CHARACTERISTICS OF THE TERATOGENIC EFFECT OF AMINOPTERIN COMPARED WITH THAT OF OTHER TERATOGENIC AGENTS

(UDC 615.739.6-092.259 : 612.64 + 616 + 007-02 : 615.739.6)

V. S. Baranov

Laboratory of Human Embryology, Institute of Experimental Medicine, Leningrad
(Presented by Acting Member of the USSR Academy of Medical Sciences D. A. Biryukov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 1,
pp. 77-82, January, 1966
Original article submitted April 19, 1965

Various developmental anomalies are known to arise among embryos of rats kept on a folic acid free diet to which has been added 9-methyl folic acid (folic acid antagonist). The nature and frequency of these anomalies is determined by the period of gestation and the duration of the period of folic acid deficiency [9].

Aminopterin is a considerably more active folic acid antagonist than 9-methyl folic acid yet the effect of aminopterin on rat embryogenesis has received insufficient study. It has been shown that aminopterin brings about the death of some embryos in pregnant rats but those surviving remain normal [7]. These findings are not in accord with the clearly expressed teratogenic activity of aminopterin on amphibian [6], avian [4] and human embryos [5]. It is possible that the teratogenic effect of aminopterin in rats has been overlooked because this substance has only been given at certain stages of pregnancy [7].

The aim of this particular research was to study the effect of aminopterin at various times during the pregnancies of white rats and to determine whether aminopterin causes teratogenic development among embryos of these animals.

EXPERIMENTAL METHODS

Experiments were carried out on rats of the Wistar strain weighing 150-200 g. Of 334 pregnant rats, 313 were treated as experimental animals, 21 as controls. Throughout their pregnancy the rats received a rich diet consisting of rat cubes, beets, and water.

The aminopterin was injected intraabdominally. Immediately before injection, the substance was dissolved in sterile physiological saline (pH 8.0-8.5). Two series of experiments were set up: in series I, the rats received a single injection of aminopterin at a dosage of 0.1 mg per kg of body weight on one day between the 1st and 13th days of pregnancy; in series II the single dose was 0.05, 0.075, 0.1, 0.1125, 0.125, 0.15, or 0.2 mg/kg body weight on which spermatozoa were found in the vaginal smear.

The rats were killed on the 13th and 19th day of pregnancy. The number of fetal implantations in the uterus and the number of corpora lutea in the ovaries was counted. The fetuses were placed in Ringer's solution an examined under a binocular lens. If the craniocaudal dimensions of the fetuses were less than the 16 and 20 mm regarded as appropriate for the 17th and 19th day of pregnancy, the fetuses were considered to have suffered arrested development. All the fetuses were fixed in 10% formalin solution or in Bouin's fluid and were retained for subsequent histological examination. The data obtained from these experiments were treated statistically; a 95% threshold of significance was used in comparing the percentage results.

EXPERIMENTAL RESULTS

As can be seen from the data set out in Table 1, aminopterin, injected between the 7th and 13th day of pregnancy resulted in a considerable mortality among the embyros, particularly when the injections occurred on the

TABLE 1. Effect of 1 mg Aminopterin on White Rat Embryos Receiving a Single Injection during the Period of the 1st to 13th Day of Pregnancy

Day of injection	No. of rats in group	No. of corpora lutea	No. of fetal implantations		Fetuses dying after implantation			Living progeny								
								Total	normal			teratoid			showing develop. arrest	
			abs.	%	abs.	%	limits of significance (as %)		abs.	%	limits of significance (as %)	abs.	%	limits of significance (as %)	abs.	%
1-st	8	78	70	89,7	7	10	4,2 — 18	63	63	100						
2-nd	8	83	77	92,7	8	9,6	4,1 — 17,1	69	69	100						
3-rd	13	124	93	75	10	10,7	5,3 — 17,7	83	82	98,8	93 — 100				1	1,2
4-th	8	87	79	91	16	7,6	2 — 12,3	73	73	100						
5-th	8	77	66	85,7	19	28,8	19,5 — 41,3	47	45	95,8	88,3 — 99,6				2	4,2
6-th	19	214	186	86,8	73	39,2	32,3 — 46,3	113	82	72,5	64,4 — 80,3	24	21,2	14 — 29,0	7	6,3
7-th	19	194	170	87,5	129	75,8	69 — 82	41	38	92,4	82,6 — 98,4				3	6,6
8-th	17	161	143	89	39	27,2	20,2 — 34,7	104	95	91,2	84,2 — 95,8	1	0,9		8	7,9
9-th	10	106	82	77,3	75	91,4	84,4 — 96,4	7	6	85,7	57,3 — 100	1	14,3			
10-th	9	86	78	90	78	100										
11-th	8	84	73	86,9	73	100										
12-th	7	71	67	94,3	50	74,5	63,5 — 87,6	17	17	100						
13-th	5	51	46	90	5	10,9	3,6 — 21	41	39	91,4	81 — 97,8				2	8,6
Control	21	197	169	85,8	15	8,8	5 — 13,5	154	154	100						

