to its antioxidant properties.[32] Thus it appears that an improvement in the antioxidant status of the heart due to probucol treatment may be an important factor in the protection seen in this study. However, the exact mechanism of improved antioxidant status is not known.

Diabetes-prone (BB/W) rats had a significant reduction in the incidence of diabetes with probucol treatment.[33] Although feeding of 1% probucol in diet had no effect on the diabetogenicity of STZ (35 or 50 mg/kg), it had a beneficial effect on the recovery of diabetic condition. In this regard, male Wistar rats maintained on a 1% probucol diet for 2 wk prior to and 2 wk after the induction of diabetes with STZ (25 mg/kg) showed significant recovery from STZ diabetes in terms of lower blood glucose levels and higher pancreatic insulin content, as compared to control diabetic rats.[34] Probucol also prolonged the time of onset in rats that eventually developed diabetes. Because a significant improvement in the serum insulin levels as well as reduction of glucose was noted in diabetic animals treated with probucol in our study, the beneficial effects with respect to cardiomyopathic changes may also involve these improvements. A study designed to examine the diabetic group with insulin treatment can shed some light on this aspect. However, these beneficial effects of probucol have also been suggested to be related to its antioxidant activity.[33,35]

In conclusion, the present study shows that a decrease in tissue antioxidant status accompanies the depressed cardiac function in diabetic cardiomyopathy. Antioxidant improvement by probucol provides evidence that protection of cardiac function may be related to the maintenance of the antioxidant status of the heart as well as improved insulin levels. This study suggests the potential usefulness of antioxidant therapy in diabetic cardiomyopathy.

ACKNOWLEDGEMENTS

Supported by a grant from the Canadian Diabetic Association. Personal support from the Medical Research Council (P.K.S.), Manitoba Health Research Council (N.K.), and University of Manitoba (N.S.I. and N.K.) is gratefully acknowledged.

