

Copper- and Zinc-containing Superoxide Dismutase and Manganese-containing Superoxide Dismutase in Human Tissues and Human Malignant Tumors¹

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ABSTRACT

Superoxide dismutases might conceivably protect against both ionizing radiation and free radical-producing antibiotic antitumor drugs. Copper- and zinc-containing superoxide dismutase (CuZn superoxide dismutase) and manganese-containing superoxide dismutase (Mn superoxide dismutase) were specifically assayed in human malignant tumors and for comparison in human tissues. The tumors possessed less CuZn superoxide dismutase than did the more metabolically active tissues, but there was a large overlap between the tissue and the tumor levels. Mn superoxide dismutase was found in all tumors, and the ratio between the activities of CuZn superoxide dismutase and Mn superoxide dismutase was not different from that of the normal tissues. Human tumors are thus different from tumors from other species which have been reported to be deficient or very low in Mn superoxide dismutase. There was no obvious relation between sensitivity to ionizing radiation and content of the enzymes among the tumors and the tissues, nor did tumor types known to be responsive to radical-producing drugs possess less CuZn superoxide dismutase or Mn superoxide dismutase than other tumors.

INTRODUCTION

The superoxide anion radical O_2^- and its corresponding acid HO_2 [pK_a , 4.88 (3)] are formed directly and through secondary reactions by ionizing radiation. The presence of oxygen in a solution greatly increases the formation of the radical by secondary reactions. Oxygen is also known to increase the biological effects of ionizing radiation. The mechanisms of the oxygen enhancement are not fully known. There are indications that toxic effects of O_2^- or products derived from the radical might contribute to it. The disproportionation of the superoxide radical, $2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$ (20), is very efficiently catalyzed by superoxide dismutases. Superoxide dismutase (EC 1.15.1.1) has been shown to protect DNA (39), proteins (11), and cell membranes (29) against ionizing radiation. Myoblasts (21), *Mycoplasma* (28), and bacteria (22, 26) are protected by superoxide dismutase in the medium. Mice given superoxide dismutase parenterally show increased radioresistance (30, 31). Variation of superoxide dismutase content in bacteria, induced by growth in media lacking the prosthetic metals of superoxide dismutase (26), has indicated the importance of endogenous enzyme in the protection against the oxygen effects. Mammalian cells in culture exposed to

diethyldithiocarbamate, which inhibits CuZn superoxide dismutase,² show increased radiosensitivity (12, 41).

Both anthracycline antibiotics and bleomycin appear to exert their toxic action on DNA by way of reactive free radicals derived from oxygen. Superoxide dismutase has been shown *in vitro* to protect DNA against bleomycin Fe^{2+} complex (13, 35, 36). The anthracyclines, daunomycin and doxorubicin, apparently take part easily in oxidation-reduction cycles producing superoxide radicals (1, 9, 10, 23), and superoxide dismutase has been shown to protect DNA (14).

In eukaryotes, 2 forms of superoxide dismutase are generally found, one cytoplasmic and mitochondrial enzyme containing copper and zinc and one mitochondrial enzyme containing manganese (40). At least in primates, the Mn superoxide dismutase is also found in the cytoplasm (19).

Against the above background, a study of the activities of CuZn superoxide dismutase and Mn superoxide dismutase in human tumors seems highly motivated. The enzymes might conceivably be of importance, for both the radiation response and the response to the radical-producing antitumor drugs. The present paper reports specific analyses of CuZn superoxide dismutase and Mn superoxide dismutase in 32 human tumors and for comparison human tissues.

MATERIALS AND METHODS

Tissues and Tumors. Human tissues from accident or suicide victims without known physical diseases were obtained within 24 hr after death from the Department of Forensic Medicine. Macroscopically homogeneous pieces of tumor (0.5 g) were cut out of operation preparations within an hr after surgery. The tissues and tumors were kept at -90° before assay. They were homogenized in an Ultra-Turrax with 20 volumes of 10 mM potassium phosphate, pH 8.0, plus 30 mM KCl. The homogenates were then sonicated with a Branson B30 under cooling with ice. After extraction for 30 min at 4° , the homogenates were centrifuged ($20,000 \times g$ for 15 min), and enzyme and protein analysis was performed on the supernatants. The procedure has been found to extract efficiently both CuZn superoxide dismutase and Mn superoxide dismutase.

