LITERATURE CITED

1. G. P. Airapet'yan, E. N. Kuz'mina, and I. N. Maiskii, Abstracts of Proceedings of the 3rd International Symposium on the Immunology of Reproduction [in Russian], Varna (1975), pp. 171-172.
2. G. E. Dobretsov, in: Progress in Science and Technology. Series "Molecular Biology," Vol. 6 (1975), p. 34.
3. T. P. Evgen'eva, Dokl. Akad. Nauk SSSR, 204, 1467 (1972).
4. I. B. Zukakova, Vopr. Onkol., No. 2, 17 (1968).
5. L. N. Lebedeva, Vopr. Onkol., No. 5, 73 (1964).
6. I. N. Maiskii, G. P. Airapet'yan, R. P. Gutkova, et al., Byull. Éksp. Biol. Med., No. 11, 86 (1974).
7. P. V. Sergeev, Yu. P. Denisov, I. N. Maiskii, et al., Biofizika, No. 2, 330 (1975).
8. F. Dumont, Ann. Immunol. (Paris), 126, 453 (1975).
9. J. Dyckman and J. K. Weltman, J. Cell. Biol., 45, 192 (1970).
10. S. T. Christian, Int. J. Neurosci., 6, 57 (1973).

INHIBITION OF THE EMBRYOTOXIC AND TERATOGENIC EFFECTS OF METHYLUREA AND SODIUM NITRITE BY VARIOUS SUBSTANCES

V. A. Aleksandrov and F. K. Dzhioev

UDC 615.9.015.4:612.64].08

After combined intragastric administration of methylurea (MU) and sodium nitrite (SN) into rats on the ninth day of pregnancy death of the embryos and a teratogenic effect were observed, due to the endogenous synthesis of nitrosomethylurea, which has a pathogenic effect. Ascorbic acid and urotropin completely blocked the embryotoxic and teratogenic effects observed after combined injection of MU and SN. Sodium sulfamate reduced the embryotoxic effect considerably and the teratogenic effect to some extent, whereas urea did not prevent the manifestation of the harmful action of MU and SN on the embryo.

KEY WORDS: pregnancy; teratogenic effect; embryotoxic effect; methylurea; sodium nitrite.

The possibility of endogenous synthesis of nitroso compounds, which is now well established, and the wide distribution of their precursors in the human environment make it necessary to look for substances which will prevent the formation of carcinogenic nitrosamines in vivo. Ascorbic acid has been suggested as an inhibitor of the endogenous synthesis of nitroso compounds from amines and nitrite [11].

When seeking substances of this kind it is very convenient to use experiments in which several amines and amides are injected at the same time as sodium nitrite into pregnant animals and then to assess the embryotoxic and teratogenic effects on their fetuses, for experiments of this type take little time and the effect can be clearly described quantitatively [2].

In this investigation the effect of ascorbic acid, urotropin, sodium sulfamate, and urea on the embryotoxic and teratogenic effects of combined administration of methylurea (MU) and sodium nitrite (SN) to rats on the ninth day of pregnancy was determined.

Laboratory of Experimental Tumors and Laboratory of Carcinogenic Agents, N. N. Petrov Scientific-Research Institute of Oncology, Ministry of Health of the USSR, Leningrad.

(Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 1, pp. 75-77, January, 1977. Original article submitted January 12, 1976.

TABLE 1. Effect of Various Substances on Manifestations of Pathogenic Action of MU and SN on Rat Fetuses When Administered Together on Ninth Day of Pregnancy

Substances given and doses	Number of pregnant rats	Number of implantations	Number of dying fetuses	Number of living fetuses	Number of fetuses with deformities	Type and frequency of deformity
MU – 30 mg/kg SN – 50 mg/kg	21	190	88 (46,3±3,6%)	102	15 (14,7±3,5%)	Exencephalus (1) Hydrocephalus (14) Hernia of the liver (1)
MU – 30 mg/kg SN – 50 mg/kg Ascorbic acid 50 mg/kg	12	112	8 (7,1±2,4%)	104	0	—
MU – 30 mg/kg SN – 50 mg/kg Urotropin 50 mg/kg	14	136	11 (8,1±2,3%)	125	0	—
MU – 30 mg/kg SN – 50 mg/kg Sodium sulfamate 50 mg/kg	17	151	25 (16,4±3,0%)	126	5 (3,9±1,7%)	Hydrocephalus (5)
MU – 30 mg/kg SN – 50 mg /kg Urea 50 mg/kg	14	130	51 (39,2±4,2%)	79	7 (8,8±3,2%)	Hydrocephalus (5) Anophthalmia (2)
Control	12	108	7 (6,4±2,4%)	101	0	—

EXPERIMENTAL METHOD

Noninbred albino rats from the "Rappolovo" nursery, Academy of Sciences of the USSR, were used. Sexually mature females weighing 160-180 g were mated in the evening, and next morning the inseminated females were identified (from the presence of spermatozoa in vaginal smears); this date was noted as the first day of pregnancy.