REFERENCES

1. Ahmed SS, Jaferi GA, Narang RM, Regan TJ. Preclinical abnormality of left ventricular function in diabetes mellitus. Am Heart J 1975;89:153
2. Jackson CV, McGrath GM, Tahiliani A, Vadiamudi RVSV, McNeill JH. A functional and ultrastructural analysis of experimental diabetic rat myocardium: Manifestations of a cardiomyopathy. Diabetes 1985;34:876
3. Pierce GN, Dhalla NS. Mitochondrial abnormalities in diabetic cardiomyopathy. Can J Cardiol 1985;1:48
4. Regan TJ, Lyons MM, Ahmed S, Levinson GE, Oldewurtel HA, Ahman M, Haider B. Evidence for cardiomyopathy in familial diabetes mellitus. J Clin Invest 1977;60:884
5. Fein FS, Sonnenblick EH. Diabetic cardiomyopathy. Prog Cardiovasc Dis 1985;27:255
6. Halliwell G, Gutteridge JMC. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. Lancet 1984;1:1396
7. Kaul N, Siveski-Iliskovic N, Hill M, Siezak J, Singal PK. Free radicals and the heart. J Pharmacol Toxicol Meth 1993;30:55
8. Sato Y, Hotta N, Sakamoto N, Matsuoka S, Ohishi N, Yagi K. Lipid peroxide level in plasma of diabetic patients. Biochem Med 1979;21:104
9. Babiy AV, Gebicki JM, Sullivan DR, Willey K. Increased oxidizability of plasma lipoproteins in diabetic patients can be decreased by probucol therapy and is not due to glycation. Biochem Pharmacol 1992;43:995
10. Wohaieb SA, Godin DV. Alterations in free radical tissue defense mechanisms in streptozotocin induced diabetes in the rat: effects of insulin treatment. Diabetes 1987;36:1014
11. Godin DV, Wohaieb SA, Garnett ME, Goumeniouk AD. Antioxidant enzyme alterations in experimental and clinical diabetes. Mol Cell Biochem 1988;84:223
12. Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. Proc Natl Acad Sci USA 1987;84:7725
13. Siveski-Iliskovic N, Kaul N, Singal PK. Probucol promotes endogenous antioxidants and provides protection against adriamycin-induced cardiomyopathy in rats. Circulation 1994;89:2829
14. Matsushita M, Yoshino G, Iwai M, et al. Protective effect of probucol on alloxan diabetes in rats. Diabetes Res Clin Pract 1989;7:313
15. Morel DW, Chisolm III GM. Antioxidant treatment of diabetic rats inhibits lipoprotein oxidation and cytotoxicity. J Lipid Res 1989;30:1827
16. Bondar RJL, Mead DC. Evaluation of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* in the hexokinase method for determining glucose in serum. Clin Chem 1974;20:586
17. Clairborne A. Catalase activity. In: Greenwald RA, ed., *Handbook of methods for oxygen radical research*. Boca Raton, FL: CRC Press, 1979:283
18. Marklund SL. Pyrogallol autoxidation. In: Greenwald RA, ed., *Handbook of methods for oxygen radical research*. Boca Raton, FL: CRC Press, 1979:243
19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158
20. Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malondialdehyde) in biochemical systems. Anal Biochem 1966;16:359
21. Aust SD. Lipid peroxidation. In: Greenwald RA, ed., *Handbook of methods for oxygen radical research*. Boca Raton, FL: CRC Press, 1979:203
22. Lowry OH, Rosebrough NJ, Farr AL, Randall AJ. Protein measurement with the folin phenol regent. J Biol Chem 1951;193:265
23. Hodgson EK, Fridovich I. The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: chemiluminescence and peroxidation. Biochemistry 1975;14:5399
24. Searle AJ, Willson RL. Glutathione peroxide: effect of superoxide, hydroxyl and bromine free radicals on enzyme activity. Int J Radiat Biol 1980;37:213
25. Schwartz CJ. The probucol experience: a review of the past and a look at the future. Am J Cardiol 1988;62:1B
26. Zimetbaum P, Eder H, Frishman W. Probucol: pharmacology and clinical application. J Clin Pharmacol 1990;30:3
27. Taylor HL, Nolan RB, Tedeschi RE and Maurath CJ. Combined results of the study of probucol at 1 gm/day in eight centres. Clin Pharmaco Ther 1978;23:131
28. Marshall RN. Pharmacology and toxicology of probucol. Artery 1982;10:7
29. Yamamoto A, Matsuzawa Y, Yokoyama S, Funahashi Y, Yamamura T, Kishino B. Effects of probucol on xanthomata regression in familial hypercholesterolemia. Am J Cardiol 1986;57:29H
30. Wissler RW, Vesselinovitch D. Combined effects of cholestyramine and probucol on regression of atherosclerosis in rhesus monkey aortas. Appl Pathol 1983;1:89
31. Pryor WA, Strickland T, Church DF. Comparison of the efficiencies of several natural and synthetic antioxidants in aqueous sodium dodecyl sulfate micelle solutions. J Am Chem Soc 1988;110:2224
32. Schneider JE, Berk BC, Gravanis MB, et al. Probucol decreases neointimal formation in a swine model of coronary artery balloon injury. Circulation 1993;88:628
33. Drash AL, Rudert WA, Borquaye S, Wang R, Lieberman I. Effect of probucol on development of diabetes mellitus in BB rats. Am J Cardiol 1988;62:27B
34. Yoshino G, Matsushita M, Maeda E, et al. Effect of probucol on recovery from streptozotocin diabetes in rats. Horm Metab Res 1992;24:306
35. Bird JE, Milhoan K, Wilson CB, et al. Ischemic acute renal failure and antioxidant therapy in the rat: the relation between glomerular and tubular dysfunction. J Clin Invest 1988;81:1630

Radioprotective and Antioxidant Effects of Zinc Aspartate and Bio-Normalizer in Children with Acute Myelo- and Lympholeukemias

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INTRODUCTION

The combined use of irradiation and chemotherapy is a fundamental principle in modern cancer management, especially in children.[1] The only limitation is that cytotoxic drugs enhance the radiation damage to normal tissues.[2] Radiation therapy can induce acute and late reactions of normal tissue. Although acute radiation injury is mostly expressed in the rapidly proliferating tissues such as skin, mucous membranes, spermatogonia, and hematopoietic cells, the late deleterious effects of irradiation occur in the central nervous system. The encephalopathy syndrome has been documented in about 50% of children with acute lympholeukemia (ALL) subjected to cranial and spinal cord irradiation for 4 to 8 wk.[3] This includes headache, somnolence, anorexia, nausea, vomiting, and sometimes unconsciousness.

