A Preliminary Study of the Superoxide Dismutase Content of Some Human Tumors

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ABSTRACT

The cell cytosol superoxide dismutase (SODase) content of 46 human tumors was investigated. The extraction procedure of McCord and Fridovich was used with the epinephrine assay of Misra and Fridovich (J. Biol. Chem., 247: 3170–3175, 1975). The purpose of the study was to determine whether SODase could be reliably assayed from small, biopsy-sized pieces of tumor (0.5 to 1.0 g). In most cases it was possible to examine larger masses of tumor, which served as a control of the methodology. In this preliminary study SODase values, calculated from a standard curve derived from purified bovine blood SODase with a specific activity of 2584 units/mg, ranged from as little as 0.23 to as much as 160.5 units/g of tumor. These findings suggested that the procedures used might be feasible on a routine basis to determine the SODase content of tumors and its possible relationship to the radiation sensitivity of the tumors.

INTRODUCTION

The function of SODase² in the dismutation of metabolically or photochemically generated superoxide free radical anions was first described by McCord and Fridovich (8). Since then, the chemistry, structure, and protective effects of the enzyme have been studied extensively (4, 14), but only scanty information is available on its clinical importance. A study of the SODase levels in the blood of elderly patients correlated high levels of the enzyme with physical and mental senility (10). Fridovich (3) has suggested the possible role of superoxide in the free radical processes associated with aging. McCord (7) has theorized that injection of SODase into the joints of patients with rheumatoid arthritis might be used to reduce the inflammation and pain caused by the superoxide-mediated oxidation of synovial fluid. This theory has been shown to have merit by the double blind studies carried out by Lund-Olsen and Mander (6), who injected the enzyme into the arthritic knee or hip joints of 22 patients with very promising results. Recently, Petkau et al. (13) showed that the dose lethal to 50% of mice given whole-body irradiation was markedly increased by prior inoculation of the animals with 35 μg of SODase per g of body weight.

This report details preliminary studies on the cell-cytosol cupri-zinc SODase in units/g extracted from different human tumors. The purpose of these studies was to determine the SODase content of small biopsy specimens of tumors with the use of the epinephrine assay (11), inasmuch as it had been determined previously that this assay was simpler and slightly more sensitive than other described photometric assays. If these studies proved successful, then the method would be used in later studies designed to determine whether the SODase content of a tumor might be related to its sensitivity to radiation.

MATERIALS AND METHODS

Freeze-dried SODase [specific activity, 2584 units/mg (9)] derived from bovine erythrocytes was used to prepare SODase standards. All other reagents were of analytical grade. Doubly glass-distilled water was used for the preparation of all solutions. The standard solutions of SODase were prepared in distilled water with enzyme content ranging from 0.25 to 25.5 units/ml, and they were stored at −20°. The epinephrine solution used in the assay was a 0.55% (w/v) solution in distilled water acidified with concentrated HCl (0.05 ml in 10 ml) to prevent autooxidation. The solution was stored at 5°. The SODase standards and the epinephrine solution were discarded after 8 weeks.

Forty-six tumors were studied, of which 7 were from the ovary, 10 were from the breast, 3 were from the lung, 18 were from the bowel, and 8 were from various anatomical sites and were classified as miscellaneous. The majority of the tumor specimens were received frozen with dry ice and on receipt were stored at −80° prior to the enzyme extraction procedure. At the start of the study, a few tumors were received unfrozen; a part of each of these tumors was immediately frozen at −80° for later examination, and the remainder were extracted and assayed. Two to 3 weeks later, the frozen aliquots were assayed, and the results were compared to those obtained from the fresh tumor specimens. No differences were detected; therefore, as a matter of convenience, for the remainder of the study tumor material that had been kept frozen at −80° was utilized.

Extracts of tissue prepared by homogenization in 0.05 M K₂HPO₄·1 × 10⁻⁴ M EDTA buffer at pH 7.8 and then clarified by centrifugation at 30,000 × g were found frequently to be too highly colored by hemoglobin and other colored molecules to permit direct assay for SODase by the epinephrine or xanthine-xanthine oxidase-nitro blue tetrazolium tests. A similar problem was also encountered in our preliminary studies of serum samples.

