ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LIII, 11 SECTIO DD 1998

Katedra Patofizjologii Wydziału Medycyny Weterynaryjnej AR w Lublinie
Katedra Patofizjologii Wydziału Lekarskiego AM w Lublinie

ANDRZEJ LEDWOŻYW, KRZYSZTOF LUTNICKI

Antioxidant enzyme activities in human brain tumors

Aktywności enzymów antyoksydacyjnych w guzach mózgu u człowieka

Lipid peroxidation processes are the deteriorative changes of unsaturated fatty acids in cell membranes. It is an autocatalytic chain reaction initiated and propagated by free radicals.

It has long been know that the rate of lipid peroxidation is often low in tumor tissues as compared to the corresponding normal tissues. This has been shown by D o n n a n (7) for hepatoma and S h u s t e r (33) for Ehrlich ascites tumor cells. The work of this author was perhaps for the first time evidence that lipid peroxidation had been proposed to be a regulator of cell division. In the intervening years these reports have been confirmed and extended (18, 37, 38).

At the same time other pertinent properties of tumor cells have been studied, notably their content of enzymes that can stimulate or inhibit lipid peroxidation. As a result, a reasonably clear pattern has emerged as follows. Firstly, the rate of lipid peroxidation is lower in tumor tissues than in corresponding normal tissues. Secondly, tumor cells and its organelles often have an abnormal lipid composition: usually a decreased phospholipid content and a low level of polyunsaturated fatty acids with occasionally increased cholesterol content (8, 29). Thirdly, tumor cells generally contain low, often undetectable levels of the enzymes of cytochrome P-450 system, which can initiate and propagate lipid peroxidation (13, 32). In addition, related observations have been made in dividing normal cells (1, 15) and in developing foetuses (19).

Increased production of free radicals can inhibit DNA synthesis and cell division (9, 36), deteriorate the cell membranes structurally and functionally (5, 16), induce the changes in lipid microenvironment of membrane-bound enzymes and receptors (40). Free radicals can also induce the appearance of new channels in the cell membrane (11, 14) and inactivation of enzymes (17, 28). The excess of free radicals is neutralized by specialized enzymes, such as superoxide dismutase and glutathione peroxidase (10, 26).

The aim of this study was to investigate the antioxidant enzyme activities and malondialdehyde (MDA) levels in human meningioma and glioma tissues, as indicators of lipid peroxidation processes intensity.

PATIENTS AND METHODS

18 brain tumors, including 8 gliomas (5 glioblastomas and 3 astrocytomas) and 10 meningiomas (3 meningotheliomatous, 5 transitional and 2 fibroblastic) were obtained during surgery. The average age of patients was 45 years. The diagnosis of each case was confirmed histologically in Department of Pathology, Medical Academy in Lublin. Portions of adjacent normal-appearing cortex and white matter were obtained for control study.

Tissues were homogenized in 50 mM Tris-HCl buffer, pH = 7.8 in a Waring Blendor homogenizer. The homogenate was centrifuged for 10 min at 2 000 g and in supernatant the activities of superoxide dismutase, glutathione peroxidase and glutathione reductase were estimated as described previously (19). Cytochrome c oxidase was estimated as described by W h a r t o n and T z a g a l o f f (41), protein content was assayed with L o w r y' s et al. (24) method, DNA content was estimated according to R i c h a r d s (30) with diphenylamine.

In parallel experiment, a suitable amount of supernatant was incubated in 37^0 C with or without peroxidation-initiating systems. Three systems were tested, in which peroxidation was stimulated either by NADPH/CCl_4 which requires both cytochrome c reductase and cytochrome P-450 (34); NADPH/ADP/Fe^{2+} which requires NADPH : cytochrome c reductase and by ascorbate/Fe^{2+} which induce lipid peroxidation by the nonenzymatic way (2). After 60 min of incubation, a suitable amount of incubation mixture was removed and MDA concentration was estimated, as described previously (21).

Statistical significance of differences was analyzed by t-Student's test for unpaired data using computer program Statistica, Satsoft Inc., Tulsa, USA.

RESULTS

Table 1 summarizes the activities of antioxidant enzymes in brain tumors. With the exception of cytochrome c oxidase, the activities of all enzymes were higher in white matter, as compared to brain cortex. In neoplastic tissues the activities of these enzymes were significantly lower than in normal tissue.

Table 2 shows spontaneous and stimulated lipid peroxidation in normal brain tissue and in brain tumors. Spontaneous peroxidation was found to be present at much low level in normal brain tissue, but not in tumors. Induced peroxidation in tumor tissues was significantly lower, in comparison with normal brain cortex and white matter.

