HOKKAIDO UNIVERSITY

Title	Some Aspects of the Effect of Endoxan on Developing Mouse Embryos (With 3 Text-figures and 1 Table)
Author(s)	SHOJI, Ryujiro; OHZU, Eiji
Citation	北海道大學理學部紀要, 15(4), 662-665
Issue Date	1965-12
Doc URL	https://hdl.handle.net/2115/27409
Type	departmental bulletin paper
File Information	15(4)_P662-665.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Some Aspects of the Effect of Endoxan on Developing Mouse Embryos[1),2),3)]

By

Ryujiro Shoji and Eiji Ohzu[4)]

Zoological Institute, Hokkaido University

(With 3 Text-figures and 1 Table)

Teratogenic effects of alkylating agents, derivatives of the prototype of which is nitrogen mustard, have increasingly been becoming the subject of special attraction among recent investigators (Gillitte and Bodenstein, 1946; Bodenstein, 1947; Haskin, 1948; Murphy, 1959; Cardinali *et al.*, 1961; Ferm, 1963). Because of the fact that some of those alkylating agents have been used therapeutically, it may not be insignificant to examine their teratogenic effects in mammals.

The present authors have been working on effects of alkylating anti-tumor agents on developing mouse embryos. In the previous study were reported certain injurious effects of Velban on pregnant mice inducing dead embryos together with a few malformed ones. The present study deals with the effect of Endoxan, a new alkylating anti-tumor derivative from nitrogen mustard, on developing mouse embryos.

The authors wish to express their sincere gratitude to Professor Sajiro Makino for his direction and for going through the manuscript. Further cordial thanks are offered to Drs. Eizi Momma, Akio A. Awa and Yanagi Kimura for their valuable advice and important criticism. Endoxan was kindly supplied through the courtesy of Mr. Tsuyoshi Takahashi, Shionogi and Co. Ltd., to whom the authors are grateful.

Materials and methods

Mice aged nearly 60 days, of DM/Mk strain were used exclusively for experiments. Oestrous females inspected by means of vaginal smears were mated to male mice of the same strain. The females were examined for the presence of vaginal plugs as a sign of successful

1) Contribution No. 717 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

2) This paper is dedicated to Professor Sajiro Makino, Zoological Institute, Hokkaido University, Sapporo, in honor of his sixtieth birthday, June 21, 1966.

3) Supported by a grant from Ministry of Education for the Co-operative Research (Cancer), No. 956105, 1965,

4) Present address: Research Laboratory, Kyowa-Hakko Kogyo Co. Ltd., Nagaizumi, Shizuoka, Japan.

copulation. Pregnant females were divided into three groups. Each group received subcutaneous injection during a period of the 11th to 14th day of gestation at daily doses of 100 mg/kg, 50 mg/kg, and 25 mg/kg in 0.5 ml saline solution, respectively. Control pregnant females also received subcutaneously a similar volume of saline solution during the same period of gestation.

All females were killed on the 18.5th day of gestation in order to examine the condition and development of fetuses. Dead fetuses were classified grossly in the following two groups: i) fetuses died before the 10th day of gestation (early death), and ii) fetuses died after the 11th day of gestation (late death). All fetuses preserved in 70 per cent alcohol were inspected for malformations.

Results

Data presented by the present experiments are summarized in Table 1. It was shown that abnormal fetuses occurred mostly in the experimental groups. Abnormalities observed are as follows; cleft palate, syndactyly, and short kinky tail, though the latter anomaly occurred even in the control group. Two abnormalities, cleft palate (Fig. 1) and syndactyly (Figs. 2 and 3), occurred only in the experimental groups. The control and experimental groups were compared in terms of the number of animals with malformations. The difference was statistically significant between the control and the experimental groups: $t=2.39$, $p=0.02$. The third anomaly, short kinky tail showed a low frequency of occurrence in both control and experimental groups. The tail abnormality varied in degree of shortening. The difference between the control and the experimental groups was not statistically significant: $t=0.4$, $p>0.5$. On this basis, it is difficult to conclude that the third anomaly was induced by the drug.

Table 1. Abnormal fetuses obtained from females administered by Endoxan

Dose (mg/kg)	No. of pregnancy	No. of implantation	No. of early death	No. of late death	No. of cleft palate	No. of syndactyly	No. of short tail
100	20	172	3 (1.7)	21 (12.2)	24 (14.0)	10 (5.8)	0
50	18	141	5 (3.5)	14 (9.9)	6 (4.3)	18 (12.8)	0
25	20	158	7 (4.4)	18 (11.4)	0	0	3 (1.9)
Total	58	471	15 (3.2)	53 (11.3)	30 (6.4)	28 (5.9)	3 (0.6)
Control	20	145	5 (3.4)	4 (2.8)	0	0	2 (1.4)

It was also shown that dead fetuses involving early and late death were observed in both control and experimental groups. It appears that the incidence of late death was high in the experimental groups, and increased with

doses. The difference in number of late death between the control and the experimental groups was statistically significant: t=2.71, p=0.01. On the other hand, the frequency of early death was not statistically significant between the two groups: t=1.21, p=0.2.

