THE TERATOGENIC EFFECT OF THALIDOMIDE IN EXPERIMENTS ON CHICK EMBRYOS

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One of the well-known teratogenic drugs is thalidomide (contergan, distaval, kevadone, softenone) which chemically is an Nα-phthalimido-glutarimide. The use of thalidomide against insomnia during the first three months of pregnancy has led, as a rule, to the birth of defective infants, among whom amelia, phocomelia, and malformations of fingers and of intestine were most common. Malformations of the nose, upper lip, external ear, eyes, heart, and the urogenital system were much less common [10,16,23,25,26].

Experiments on different animals have been conducted in order to establish an adequate model for the testing of new substances and for the study of the teratogenic effect of thalidomide on embryos. However, the results of numerous experiments on chick embryos [1,4,7,17,27], rats [3,5,6,8,15,18], rabbits [3,5,18,21], mice [5,6,10,14,18] and monkeys [2,9] have shown that it is very difficult to reproduce the defects observed in man, and that these defects are only relatively specific. The negative results obtained were apparently due to the species of experimental animals and to the experimental conditions used [5,14,15,19].

The purpose of this investigation was to study the teratogenic effect of thalidomide on chick embryos at different stages of development and to compare the defects produced with those described in mammals.

METHODS

Thalidomide was specially synthesized for these experiments in the Department of Organic Synthesis of our Institute by A. P. Skoldinov and A. I. Ivanov.

Experiments were conducted on fresh fertilized hens' eggs. Thalidomide was injected in one dose prior to incubation of the eggs as a suspension in normal saline or dissolved in dimethylformamide, in a volume of 0.1 ml, into the yolk sac. The doses varied from 0.001 to 1.0 mg. The eggs were incubated at 38° C at a relative humidity of 60%. For periods up to 4 days of incubation, Sorokin's chamber was used by means of which it was possible to examine and photograph the embryos and to count the somites. After the 5th day of incubation, the embryos were studied macroscopically.

RESULTS

As seen in Table 1, in the contr ›l group of 17 embryos, the mortality constituted 5% and there were no deformed embryos. The introduction of dimethylformamide in doses of 1 to 100 mg per egg was accompanied by an increase of mortality to 81% and the appearance of different defects in 12% of the embryos. Following introduction of normal saline, defects were seen in 7% of the embryos. Thalidomide, introduced as a suspension, caused the death of 42% of the embryos and defects in 9%. Thalidomide solution in dimethylformamide led to the death of 73% of the embryos and to production of defects in 15%.

The introduction of dimethylformamide led to a general inhibition of development, upset in the development of the brain and eyes, and sometimes to an inversion of the position of the embryos. Normal saline interfered with

The data presented indicate that thalidomide produced a teratogenic effect at the earliest stages of development of chick embryos. However, this effect was non-specific, because similar changes were produced following introduction of dimethylformamide and even of normal saline.

Later we studied the teratogenic effect of thalidomide at later stages of development (5-14 days of incubation). It is shown in Table 2 that in the control group the mortality reached 24% and defects of development were noted in 3% of the embryos. For dimethylformamide, these figures were 79% and 12%, respectively, and for normal saline 61% and 7%, respectively. Following introduction of thalidomide suspension mortality and defects of development were noted in 83% and 20% of embryos, respectively, and following introduction of thalidomide solution, these figures were 80% and 12%, respectively. Between the 5th and the 14th days of incubation, the defects of development were easily seen macroscopically. The most characteristic defects are shown in the figure. Thus, defects in the development of the brain were represented by protuberances from different parts of the brain. Defects in the development of eyes consisted in the absence of one or of both eyes, changes in the size and shape of eyes, or protrusion of the cornea with the production of a cyst filled with a transparent fluid. Defects in the development of the beak consisted in a curvature or shortening of the entire beak or of its upper or lower part, and in the bifidy of the upper part. The most common defect was failure of closure of the anterior abdominal wall, which led to protrusion and displacement of internal organs. Defects in extremities consisted of curvature, shortening and thickening of legs and in atrophy of digits. Defects in the spine led to a curvature of the body axis, shortening of the body, and a reduction or complete absence of the coccyx.

Table 2 shows the frequency of finding of defects and the number of embryos with defects. The ratio of the first value to the second yields a coefficient of the degree of the upset of development in individual embryos. The higher the coefficient, the greater the upset of development. It will be seen from Table 2 that,for controls, this coefficient was equal to 1.5, for thalidomide in suspension, 3.5, and for thalidomide in solution, 4.3.

These experiments have shown that introduction of thalidomide into chick embryos, in 20% of the cases led to a production of different faults of development. Our results differ from those of other authors [1,7,17,27] who found higher frequencies of defects in chick embryos (35-50%). This difference may be due to differences in the doses of thalidomide or to different times and routes of introduction of the drug.

It is of interest that, under the effect of thalidomide, the total number of defects of mesodermal origin was equal to 62 among 27 embryos, while that of defects of ectodermal origin was equal to 38 among the same embryos. The upset in differentiation and organogenesis of formations of ectodermal origin (brain, eyes, beak) is apparently related to a direct effect of thalidomide on these structures. This is a strong possibility, as it was shown [11] that carbon-labelled thalidomide was observed in brains of rats 30 min after parenteric inoculation. As regards the mechanism of action of thalidomide, there is an opinion [20,24] that it is able to block the metabolism of B-vitamins at different stages.

Thalidomide produces relatively specific defects, since similar changes were observed, although considerably less frequently, following introduction of dimethylformamide and even of normal saline. This is in accordance with a well-known fact that different agents at the same stage of development may produce similar changes [22].

A wide use of developing chick embryos in the study of teratogenic effects of new drugs would be well justified because defects of development, such as seen in mammals, can be reproduced in chick embryos, and they are convenient to use in mass experiments.

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