DISCUSSION

The decrease of superoxide dismutase activity in neoplastic tissues has been observed by several authors (6, 23, 31). Others have found the decrease of catalase activity in mouse hepatomas (3). Low level of superoxide dismutase in liver, spleen, lung and kidney in mice with implanted Ehrlich tumor was observed by L e u t h -a u s e r et al. (22) and V a n B a l g o o y and R o b e r t s (39). At present, the cause of these changes is not known. The fall in antioxidant enzyme activities

Table 1. Antioxidant enzyme activities (units per mg of DNA) in human brain tumors

	White matter	Gray matter	Glioma	Meningioma
Cu, ZN-Superoxide dismutase	41± 3	56 ± 6^{a}	$22 \pm 2^{b,c}$	$14 \pm 2^{d,e}$
MN-Superoxide dismutase	21 ± 2	23 ± 3	$12 \pm 2^{b,c}$	$7 \pm 1^{d,e}$
Glutathione peroxidase	0.26 ± 0.04	1.7 ± 0.2^{a}	$0.4 \pm 0.1^{b,c}$	$0.4 \pm 0.1^{d,e}$
Glutathione reductase	1.1 ± 0.2	0.37 ± 0.04^{a}	$0.12 \pm 0.02^{b,c}$	$0.06 \pm 0.01^{d,e}$
Cytochrome c oxidase	0.26 ± 0.9	10.6 ± 0.8	$4.4 \pm 0.5^{b,c}$	$2.7 \pm 0.3^{d,e}$

Mean values ± S.D. t-Student test for unpaired data: a – $p < 0.01$ gray matter vs white matter, b – $p < 0.001$ glioma vs white matter, c – $p < 0.001$ glioma vs gray matter, d – <0.001 meningioma vs white matter, e – $p < 0.01$ meningioma vs gray matter

may be connected with the changes in cell proliferation rate. However, the fall in Mn-superoxide dismutase activity is not correlated with accelerated cell proliferation. Oberley et al. (27) have shown the increase in activity of this isoenzyme in mouse liver, and this event was correlated with the peaks of cell division. The decrease in antioxidant enzyme activities in neoplastic tissues is not a rule.

In 1,210 leukemia cells as well as in human leukemia cells Cu,Zn-superoxide dismutase activity was significantly higher as compared to normal cells of similar types (42, 43). Gonzales et al. (12) have shown the lack of any changes in catalase and glutathione peroxidase activities in red blood cells isolated from patients with acute myelogenous leukaemia, chronic lymphocytic leukaemia, Hodkins disease, lymphosarcoma and various visceral cancers. These authors have also observed a significant increase in red blood cells superoxide dismutase activity in patients with acute myelogenous leukaemia and lymphoproliferative disorders.

In the present study, the lower activities of antioxidant enzymes in human gliomas and meningiomas were observed. Activities of these enzymes were expressed in units per mg of DNA. This manner of enzyme activity expression proves that in human brain tumors a real decrease in activity of these enzymes exists. Contrary to Sun and Cederbaum (35), we have observed the lower activity of cytochrome c oxidase in brain tumors as compared with normal brain tissue.

Cheeseman et al. (4) have shown the low rate of lipid peroxidation in rat Novikoff hepatoma. In the case of enzymatic systems which were used to initiate lipid peroxidation, this event may be due to low activity of initiating enzymes *per se*. In the Cheeseman's Novikoff hepatoma preparation the NADPH : cytochrome c oxidase was found to be only 10% of the control level. However, the exceedingly low level of ascorbate/iron-induced lipid peroxidation in glioma and meningioma tissues could not be wholly ascribed to low enzyme levels. This suggests that membranes of glioma and meningioma cells contained low levels of polyunsaturated fatty acids and/or elevated levels of free radical scavengers, such as antioxidants.

In fact, Cheeseman et al. (4) have found elevated α-tocopherol concentrations in rat Novikoff hepatoma cells.

The significance of these findings remains to be elucidated. In another paper (20) we have investigated phospholipid and fatty acid composition of gliomas and meningiomas. In this way it is hoped that the true significance of lipid peroxidation in tumor cells can be assessed.

CONCLUSIONS

1. With the exception of cytochrome c oxidase, the activities of all antioxidant enzymes were higher in white matter, as compared to brain cortex. In neoplastic tissues the activities of these enzymes were significantly lower than in normal tissue.

