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INHIBITION OF TRANSPLACENTAL CARCINOGENIC EFFECT OF N-NITROSOMETHYLUREA IN RATS BY BUFORMIN

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UDC 615.277.4:547.495.2].032.013.85

KEY WORDS: N-nitrosomethylurea; transplacental carcinogenesis; latent diabetes; buformin.

An important but little studied aspect of the problem of transplacental carcinogenesis is the search for ways of preventing the onset of tumors in a progeny exposed to the action of chemical carcinogens during the period of embryogenesis [7]. In the writers' opinion, the approach based on the view that during the action of carcinogens in the body, long before clinical manifestation of the tumor, various hormonal-metabolic disturbances develop which, on the one hand, facilitate the development of malignant cells and on the other hand, depress the system of antitumor immunity [5], is very promising.

The aims of the present investigation were to detect some of these disturbances arising during transplacental carcinogenesis and to attempt to use antidiabetic agents to overcome them with a view to inhibiting tumor growth.

EXPERIMENTAL METHOD

On the 21st day of pregnancy female rats were given an intraperitoneal injection of N-nitrosomethylurea (NMU) in a dose of 20 mg/kg. All the F_1 offspring and also the intact rats used as a control were divided into three groups at the age of 3 months. In the rats of group 1, after starvation for 18 h, determinations were made of the blood sugar (by the o-toluidine method) immunoreactive insulin (IRI), cholesterol, triglycerides, and somatomedin, as described previously [3, 4]; The blood sugar, IRI, and somatomedin levels also were determined 30, 60, and 120 min after peroral administration of glucose in a dose of 3 g/kg. Hemicastration was performed on the female rats of group 2 and the ability of diethylstilbestrol propionate (DESP), injected subcutaneously in a dose of 0.57 μg over a period of one week, to depress compensatory hypertrophy of the ovary (CHO) was studied in these animals [2]. The remaining animals, exposed to the transplacental action of NMU and constituting group 3, were divided into subgroups, and until the end of life five times a week they were given perorally through a tube 1 ml of tap water or 5 mg buformin (N-butylbiguanide hydrochloride; "Adebit," from "Chinoin," Hungary), in the same volume of water. Neoplasms discovered were studied microscopically.

EXPERIMENTAL RESULTS

The ability of DESP to inhibit CHO induced by hemicastration was significantly depressed in rats exposed to the transplacental action of NMU (Fig. 1); this may be interpreted as a manifestation of a raised threshold of sensitivity of the hypothalamic-hypophyseal complex to inhibition by estrogen. This corresponds to observations made in experiments in which carcinogens were administered to sexually mature rats [2].

In three-month-old rats whose mothers were exposed to the action of NMU on the 21st day of pregnancy, a decrease in glucose utilization was observed after 1 and 2 h in the glucose tolerance test compared with the control (Fig. 2). The insulin level in the control and experimental animals did not differ significantly either before or after administration of glucose, whereas the somatomedin concentration was significantly depressed in rats exposed to transplacental action of NMU (Fig. 2). The insulin–glucose index in the control rats 2 h

Laboratory of Endocrinology and Laboratory of Experimental Tumors, N. N. Petrov Research Institute of Oncology, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR L. N. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 7, pp. 88-90, July, 1980. Original article submitted July 21, 1979.

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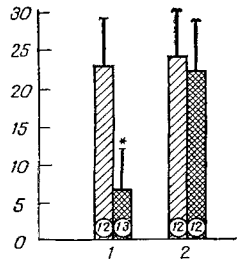