Superoxide Dismutase Analysis. Superoxide dismutase was determined in terms of its ability to catalyze the disproportionation of O_2^- in alkaline aqueous solution. The disproportionation was directly studied in a spectrophotometer, essentially as described before (16), the difference being that both CuZn superoxide dismutase and Mn superoxide dismutase were

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² The abbreviations used are: CuZn superoxide dismutase, copper- and zinc-containing superoxide dismutase; Mn superoxide dismutase, manganese-containing superoxide dismutase.

assayed at pH 9.50. One unit in the assay is defined as the activity that brings about a decay in O_2^- concentration at a rate of $0.1 s^{-1}$ in 3 ml buffer. It corresponds to 8.3 ng human and to 4.1 ng bovine CuZn superoxide dismutase and 65 ng bovine Mn superoxide dismutase. The pure human enzyme has not been investigated with this assay, but its specific activity is probably similar to that of the bovine enzyme. The xanthine oxidase-cytochrome *c* assay for superoxide dismutase works at physiological conditions, neutral pH and low O_2^- concentration (20). When bovine and human enzymes are analyzed, one unit in the present assay corresponds to 0.024 units CuZn superoxide dismutase and 0.24 units Mn superoxide dismutase, respectively, in the "xanthine oxidase" assay. The present assay is thus about 10 times more sensitive for CuZn superoxide dismutase activity than for Mn superoxide dismutase activity.

Staining for superoxide dismutase in agarose gel plates was performed with slight modifications of the methods of Beauchamp and Fridovich (2) and Bohnenkamp and Weser (4).

Protein Analysis. For protein analysis, Coomassie Brilliant Blue G-250 was used (6). Human serum albumin was used for standardization. This sensitive convenient method was compared with the more established technique of Lowry *et al.* (15). Human tissue homogenates (pancreas, lymphatic node, muscle, lung, heart, renal cortex, renal medulla, thyroid gland, liver, brain white matter, brain gray matter, adipose tissue, and spleen) were analyzed with both methods standardized the same way. The results were very similar, the ratio between the results of Lowry *et al.* and the results of the present method being 1.12 ± 0.14 (S.D) (range, 0.98 to 1.42).

RESULTS

Table 1 collects the results of CuZn superoxide dismutase and Mn superoxide dismutase analysis in human tissues. Table 2 presents the results of analysis on tumors.

As seen, the activity of Mn superoxide dismutase was in general almost as large as that of CuZn superoxide dismutase if one takes into account the fact that the method used was 10 times less sensitive for Mn superoxide dismutase. The enzymic activities were also studied after electrophoresis in agarose gel plates and subsequent staining for superoxide dismutase. The results of the semiquantitative evaluation of the plates agreed very well with the results of the direct enzymic analysis presented in Tables 1 and 2.

DISCUSSION

The tumors in general appear to possess less CuZn superoxide dismutase than do tissues, at least when compared with metabolically active organs such as liver, kidney, and adrenal gland. However, there is a large overlap between the levels of tissues and tumors. Only in a few cases can tissues and tumors derived from the tissue be compared. There is a large difference between kidney and the renal carcinoma and between testis and the testis embryonal carcinoma. On the other hand, lymphatic nodes and spleen do not possess more superoxide dismutase than do corresponding tumors. When making this type of comparison, one must take into consideration the fact that the tumors in general are derived from one cell type, whereas the tissues are composed of several cell types. The

Table 1

Superoxide dismutase in human tissues

The tissues were analyzed as described in "Materials and Methods." Note that the activity figures for Mn superoxide dismutase should be multiplied by 10 in order to make them comparable with the CuZn superoxide dismutase figures on a physiological activity basis.