2. Spontaneous peroxidation was found to be present at much low level in normal brain tissue, but not in tumors. Induced peroxidation in tumor tissues was significantly lower, in comparison with normal brain cortex and white matter.

REFERENCES

1. Burlakova E. B., Molochkin B. M., Palmina N. P.: Role of membrane lipid oxidation in control of enzymatic activity in normal and cancer cells. Adv. Enzyme Regul. **18**, 163, 1980.
2. Burton G. W., Joyce A., Ingold K. U.: Is vitamin E the only lipid-soluble chain-breaking antioxidant in human blood plasma and erythrocyte membranes? Arch. Biochem. Biophys. **221**, 281, 1983.
3. Busch H.: Toxohormone. In: An introduction to the biochemistry of the cancer cell. Academic Press, New York 1962.
4. Cheeseman K. H., Burton G. W., Ingold K. U., Slater T. F.: Lipid peroxidation and lipid antioxidants in normal and tumor cells. Toxicol. Pathol. **12**, 235, 1984.
5. Corr P. B., Gross R. W., Sobel B. E.: Amphipathic metabolites and membrane dysfunction in ischemic myocardium. Circ. Res. **55**, 135, 1984.
6. Dionisi D., Galeotti T., Terranova J., Azzi A.: Superoxide radicals and hydrogen peroxide formation in mitochondria from normal and neoplastic tissues. Biochim. Biophys. Acta **403**, 292, 1975.
7. Donnan S. K.: The thiobarbituric acid test applied to tissues from rats treated in various ways. J. Biol. Chem. **182**, 415, 1975.
8. Feo F., Canuto R. A., Bertone G., Garcer R., Pani P.: Cholesterol and phospholipid composition of mitochondria and microsomes isolated from Morris hepatoma 5123 and rat liver. FEBS Lett. **33**, 229, 1973.
9. Frankel E. N.: Lipid oxidation. Prog. Lipid Res. **19**, 1, 1980.
10. Fridovich I.: Superoxide dismutase. Adv. Enzymol. **41**, 35, 1974.
11. Goldstein I. M., Weissman G.: Effects of the generation of superoxide anion on permeability of liposomes. Biochem. Biophys. Res. Commun. **75**, 604, 1977.

12. Gonzales R., Auclair C., Voisin E., Gautero H., Dhermy D., Boivin P.: Superoxide dismutase, catalase and glutathione peroxidase in red blood cells from patients with malignant diseases. Cancer Res. **44**, 4137, 1984.
13. Gravela E., Feo F., Canuto R. A., Garcea R., Gabriel L.: Functional and structural alterations of liver ergastoplasmic membranes during DL-ethionine hepatocarcinogenesis. Cancer Res. **35**, 3041, 1975.
14. Guskova R. A., Ivanov I. I., Koltover V. K., Akhobadze V. V., Rubin A. B.: Permeability of bilayer lipid membranes for superoxide radicals. Biochim. Biophys. Acta **778**, 575, 1984.
15. Henderson P. T., Kerster K. J.: Metabolism of drugs during rat liver regeneration. Biochem. Pharmacol. **19**, 2343, 1970.
16. Herbette L., Messineo F. C., Katz A. M.: The interaction of drugs with the sarcoplasmic reticulum. Annu. Rev. Pharmacol. Toxicol. **22**, 413, 1982.
17. Hochstein P., Jain S. K.: Association of lipid peroxidation and polymerization of membrane proteins with erythrocyte aging. Fed. Proc. **40**, 183, 1981.
18. Lash E. D.: The antioxidant and pro-oxidant activity in ascites tumors. Arch. Biochem. Biophys. **115**, 332, 1966.
19. Ledwożyw A., Kądziołka A.: Ontogenesis of antioxidant enzymes in pig. Pol. Arch. Wet. **29**, 77, 1989.
20. Ledwożyw A., Lutnicki K.: Phospholipids and fatty acids in human brain tumors. Acta Physiol. Hung. **79**, 381, 1992.
21. Ledwożyw A., Michalak J., Stępień A., Kądziołka A.: The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. Clin. Chim. Acta **155**, 275, 1986.
22. Leuthauser S. W. C., Oberley L. W., Oberley T. D., Loven D. P.: Lowered superoxide dismutase activity in distant organs of tumor-bearing mice. J. Natl. Cancer Inst. **72**, 1065, 1984.
23. Leuthauser S. W. C., Oberley L. W., Oberley T. D., Sorenson J. R. Ramakrishna K.: Antitumor effect of a copper coordination compound with superoxide dismutase-like activity. J. Natl. Cancer Inst. **66**, 1077, 1981.
24. Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265, 1951.
25. Malvy C., Paoletti C., Searle A. J. F., Wilson R. L.: Lipid peroxidation in liver: hydroxydimethylcarbazole, a new potent inhibitor. Biochem. Biophys. Res. Commun. **95**, 734, 1980.
26. McCord J. M., Fridovich I.: Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein). J. Biol. Chem. **244**, 6045, 1969.
27. Oberley L. W., Bize I. B., Sahu S. K., Leuthauser S. W. C., Gruber H. E.: Superoxide dismutase activity of normal murine liver, regenerating liver and H6 hepatoma. J. Natl. Cancer Inst. **61**, 375, 1978.
28. Post J. A., Leunissen-Bijvelt J., Ruigrok T. J. C. Verkleij A. J.: Ultrastructural changes of sarcolemma and mitochondria in the isolated rabbit heart during ischemia and reperfusion. Biochim. Biophys. Acta **845**, 119, 1985.
29. Reitz R. C., Thompson J. A., Morris H. P.: Mitochondrial and microsomal phospholipids of Morris hepatoma 7777. Cancer Res. **37**, 561, 1977.
30. Richards G. M.: Modifications of the diphenylamine reaction giving increased sensitivity and simplicity in the estimation of DNA. Anal. Biochem. **57**, 369, 1974.
31. Sahu S. K., Oberley L. W., Stevens R. H., Riley E. F.: Superoxide dismutase activity of Ehrlich ascites tumor cells. J. Natl. Cancer Inst. **58**, 1125, 1977.