Radiation-induced injury can be reduced by the administration of radioprotective agents. As the injury following the ionizing irradiation exposure is the most spectacular example of free-radical-mediated pathology,[4] it is not surprising that radioprotective agents usually possess free-radical scavenging properties. Thus, different types of superoxide dismutase (SOD) have been widely used[5,6] as well as α-lipoic acid,[7] *N*-acetylcystein,[8] and vitamin E.[9] Zinc is known to be one of the most important trace elements with antioxidant properties.[10] In addition, zinc aspartate was found to reduce significantly the irradiation toxicity against hematopoietic tissue[11] and does not affect the antitumor activity of therapeutic irradiation.

There is evidence that α-TNF, capable of inducing the overexpression of mitochondrial Mn-SOD, increases the resistance of cells to radiation.[12] Earlier, we showed[13] that Bio-Normalizer (BN), a nontoxic natural food supplement produced by yeast fermentation of *Carica papaya linn*, is a powerful immunomodulator that stimulates macrophages and lymphocytes to produce α-TNF and interferons. In addition, BN exhibits free-radical scavenging[14] and antioxidant[15,16] activities. Therefore, it seems interesting to study the effects of the zinc aspartate and BN administration to children suffering from acute myeloleukemia (AML) and ALL in order to prevent the late encephalopathy syndrome. Effects of these drugs on childrens' free-radical status, namely the oxygen radical production by blood leukocytes, the leukocyte CuZnSOD and MnSOD activities, and the erythrocyte-reduced glutathione content, have been estimated.[17]

PATIENTS, MATERIALS, AND METHODS

Children aged between 2 and 15 yr who suffered from ALL (primary and relapse, 19 patients) and AML (9 patients) were studied. All children were examined and treated at the Russian Institute of Pediatric Hematology. They were treated by conventional chemotherapy (details of antileukemic chemotherapy can be found elsewhere in the literature) using cranial and spinal cord irradiation in dosages of 12–18 Gy for ALL patients and 18–22 Gy for AML patients. Children fulfilling all the eligibility criteria were randomized in three groups. The control group children (5 ALL and 4 AML patients) were on a conventional maintenance therapy for 1 mo after irradiation course. The second group of children (7 ALL patients) were additionally given 150 mg of zinc aspartate daily for 30 d. The third group of children (7 ALL and 5 AML patients) were administered 9 g zinc instead of BN. Used as normal subjects were 39 healthy Moscow children from whom informed consent was granted. This pilot clinical trial was performed in accordance with the protocol approved by the Ethics Committee of the Institute of Pediatric Hematology. (Informed consent of a patient or his/her parents was obtained before the beginning of clinical trials.)

Zinc aspartate (Unizinc) was a gift of F. Kohler Chemie GmbH (Germany). Bio-Normalizer was a gift of Sun-O International (Gifu, Japan). The other reagents for the cell isolation and the free-radical assays were purchased from Sigma

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TABLE I.

TOTAL ENCEPHALOPATHY SCORE AFTER UNIZINC AND BIO-NORMALIZER ADMINISTRATION

Group	Total score after the irradiation course	Total score 1 mo later
Control	0.8 ± 0.1	1.3 ± 0.1
Unizinc	0.9 ± 0.2	0.17 ± 0.08
Bio-Normalizer	1.7 ± 0.2	0.18 ± 0.06

TABLE II.

REDUCED GLUTATHIONE (GSH) CONTENT IN THE ERYTHROCYTES (μmol/g Hb)

Group	GSH content after irradiation course	GSH content 1 mo later
Control	6.1 ± 1.0	6.6 ± 1.0
Unizinc	6.2 ± 0.9	6.6 ± 1.1
Bio-Normalizer	6.0 ± 1.1	8.7 ± 1.5*

*$p < 0.05$.