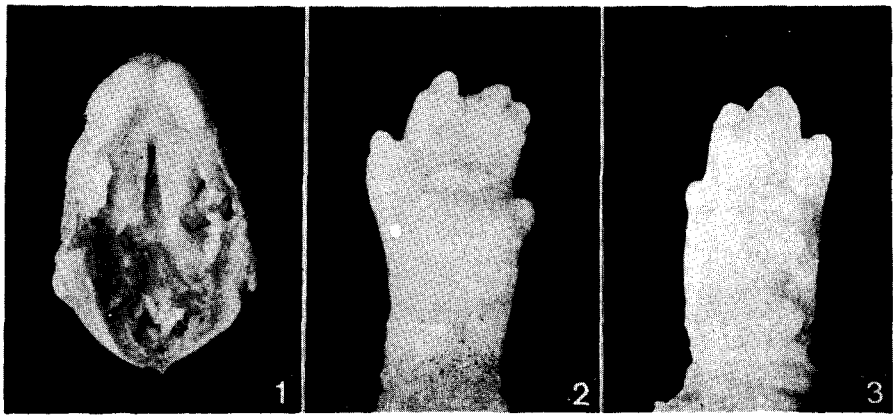

Fig. 1. Cleft palate, from an Endoxan-treated fetus.
Figs. 2 and 3. Syndactyly from Endoxan-treated fetuses. Fig. 2: the 2nd and 3rd fingers fused. Fig. 3: one of fingers absent.

Discussion

Murphy (1959) studied teratogenic effects of various alkylating agents on rat embryos 12 days after gestation under various doses, and reported that the high incidence of cleft palate and syndactyly occurred together with some other external and skeletal abnormalities in the experimental groups. He further described that there was a similarity in the type of abnormalities produced by those agents, though some distinct differences in skeletal abnormalities are noted. The results obtained in the present experiments with Endoxan on mouse embryos indicated that the high incidence of cleft palate and syndactyly occurred in the experimental groups. The present results seem to supplement those of Murphy, so far as induced external abnormalities are concerned.

Ohzu and Shoji (1965) studied effects of Velban on the mouse embryos of MT and dd strains, and reported that the incidence of anomalies with harelip and hindfoot polydactyly occurred exclusively in the experimental groups of MT strain mice. Anomalies reported by them differ from those by the present experiments and also by Murphy (1959). The situation may be attributable to the difference in sensitivity by different mouse strains. Similar features were presented by Cardinali *et al.* (1961) in mice, rats and some other animals after alkylating agent treatments.

Ferm (1963), working on the effect of anti-tumor alkylating agents on pregnant hamsters, concluded that the common teratogenic action of those agents might be caused by their mitosis-arresting activity on developing embryonic tissues. Shoji and Kimura (1964) studied the antimitotic activity of Endoxan on HeLa cells *in vitro*, reporting that Endoxan exerted remarkable growth-inhibiting effects upon HeLa cells when exposed just after cultivation. The results obtained in the present experiments seem to be in favor of the view of Ferm (1963).

Summary

Effects of Endoxan on mouse embryos were studied with special regard to the incidence of abnormal embryos and death of fetuses. Three different doses of Endoxan were given subcutaneously to pregnant females at 11- to 14-day-gestation.

Abnormalities, such as cleft palate and syndactyly, occurred exclusively in the experimental groups. The ratio of early death of fetuses to treated females was nearly the same as that of the control groups, whereas the frequency of late death was statistically higher in the experimental groups than in the control groups.

References

Bodenstein, D. 1947. The effects of nitrogen mustard on embryonic amphibian development. J. Exp. Zool. **104**: 311–342.

Cardinali, G., G. Cardinali, and J. Blair 1961. The stathmokinetic effect of vincaleukoblastine on normal bone marrow and leukemic cells. Cancer Res. **21**: 1542–1544.

Ferm, V.H. 1963. Congenital malformations in hamster embryos after treatment with vinblastine and vincristine. Science **141**: 426.

Gillitte, A., and D. Bodenstein 1946. Specific developmental inhibitions produced in amphibian embryos by a nitrogen mustard compound. J. Exp. Zool. **103**: 1–32.

Haskin, D. 1948. Some effects of nitrogen mustard on the development of external body form in the fetal rat. Anat. Rec. **102**: 493–509.

Murphy, M.L. 1959. A comparison of the teratogenic effects of five polyfunctional alkylating agents on the rat fetus. Pediatrics **23**: 231–244.

Ohzu, E., and R. Shoji 1965. Preliminary notes on abnormalities induced by Velban in developing mouse embryos. Proc. Jap. Acad. **41**: 321–325.

Shoji, R., and Y. Kimura 1964. Cytological effects of Endoxan (ASTA) on HeLa cells *in vitro*. Jap. J. Genet. **39**: 276–282.