32. Saine S. E., Fang W. F., Strobel H. W.: Drug metabolism in Novikoff hepatoma. Evidence for a mixed function oxidase system and partial purification of cytochrome P-450 reductase. Biochim. Biophys. Acta **526**, 345, 1978.
33. Shuster C. W.: Effects of oxidized fatty acids on ascites tumor metabolism. Proc. Soc. Exp. Biol. Med. **90**, 423, 1958.
34. Slater T. F., Sawyer B. C.: The stimulatory effects of CCl_4 and other halogenoalkanes on peroxidative reactions in rat liver fractions *in vitro*. General features of the system used. Biochem. J. **123**, 805, 1971.
35. Sun A. S., Cederbaum A. I.: Oxidoreductase activities in normal rat liver, tumor-bearing rat liver and hepatoma HC-252. Cancer Res. **40**, 4677, 1980.
36. Tappel A. L.: Lipid peroxidation damage to cell components. Fed. Proc. **32**, 1870, 1973.
37. Thiele E. M., Huff J. W.: Lipid peroxide formation and inhibition by tumor mitochondria. Arch. Biochem. Biophys. **8**, 208, 1960.
38. Utsumi K., Yamamoto G., Inaba K.: Failure of Fe^{2+}-induced lipid peroxidation and swelling in the mitochondria isolated from ascites tumor cells. Biochim. Biophys. Acta **105**, 368, 1965.
39. Van Balgoo y J. N. A., Roberts E.: Superoxide dismutase in normal and malignant tissues in different species. Comp. Biochem. Physiol. **628**, 263, 1979.
40. Victor T., Van der Merwe, N., Benade A. J. S., La Cock C., Lochner A.: Mitochondrial phospholipids composition and microviscosity in myocardial ischemia. Biochim. Biophys. Acta **834**, 215, 1985.
41. Wharton D. C., Tzagaloff A.: Cytochrome oxidase from beef heart mitochondria. Meth. Enzymol. **10**, 245, 1967.
42. Yamanaka N., Nishida K., Ota K.: Increase of superoxide dismutase activity in various leukemia cells. Physiol. Chem. Phys. **11**, 253, 1979.
43. Yamanaka N., Ota K., Utsumi K.: Changes in superoxide dismutase activities during development, aging and transformation. In: Hayaishi O., Asada K. (eds.) Biochemical and Medical Aspects of Active Oxygen. University Park Press, Baltimore 1978.

STRESZCZENIE

Badano aktywności enzymów antyoksydacyjnych w substancji szarej i białej mózgu człowieka i tkankach gliomy i meningiomy. Aktywności dysmutazy nadtlenkowej, katalazy, peroksydazy glutationu, reduktazy glutationu i oksydazy cytochromu c były istotnie niższe w guzach, w porównaniu z tkanką nie objętą procesem nowotworowym. Niższe były też natężenia peroksydacji lipidów, zarówno spontanicznej, jak i indukowanej.