Chemical (St. Louis, MO). To estimate quantitatively the deleterious effects of irradiation on the central nervous system, the total score of encephalopathy symptoms for each patient was calculated. The following score was assumed: for somnolence, 0.5; weakness, 0.5; headache, 0.5; nausea, 0.5; convulsion, 1.5; coma, 3.0.

Early in the morning before breakfast 10 ml of venous blood was drawn by disposed syringes into three tubes: 1.5 ml was collected in a tube with 20 U/ml heparin; 0.5 ml in a tube with sodium citrate; and 8 ml in a tube without any anticoagulant. Serum was obtained from the 8-ml blood sample by centrifugation and then used for biochemical assay (Beckman Lab System CX40, USA). The 0.5-ml blood sample was used for differential cell count, and the 1.5-ml blood sample was used in the chemiluminescence, SODs, and glutathione assays.

To isolate white blood cells (WBC), the blood samples (1.5 ml) were sedimented with 1.5 ml of dextran-metrizoate mixture (50 ml 6.2% dextrane/20 ml 38% metrizoate) at 25°C for 30 min. The cell-rich supernatant was centrifuged at 150 *g* for 10 min. Cell pellets were washed twice in Hank's balanced salt solution (HBSS). The final suspension of 2–3 × 10^6 WBC was prepared in the culture medium 199. Cells were counted with a microscope, and their viability was assessed by exclusion of 0.1% trypan blue dye.

Luminol-dependent chemiluminescence (LDCL) was measured on an LKB luminometer model 1251. WBC suspension (20 μl, 2 × 10^6 cells/ml), luminol (50 μm, final concentration), and 0.85 ml of HBSS were mixed in a 1-ml cuvette at 37°C. After 5 min, the 0.1% suspension of latex (Sigma Chemical) in 0.95% NaCl solution was added, and the light emission was recorded continuously. The intensity of spontaneous-LDCL and the differences between maximal values of the cellular response to latex and of spontaneous LDCL were measured.

Superoxide dismutase (CuZnSOD) activity in WBC was determined by the adrenaline method,[18] in which the rate of superoxide production was measured by lucigenin-amplified chemiluminescence (LgCL). Briefly, a sample of isolated WBC (0.2 ml) was lysed with ice-cold water. Lysate was added to an equal volume of ethanol-chloroform mixture (1:1 v/v) and centrifuged at 1500 *g* for 30 min. Protein content in supernatant was determined by the Lowry method.[19] To measure the total SOD activity, 50 ml top clear supernatant was added to carbonate buffer (pH 10.2, a total volume of 900 μl) containing EDTA (100 μm) and lucigenin (100 μm) in a polystyrene cuvette, and the level of basal LgCL was registered continuously. Reaction was started by adding 50 μl adrenaline (100 μm) through the dispenser. LgCL light sum for 5 min was recorded and compared with that of a control sample (50 ml water:ethanol:chloroform solution [2:1:1 v/v/v]). The total SOD activity was calculated from calibration curve using commercial SOD as a standard and expressed as ng/mg protein. For the determination of MnSOD activity, CuZnSOD was inhibited by the addition of NaCN (4 mM) to the supernatant. After that, the LgCL measurement of MnSOD activity was performed as described above. The CuZnSOD activity was estimated as a difference between the total and MnSOD activities. Erythrocytes were obtained after blood sedimentation on dextran-metrizoate gradient and washed twice in the cold potassium phosphate buffer, pH 7.2. The content of reduced glutathione was determined by the Beutler method.[20]

Statistical Analysis

Nonparametric procedures were used throughout. Paired comparisons were made with Wilcoxon's signed rank test.[21] Statistical significance was assumed at $p < 0.05$. Results are expressed as mean ± SEM.

