long bones such

TABLE 3. Effects on mouse embryos of a single maternal ip injection of inosine-5'-monophosphate disodium (IMP), hypoxanthine (HX), guanosine (GR) and 4-hydroxypyrazolopyrimidine (HPP) on gestation day 13.

Group (mg/kg)	No. of mothers treated	Total implants	Dead offspring %	Live fetuses % : out of total live			
				Mean body wt	External malfor- mation %	Subcuta- neous hematoma %	Skeletal ³ malfor- mation %
IMP-500	17	217	12.4**	1.37*	0	0	0
IMP-1000	15 ¹	224	24.6*	1.31*	0.6	0	0
HX-600	14	192	18.2*	1.35*	1.9	2.5	0
HX-800	14	206	20.4*	1.30*	2.4	0.6	0
HX-1000	20	249	21.3*	1.32*	3.1	4.6	0
GR-750	17	221	21.3*	1.33*	1.1	1.7	0
GR-1000	20	268	23.1*	1.35*	7.8*	5.3*	0.5
HPP-50	17	198	11.1	1.35*	1.1	0	7.4*
HPP-100	17 ²	199	33.2*	0.97*	2.3	0	63.2*
Control	18	232	6.5	1.40	0.5	0	0

1 : One mother found dead next morning after injection.

2 : One mother only with resorptions excluded from autopsy data.

3 : Those observed externally were excluded.

* : Significant at $P < 0.01$ ** : Significant at $P < 0.05$

TABLE 4. Classified mouse fetus malformations caused by a single maternal ip injection of IMP, HX, GR and HPP on gestation day 13.

Group (mg/kg)	No. of live fetuses	Classified no. of fetuses				
		# Abnor- malities	External malfor- mations	Types ¹	Skeletal malfor- mations	Types ²
IMP-500	190	0	0	—	0	—
IMP-1000	169	1	1	CF 1	0	—
HX-600	157	5	3	CP 1 DD 3	0	—
HX-800	164	4	4	CF 1 DD 3 CP 1	0	—
HX-1000	196	10*	6	CF 1 DD 4 CP 2	0	—
GR-750	174	3	2	DD 2	0	—
GR-1000	206	19*	16*	BG 1 CP 7 CF 2 DD 12	1	WR 1
HPP-50	176	15*	2	CP 2	13*	FR 1 WR 12 BL 12
HPP-100	133	84*	3	CP 3	84*	BL 79 WR 84
Control	217	1	1	CP 1	0	—

* : Significant at $P < 0.01$

: These refer to external defects, subcutaneous hematoma and skeletal malformations.

1 : Code for type, BG : Brachygnathia, CF : Club foot, CP : Cleft palate, DD : Digital defect.

2 : Code for type, BL : Bent long bones, FR : Fused ribs, WR : Waved ribs.

as humerus, radius, ulna, femur, tibia, fibra and occasionally clavicle, ilium and ischium.

DISCUSSION

Since hypoxanthine and guanine as well as adenine inhibit xanthine oxidase (3), 4-hydroxypyrazolopyrimidine was also employed in this study as it is a potent xanthine oxidase inhibitor (4).

Chaube and Murphy (5) injected intraperitoneally inosine or hypoxanthine at 1000 or 700 mg/kg into pregnant rats on day 12 of gestation and did not find any adverse effect on pregnancy and fetuses. But in this study with mice, these metabolites as well as guanosine were all teratogenic when comparable doses of each compound was similarly injected either on day 10 or on day 13 of gestation.

Compounds examined in this study can be classified into three categories according to effects on developing mouse embryos: 4-hydroxypyrazolopyrimidine affected embryos differently from metabolites in terms of types of abnormalities, consequently, teratogenic action of the metabolites may not be related to their action as an inhibitor of xanthine oxidase. With respect to the action of the metabolites, classification into two categories may be possible; the effect of inosine-5'-monophosphate disodium was different from that of hypoxanthine and guanosine in terms of external malformations and subcutaneous hematoma. In skeletal defects induced by inosine-5'-monophosphate disodium a "ceiling effect" was shown when given on day 10. In groups treated with other metabolites, however, a dose-effect relation was clearly demonstrated.

The effects of hypoxanthine and guanosine on developing mouse embryos found in this experiment were similar to that of adenine which also induced left forelimb defects (6), vertebral and rib malformations (1), cleft palate, digital defects and subcutaneous hematoma (2). Considering that a large dose of adenine results in nephrotoxic effects on adult rats by formation of sparingly soluble 2,8-dioxyadenine (7-9), while guanosine is presumed to have no such effect, teratogenic effects of both compounds may not be related to this toxicity in the maternal kidney.

SUMMARY

Purine metabolites inosine-5'-monophosphate disodium (IMP), hypoxanthine (HX), guanosine (GR) and 4-hydroxypyrazolopyrimidine (HPP), a xanthine oxidase inhibitor, were each intraperitoneally injected to female mice on day 10 or 13 of gestation. Pregnancy was terminated on day 19 of gestation and fetuses were examined for external and skeletal abnormalities as well as embryonic deaths. Noted were the following: 1) All purine metabolites were embryotoxic and teratogenic inducing mainly vertebral and rib malformations, when large doses were administered on day 10 of gestation. 2) HPP was far more embryotoxic than purine metabolites when given on day 10. Malformations were mainly harelip and cleft palate. 3) When injected on day 13, HX and GR caused embryonic deaths and malformations such as cleft palate and digital defects as well as subcutaneous hematomas, whereas IMP resulted in embryonic death. 4) HPP was also embryotoxic and teratogenic

when injected on day 13. The types of malformations were quite different from those observed with HX and GR in that HPP induced high frequency of waved ribs and bent long bones.

REFERENCES

- 1) FUJII, T.: *Teratology* **3**, 299 (1970)
- 2) FUJII, T. AND NISHIMURA, H.: *Jap. J. Pharmac.* **20**, 445 (1970)
- 3) BAKER, B.R. AND HENDRICKSON, J.L.: *J. Pharm. Sci.* **56**, 955 (1967)
- 4) ELLION, G.B., KOVENSKY, A. AND HITCHINGS, G.H.: *Biochem. Pharmac.* **15**, 863 (1966)
- 5) CHAUBE, S. AND MURPHY, M.L.: *Biochem. Pharmac.* **18**, 1147 (1969)
- 6) FUJII, T. AND NISHIMURA, H.: Unpublished
- 7) BENDICH, A., BROWN, G.B., PHILIPS, F.S. AND THIERSCH, J.B.: *J. biol. Chem.* **183**, 267 (1950)
- 8) PHILIPS, F.S., THIERSCH, J.B. AND BENDICH, A.: *J. Pharmac. exp. Ther.* **104**, 20 (1952)
- 9) PHILIPS, F.S., STERNBERG, S.S., HAMILTON, L. AND CLARKE, D.A.: *Ann. N.Y. Acad. Sci.* **60**, 283 (1954)