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Embryotoxicity and Teratogenicity of Some Derivatives of Chloroethylaminophenylacetic Acid

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Embryotoxic and teratogenic properties of Lophenal, Phenalon, Pharanox and Pharanoxi selenate were investigated experimentally. All examined antitumour agents showed embryotoxic effects. Lophenal, Phenalon and Pharanox had teratogenic effects. By modifying the structure of Pharanox with selenium a reduction in teratogenic effect was achieved. (Pathology Oncology Research Vol 4, No 1, 27–29, 1998)

Key words: embryotoxiciy, terotogenicity, antitumor agents

Introduction

Lophenal, Phenalon, Pharanox and its derivative, Pharanoxi selenate, are all chloroethylaminophenylacetic acid variants produced in Lithuania.[1,2] Lophenal is used for combination therapy of lymphoproliferation diseases, ovarian cancer and other tumors.[3] The transport component of the cytotoxic group DL-phenylalanine, which was included in Lophenal, was changed to physiologically active L-phenylalanine in Phenalon. In clinical trials, Phenalon demonstrated activity against a broad spectrum of experimental tumors. In testing against 18 different transplantable tumors, Phenalon inhibited the growth of epithelial and other tumors and prolonged the life span of animals with hemoblastosis.[1] In humans the most sensitive to Phenalon are malignant lymphomas, especially in cases with chemoresistancy.[4]

In order to achieve more effective and selective antitumor action, Pharanox was developed. Pharanox was further modified on the cytotoxic group by oxygen, and later by selenious acid obtaining a corresponding alkylating agent, Pharanoxi selenate.[2] Pharanox was shown to be an effective antitumor agent in experimental studies and it is now introduced into clinical trials. Most sensitive to Pharanox is metastasic melanoma; less susceptible are lymphomas and rectal cancer.[5] It should be noted that Pharanox improves the therapeutic effect of radiotherapy in the treatment of advanced spinocellular lung cancer.[6] The antitumor activity of the new selenium containing alkylating agent is similar or higher than that of the agent without selenium.[7]

Alkylating antitumor drugs such as sarcolysin, leukeran and others used in clinical oncology are known to have embryotoxic and teratogenic effects.[8,9] The aim of this study was to establish embryotoxic and teratogenic properties of the following new antitumour drugs: Lophenal, Phenalon, Pharanox and Pharanoxi selenate.

Materials and Methods

Lophenal, Pharanox and Pharanoxi selenate have been synthesized at the Lithuanian Oncology Center. Phenalon was made at the Lithuanian Biochemistry Institute. Drugs were given in therapeutic doses: Lophenal – per os, 25 mg/kg, Phenalon – 20 mg/kg, Pharanox and Pharanoxi selenate – 10 mg/kg. Experiments were performed on 432 pregnant Wistar rats, 200-250 g, (on 3834 embryos); while 108 pregnant rats (1543 embryos) were used in control groups. Three series of experiments were performed. Animals of every series were divided into 18 groups (6 mated females per group). Female rats were mated with male rats in the evenings. The following morning vaginal smears were examined and the females were considered

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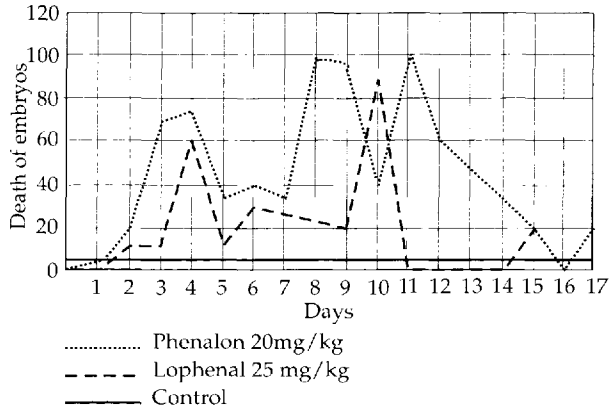

Figure 1. *Embryotoxicity of Lophenal and Phenalon*

mated if sperm was found in the smear. That day was designated as day 0 of pregnancy.

Antitumor agents were given once from 1th to 16-18th days of embryogenesis. On the 21th day of pregnancy the rats were sacrified in the state of neuroleptoanalgesia and autopsy was performed identifying number of corpora lutea, number of implantation sites, number of resorption sites, number of live or died fetuses per litter. The fetuses were also weighed and examined for gross malformations. Percent of postimplantation death as well as percent of fetuses with external malformations were calculated.[10-12] One half of the fetuses were eviscerated and fixed in 70% ethanol for skeletal examination[13] and the other half of the fetuses were fixed in Bouins fluid for histological evaluation.[14] Statistical evaluation was performed by using the Student t-test.

Results and Discussion

Embryotoxicity

Lophenal and Phenalon both showed embryotoxic effects, however Lophenal less so than Phenalon. The embryotoxic effect of Phenalon was pronounced at the early stage of implantation as well as during placenta formation and at the stage of active organogenesis. The embryotoxic effect of Lophenal was detected at the stage of implantation and during neurulation *(Figure 1)*. Pharanox and its derivative containing selenium showed the same embryotoxic effect as Lophenal and Phenalon. Pharanox was embryotoxic at the stages of implatation and active organogenesis; while Pharanoxi selenate at the stages of placenta formation and active organogenesis *(Figure 2)*.

