EFFECT OF REGENERATION STIMULATORS ON CELLULAR AND INTRACELLULAR ORGANELLE FUNCTION IN THE EARLY STAGES OF THE MEMBRANOTROPIC EFFECT OF ENVIRONMENTAL CHEMICAL FACTORS

R. V. Merkur'eva, G. L. Bilich, and N. N. Litvinov

UDC 612.6.03.014.2.014.46-063

KEY WORDS: alveolar macrophages; nitrosodimethylamine; regeneration stimulator; lysosomes; endoplasmic reticulum.

Numerous external environmental factors, including chemical pollutants, have a membranotropic action [3, 7, 8, 12]. Modern views on the membrane organization of the cell reflect the unique functions performed by membranes in the body, including a barrier function, connected with the regulation of permeability, active transport of metabolites, protection of the integrity of cell structures, and in the mechanism of receptor interaction [2, 13]. The similarity in principle of the chemical composition and the common pattern of physical organization of different biological membranes suggests that there are general rules which govern the biochemical mechanisms of manifestation of the membrane-damaging effect of external environmental chemical factors [4, 10]. These results, described in detail in the publications cited above, are manifested as systematic enzymic disorganization of cell organelles, accompanied by increased permeability and destabilization of the plasma membrane, lysosomes, endoplasmic reticulum, and mitochondria of the liver, lungs, brain, and so on. Membrane transformation of this kind takes place against the background of marked changes in the carbohydrate moiety of membrane-structured sialoglycoproteins, and this may be one manifestation of the metabolic mechanisms of the membranotropic effect [4, 7].

The metabolic mechanisms of the membrane-damaging effect described above are characteristic of the early stages of development of biological effects, including during exposure to a chemical carcinogen [14]. The role of sialocontaining glycoproteins, which are found not only on the surface of the plasma membrane, but also in all cellular organelles, in neoplastic transformation of the cell is particularly important. Sialic acids modify the antigenic properties of tumor cells, thereby preventing reactions of immunity of the host organism as a whole [11].

Considering the ability of membranes to undergo reconstruction and repair, it was considered important to discover the stability of this membrane-damaging effect, especially in the early stages of its appearance. Is it possible to prevent the membranotropic action of chemical pollutants by increasing the nonspecific resistance of the body, including with the aid of oriented regulation of membrane repair processes?

This paper gives the results obtained by the use of a pyrimidine derivative, substances which are regeneration stimulators and which participate in the regulation of reduction processes in the lungs [1]. Under the influence of several pyrimidine derivatives, it is possible to prevent unfavorable metabolic effects arising at the cell level (alveolar macrophages of the lungs) and in cell organelles (lysosomes) and the endoplasmic reticulum (liver and macrophages) in the early stages of the membrane-damaging action of the widely distributed chemical carcinogen nitrosodimethylamine (NDMA) [6].

Laboratory of Biochemistry, Department of Medico-Biological Research, A. N. Sysin Research Institute of General and Communal Hygiene, Academy of Medical Sciences of the USSR, Moscow. Research Problem Laboratory, Mari University, Ioshkar-Ola. (Presented by Academician of the Academy of Medical Sciences of the USSR G. I. Sidorenko.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 1, pp. 98-100, January, 1984. Original article submitted January 14, 1983.

 0007-4888/84/9701-0124$08.50

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 250-300 g, divided into seven groups (six animals in each group): group 1) intact animals, 2) animals exposed once to NDMA by the intragastric route (30 mg/kg body weight), 3) animals receiving one derivative of the pyrimidine series in a single dose of 100 mg/kg by the intragastric route, 4) animals undergoing left-sided pneumonectomy, 5) animals exposed to a combination of NDMA and the pyrimidine derivative, 7) animals exposed to NDMA, left-sided pneumonectomy, and the regeneration stimulator. All these procedures were carried out on the animals 24 h before isolation of the alveolar macrophages.

The state of function of the lung macrophages and hepatocytes was assessed by a combination of biochemical methods of determining enzyme activity of various subcellular organelles. Alveolar macrophages were isolated and counted, and activity of the enzymes β-galactosidase, N-acetyl-β-D-glucosaminidase, and nonspecific esterase was determined by the methods described previously [5]. The morphologic and morphometric study of lung tissue followed the method developed by the Research Problem Laboratory for the Study of Pharmacologic Regulation of Repair Processes in Organs and Tissues, Mari University [9].

EXPERIMENTAL RESULTS

Injection of the pyrimidine derivative into healthy animals caused an increase in the number of alveolar macrophages, a significant ($P < 0.05$) increase in activity of lysosomal enzymes located in them — the matrix enzyme β-galactosidase on average to 10.35 ± 1.36 nmoles/min/10^6 cells (by 2.8 times), and N-acetyl-β-D-glucosaminidase, partially structurally bound with the lysosomal membranes on average to 6.50 ± 1.07 nmoles/min/10^6 cells compared with the control (4.83 ± 0.67 nmoles/min/10^6 cells). Meanwhile a statistically significant increase in activity of microsomal nonspecific esterase was observed to 6.3 ± 0.78 nmoles/min per 10^6 cells compared with the control (1.8 ± 0.11 nmoles/min per 10^6 cells), which is interpreted as the favorable effect of this compound, directed toward activation of the protective reactions of the host.