TABLE 2. Effect of Different Doses of Aminopterin Injected on 6th Day of Pregnancy

Dose of aminopterin (in mg/kg)	No. of rats in group	No. of corpora lutea	No. of fetal implantations		Fetuses dying after implantation			Living progeny									
								Totals	normal			teratoid			showing develop. arrest		
			abs.	%	abs.	%	Limits of significance (as %)		abs.	%	Limits of significance (as %)	abs.	%	Limits of significance (as %)	abs.	%	Limits of significance (as %)
0,05	8	75	65	86,9	6	9	3,3—17,2	59	55	93,2	85,3— 98,2	1	1,7	0,1 — 6,5	3	5,1	1 —12,1
0,075	8	76	70	90,7	14	20	11,6—30,1	56	50	89,4	76,4— 95,6	6	10,8	4,1 —20,2			
0,100	39	353	316	89,5	104	32,9	28 —38,1	212	176	82,2	74,4— 89,3	29	13,7	9,4 —18,6	7	3,3	1,2— 6,4
0,1125	30	395	253	85,7	120	47,4	41,3—53,5	133	107	81,2	74,2— 87,4	5	3	0,79— 6,6	21	15,7	10,1—22,4
0,125	36	366	334	91,9	197	58,9	53,7—63,9	137	100	72,8	65,3— 79,8	19	13,8	8,6 —20	18	13	10,8—19,1
0,150	42	420	360	85,7	265	73,3	67,7—78,4	91	68	74,7	65,3— 83	17	18,6	11,4 —27,2	6	6,7	2,5—12,7
0,200	9	98	81	82,6	70	86,4	78,2—93	11	10	90,9	80,9—100	1	9,1	0,1 —33,4			
Control	21	197	169	85,8	15	8,8	5—13,5	154	154	100							

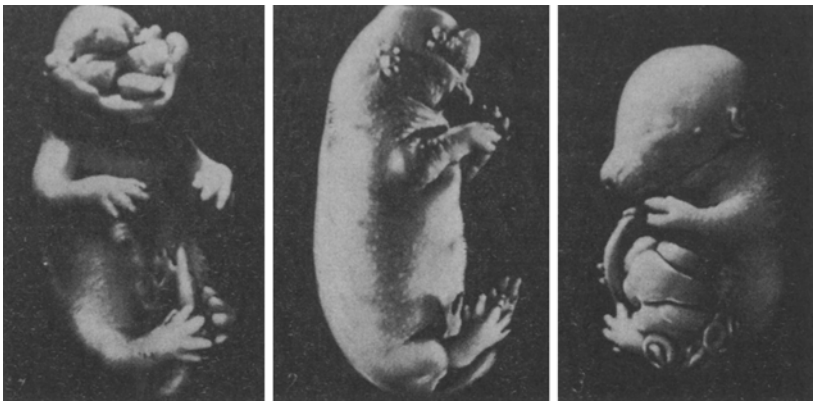

Fig. 1. Teratogenic effect of aminopterin injected on the 6th day of pregnancy. Embryos on 19th day of development. 1) Absence of facial region to skull, complete failure of the palatine processes to close; 2) strong reduction of maxilla and mandible, anophthalmia; 3) ectrodactyl, exophthalmia.

