SUPEROXIDE RADICAL FORMATION BY NUCLEAR MEMBRANES OF HUMAN BRAIN TUMORS

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Among the possible causes of transformation of cells and distortion of normal proliferation and differentiation, some investigators include a disturbance of the balance of intracellular processes of formation and detoxication of free oxygen radicals [3, 10, 11]. The basis of such an imbalance in the cells of most experimental tumors may be low superoxide dismutase activity, on the one hand [3, 7, 8, 10-12], and an unchanged or increased rate of formation of superoxide radicals (O_2^-), on the other hand. A widely used method of determining the effectiveness of formation of superoxide radicals by membranes is the adrenalin method, based on inhibition of oxidation of adrenalin by superoxide dismutase (SOD) into adrenochrome in the presence of electron donors [6, 7, 9] although, as was stated previously [4], this method cannot be regarded as unequivocal proof of O_2^- formation. It was shown previously [1] that the nuclear membranes of hepatoma 22a posses much higher SOD-inhibited NADPH-adrenaline oxidase activity than the membranes of normal cells. Hepatoma nuclear membranes were characterized by high cyanide-sensitivty and ability to utilize not only NADPH, but also NADH as electron donors [4, 13]. These results were later confirmed by investigations on several other experimental tumors of rats [5].

Since all the data mentioned above were obtained on experimental models of tumor growth, the study of oxidation of adrenalin in the presence of SOD-inhibited NADPH in human tumors is of considerable interest. In the present investigation this process was studied in preparations of nuclear membranes isolated from human brain tumor tissue.

EXPERIMENTAL METHOD

Human brain tumor tissue obtained during operations at the Kiev Research Institute of Neurosurgery, Ministry of Health of the Ukrainian SSR, was used. Immediately after removal the tumor was washed in isotonic NaCl solution and placed in a container with ice, in which it was kept until required for investigation (1.5-2 h). Pieces of brain adjacent to the tumor but not affected by it, resected during the operation, and also brain tissue from people dying from craniocerebral trauma served as the control.

The nuclear membranes were isolated and all determinations carried out as described previously [5].

EXPERIMENTAL RESULTS

The results showed that nuclear membranes isolated from the tissues of most tumors investigated were able to catalyze oxidation of adrenaline (which was inhibited by SOD) in the presence of both NADPH and NADH (Table 1). Mitochondria were above to form O_2^- [2], and in principle their high content in the nuclear membrane preparation might have affected the results of the measurements. To study this possibility, preparations of nuclear membranes

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TABLE 1. SOD-Sensitive NADPH-Dependent Oxidation of Adrenalin by Nuclear Membranes (nmoles adrenochrome/min/mg protein) Isolated from Human Normal Brain Tissue and Brain Tumors

Case No.	Tissue	NADPH	NADH	Succinate + antimycin A
	I-II degree of malignancy:			
1	Astrocytoma	18	3	<1
2		9	<1	<1
3		19	2,5	2
4	Neurinoma	<1	<1	<1
5	Ependymoma	10	<1	<1
6	Pituitary adenoma	<1	3	4
7	Meningioma	4	7	3
8		12	5	4
	III-IV degree of malignancy:			
9	Anaplastic astrocytoma	21	16	<1
10	Same	9	4	<1
11	» »	14	12	<1
12	» »	20	14	<1
13	Glioblastoma	5	<1	<1
14	»	<1	<1	<1
15	Gliosarcoma	8	2	<1
16	Medulloblastoma	<1	<1	<1
17	Polymorphocellular sarcoma	75	20	<1
	Normal brain tissue*			
1		<1	<1	<1
4	Same	<1	<1	—
9, 12, 16, 17	» »	<1	<1	<1
	Normal brain tissue†	<1	<1	<1

Legend. Degree of malignancy defined according to WHO nomenclature [1]. *) Brain tissue adjacent to tumor but not involved by it (serial number of corresponding case is shown). †) Brain tissue from people dying from craniocerebral trauma (three cases).

were tested for their ability to oxidize adrenaline in the presence of succinate and antimycin A, i.e., under optimal conditions for mitochondria [2]. Only in four cases (Table 1) could NADH-dependent oxidation of adrenaline by the nuclear membranes of the tumors have been due to contamination by mitochondria.

One of the most important distinguishing features of SOD-inhibited adrenaline oxidation in the presence of electron donors in tumor nuclear membranes is cyanide sensitivity [4, 5]. The experiments showed that as in the case of experimental tumors, cyanide, in a concentration of 2 mM, completely inhibited NADPH-dependent adrenalin oxidase in human brain tumor nuclear membranes.

