CASE REPORT

Breast tumor developed in a pregnant rat after treatment with the teratogen Cycloheximide

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Abstract

Aim: To describe histochemically and immunohistochemically a breast tumor presented in a pregnant rat given Cycloheximide to examine its teratogenic and embryotoxic effect on the embryos.

Methods: Cycloheximide was injected in pregnant Wistar rats at a dose of 3 mg/kg.b.w. on both 10th and 11th gestational days. In one of the rats, a large breast tumor developed rapidly. Histochemical staining with Hematoxylin-Eosin and Masson Trichrome and immunohistochemical identification with mouse monoclonal antibodies: a) Estrogen Receptor A and b) Estrogen Receptor B was performed.

Results: Analysis with A-receptor and B-receptor showed that the breast tumor which was developed after treatment with Cycloheximide was malignant. Positive immunohistochemical reaction was evident especially with A-receptor indicating the malignancy of the tumor.

Conclusions: Cycloheximide is a known toxic and teratogenic agent and potentially a carcinogenic drug. Thus it should be used with extreme caution as a pesticide. Hippokratia 2010; 14 (2): 136-138

Key words: cycloheximide, toxicity, teratogenesis, estrogen receptors, breast tumor.

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The present study was designed in order to analyze histochemically and immunohistochemically a sizeable tumor which was developed at the abdominal region of a white rat, which was treated during pregnancy with the teratogenic and embryotoxic agent Cycloheximide. This study was undertaken in order to examine and determine the role of Cycloheximide in the pathogenesis of this certain tumor and to define the tumor's identity through immunohistochemical identification of estrogen receptors A and B.

Cycloheximide is a toxic agent and a protein synthesis inhibitor and exerts its effect by blocking protein translation progress[1-3]. Because of significant toxic side effects, it is generally used only for in vitro research applications. However it is widely used as a fungicide. It is considered to be a highly teratogenic agent, expressing its teratogenic activity mainly on the mammalian skeleton. Considering that this chemical substance is implicated in the cell cycle and the cell apoptosis, it is appropriate for the study of molecular teratology[4].

Cycloheximide (CHX) has also been studied in breast cancer cell lines. Therefore, studies from Mullauer et al[5] on breast cancer epithelial cell lines on Fas (CD95/Apo-1) ligand, which is a cell membrane receptor that upon binding by its ligand (FasL) triggers a signal, resulting in apoptotic cell death, indicated that co-treatment of breast cancer cell lines with Cycloheximide rendered the resistant cell line sensitive.

Description

Ten female white rats which were mated for 24 hours. Female rats were examined for vaginal plug after this period of time. All female rats which were found positive for a vaginal plug were divided into two groups of five animals each (Groups 1 and 2). Group 1 was treated with Cycloheximide while Group 2 remained untreated (control group). The guide for the care and use of laboratory animals of the National Academy Press (1996) was taken into account.

Cycloheximide (Sigma) was administered to 5 pregnant Wistar white rats at a dose of 3 mg/kg.b.w. (injected intramuscularly in isotonic solution NaCl 9‰) on 10th and 11th gestational days. Pregnant animals were examined on a daily basis in terms of body weight, vaginal hemorrhage, convulsions, light-darkness sensitivity and haemopthalmus. These parameters are indicative of the toxicity of the drug. Pregnancy on most of the rats resulted in pregnancy regressions, abortions and teratomas

development. Three of the animals were sacrificed on the 18th gestational day and from their uterus, after vertical laparotomy, unformed embryonic masses and teratomas were removed. The remaining two animals were retained in order to be followed up for their gestation and delivery results. None of the two animals' pregnancy reached the gestation term and labor, as spontaneous abortion took place. Nevertheless it was decided to keep both the animals alive in order to be followed up. Two months later one of the animals died while on the other one, which the present study refers to, the presence of a subcutaneous mass at the abdominal region was observed. The rat was kept alive and there was a two month follow up of this mass which rapidly developed into a large subcutaneous tumor around the breast areas. Except for the presence of the mass, the animal presented with intense emaciation. Three months after the appearance of the tumor, the rat was anaesthetized and the toumor was resected surgically.

Selected tissue segments were fixed in neutral formalin 10% for histochemical and immunohistochemical identification of A and B estrogen receptors. Macroscopical examination of the tumor showed tissue unevenness, consisting mainly of large parts of connective tissue, calcified parts and large parts of glandular parenchyma. The tumor was surrounded by abundant large blood vessels (Figure 1).

