Carcinogenic Effect of an Antitumor Drug Etoposide in Laboratory Animals

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Intravenous injection of antitumor drug etoposide in the maximum tolerated dose was followed by the development of breast cancer in 38.1% female Wistar rats and 6.7% female outbred mice.

Key Words: *etoposide; laboratory mice and rats; breast cancer*

The development of secondary diseases is one of the most severe complications of chemotherapy. The risk for secondary malignant tumors after therapy is 10-20 times higher than in the general population [1,2,10]. The frequency of acute leukemia is highest [1,2,5,6,12]. The development of urinary bladder cancer, cervical carcinoma, brain tumor, and malignant histiocytosis in various periods after chemotherapy was also reported [1,2, 7,13]. Alkylating agents, topoisomerase inhibitors, and platinum drugs are cytostatic agents with carcinogenic activity [1,2,6].

Here we studied the long-term effects of animal exposure to toxic activity of an antitumor drug etoposide.

MATERIALS AND METHODS

Experimental rats and mice were obtained from the collection fund of the Laboratory of Experimental Biological Modeling (Institute of Pharmacology, certificate). The animals were kept in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). Before and during the study, all animals were maintained in a vivarium at 20-23°C and 1:1 light/dark conditions; humidity did not exceed 50%; air exchange rate (out/in) was 8:10. The animals were housed in VELAZ standard plastic cages for rats (57.5×35.0×18.5 cm, 5 specimens per cage) and mice (42×25×14 cm, 10 specimens per cage) with fine wood chips. The animals were kept in the open system and fed a standard diet.

Female Wistar rats received single intravenous injection of etoposide (vepeside, Bristol-USA, Teva- Israel; etoposide, Lens-Moscow) in a maximum tolerated dose of 40 mg/kg. This dose was estimated by the graphic probit method. The study involved 42 rats aging 2.0-2.5 months and weighing 200-250 g.

Etoposide was tested for specificity of the toxic effect. Female outbred mice (n=30) and female CBA/CaLac mice (n=30) received single intravenous injection of etoposide in the maximum tolerated dose of 60 mg/kg. Experiments were conducted on the animals aging 2-3 months and weighing 20-22 g. The animals were monitored for 6 months after cytostatic treatment.

Tumors were fixed in formalin and embedded into paraffin. Deparaffinized sections were stained with hematoxylin and eosin. The classification of breast cancers in rats was used to describe the histological samples and to make the diagnosis [11].

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The results were analyzed by Fisher's angular transformation.

RESULTS

Breast cancers in rats were first revealed 3 months after etoposide injection. Six months after treatment, tumors were found in 19 animals (45.2%). The long-term effects of toxic cytostatic agents (anthracyclines, platinum drugs, taxanes, and alkylating compounds) on various organs and tissues were recently studied at the Institute of Pharmacology. These pathological changes were not found in laboratory animals maintained under standard conditions.

Macroscopically, the tumor looked like a round node (diameter 1-7 cm) with irregular surface located subcutaneously but not adhering to the skin. Necrosis in the tumor node spread to the skin in 30% animals. Two tumor nodes were found in 2 rats. None animals had metastasis to regional lymph nodes.

Histological types of breast tumors in etoposide-treated rats (Table 1, Fig. 1) corresponded to