Unlike nuclear membranes of most brain tumors studied, nuclear membranes isolated from normal brain were unable to oxidize adrenaline into adrenochrome in the presence of the two substrates. It will be noted that in some cases nuclear membranes of tumor cells likewise did not oxidize adrenaline into adrenochrome (glioblastoma, neurinoma, medulloblastoma, pituitary adenoma). Differences in the rates of NADPH-dependent oxidation of adrenalin into adrenochrome within a group of tumors of similar histogenesis will be noted and is evidence of the metabolic heterogeneity of human brain tumors.

The results are evidence that the unique properties of nuclear membranes, namely ability to oxidize adrenalin into adrenochrome by a cyanide-sensitive mechanism in the presence of both NADPH and NADH, are possessed by cells not only of experimental tumors in animals, but also by human malignant brain tumors. The study of the connection between the degree of malignancy of the tumor and the presence of the above-mentioned properties of the nuclear membranes is an interesting topic for study. Comparison of the velocity of oxidation of adrenalin into adrenochrome catalyzed by brain tumor nuclear membranes in the presence of NADPH, and inhibited by SOD, with the degree of their malignancy defined as in [1], suggest the existence of positive correlation between them.

LITERATURE CITED

1. A. P. Avtsyn, T. M. Vikhert, and S. Yu. Kasumova, Vopr. Neirokhir., No. 2, 45 (1981).
2. I. V. Girgolava, M. Yu. Ksenzenko, A. A. Konstantinov, et al., Biokhimiya, 45, 757 (1981).
3. A. V. Peskin, I. B. Zbarskii, and A. A. Konstantinov, Dokl. Akad. Nauk SSSR, 229, 751 (1976).

4. A. V. Peskin, I. B. Zharskii, and A. A. Konstantinov, Biokhimiya, 46, 579 (1981).
5. A. V. Peskin, A. M. Tarakhovskii, V. A. Shlyakhovenko, et al., Dokl. Akad. Nauk SSSR, 263, 1270 (1982).
6. S. D. Aust, D. L. Roering, and T. C. Pederson, Biochem. Biophys. Res. Commun., 47, 1133 (1972).
7. I. B. Bize, L. W. Oberley, and H. P. Morris, Cancer Res., 40, 3686 (1980).
8. I. D. Capel and A. Thornley, Eur. J. Cancer, 18, 507 (1982).
9. O. Dionisi, T. Galeotti, and T. Terranova, Biochim. Biophys. Acta, 403, 292 (1975).
10. T. A. Fernandez Pol, P. D. Hamilton, and D. T. Klos, Cancer Res., 42, 69 (1982).
11. L. W. Oberley, T. D. Oberley, and G. R. Buettner, Med. Hypothes., 6, 249 (1980).
12. A. V. Peskin, Y. M. Koen, I. B. Zbarskii (I. B. Zbarsky), et al., FEBS Lett., 78, 41 (1977).
13. A. V. Peskin, I. B. Zbarskii (I. B. Zbarsky), and A. A. Konstantinov, FEBS Lett., 117, 44 (1980).

INDUCTION OF SARCOLYSIN RESISTANCE IN PLASMACYTOMA MOPC/406

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Sarcolysin is highly effective in the treatment of multiple myeloma [1. 4]. However, after several successful courses of treatment with this agent resistance has been observed to develop. In recent years a number of transplantable mouse plasmacytomas have been obtained [3, 4].

Plasmacytoma MOPC/406 was obtained in 1967 in BALB/c mice by injection of mineral oil and immunization with sheep's and bovine red blood cells. It is an experimental model which is similar in histogenesis and morphology to the corresponding human tumors. At the same time, this strain is sensitive to sarcolysin and cyclophosphamide, both of which are widely used clinically for the treatment of multiple myeloma.

The aim of this investigation was to obtain an adequate experimental model of a sarcolysin-resistant plasmacytoma.

EXPERIMENTAL METHOD

Experiments were carried out on 250 BALB/c mice. Plasmacytoma was transplanted by intraperitoneal injection of a suspension of ascites cells. Transplantations were 100% successful after 14 days. Sarcolysin began to be administered 72 h after transplantation.

Induction of resistance to sarcolysin began after the fifth dose of the drug per os or subcutaneously in doses of 0.25, 0.5, and 1 mg/kg. The dose per course was gradually increased, to reach 7.5 mg/kg by the 15th generation, 15 mg/kg by the 17th, and 20 mg/kg by the 18th-20th generations.

The antileukemic effect was assessed by the increase in mean duration of survival. During induction the leukemic cells were preserved by freezing in stages in liquid nitrogen, using a 5% solution of diethyl sulfoxide in the ratio of 1:1 with ascites fluid as the protective agent. The material was poured into polyethylene ampuls, previously sterilized by irradiation. The cells were frozen according to the following program: at the rate of 1° C/min to −40°C, 5°C/min to −80°C, and 20°C/min to −130°C, and then transferred into liquid nitrogen.

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