BIOLOGICAL ACTION OF NITROSODIMETHYLAMINE ON VARIOUS EXPERIMENTAL MODELS STUDIED *in vivo* and *in vitro*

G. I. Sidorenko, V. Yu. Akhundov,
N. N. Litvinov, R. V. Merkur'eva,
A. B. Shekhtman, S. I. Delinskaya,
N. I. Bagirova, and N. P. Burmantova

UDC 615.277.4.076.9

KEY WORDS: nitrosodimethylamine; lysosomes; cytochromes P-450 and b_5; cell culture; embryotoxic effect.

An urgent problem in experimental biology and medicine in general and in environmental hygiene in particular is to improve techniques used to assess the biological action of chemical contaminants involving the use of various experimental models *in vivo* and *in vitro*. The study of this problem by means of modern biochemical and cytological methods of investigation is worthwhile and necessary, for the essential role of enzymic disorganization of intracellular structures in the development of unfavorable sequelae, namely embryotoxic, carcinogenic, and other effects arising under the influence of chemical substances, has been demonstrated in recent years [6, 12].

Meanwhile, despite much research in this field, the comparative evaluation of the biological action *in vivo* and *in vitro* of such widespread chemical carcinogens as the nitroso compounds [8, 9], which cause transplacental carcinogenesis [1, 2], is a very difficult task for the experimenter.

This paper describes the results of a comparative study of the biological action of nitrosodimethylamine (NDMA) on various experimental models: on male rats and pregnant mice, with exposure to the compound *in vivo* followed by the study of embryonic cell cultures *in vitro*.

EXPERIMENTAL METHOD

Experiments were carried out on 240 noninbred male albino rats (40 control and 200 experimental), in which biochemical investigations of the liver tissue were undertaken during the development of the biological effect of NDMA 12, 24, 48, and 72 h after a single dose of the carcinogen (30 mg/kg) administered by the intragastric route, and also after continuous intake of NDMA for 2 and 5 months with the drinking water (0.005, 0.05, and 0.5 mg/kg). Total and free activity of lysosomal hydrolases — N-acetyl-β-D-glucosaminidase and β-galactosidase — was determined in rat liver homogenates by methods described previously [12]. Total activity of acid glycosidases was calculated in micromoles p-nitrophenol liberated by enzymic hydrolysis of 1 g tissue per minute (μmoles/g/min) and free activity was expressed as a percentage of total activity. The permeability of the lysosomal membranes and the possibility of their labilization were judged from the ratio between total and free activity.

To compare the enzymic organization of the lysosomes with NDMA detoxication and metabolism the concentrations of cytochromes P-450 and b_5 were determined in nanomoles/mg microsomal protein [4] and excretion of amidopyrine metabolites (4-aminoantipyrin and N-acetyl-4-aminoantipyrin) with the urine was determined as a percentage of the quantity of amidopyrine administered [7], in parallel tests on the fraction of rat liver microsomes. Protein was determined by Lowry's method [11].

In another series of experiments 300 pregnant mice were used: 200 experimental (1680 embryos) and 100 control (750 embryos). During the last third of pregnancy the pregnant

A. N. Sysin Institute of General and Communal Hygiene, Academy of Medical Sciences of the USSR, Moscow. G. M. Musabekov Azerbaijan Research Institute of Virology, Microbiology, and Hygiene, Baku. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 6, pp. 101-103, June, 1982. Original article submitted September 21, 1980.

 0007-4888/82/9306-0820$07.50

mice were given NDMA solution (30 mg/kg) through a special cannula *per os* directly into the stomach. The animals were killed 2-3 days before parturition and a primary trypsinized tissue culture was prepared from the skin and muscle tissue of the mouse embryos. The seeding dose was 500,000 cells/ml and trypisnization was carried out by the standard method. When a monolayer of cells had formed it was removed and washed several times with Hanks' solution. The thoroughly washed cell monolayer, separated from the glass into a small quantity of physiological phosphate buffer (mechanically or with versene), was centrifuged for 10 min at 700g. The residue was resuspended in 0.25M sucrose solution at the rate of $3 \cdot 10^6$ to $5 \cdot 10^6$ cells/ml sucrose. The cells were then homogenized in a homogenizer of Potter-Elvhjem type with Teflon pestle for 5 min at 3000 rpm. The volume of homogenate was adjusted to 5 ml and cell fragments were removed by centrifugation for a short time at 800g; the completeness of destruction of the cells was verified in the phase-contrast microscope. All manipulations were carried out at 4°C in cold solutions. The supernatant was then centrifuged for 20 min at 10,000g in a refrigeration centrifuge. Enzyme activity and protein concentrations were determined in the supernatant after removal of nuclei and mitochondria. Activity of N-acetyl-β-pre-glucosaminidase and β-galactosidase was determined by the method mentioned above and expressed in micrograms nitrophenol liberated by enzymic hydrolysis of 1 mg protein in 1 min. The results were subjected to statistical analysis by Student's t test at a level of significance of $P < 0.05$.

EXPERIMENTAL RESULTS

Analysis of the data showed that during development of the biological effect of NDMA in male rats *in vivo* the earliest and most significant changes were observed in respect of β-galactosidase, an enzyme located in the matrix of the liver lysosomes. For instance, 12 h after administration of NDMA a significant increase was found in β-galactosidase activity, on average to 0.34 ± 0.01 μmole/g/min, which is 42% higher ($P < 0.01$) than in the control group (0.25 ± 0.02 μmole/g/min). During further development of the biological action of NDMA (24 h after administration) free β-galactosidase activity in the rat liver was increased on average by 45%, evidence of labilization of the lysosomal membranes. Liver β-galactosidase activity in the experimental rats was still increased 72 h after administration of NDMA, when it averaged 0.31 ± 0.03 μmole/g/min. Similar statistically significant activation of liver β-galactosidase also was found after administration of a smaller dose (0.05 mg/kg) of NDMA for a long period (2 months).