A statistically significant decrease in the number of alveolar macrophages and a decrease in activity of nonspecific esterase in these cells on average to 1.4 ± 1.0 nmoles/min per 10^6 cells and of N-acetyl-β-D-glucosaminidase to 1.5 ± 0.2 nmoles/min per 10^6 cells was observed 1 day after injection of NDMA; this reflects the unfavorable biological action of the chemical carcinogen on alveolar macrophage function. Left-sided pneumonectomy led after 1 day to similar changes in macrophage function, accompanied by an increase in volume of the alveoli. In animals exposed to pneumonectomy and NDMA, a significant decrease was found in the number of alveolar macrophages on average by 38% ($P < 0.05$) together with a decrease in activity of N-acetyl-β-D-glucosaminidase compared with the control.

By contrast, combined exposure to NDMA and the pyrimidine derivative led to a considerable increase (on average twofold) in the number of alveolar macrophages and to a marked increase in activity of the enzymes studied in them: β-galactosidase to 7.17 ± 1.05 nmoles/min per 10^6 cells, and nonspecific esterase to 13.6 ± 2.2 nmoles/min per 10^6 cells compared with the control. The corrective action of the pyrimidine derivative was thus exhibited as an increase in the number of alveolar macrophages, performing the function of biological protection of the organism, and increasing activity of enzymes participating in metabolic reactions in the cell. Morphologic and morphometric studies revealed an increase in volume of the parenchyma and in the total alveolar surface area, a decrease in the volume of tissue of the alveolar septa, evidence of the antiedematous action of the pyrimidine derivative, and also a decrease in the volume of the alveoli, from which the protection of acute emphysema after pneumonectomy could be judged.

The effect of a combination of increased enzyme activity in different parts of the alveolar macrophages and simultaneous accumulation of the cells themselves in response to both the isolated and the combined action of the regeneration stimulator and chemical carcinogen, evidently reflects the favorable effect of the pyrimidine derivative used, for it prevents the early metabolic manifestations of the harmful action of NDMA at the cellular and subcellular levels.

The results of these experiments also showed that the regeneration stimulator used normalizes to a certain extent the disturbed metabolic processes in the rat liver in the early stages of action of NDMA. The most marked (completely normalizing) effect of the py-

rimidine derivative was found against β-glucuronidase, whose activity in rat liver tissue in the early stages of exposure to NDMA was significantly increased on average by 31% compared with the control. This fact evidently points to activation of glucuronide metabolism in the course of detoxication of the chemical carcinogen. A similar normalizing action of the pyrimidine derivative also was discovered on the microsomal enzyme of the liver — nonspecific esterase.

As regards sialic acids, unlike the unfavorable action of the carcinogen, when their level in rat liver tissue was significantly lowered on average by 25% ($P < 0.05$), after injection of the pyrimidine derivative the content of sialoglycoproteins increased by 46% ($P < 0.01$), significantly higher than the control level. This last effect is evidently due to activation of biosynthesis of N-acetylneuraminic acid in the liver under the influence of the regeneration stimulator. The results suggest that the onset of metabolic changes at the cellular level (alveolar macrophages) and in the intracellular organelles (lysosomes, microsomes) under the influence of chemical carcinogens may be due to a certain extent to a disturbance of the regulatory mechanisms taking place in particular with the participation of pyrimidine derivatives. Prospects for a further oriented search for biologically active substances which participate in regulation of the biochemical mechanisms of the membrane-damaging effect, with the aim of increasing pathogenetic resistance and preventing early metabolic manifestations of unfavorable biological effects of external environmental chemical factors, including those of carcinogenic nature, are accordingly revealed.

LITERATURE CITED

1. G. L. Bilich and V. É. Kolla, in: Current Problems in Regeneration [in Russian], Ioshkar-Ola (1982), pp. 25-35.
2. E. I. Volkov and A. S. Chernavskii, Izv. Akad. Nauk SSSR, Ser. Biol., No. 1, 29 (1981).
3. U. A. Kuz'minskaya, L. V. Bersan, and L. M. Veremenko, Vopr. Pitan., No. 5, 48 (1978).
4. R. V. Merkur'eva, Gig. Epidemiol. (Prague), 22, No. 4, 367 (1978).
5. R. V. Merkur'eva, B. V. Aulika , N. N. Skvortsova, et al., Gig. Sanit., No. 9, 75 (1978).
6. R. V. Merkur'eva, N. N. Litvinov, B. V. Aulika, et al., in: Pharmacologic Regulation of Regenerative Processes in Experimental and Clinical Practice [in Russian], Ioshkar-Ola (1981), pp. 11-20.
7. R. V. Merkur'eva, Vopr. Med. Khim., No. 2, 35 (1982).
8. A. O. Puzikov and V. N. Otmakhov, in: Pharmacologic Regulation of Regenerative Processes in Experimental and Clinical Practice [in Russian], Ioshkar-Ola (1978), pp. 59-67.
9. G. I. Sidorenko and R. V. Merkur'eva, Gig. Sanit., No. 8, 8 (1981).
10. C. A. Landa, S. S. Defilpo, R. Maccioni, et al., J. Neurochem., 37, 813 (1981).
11. S. D. Lee (editor), Biochemical Effects of Environmental Pollutants, Ann Arbor, Michigan (1977).
12. V. P. Lento, T. Vartio, and J. Virtanen, FEBS Lett., 24, 289 (1981).
13. R. W. Merkurjewa, N. N. Litwinow, N. P. Burmantowa, et al., Z. Ges. Hyg., 26, 197 (1980).