| Tissue | | CuZn superoxide dismutase | | Mn superoxide dismutase | |
|--------------------|----------------|---------------------------|------------------|-------------------------|------------------|
| | | Units/g wet wt | Units/mg protein | Units/g wet wt | Units/mg protein |
| Liver | A ^a | 79,000 | 1,200 | 2,100 | 31 |
| | B | 120,000 | 1,500 | 2,900 | 35 |
| Renal cortex | A | 22,000 | 350 | 1,600 | 26 |
| | B | 37,000 | 470 | 3,200 | 40 |
| Adrenal gland | A | ND | ND | ND | ND |
| | B | 38,000 | 850 | 2,000 | 45 |
| Renal medulla | A | 16,000 | 420 | 1,100 | 30 |
| | B | 13,000 | 310 | 590 | 28 |
| Erythrocytes | A | 23,000 | 67 | 0 | 0 |
| | B | 21,000 | 61 | 0 | 0 |
| Heart | A | 13,000 | 340 | 1,000 | 26 |
| | B | 13,000 | 330 | 2,700 | 67 |
| Uterus | X | 18,000 | 264 | 410 | 6 |
| Brain gray matter | A | 12,000 | 540 | 350 | 16 |
| | B | 13,000 | 480 | 660 | 24 |
| Spleen | A | 11,000 | 95 | 260 | 2.2 |
| | B | 14,000 | 200 | 510 | 7.4 |
| Skeletal muscle | A | 11,000 | 280 | 140 | 3.7 |
| | B | 11,000 | 200 | 380 | 7.2 |
| Brain white matter | A | 11,000 | 730 | 130 | 9 |
| | B | 9,800 | 570 | 220 | 13 |
| Thyroid gland | A | 9,500 | 79 | 110 | 0.9 |
| | B | 10,000 | 91 | 140 | 1.2 |
| Testis | X | 9,300 | 340 | 330 | 12 |
| Pancreas | A | 9,800 | 160 | 650 | 11 |
| | B | 9,600 | 260 | 1,000 | 30 |
| Lung | A | 8,700 | 170 | 260 | 5 |
| | B | 8,600 | 160 | 580 | 11 |
| Lymphatic node | A | 9,200 | 250 | 640 | 18 |
| | B | ND | ND | ND | ND |
| | X | 7,200 | 130 | 1,100 | 19 |
| Adipose tissue | A | 1,200 | 200 | 54 | 9.1 |
| | B | 800 | 220 | 39 | 11 |

^a A, apparently healthy male accident victim, age 31; B, apparently healthy male accident victim, age 54; ND, not determined; X, supplementary tissues taken from other individuals.

difference between tissues and tumors is more pronounced when the results are compared on the basis of wet weight rather than on the basis of protein content. The "wet weight" results should best describe the *in vivo* protective activity of the superoxide dismutase.

Mn superoxide dismutase was detected in all tumors investigated. Again, the activities were in general slightly smaller than in normal tissues, and again, the differences were smaller when the results were compared on the basis of protein content.

The method used for superoxide dismutase analysis is about 10 times less sensitive for Mn superoxide dismutase than for

Table 2
Superoxide dismutase in human tumors

The tumors were analyzed as described in "Materials and Methods." Note that the activity figures for Mn superoxide dismutase should be multiplied by 10 in order to make them comparable with the CuZn superoxide dismutase figures on a physiological activity basis.