The biochemical mechanisms of this activation of the lysosomal matrix enzyme during development of the biological action of NDMA *in vivo* may be determined by the need for intensified detoxication and enzymic degradation of abnormal metabolites accumulating in the cell. This view is supported by the induction of a system of NDMA metabolization discovered after administration of NDMA (0.05 mg/kg) *in vivo*. The effect was manifested after 2 months as a significant increase in the cytochrome P-450 concentration in the microsomal fraction of rat liver on average to 1.31 ± 0.12 nmole/mg ($P < 0.01$), which is 58% higher than the control value (0.83 ± 0.10 nmole/mg). Meanwhile the concentration of cytochrome b_5 in the liver microsomes was increased on average to 0.98 ± 0.11 nmole/mg ($P < 0.01$), which is 61% higher than the control level (0.61 ± 0.03 nmole/mg).

Induction of cytochrome in the liver of the experimental rats was accompanied by changes in the ratio of excretion of amidopyrine metabolites with the urine, namely an average increase of 55% in the 4-aminoantipyrin concentration and a 40% decrease in the N-acetyl-4-aminoantipyrin concentration. These data indicate changes in the relationship between different processes of amidopyrine biotransformation in the microsomes (demethylation and acetylation), evidently due to an early manifestation of the harmful action of NDMA on the detoxication system of the liver.

Biochemical investigation of lysosomal enzyme activity in experiments with mouse embryonic tissue culture *in vitro* also showed that the most significant changes affected β-galactosidase, mean activity of which was increased to 0.011 ± 0.0003 μg/mg/min, 1.5 times higher than the control (0.0072 ± 0.0003 μg/mg/min). Activity of N-acetyl-β-D-glucosaminidase in a culture of mouse embryonic skin and muscle tissue obtained from pregnant animals treated with NDMA also was increased on average by 36% to 0.074 ± 0.005 μg/mg/min (0.055 ± 0.003 μg/mg/min in the control).

Comparison of the usual parameters of the embryotoxic effect (pre-, post-, and total embryonic mortality, weight and size of the fetuses, and so on) and the results of the bio-

chemical tests showed definite correlation between embryotoxicity and activation of lysosomal enzymes.

Whereas in the control group there were no spontaneous abortions, in the experimental group abortions occurred in 30 ± 6% of cases on the 2nd day after administration of NDMA. NDMA increased the prenatal mortality more than fourfold (from 6.7 ± 5 to 32 ± 6.6%). The postnatal mortality also rose sharply (from 9 ± 6 to 37 ± 7%). The total embryonic mortality was relatively high after exposure to NDMA (0.76%). The size and weight of the fetuses showed no significant change.

Incidentally a similar pattern of change in activity of lysosomal acid glycosidases — β-galactosidase and N-acetyl-β-D-glucosaminidase — also was found in the liver and placenta of pregnant rats and the liver of newborn fetuses as a result of the embryotoxic action of several chemicals [6]. These facts are important in connection with the existence of "lysosomal diseases" in man; these are due to a genetic defect in one of the lysosomal enzymes and are accompanied by "compensatory activation" of other lysosomal acid hydrolases [3, 5, 11].

Comparative analysis of the experimental results thus shows that data obtained by the action of NDMA on different experimental models *in vivo* and *in vitro* can be successfully extrapolated.

LITERATURE CITED

1. V. A. Aleksandrov, in: Carcinogenic N-Nitroso Compounds — Action, Synthesis, Determination (First Symposium) [in Russian], Tallin (1973), pp. 84-86.
2. D. Sh. Beniashvili, in: Carcinogenic Nitroso Compounds — Action, Synthesis, Determination (Second Symposium) [in Russian], Tallin (1975), pp. 22-23.
3. G. Ya. Vidershain, in: Biochemical Diagnosis of Hereditary Diseases [in Russian], Moscow (1974), pp. 9-36.
4. I. A. Karuzina and A. I. Archakov, in: Modern Methods in Biochemistry [in Russian], Moscow (1977), pp. 49-62.
5. R. V. Merkur'eva, E. M. Meerson, V. K. Il'ina, et al., in: Biochemical Diagnosis of Hereditary Diseases [in Russian], Moscow (1974), pp. 132-145.
6. R. V. Merkur'eva, Yu. A. Rakhmanin, S. G. Davydova, et al., Vest. Akad. Med. Nauk SSSR, No. 8, 67 (1978).
7. T. A. Popov and O. B. Leonenko, Gig. San., No. 9, 56 (1977).
8. L. M. Shabad, Circulation of Carcinogens in the Environment [in Russian], Moscow (1973).
9. I. N. Shvemberg, Vopr. Onkol., No. 1, 97 (1967).
10. J. Butterworth, G. R. Sutherland, and D. M. Broadhead, Clin. Chim. Acta, 44, 295 (1973).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
12. R. V. Merkur'eva, N. N. Litvinov, G. S. Shaternikova (R. W. Merkurjewa, N. N. Litwinow, G. S. Schaternikowa), et al., Z. Ges. Hyg., 26, 197 (1980).