Selected areas from the tumor were embedded in paraffin and histological tissue sections 4-5 μm thick were placed on superfrost microscope slides positively charged. Histochemical staining was performed with Hematoxylin-Eosin and Masson Trichrome. Immunohistochemical identification was performed with Novocastra laboratories Ltd, UK mouse monoclonal antibodies:

α) Estrogen Receptor (mouse monoclonal antibody) NCL-ER-6F11 (A-receptor)

β) Estrogen Receptor (mouse monoclonal antibody) NCL-ER-beta (B-receptor)

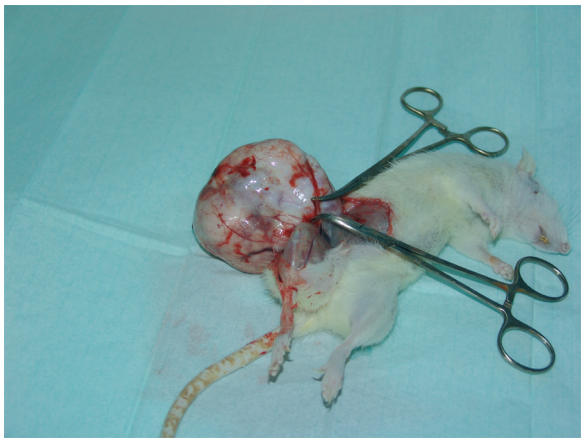

Figure 1: Female rat under general anesthesia and during operation for surgical resection of the tumor. The sizeable tumor is enucleated and extends to the anterior thoracic-abdominal region.

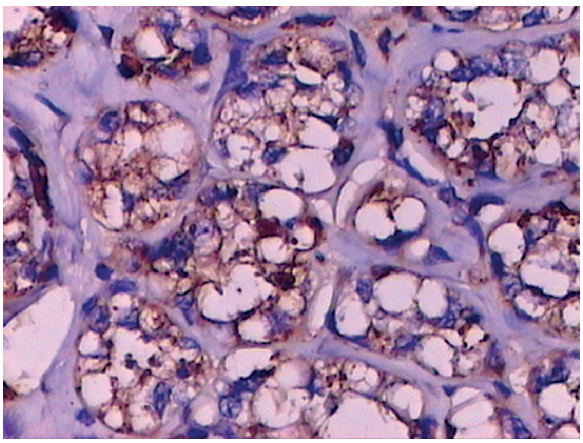

Figure 2: Strongly positive estrogen A detection in the glandular parenchyma of the tumor. Magnification X400.

For Immunohistochemical techniques, a microwave oven was used for the pretreatment of tissues sections.

The same methodology was used for histochemical and immunohistochemical staining for tissue samples of the animals from the second group. Tissue samples from control group animals were also collected from the abdominal region respectively.

With Hematoxylin-Eosin and Masson Trichrome histochemical staining, glandular parenchyma and connective tissue matrix were identified. Those specific areas gave positive response to estrogen receptors B immunohistological staining. More specifically, we observed abundance of fibroblasts into the tumor matrix. Lymphocytes were localized among fibroblasts and collagen fibers and expressed a positive response to estrogen receptors B. Glandular cells of the deferent ducts were moderately positive to immunohistochemical staining for estrogen receptors B. Strongly positive response to the immunohistochemical staining of estrogen receptors B were seen in the small deferent ducts as well as in the final secretory glandular parts.

Strongly positive reaction was observed for estrogen receptors A at the glandular cells of the deferent ducts (Figure 2). These data, according to the international literature as well as the data given from Novocastra Labs, referring to Cycloheximide, suggest that treatment with this agent may contributed to the development of a malignant breast tumor, as it has been shown by the NCL-ER-6F11 antibody (A Estrogen Receptor) in particular, which is used for determining the status of estrogen receptor A in breast cancer tissue[6].

Discussion

Various research studies correlate cycloheximide's activity to the estrogen receptors' expression along with the development of malignant diseases, in particular those of the breast[7,8].

Estrogen expression has been closely linked with breast oncogenesis and malignancy. Estrogen receptors (ER) and their key role in breast cancer progression have

been studied by Swami et al[9]. Estrogens and androgens keep an important role on breast cancer cells development, proliferation and evolution.

Our results support that for the time period and the specific dosage level that the drug was administered, Cycloheximide influenced noxiously the rat's mammary gland and a large malignant breast tumor was developed due to the potential oncogenic action of the drug. The oncogenic activity of cycloheximide was expressed as a side effect after cycloheximide administration in rat pregnancy.

Finally, it should be mentioned that the role of cycloheximide in the modification and development of estrogen receptors still remains under study and discussion for further clarification.

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