The adopted extraction procedure for the cell cytosol SODase was therefore, with very minor exceptions, that of McCord and Fridovich (9). The weighed tissue was finely minced while thawing, and the tissue fragments were suspended in 10 volumes (w/v) of the K₂HPO₄·EDTA buffer.
Tumors weighing in excess of 5 g were homogenized in a Waring Blender during 4 periods of 15 sec with pauses of 15 sec. Small samples were kept chilled at 0° with crushed ice during sonic dispersion with a Branson sonifier at a power setting of 110 to 115 watts. The homogenates were centrifuged at 30,000 × g for 30 min at 5° to remove cell particulates. The supernatant fluid from this centrifugation was carefully removed and treated with 0.25 volume of ethanol and 0.15 volume of chloroform, both chilled at 5°, to precipitate the hemoglobin complexes, which were then removed by centrifugation of the mixture at 30,000 × g for 30 min at 5° (this procedure is known to destroy the manganese enzyme of the mitochondria, and no attempt was made to assay separately for this enzyme). The supernatant fluid from this centrifugation was transferred to a beaker, allowed to warm to room temperature (27°), and 30% (w/v) solid, dibasic potassium phosphate was added. The mixture was gently stirred until all the salt had gone into solution. The mixture was centrifuged at 1000 × g for 20 min at 5° to bring about a clean separation of the phases. The lighter phase was essentially water:ethanol, as described by McCord and Fridovich (9), and contained the bulk of the enzyme. This supernatant fluid was transferred to a chilled test tube, and the enzyme was precipitated by the addition of 0.75 volume of cold acetone (−20°). The SODase precipitate was sedimented by centrifugation at 1000 × g at 5° for 30 min. The acetone supernatant was removed as completely as possible, and the precipitate was resuspended in double-distilled water (3.0 ml for extracts from tumors weighing 5 g or more and 1.0 ml for samples of less than 5.0 g). The solution containing the SODase was allowed to stand at 5° overnight to ensure complete solubilization of the enzyme. The following day the solution was clarified by centrifugation at 1000 × g at 5° for 30 min to remove any traces of flocculated material. The water-clear supernatant fluid constituted the tumor extract and was removed to a fresh tube and stored at −20° until assayed. The DE32 column used by McCord and Fridovich (8) in their preparative procedure was omitted.

Spectrophotometric analyses were made with the use of a programmable Zeiss PM6 spectrophotometer with timed advance. The assay procedure based on the autoxidation of epinephrine to an adrenochrome at pH 10.2 followed at 30-sec intervals by measurement of the changes in absorption at a wavelength of 480 nm (11). This autoxidation of epinephrine is thought to produce O$_2^-$ as an intermediate that can be scavenged by SODase. The reduction in the rate of formation of the adrenochrome due to the presence of SODase provided the basis for this assay. Each cuvet contained the following solutions, listed in the order in which they were added to the cuvet: 0.025 ml of 0.55% epinephrine solution, 0.20 ml of SODase standard solution or tumor extract (to Cuvets 2 to 4 only), and 2.0 ml of 0.05 M Na$_2$CO$_3$ × 10$^{-4}$ M EDTA buffer. Buffer (2.2 ml) was added to Cuvet 1, as neither SODase nor tumor extract was placed in this cuvet, the epinephrine control. The buffer was always added to the cuvets moving from left to right (from 1 to 4) and this operation never took more than 7 sec. A standard curve for ox blood SODase (Chart 1) was plotted as percentage of inhibition of the absorption at 480 nm of the adrenochrome by SODase concentrations ranging from 0.25 to 5.0 units/ml. All test samples derived from tumors were diluted as necessary to give readings within this range.

RESULTS

The assay procedure consisted of extraction of the enzyme from a known mass of tumor tissue; its solution in a specified volume of doubly distilled water; the spectrophotometric determination of the enzyme measured by its effect on the rate of autoxidation of epinephrine solution at a pH of 10.2 at a standard elapsed time of 2.5 min. The calculation of the SODase content of the tumor specimen was performed in terms of units of the enzyme (9) per g of tumor by reference to the standard SODase curve (Chart 1). There was a wide spread in the amount of SODase present in the studied tumors, ranging from less than 0.025 unit/g to as much as 160 units/g of tissue. The extraction and assay procedures used gave similar results when 5 g or more of a given tumor were compared to 1.0 g or less from the same tumor. Tumor masses of less than 0.5 g were not investigated. Anomalous results were occasionally obtained in repeat examinations of the same tumor. Investigation showed that these anomalies could be attributed to residual traces of acetone by undetected heating of the sample during homogenization and by excess amounts of fatty and fibrous material in the specimen. Flowing nitrogen through the SODase extract at 5° for a period of 4 to 6 hr was found to resolve the acetone problem, and strict temperature control and careful dissection of the tumor before processing lessened the anomalous results previously obtained with replicate samples of the tumors. Samples that had not...
been maintained frozen during transit to the laboratory yielded no enzyme.