Fig. 1

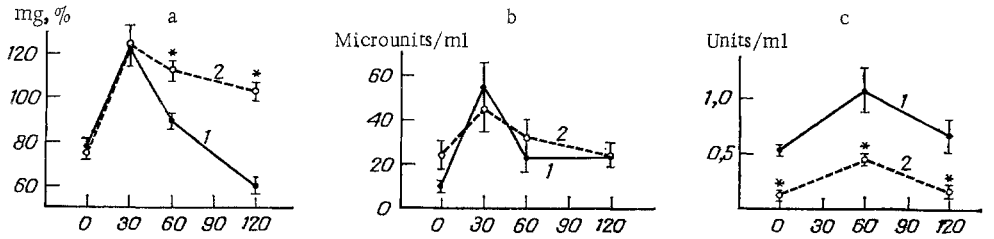

Fig. 2

Fig. 1. Effect of DESP on CHO induced by hemicastration in rats exposed to transplacental action of NMU. Ordinate, degree of CHO (in %). 1) Control, 2) NMU. Oblique shading – injection of oil; cross-hatching – injection of DESP. Circled numbers show number of animals in groups. Values of M ± m given. *) Difference from values in rats receiving oil is significant ($P < 0.05$).

Fig. 2. Dynamics of blood sugar (a), insulin (b), and somatomedin (c) in rats exposed to transplacental action of NMU, in glucose tolerance tests. Abscissa, time (in min) after peroral administration of glucose solution (3 g/kg). 1) Control, 2) NMU. *) Here and in Fig. 3, difference from control is significant ($P < 0.05$).

after glucose loading was 1.5 times higher than in the experimental animals (0.38 and 0.23 respectively), evidence of a fall in the reserve capacity of the islet-cell system and of a relative deficiency of insulin in rats exposed to the action of the carcinogen during embryonic development. The blood cholesterol level of these rats was 1.4 times higher than in the control ($P < 0.05$), but the triglyceride concentration was the same in both groups (Fig. 3).

NMU is known to cause hyperglycemia in Chinese hamsters, an effect which is attributed to its selective harmful action on the β-cells of the pancreas [8, 9]. In mice, however, NMU does not induce hyperglycemia [9]. The results now obtained showed that a disturbance of carbohydrate tolerance of the latent juvenile diabetes mellitus type develops in rats exposed to the transplacental action of NMU. A similar disturbance also has been found after administration of NMU to sexually mature rats [4].

Administration of the antidiabetic drug buformin gave a distinct inhibitory effect on realization of the transplacental carcinogenic action of NMU (Table 1), especially with respect to the frequency of malignant tumors of the nervous system, which was reduced by 3.5 times. An anticarcinogenic effect of the biguanides was observed previously in relation to the carcinogenic action of 7,12-dimethylbenz(a)anthracene and 1,2-dimethylhydrazine on sexually mature animals [4, 6]. On the other hand, the transplacental carcinogenic effect

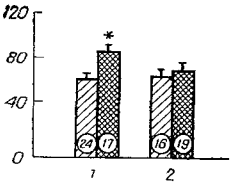

Fig. 3. Blood cholesterol and triglyceride levels (in mg%) of rats exposed to transplacental action of NMU. 1) Cholesterol, 2) triglycerides. Oblique shading – control; cross-hatching – NMU. Circled numbers indicate number of determinations. Values of M ± m given.

TABLE 1. Effect of Buformin on Frequency and Location of Neoplasms in Rats Exposed to Transplacental Action of N-nitrosomethylurea

Exposure to	Number of rats	Number of rats with all tumors	Number of rats with malignant tumors of			Number of rats with benign tumors	Total number of tumors	Mean latent period of tumors, days
			nervous system	kidney	other locations			
NMU transplacentally plus water after third month of life	48	26 (54,2%)	16 (33,4%)	6 (12,5%)	5 (10,4%)	3 (6,3%)	30	458±22
NMU transplacentally plus buformin after third month of life	63	17 (23,6%)*	6 (9,5%)*	8 (12,7%)	1 (1,6%)	2 (3,2%)	17	434±38

*Difference from control significant ($P < 0.05$).

of NMU was potentiated in rats in which a state of permanent estrus was induced at the age of 3 months, accompanied by a lowering of carbohydrate tolerance and by other disturbances of carbohydrate and fat metabolism [1, 3].

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