MU and SN, and also ascorbic acid, urotropin, sodium sulfamate, and urea were dissolved in water and given to the animals by gastric tube, one substance after the other, in a fasting state on the ninth day of pregnancy. MU was given in a dose of 30 mg/kg and all the other substances in a dose of 50 mg/kg. This time of pregnancy was chosen on the basis of the results of the writers' previous experiments with N-nitrosomethylurea (NMU), which showed that rat embryos at the fifth day of development were most sensitive to embryotoxic and teratogenic effects. The animals were killed on the 21st day of pregnancy, laparotomy was performed, and the number of living (normal and abnormal) and dead fetuses was counted. Each fetus was carefully inspected, external abnormalities were recorded, and the fetus was then fixed in Bouin's fluid for 7-10 days. After fixation a microdissection of the fetuses was carried out and all developmental defects discovered were finally counted.

EXPERIMENTAL RESULTS

The experiments showed (Table 1) that after intragastric administration of MU in a dose of 30 mg/kg together with SN in a dose of 50 mg/kg to rats on the 9th day of pregnancy the number of fetuses which died was 46%, and 15 of the 102 surviving fetuses had deformities such as hydrocephalus (14), exencephalus (1), and hernia of the liver (1).

The substances indicated above, when given together with MU and SN, differed in their effects on the action of MU and SN. Ascorbic acid and urotropin, for instance, completely blocked the embryotoxic and teratogenic action of MU and SN. Sodium sulfamate reduced the embryotoxic effect greatly and the teratogenic effect to some degree, whereas urea, in the dose used, did not prevent the manifestation of the embryotoxic action and did not reduce the teratogenic effect.

To decide whether the substances tested can modify the effect of NMU when already formed in vivo from the exogenous precursors a special series of experiments was carried out. After intragastric administration of a single dose (10 mg/kg) of NMU to rats on the ninth day of pregnancy the mortality among fetuses was 42%, and of 62 living fetuses deformities of the brain hernia (in one case), hydrocephalus (in six), and anophthalmia (in three) type were observed in 10 (16.1%). Additional administration of ascorbic acid, urotropin, sodium sulfamate, or urea in a dose of 50 mg/kg (by mouth) together with NMU had no significant effect on the level of embryonic mortality or on the frequency or character of the deformities. Consequently, the substances tested can be considered to modify endogenous synthesis of NMU before its formation from its precursors.

Evidence has been obtained of a carcinogenic effect manifested in rats, mice, and hamsters exposed to combined treatment with alkylurea and SN both in the postnatal [9, 15] and in the antenatal (transplacental action) period of their development [6, 13, 14]. Finally, direct evidence has now been obtained of the formation of nitrosamides in the gastrointestinal tract of rats and in a model system in vitro from the corresponding alkylureas and SN [8, 12]. When rats were given MU in a dose of 21.5 mg together with SN in a dose of 10 mg as aqueous solutions, about 27% of NMU was found to be formed in the gastric contents after 45 min [10].

Experiments on model systems have repeatedly shown that ascorbic acid is an effective inhibitor of the endogenous synthesis of nitroso compounds from precursors, for it forms an ascorbate which is then oxidized to a dehydroascorbate. The velocity constant of this reaction under the conditions chosen was greater than the velocity constant of the reaction of introducing the nitroso group into amines. As a result, of the two reactions of formation of dihydroascorbate and nitrosamine, competing for the nitrosifying agents, the first took place faster and so prevented the formation of the carcinogenic nitroso compounds [3-5, 11].

It has been reported that in the presence of ascorbic acid the hepatotoxic effect observed after combined administration of amidopyrine and SN to mice [5] and the teratogenic effect after administration of ethylurea with SN to rats [7] are weakened. In the present experiments urotropin and sodium sulfamate, as well as ascorbic acid, were sufficiently effective as inhibitors. These results must be taken into consideration when methods are developed for preventing the possible formation of carcinogenic nitroso compounds in the human body.

LITERATURE CITED

1. V. A. Aleksandrov, Vopr. Onkol., No. 12, 76 (1974).
2. V. A. Aleksandrov (V. A. Alexandrov) et al., Experientia, 27, 538 (1971).
3. M. C. Archer, S. R. Tannenbaum, T. Y. Fan, et al., J. Nat. Cancer Inst., 54, 1203 (1975).
4. C. A. Bunton, H. Dahn, and L. Loewe, Nature, 183, 163 (1959).
5. M. Greenblatt, J. Nat. Cancer Inst., 50, 1055 (1973).
6. S. Ivankovic and R. Preussmann, Naturwissenschaften, 57, 460 (1970).
7. S. Ivankovich, W. J. Zeller, D. Schmähl, et al., Naturwissenschaften, 60, 525 (1973).
8. S. S. Mirvish, J. Nat. Cancer Inst., 46, 1183 (1971).
9. S. S. Mirvish, M. Greenblatt, and V. R. C. Kommineni, J. Nat. Cancer Inst., 48, 1311 (1972).
10. S. S. Mirvish and C. Chu, J. Nat. Cancer Inst., 50, 745 (1973).
11. S. S. Mirvish, L. Wallcave, M. Eagen, and P. Shubik, Science, 177, 64 (1972).
12. R. Montesano and P. N. Magee, Int. J. Cancer, 7, 249 (1971).
13. G. Osske, R. Warzok, and J. Schneider, Arch. Geschwulstforsch., 40, 244 (1972).
14. M. Rustia, Cancer Res., 34, 3232 (1974).
15. J. Sander, Arzneim. Forsch., 21, 1707 (1971).