EFFECT OF CHLORIDIN ON CHICK EMBRYOGENESIS

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Chloridin (2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine [Daraprim]) is a drug which is widely used for the treatment and prevention of malaria. Furthermore, this preparation has been recommended for use in treating toxoplasmosis of pregnant women [1-3].

It has been demonstrated that chloridin has a high teratogenic activity with respect to the embryos of white rats. There are no data in the literature on the effect of chloridin on the embryogenesis of other types of laboratory animals, in particular chicks.

The purpose of the present study was to elicit the effect of chloridin on the development of the chick embryo.

EXPERIMENTAL METHOD

The work was performed on fertilized chicken eggs of the Russian White variety incubated at 38° and 60% relative humidity. We studied the effect of chloridin on the development of the chick embryo at various stages when the main processes of organogenesis occur (up to the 4th day). Chloridin was injected in doses of 0.1-1.5 mg in a volume of 0.1-0.2 ml as a solution in polyethylene glycol. The injection was made in the yolk through a puncture on the blunt end of the egg. In the control we injected polyethylene glycol in the same volume as in the experiment. In all, we used 600 embryos in the experiments and 126 in the control. Every day, beginning with the 4th day,

TABLE 1. Teratogenic Activity of Chloridin

Experimental conditions			Results of experiment by 10th day of incubation		
time of injection of chloridin	dose (in mg)	no. of embryos	No. of embryos with malformations		
			abs.	%	confidence limits
Before incubation	0,1	45	11	24,45	13,1—37,8
	1,25	43	33	76,75	63,1—87,9
	0,5	48	35	72,92	59,6—84,4
1st day	0,5	45	23	51,12	36,6—65,5
	1,0	50	30	60,0	46,3—73,1
2nd day	0,5	52	27	51,92	38,0—65,2
	1,0	61	33	54,09	40,9—66,8
	1,5	53	32	60,38	46,6—72,7
3rd day	0,5	47	14	29,79	17,7—43,7
	1,0	54	25	46,29	33,3—59,4
4th day	0,5	54	19	35,18	23,1—48,1
	1,0	48	17	35,41	22,6—49,3
Total		600	299		
Control (injection of polyethylene glycol on 1-4th day)	0,1—0,2 ml	126	6	4,76	1,8—8,8

Fig. 1. Sensitivity of chick embryos to teratogenic (1) and lethal (2) effect of chloridin (0.5 mg on 0-4th day of incubation). On the y-axis is the percent of malformed and dead embryos, on the x-axis are the days of incubation.

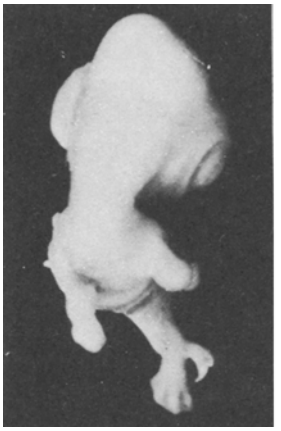

Fig. 2

Fig. 2. Chick embryo (7 days incubation). Fused proximal parts of lower extremities, underdevelopment of distal part of one of them.

Fig. 3

Fig. 3. Chick embryo (7 days incubation). Defect in the development of the anterior pectoral wall, hydropericardium.

the eggs were examined through an egg tester, the dead embryos were extracted, and the anomalies recorded. The results were calculated on the 10th day of incubation. The numerical data was subjected to statistical analysis of the variance.

EXPERIMENTAL RESULTS

The results of our investigations showed that chloridin has a high teratogenic activity with respect to the chick embryo (Table 1).

The teratogenic activity of chloridin varied depending upon the developmental stage at which the drug was injected (Fig. 1). The shape of the curve of Fig. 1 shows that the teratogenic activity of chloridin decreased with an increase of age of the chick embryos. The teratogenic activity of this drug also depended on its dose.

The injection of chloridin led to the appearance of multiple monstrosities in the chick embryos: a decrease of body size combined with shortening of the extremities (micromelia), deformation of the femur and shin, shortening of the toes, reduction of the lower part of the beak, and absence of scleral papillae. In individual cases, we found malformations of the brain (cerebral hernias), of the eyes (anophthalmia and microphthalmia), defects in the development of the anterior pectoral and abdominal walls with eventration of the viscera. The greatest number of malformed embryos died on the 5th-6th day. In these embryos, we frequently noted, in place of the lobes which form at this stage of development, a constriction of the distal portion of the extremity resembling a stump. Figure 2 shows a rare type of anomaly of the extremities: fused proximal ends of the lower extremities, the distal portion of one of them is underdeveloped, the wings are displaced to the dorsal side. A defect in the development of the anterior pectoral wall with hydropericardium and ectopia cordis was a frequent type of anomaly (Fig. 3).

The distribution of these anomalies in relation to the period of injection and dose of the drug is shown in Table 2. In the embryos that died at early stages (3rd-4th day) the area of the vascular field was smaller than in the norm; sometimes in place of the vessels we found blood islands. In these embryos we could see the underdevelopment of the trunk fold, as a result of which the embryo was flattened on the yolk and was not raised above it; the embryos had an irregularly formed cephalic end, the somites were reduced in size, sometimes asymmetric. In individual cases the embryos were autopsied on the 4th-5th day of incubation, in which case we noted a decrease in the area of the vascular field and the paucity of its vessels in comparison with the control.

TABLE 2. Characteristics of Monstrosities Caused by Chloridin (calculation of results on 10th day of incubation)

Time of injection	Dose (in mg)	Number of embryos	Number of embryos with malformations	Type of malformation								
				general underdevelopment	hydrops	brain malformations	eye malformations	ectopia cordis	malformations of extremities	reduction of tail	reduction of lower jaw	deformation of body axis
Before incubation	0,1	45	11	3	2	2	1	0	3	1	4	1
	0,25	43	33	6	0	5	9	9	11	5	4	2
	0,5	48	35	16	0	9	20	3	11	1	3	7
1-st Day	0,5	45	23	7	1	4	13	3	5	1	0	2
	1,0	50	30	7	3	7	13	0	11	0	4	5
2-nd Day	0,5	52	27	8	3	2	13	7	8	1	0	2
	1,0	61	33	10	3	5	7	12	21	4	3	0
	1,5	53	32	4	0	6	7	11	18	0	14	2
3-rd Day	0,5	47	14	4	1	0	4	2	10	1	7	1
	1,0	54	25	14	2	5	5	2	18	4	5	2
4-th Day	0,5	54	19	4	1	1	3	4	2	5	4	0
	1,0	48	17	3	4	1	2	3	8	1	2	0

The peaks of sensitivity to chloridin of a particular anlage was somewhat lengthy. Thus, malformations of the extremities occurred with the greatest frequency upon injection of the drug on the 2nd-3rd day of incubation, malformations of the brain on the 0-1st day.

In the control with injection of polyethylene glycol 4.76% malformations were noted.

Chloridin is toxic for chick embryos (see Fig. 1). The highest percent of lethality was upon the injection of the drug before incubation (97.9% at a dose of 0.5 mg). Lethality changed with relation to the stage at which the drug was administered, within 72-88%.

Apparently, the teratogenic activity of this drug [4] is due to its direct effect on the embryonic cells and not by any shifts in the maternal organism. Since this effect is found in mammals and birds we can assume that chloridin affects any important processes of embryogenesis.

LITERATURE CITED

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