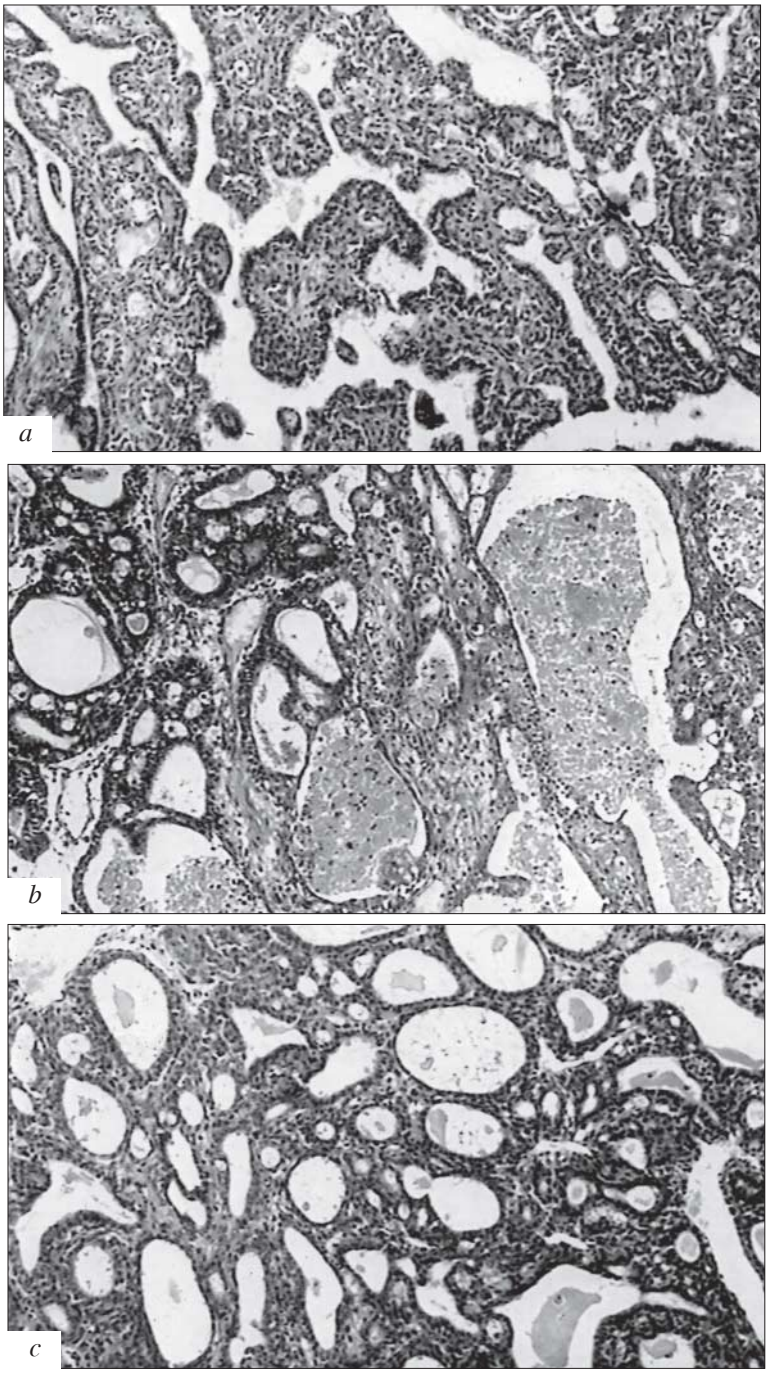

Fig. 1. Breast cancer in rats 6 months after etoposide injection: infiltrating papillary cancer (*a*); infiltrating comedo-type cancer (*b*); and infiltrating cribriform cancer (*c*). Hematoxylin and eosin staining, ×100.

TABLE 1. Histological Examination of Breast Tumors in Rats (n=42) in the Delayed Period after Etoposide Injection

Histological diagnosis	Number of animals with tumors	Percent of tumors
Benign tumors		
tubular adenoma	2	4.8
intraductal papilloma	1	2.4
total	3	7.1
Malignant tumors		
infiltrating cribriform cancer	4	9.5
infiltrating papillary cancer	7	16.7
intraductal papillary and cribriform cancer	2	4.8
infiltrating comedo-type cancer	2	4.8
carcinosarcoma with structures of intraductal papillary cancer and leiomyosarcoma	1	2.4
total	16	38.1

the classification [11]. The animals with breast cancer had inflammatory infiltration of the tumor stroma of different severity. The desmoplastic tumor response was found in 3 rats (18.7%).

Regular examination of mice showed that tumors develop only in 2 outbred animals (6.7%) 3 months after etoposide injection. Infiltrating cancer of solid or tubular-and-solid structure was accompanied by stromal infiltration.

Breast cancer in some rats and mice was associated with the development of benign tumors (adenoma). It can be hypothesized that etoposide-induced carcinogenesis develops in several stages and results in malignant transformation.

In the delayed period after etoposide injection, breast cancer (secondary tumors) was more often found in Wistar rats (38.1%, $p<0.001$) than in outbred (6.6%) and CBA/CaLac mice (10%).

Recent studies showed that one of the risk factors for secondary tumor growth is genetic predisposition, which has a synergistic effect on carcinogenic activity of cytostatic drugs [2]. Similarly to all antitumor drugs, etoposide is a genotoxic compound that causes mutations in gametes and somatic cells [6,9]. The mutagenic effects of this drug are related to the inhibition of topoisomerase activity [3,9]. Moreover, they can be associated with prooxidant activity of etoposide metabolites (*e.g.*, quinones and derivatives) [8]. Published data show that the genotoxic effect of estrogen metabolites, especially, quinones, results in the development of breast cancer [4].

The frequency of breast cancer significantly differs in rats and mice receiving equivalent toxic doses of etoposide. Etoposide chemotherapy does not result in the development of breast cancer in humans. These data suggest the existence of species sensitivity to the cytostatic drug.

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