| Patient Age (yr) | Tumors | CuZn superoxide dismutase | | Mn superoxide dismutase | |
|------------------|----------------------------------------------------|---------------------------|------------------|-------------------------|------------------|
| | | Units/g wet wt | Units/mg protein | Units/g wet wt | Units/mg protein |
| | Mammary | | | | |
| K. D. 86 | Moderately differentiated carcinoma | 7,000 | 160 | 270 | 6.1 |
| I. K. 79 | Small-cell lobular carcinoma | 9,200 | 290 | 140 | 4.2 |
| H. A. 78 | Low-differentiated ductal carcinoma | 6,000 | 200 | 250 | 8.1 |
| B. H. 53 | Low-differentiated ductal carcinoma | 7,000 | 220 | 480 | 15 |
| G. P. 50 | Low-differentiated ductal carcinoma | 5,000 | 100 | 960 | 19 |
| S. J. 44 | Moderately differentiated ductal carcinoma | 15,000 | 290 | 390 | 7.5 |
| G. B. 41 | Moderately differentiated ductal carcinoma | 6,000 | 140 | 120 | 2.6 |
| T. K. 36 | Moderately differentiated ductal carcinoma | 8,800 | 200 | 220 | 4.8 |
| E. S. 19 | Fibroadenoma | 5,300 | 120 | 290 | 6.6 |
| | Mammary fibroadenosis | | | | |
| M. K. 50 | Fibrous fibroadenomatosis | 3,700 | 140 | 160 | 6.1 |
| E. J. 43 | Fibroadenosis | 2,300 | 85 | 80 | 3 |
| | Cancer ventriculi | | | | |
| V. J. 71 | Anaplastic adenocarcinoma | 4,600 | 88 | 640 | 12 |
| G. L. 71 | Low-differentiated adenocarcinoma | 7,100 | 170 | 390 | 9 |
| | Cancer recti | | | | |
| R. M. 68 | Low to moderately differentiated adenocarcinoma | 5,100 | 130 | 700 | 18 |
| S. E. 65 | Moderately differentiated adenocarcinoma | 18,900 | 150 | 970 | 12 |
| I. M. J. 32 | Low-differentiated adenocarcinoma | 5,300 | 180 | 450 | 15 |
| | Cancer vesicae urinae | | | | |
| V. P. 66 | Well-differentiated papillary carcinoma | 10,600 | 230 | 490 | 8.8 |
| | Cancer renis | | | | |
| I. K. 44 | Moderately differentiated adenocarcinoma | 6,100 | 110 | 830 | 14 |
| I. K. 44 | Adjacent normal kidney | 20,500 | 230 | 2,200 | 24 |
| | Cancer thyroideae | | | | |
| E. N. 70 | Large-cell low-differentiated | 4,600 | 94 | 45 | 0.9 |
| | Malignant melanoma s.c. metastase | | | | |
| T. F. 76 | | 7,300 | 130 | 200 | 3.5 |
| | Leiomyosarcoma | | | | |
| K. J. 75 | Moderately differentiated | 18,700 | 430 | 2,200 | 49 |
| | Chondrosarcoma | | | | |
| S. J. 52 | | 3,900 | 290 | 90 | 6.6 |
| | Ewing's sarcoma | | | | |
| A. L. 19 | | 9,200 | 320 | 37 | 1.0 |
| | Teratoma of testis with embryonal carcinoma | | | | |
| K. J. 31 | | 4,300 | 87 | 72 | 1.5 |
| | Benign lipoma | | | | |
| A. N. 56 | | 1,300 | 120 | 67 | 6.4 |
| | Lymphoma | | | | |
| L. S. 32 | Diffuse well-differentiated lymphocytic lymphoma | 11,600 | 180 | 840 | 13 |

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Table 2—Continued

| Patient Age (yr) | Tumors | CuZn superoxide dismutase | | Mn superoxide dismutase | |
|---------------------|------------------------------------------|---------------------------|------------------|-------------------------|------------------|
| | | Units/g wet wt | Units/mg protein | Units/g wet wt | Units/mg protein |
| A. M. L. 69 | Hodgkin's Disease | | | | |
| | Lymphocytic predominance, infiltrates in | | | | |
| | Spleen | 2,000 | 78 | 150 | 5.7 |
| | Lymphatic node | 7,600 | 350 | 941 | 42.8 |
| O. G. 17 | Liver | 10,500 | 110 | 872 | 9.1 |
| | Nodular sclerosis, spleen infiltrate | 9,600 | 110 | 550 | 6.4 |
| R. P. 21 | Mixed cellularity | | | | |
| | Lymphatic node | 9,800 | 170 | 880 | 15.6 |
| | Spleen infiltrate | 10,000 | 110 | 650 | 7.1 |

CuZn superoxide dismutase. If that is taken into consideration, it is found that Mn superoxide dismutase constitutes a large part of the superoxide dismutase activity of tissues and tumors, in several cases more than half. It is obvious that both enzymes must be considered in an evaluation of the superoxide radical scavenging capacity of human tissues and tumors. The enzymes should be determined separately; because of their partially different localizations, their role in protection of various targets in the cells against various sources of superoxide may differ. It has been reported that the CuZn superoxide dismutase (24, 25, 27, 34) and the total superoxide dismutase (5, 38) activities of various animal tumors are lower than those of normal tissues. The present findings do not contradict those results, although, as discussed above, a general comparison between organs and tumors is difficult to make.