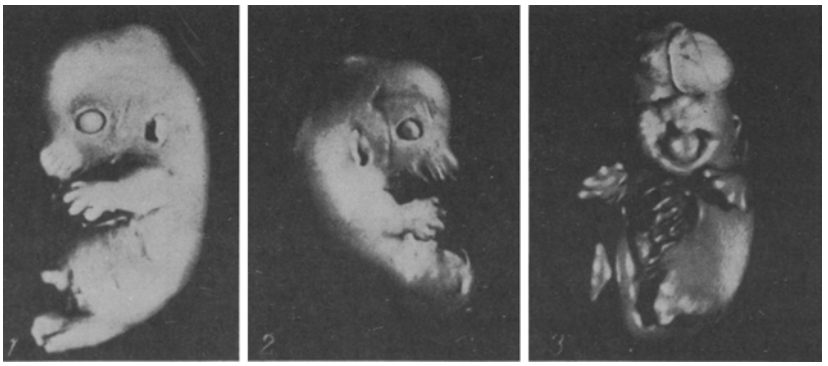

Fig. 2. Teratogenic effect of aminopterin injected on 6th day of pregnancy. Embryos on 17th day of development. 1) Coalescence of posterior extremities (siren effect); 2) reduction of pelvic region, absence of posterior extremities, tail, hepatic hernia; 3) exencephaly, dextral hare-lip, bilateral anophthalmia.

7th and the 10-11th days of pregnancy; moreover, aminopterin gave rise to teratogenic development. The teratogenic effect of aminopterin occurred only on the 6th day of pregnancy, when 24 of the 113 surviving embryos (21.2%) showed some anomaly of development. Single embryos were encountered in which the teratogenic effect of aminopterin was associated with the 8th and 9th day of pregnancy. Thus, on the 8th day 1 teratoid fetus out of 104 was encountered and on the 9th day 1 out of 7 surviving embryos. However, this weak teratogenic effect of aminopterin on the 8th and 9th days of pregnancy was not reproduced in further series of experiments, i.e., it appears to depend on uncontrollable factors and must be regarded as fortuitous. For this reason, we have only analyzed the teratogenic effect of aminopterin on the 6th day of pregnancy.

The results of series II experiments are set out in Table 2. From this table it is evident that the lethal effect of aminopterin injected on the 6th day of pregnancy is in direct proportion to the size of the dose. The teratogenic effect of aminopterin increases as the dose increases from 0.05 to 0.1 mg, attaining a maximum 13.7%, at the latter value, higher doses do not result in greater mortality. In addition, the teratogenic effect of aminopterin injected on the 6th day of pregnancy is clearly manifested in all experiments of the series, i.e., it is completely constant.

The main types of ontogenetic anomalies evoked by aminopterin are listed in Table 3. From among the whole series of experiments we obtained 78 deformed (teratoid) embyros (out of 699 living progeny). The most frequently encountered abnormalities were unilateral or bilateral anophthalmia (46.5%); partial or complete nonclosure of the

TABLE 3. Characteristics of Abnormal Development Associated with Various Doses of Aminopterin on the 6th Day of Pregnancy

Organ affected	Type of malformation	No. of embryos with that type of malformation	
		absolute	% (of number of corresponding organs in all the progeny)
Eye	Anophthalmia	48	46.5
	Microphthalmia	10	6.6
Brain	Cephalic hernia	14	17.9
	Exencephaly	3	3.8
	Microcephaly	4	5.1
Front of skull	Hare lip	14	12.5
	Wolf muzzle	4	5.1
	Reduction of jaws	7	6.6
Anterior abdominal wall	Hepatic hernia	24	30.5
	Exomphalia	10	12.6
Anterior limbs	Electrodactyly	3	1.9
Posterior limbs	Coalescence of posterior extremities (siren effect)	2	2.5
	Absence of posterior extremities	3	3.8
Tail	Absence of tail	6	7.6
Pelvis	Reduction of posterior part of body	3	3.8