The results obtained from the studied tumors are listed in Table 1 and indicate that the ovarian and breast tumors contained relatively small amounts of SODase while tumors from the lung and bowel showed generally higher values. Of the 7 ovarian tumors examined, 1 had a high SODase content of 23 units/g, 4 had SODase content of 3.0 to 5.3 units/g, and 2 had less than 2.6 units/g of tumor. Of 10 breast tumors studied, only 2 tumors had high SODase values of 13.0 and 46.0 units/g, respectively, and the remaining breast tumors had values between 0.25 and 4.6 units/g. The 3 lung tumors studied had high values of SODase ranging from 15 to 71 units/g. The 18 bowel tumors could be divided into 2 groups on the basis of their SODase content; 8 tumors had values of less than 2.54 units/g while the remaining 10 tumors had much higher values ranging from 11.5 units/g to a high of 161 units/g. No comparisons could be made between the tumors in the miscellaneous group, inasmuch as all came from different anatomical sites.

**DISCUSSION**

SODase plays a key role in the viability of aerobic and aerotolerant cells in the presence of atmospheric oxygen and ionizing radiation (13). Human tissues high in SODase activity, such as brain, liver, kidney, thyroid, pituitary, and cardiac muscle, have been shown to be generally more resistant to damage by ionizing radiation than are those tissues with a low SODase content, such as spleen and bone marrow, which are radiation sensitive (5, 15). Hyperbaric oxygen radiotherapy for anoxic cancerous growths has been shown to be up to 3 times as effective as conventional radiotherapy (16) and is thought to be indicative of superoxide-mediated cellular damage. It has also been shown that injection of peroxide, which inactivates SODase (2), greatly enhanced the destructive effect of irradiation. The reverse of this, wherein mice given SODase before lethal whole-body irradiation had a significantly higher survival rate (13), suggests that our proposal that high intrinsic cellular cytosol levels of SODase in some tumors might be associated with a high radiation resistance may have some merit. If, therefore, the radiation sensitivity of a tumor could be predicted by determination of its content of SODase, then an invaluable diagnostic tool would be available to the clinician. For such a test to be clinically useful, the assay procedure for the enzyme content of a tumor extract would have to be not only sensitive and specific but also simple to perform. At the moment we feel that the rather harsh but effective extraction of cell cytosol SODase according to the method of McCord and Fridovich (8) and the epinephrine assay used in our study satisfy these criteria. Obviously, the methodology can be improved in both its simplicity and its accuracy so that it will include the mitochondrial SODase, for example, and so that it will lower the limit presently attainable by use of the epinephrine and nitro blue-tetrazolium xanthine-xanthine oxidase assays. Until a better method can be devised, the assay procedure, as reported, does permit preliminary studies that determine whether the cell content of SODase is related to radiation sensitivity. Other methods, perhaps more adaptable to human tissues, have been reported (14), but they appear to be too complex for use in a routine clinical laboratory. The reduction of nitro blue tetrazolium to the colored formazan by the superoxide radical generated by the xanthine-xanthine oxidase system.
thine oxidase system (1) has been used as an alternative assay in this laboratory but did not give different results or have a greater sensitivity than the simpler epinephrine assay. It is possible that the more recently described, more involved technique with the use of horseradish peroxidase (12) may be less susceptible to sample impurities, but further study is required to simplify this procedure. Further investigation of these and other possible assay and extraction procedures is being planned.

The described results have been in general agreement with the theory that there may exist a relationship between radiation sensitivity and tumor SODase content. Ovarian and breast tumors, which are frequently responsive to radiation therapy, generally had small SODase values of 5.6 units/g and 12 units/g, respectively. The 3 lung tumors, however, had high SODase values of more than 34 units/g, which is in agreement with the known resistance of many lung tumors to radiation. Of the examined bowel tumors, 56% had a high SODase content and presumably would have been more resistant than the remaining 44%. Radiation therapy alone is not frequently used for the treatment of bowel tumors, and it would be of interest to examine a series of cases in which this type of therapy was used and then to relate the response to the SODase content of the tumor. Such a study would be of particular interest, inasmuch as it is known that colonic mucosal cells are rather radiation sensitive, despite the fact that they contain a fairly high level of SODase (5), but comparable data are available for colonic tumor cells. The SODase studies of Michelson (10) on the blood of elderly patients and on those with mental illness demonstrate the need for base line studies of blood SODase levels in normal healthy persons and in persons with different disease processes. Such studies might provide a convenient method for determining the optimal therapy and the response to that therapy, particularly in patients with tumors.

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