Several investigations have found none (24, 34) or very little Mn superoxide dismutase (25, 27, 38) in various animal tumors. The idea has been advanced that loss of Mn superoxide dismutase is intimately related to the cancerous phenotypes (25). Diethylthiocarbamate, which inhibits CuZn superoxide dismutase, sensitizes against ionizing radiation (12, 41). It has been suggested that the compound would sensitize malignant cells more than normal cells because of their lack of Mn superoxide dismutase activity. Obviously, the situation in human tumors is different; here, large amounts of Mn superoxide dismutase are found. The difference may be due to the fact that Mn superoxide dismutase is apparently found in both cytoplasm and mitochondrial matrix in primates (19), whereas in other animals, Mn superoxide dismutase is found only in mitochondrial matrix. The number of mitochondria is often decreased in malignant cells, and mitochondria from malignant cells have been reported to be devoid of Mn superoxide dismutase (8). Our investigation does not rule out the possibility that human "malignant" mitochondria possess less Mn superoxide dismutase than do "normal" mitochondria.

One investigation of CuZn superoxide dismutase in human tumors has been reported (37). Much larger differences in enzyme content were found between different tumors than in the present investigation. The enzymic analyses were done on chloroform-ethanol extracts of tumor homogenates. When working with hemolysates, we have found that this procedure is unreliable for human CuZn superoxide dismutase, whereas it works well with the bovine enzyme (17). This fact may explain the differences found.

Tissues with high metabolism, e.g., liver and kidney, appear to possess more superoxide dismutase than do others, but

there is no obvious relationship between radioresistance and content of superoxide dismutase. For example, liver tissue is more radiosensitive than is skeletal muscle (33), which contains much less superoxide dismutase. Among the analyzed malignant tumors, it can be seen that the thyroid carcinoma and the leiomyosarcoma, although both usually very radioresistant tumors, differ by a factor of 8 with respect to enzyme activities. In our material of breast cancer, the enzyme activities range from 7,200 to 18,900 units/g, wet weight (total activity of Mn superoxide dismutase plus CuZn superoxide dismutase, with the activity of Mn superoxide dismutase multiplied by a factor of 10), which is not very different from the range of our lymphomas, although breast cancer is a much more radioresistant tumor type. Inside the breast cancer group, there is no obvious correlation between degree of differentiation and enzyme content. However, the mechanisms that lead to a destruction of parenchymatous tissues and tumors after irradiation are complex. The proliferative state of the cells is of importance. Damage to the vascular cells may also contribute to the destruction (7). This may explain the lack of obvious correlation between generally assumed radiation sensitivities of tissues and tumors and the overall enzymic activities found in the present investigation.

As for the radical-producing antitumor drugs, we see no obvious relation between the response to treatment of various tumor classes and their content of the superoxide dismutases. It is premature to assess the value of CuZn superoxide dismutase and Mn superoxide dismutase analysis in tumors for prediction of radiation response and response to radical-producing antibiotics. Tumors of different origin are biologically very different, and this may lead to differences in the superoxide dismutase activities. It is possibly easier to find a correlation between CuZn superoxide dismutase and Mn superoxide dismutase activity and response to treatment within a class of tumors. A prospective follow-up of response to treatment versus superoxide dismutase activity may give an answer. Attempts to correlate the enzymic activities to various morphological and biological criteria of malignancy may also give a clue.

The method for superoxide dismutase analysis used in the present investigation is very sensitive. Less than 10 mg of tissue or tumor are enough for double specific analysis of CuZn superoxide dismutase and Mn superoxide dismutase. It should be suitable for analysis of, e.g., needle biopsies. Except for homogenization by sonification and centrifugation, no other treatment is necessary before assay. It has an advantage over

radioimmunological methods in that the enzymic activities and not the amounts of protein are measured. The specific activity of superoxide dismutase protein has been shown to differ in some instances (32). Because the method is direct, it should be less liable to interference than the commonly used indirect assays. Before the present method was devised, we used 2 indirect methods in parallel for analysis on tissues and tumors, the xanthine oxidase-cytochrome *c* assay (20) and the pyrogallol autoxidation assay (18). Even though the homogenates were dialyzed before assay, we were unable to obtain a constant ratio between the results, and hence, reliable estimates of the Mn superoxide dismutase and CuZn superoxide dismutase activities could not be obtained.

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