anterior abdominal wall with resultant hepatic hernia (30.5%), or exomphalia – visceral prolapsus (12.6%), as illustrated in Fig. 1, picture 3, together with cephalic hernia (17.9%). Skull malformations were encountered fairly frequently, e.g., unilateral or bilateral hare-lip in 12.5% of cases (Fig. 2, picture 3); reduction of the mandible and maxilla in 6.6% of embryos (fig. 1, picture 2); complete or partial failure of the palatine processes to close (wolf muzzle effect) in 5% of cases (Fig. 1, picture 1). Embryos were also encountered with malformation of the digits, e.g., ectrodactyly – reduction in the number of digits (Fig. 1, picture 2); complete absence of the pelvic region and the posterior extremities (Fig. 2, picture 2). Sometimes the posterior extremities coalesced in the form of a "mermaid's tail" and gave rise to the "siren effect (Fig. 2, picture 1). The injection of aminopterin on the sixth day of pregnancy appears to produce all the main types of ontogenetic abnormalities in the organs of the embryos (Table 3).

In addition to its teratogenic effect aminopterin causes a general retardation of embryonic development. The greatest number of embryos with retarded development was associated with doses of 0.1125 mg (15.7%) and 0.125 mg (13%).

Thus, aminopterin is able to cause teratogenic development in rat embryos. However, whereas in frog and bird embryos this teratogenic effect of aminopterin can be produced by injection at various stages of embryogenesis, in rats it is confined to injection on the 6th day of pregnancy.

Other noxious substances cause malformation in rat embryos when injected at the time of organogenesis, i.e., from the 8th to the 14th day of pregnancy, and in such cases the nature of the malformation depends on the particular stage of embryogenesis when the agent exerts its effect. It is presumed that malformation of the organ arises as a consequence of the noxious agent affecting the development of the organ rudiment at a critical period of development [2, 3]. The teratogenic effect of aminopterin in rats, however, manifests itself on the 6th day of pregnancy, i.e., just before the commencement of organogenesis, and consequently at some time before any critical period in

the development of the affected organs. On the 6th day of pregnancy the rat embryo is represented by a vesicle (blastocyst) consisting of 300-350 blastomeres, and in which the differentiation of the embryoblast has not even begun [1]. The teratogenic effect of aminopterin cannot therefore be explained in terms of its direct influence on organ rudiments at a critical period of their development. Furthermore, during the critical phase of development of the various organs, from the 8th to the 14th day of pregnancy, aminopterin evokes no malformations. It is possible to suppose that this substance, by blocking folic acid metabolism, also disrupts the metabolism of purine and pyrimidine compounds and thus exerts a direct effect on the genetic apparatus of the embryonic cells at the 6th day of pregnancy, i.e., it induces somatic mutations which ultimately result in the appearance of developmental anomalies. This hypothesis requires further experimental verification.

SUMMARY

Applied on the 6th day of gestation, aminopterin causes in rat embryos various malformations of the eyes, facial skull, brain, extremities, abdominal wall, tail, and spine. Aminopterin application somewhere later in pregnancy causes a high embryonic mortality but no malformations in surviving embryos.

The lethal effect of aminopterin on the 6th day of gestation is in direct proportion to the dose applied, whereas no such relationship exists for the teratogenic activity of aminopterin.

LITERATURE CITED

1. N. A. Somoshkina, Tsitologiya, No. 1 (1961), p. 75.
2. P. G. Svetlov and G. F. Korsakova, In the book: Influence of Ionizing Radiation During Pregnancy on the Condition of the Fetus and the Newborn [in Russian], Leningrad (1960), p. 37.
3. P. G. Svetlov, In the book: Problems of Cytology and General Physiology [in Russian], Moscow – Leningrad (1960), p. 263.
4. I. Bellaris, In the book: Ciba Foundation Symposium on the Chemistry and Biology of Pteridines, London (1954), p. 113.
5. C. Goeth, Am. J. Obstet. Gynec., Vol. 83 (1962), p. 1474.
6. J. Grant, Develop. Biol., Vol. 2 (1960), p. 197.
7. M. L. Murthy and D. A. Karnofsky, Vol. 9 (1956), p. 955.
8. M. M. Nelson, In the book: Ciba Foundation Symposium on Congenital Malformations, London (1960), p. 134.
9. J. B. Thiersh and F. J. Philips, Proc. Soc. Exp. Biol., New York, Vol. 77 (1950), p. 204.

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