ROLE OF THE SYSTEM FOR SUPEROXIDE RADICAL FORMATION AND DETOXICATION IN THE MECHANISM OF THE ANTITUMOR EFFECT OF ADRIAMYCIN

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A distinguishing feature of antitumor antibiotics of the anthracycline series, which includes adriamycin, is their ability to increase the intensity of formation of free oxygen radicals by the electron-transport chains of mitochondria [13], microsomes [6], and nuclear membranes [7]. The superoxide ($O_2^{\cdot-}$) and hydroxyl ($OH^{\cdot}$) radicals thus formed can interact with virtually any biomolecules and cause changes in their properties, followed by disturbances of cellular homeostasis [3]. Radicals formed in the immediate proximity of the genetic apparatus of the cell, i.e., on the nuclear membrane, are evidently particularly important. There is convincing proof that free oxygen radicals can cause disturbances of DNA structure [3].

Adriamycin is widely used at the present time in clinical neuro-oncology [2]. However, the molecular mechanisms of sensitivity of malignant brain tumor cells to this antibiotic have virtually not been studied.

The aim of the present investigation was to compare the sensitivity of two strains of experimental brain tumors to the action of adriamycin and to study the intensity of $O_2^{\cdot-}$ formation by nuclear membranes in them and the activity of superoxide dismutase (SOD), an enzyme which inactivates $O_2^{\cdot-}$ [3].

EXPERIMENTAL METHOD

Experiments were carried out on 150 noninbred rats of both sexes aged 2 months. Experimental brain tumors (strain Nos. 35 and 221 [1]) were subjected to passage by a strictly standardized method, by intracerebral inoculation. Nuclei and their membranes were isolated from the brain of intact rats and from the tumor tissue [9, 10]. The intensity of NADPH-dependent $O_2^{\cdot-}$ formation by nuclear membranes was determined by the method in [5]. Total SOD activity and the activity of its isozymes (Cu,Zn-SOD and Mn-SOD) was determined by the method in [8] in tissue extracts prepared by the method in [11]. The protein content in the extracts was determined by Lowry's method.

Adriamycin (Doxorubicin, from Pharmitalia) was injected intravenously in a dose of 3 mg/kg on three consecutive days, starting with the 3rd day (strain No. 35) and the 6th day (strain No. 221) after transplantation of the tumors. As preliminary microscopic studies showed, by this time the tumors measured about 1 mm^3. To assess the effectiveness of therapeutic action, the criteria χ_1, χ_2, χ_3 were calculated [1, 4].

EXPERIMENTAL RESULTS

As the experiments showed, the test tumors differed in the intensity of $O_2^{\cdot-}$ formation by their nuclear membranes, SOD activity, and life span of animals inoculated with the tumors (Table 1).

Assuming that the life span of tumor-bearing animals reflects the degree of malignancy of the tumor, it can be concluded from the results in Table 1 that the intensity of $O_2^{\cdot-}$ formation by the nuclear membranes was directly proportional, whereas SOD activity was inversely proportional, to the degree of malignancy of the tumors studied. The imbalance in cells of

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TABLE 1. Activity of SOD and Its Isozymes and Intensity of Oxygen Formation in Rat Brain and in Tissues of Transplanted Brain Tumors (M ± m)

Test object	Life span of animals after transplantation of tumors, days	Intensity of oxygen formation, nmoles adrenochrome/min/mg protein	Activity of SOD and its isozymes, units/mg protein		
			total	Cu, Zn-SOD	Mn-SOD
Brain of intact rats	—	1,6±0,1 (4)	68,0±6,0 (5)	56,0±6,0 (5)	12,0±1,0 (5)
Strain No. 35	7±2	8,0±1,0 (3)	22,0±1,0 (5)	18,0±2,0 (5)	4,0±0,8 (5)
Strain No. 221	24±7	2,1±0,2 (3)	42,0±2,0 (5)	36,0±2.0 (5)	6,0±1,5 (5)
		$P_{1-2}<0,05$ $P_{1-3}>0,05$ $P_{2-3}<0,05$	$P_{1-2}<0,05$ $P_{1-3}<0,05$ $P_{2-3}<0,05$	$P_{1-2}<0,05$ $P_{1-3}<0,05$ $P_{2-3}<0,05$	$P_{1-2}<0,05$ $P_{1-3}<0,05$ $P_{2-3}>0,05$

Legend. Number of experiments given in parentheses.

TABLE 2. Antitumor Effectiveness of Adriamycin (in percent)

Tumor strain	$\varkappa_1$	$\varkappa_2$	$\varkappa_3$
№ 35	72	160	1,46
№ 221	0	94	0,86

Legend. $\varkappa_1$) fraction of surviving animals of experimental groups at time of death of all control animals, $\varkappa_2$) relative increase in life span (after beginning of treatment) of animals of experimental group, $\varkappa_3$) ratio between time of death of 50% of animals in experimental and control groups.

strain No. 35 between $O_2^{\cdot-}$ formation on the nuclear membrane and enzymic detoxication of these radicals was much stronger than in cells of strain No. 225. When these results are interpreted, the absence of strictly compartmentalized SOD actually in the cell nucleus must also be taken into account [12]. Detoxication of the $O_2^{\cdot-}$ radicals formed with the participation of the nuclear membrane is effected by cytosol SOD (Cu,Zn-SOD). Accordingly, special attention must be paid to the considerable reduction in the activity of this isozyme in cells of the tumors studied compared with brain tissue (Table 1).

In the writers' view, it is the initial imbalance between processes of formation and detoxication of $O_2^{\cdot-}$ in cells of strain No. 35 that is responsible for the higher sensitivity of the cells of this tumor to the action of adriamycin than those of strain No. 221: administration of adriamycin prolonged the life of animals with these tumors by more than 1.5 times (χ_2 = 160), whereas strain No. 221 was practically insensitive to the action of this antibiotic (Table 2).

The results of these experiments thus suggest that the intensity of formation and detoxication of $O_2^{\cdot-}$ in tumor cells can be used as parameters for assessing their sensitivity to the action of antibiotics of the anthracycline series.

LITERATURE CITED

1. E. N. Zhmareva, in: Current Problems in Experimental Tumor Chemotherapy [in Russian], Chernogolovka (1982), pp. 189-191.
2. A. P. Romodanov, in: Neurosurgery [in Russian], No. 14, Kiev (1981), pp. 3-11.
3. I. Fridovich, in: Free Radicals in Biology [Russian translation], Moscow (1979), pp. 272-308.
4. N. M. Émanuél', Kinetics of Experimental Tumor Processes [in Russian], Moscow (1977).
5. S. D. Aust, D. L. Roerig, and T. C. Pederson, Biochem. Biophys. Res. Commun., 47, 1133 (1972).
6. N. R. Bachur, S. L. Gordon, and M. V. Gee, Cancer Res., 38, 1745 (1978).
7. N. R. Bachur, M. V. Gee, and R. D. Friedman, Cancer Res., 42, 1078 (1982).
8. C. Beauchamp and I. Fridovich, Anal. Biochem., 44, 276 (1971).
9. M. Bornens, Methods Cell. Biol., 15, 163 (1977).
10. A. Di Girolamo, F. C. Henshaw, and H. H. Hiaff, J. Mol. Biol., 8, 479 (1964).
11. L. W. Oberley and I. B. Bize, J. Natl. Cancer Inst., 61, 375 (1978).
12. S. E. Patton, G. M. Rosen, and E. S. Rauckman, Mol. Pharmacol., 18, 588 (1980).
13. W. S. Thayer, Chem. Biol. Interact., 19, 265 (1977).