Teratogenicity

Lophenal, Phenalon and Pharanox showed highly expressed teratogenetic effect, especially in the stage of organogenesis *(Figure 3)*. Lophenal is less teratogenic than Phenalon and Pharanox. In cases of Lophenal, Phenalon and Pharanox treatment the following abnormalities appeared: exencephaly, exophtalmia, ectrodactylia, reduction of mandible and tail and others *(Figure 4)*. It is important to note that Pharanoxi selenate is not teratogenic, which is probably due to the antioxidant properties of selenium. There are data in the literature that of free-oxygen radicals can mediate teratogenic effect.[15] It seems that the antioxidant selenium in Pharanoxi selenate counteract with the production of free-oxygen radicals induced by alkylating capacity of Pharanoxi.

Embryotoxic and teratogenic drugs belong mostly to the family of alkylating agents. Therefore Myelosan, Sarcolysin, Cyclophosphamide, Chlorambucyl and others were found to be embrytoxic and teratogenic.[8,9,16] Lophenal and Phenalon showed such embryotoxicity on the 4th day of embryogenesis, similar to the embryotoxicity of Sarcolysin.[17] However, increased toxicity at the 10th and 11th day of embryogenesis by Lophenal and Phenalon is typical to Myelosan.[16] The embryotoxicity of Pharanox appeared on the 7th and 10th day of embryogenesis, similar to the embryotoxicity of Lophenal and Phenalon. However the embryotoxicity of Pharanox is less

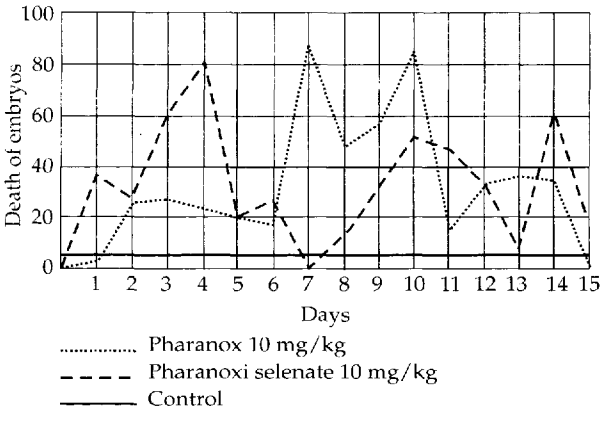

Figure 2. *Embryotoxicity of Pharanox and Pharanoxi selenate*

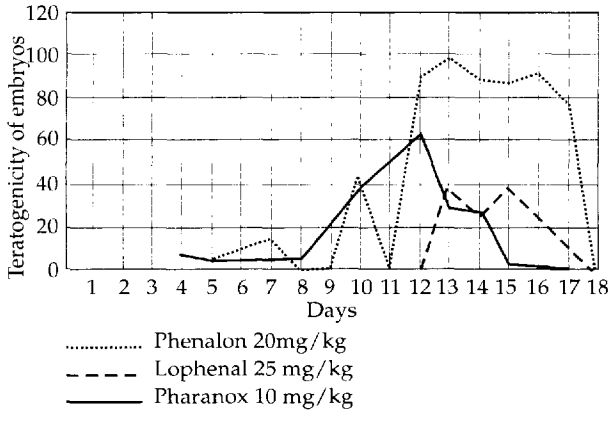

Figure 3. *Teratogenicity of Lophenal and Pharanox*

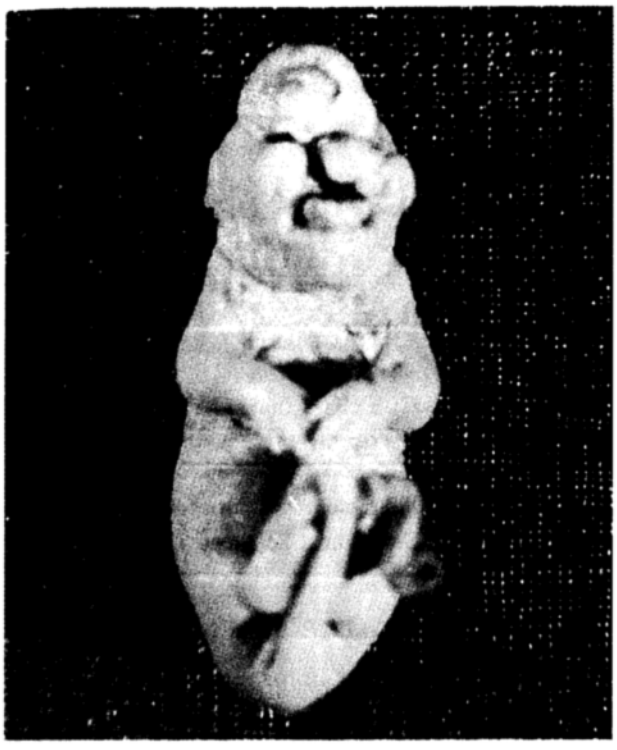

Figure 4. Rat fetus with malformed jaw and snout.

pronounced than Lophenal and Phenalon. Pharanoxi selenate is embryotoxic on 4th day of embryogenesis which is characteristic of Sarcolysin.[17] Lophenal, Phenalon and Pharanox, as with many alkylating agents, showed teratogenic effects during the stage of active organogenesis.[16]

Skeletal examination of fetuses

It was proved that Lophenal, Phenalon and Pharanox have negative effect on the ossification process of the bones.

Conclusions

(a) All alkylating antitumor agents examined, Lophenal, Phenalon, Pharanox and Pharanoxi selenate are embryotoxic, however the degree of embryotoxicity depends on the structure of the compounds and stages of embryogenesis;

(b) modifying the structure of Pharanox with selenium leads to the reduction in teratogenetic effects.

(c) Lophenal, Phenalon and Pharanox have negative effect on bone ossification.

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