RESULTS AND DISCUSSION

Examination of children by pediatricians during and after the completion of clinical trial has shown that there were no toxic side effects, allergic reactions, or other adverse events during the Unizinc and BN administration. Biochemical and immunological analyses confirmed these observations (data not shown). Six of 9 children (66%) of the control group exhibited some neurological symptoms immediately following irradiation exposure. The average score characterizing these symptoms was equal to 0.8 (Table I). One month later, this value became 1.3, which showed an increase in encephalopathy symptoms. These findings are in good agreement with previous observations that the most severe neurotoxic effects of therapeutic irradiation occurred 4-6 wk after the irradiation course was stopped. At the same time, only one of seven children (14%) treated with Unizinc exhibited moderate encephalopathy; the average score of CNS damage immediately after the irradiation cycle was 0.93 and dropped to 0.17 in 1 mo. BN also was found to significantly suppress neurotoxicity of therapeutic irradiation (Table I; the average score after irradiation was 1.7, and 1 mo later it was 0.18).

It is well established now that glutathione is one of the major blood antioxidants responsible for the rapid inactivation of various oxidants formed during the metabolism of drugs or other xenobiotics or as the result of environmental hazard.[22] In the present work it was found that the irradiation cycle slightly (statistically insignificant) decreased the level of reduced glutathione (GSH) in the erythrocytes (Table II). Unizinc administration had little effect, but BN administration substantially increased the content of GSH. It should be stressed that BN itself contains the comparatively small amount of SH groups (about 12 μmol/g, unpublished data). Furthermore, we

TABLE III.

INTENSITIES OF SPONTANEOUS AND LATEX-ACTIVATED LUMINOL-DEPENDENT CHEMILUMINESCENCE (CL, mV)

Group	No. of patients	Spontaneous CL	Latex-activated CL
Control	39	290 ± 132	5287 ± 1267
ALL and AML	13	30 ± 11	460 ± 157
AML after chemotherapy	9	1445 ± 695	16700 ± 4309
ALL after chemotherapy	13	792 ± 403	9193 ± 2512
AML after irradiation	5	646 ± 401	12168 ± 8359
ALL after irradiation	13	1119 ± 486	13019 ± 5074
ALL after Unizinc administration	7	616 ± 413	7571 ± 3049
ALL after Bio-Normalizer administration	7	891 ± 164	9033 ± 4276
AML after Bio-Normalizer administration	5	214 ± 46	3335 ± 2397

found that blood-circulated leukocytes of ALL and AML patients produced surprisingly low intensities of spontaneous and latex-activated LDCL and had high activities of both CuZnSOD and MnSOD before the beginning of intensive anticancer therapy (Tables III and IV). Similar results were obtained for bone marrow leukocytes (data not shown). However, chemotherapy and the following irradiation exposure sharply increased the intensities of spontaneous and latex-activated LDCL in the blood leukocytes (compared with donors and the leukemia patients before the treatment), indicating that in this case hydroxyl radical overproduction occurred (Table III).

At the same time, both chemo- and radiotherapy inhibited dramatically the main leukocyte antioxidant enzymes CuZnSOD and MnSOD (Table IV). The level of MnSOD in patients after chemotherapy became significantly lower than even a normal value. On the other hand, a 1-mo Unizinc and BN administration led to decreasing spontaneous and activated LDCL (Table III). The biggest inhibitory effect was found for BN administration in AML patients when the values of spontaneous and activated LDCL achieved normal levels. Zinc therapy decreased insignificantly CuZnSOD and MnSOD in leukocytes, whereas BN administration enhanced both CuZnSod in ALL patients and MnSOD in AML patients.

These data suggest that the strong protocol treatment of lympho- and myeloproliferative malignancies caused the suppression of main intracellular antioxidant enzymes and drastically induced hydroxyl radical release from leukocytes, which can be the source of the CNS oxidative damage induced by therapeutic irradiation. Unizinc can treat such deleterious effects of irradiation by the inhibition of oxygen radical overproduction by circulated leukocytes. This inhibitory effect may be a consequence of the leukocyte NADPH-oxidase inactivation and/or the direct scavenging activity of zinc ions.

Moreover, we supposed that the aspartate ligand, a neurotransmitter amino acid, can accelerate zinc penetration through the blood-brain barrier via binding to the specific aspartate receptors on the surface of the neuronal membranes. Therefore, zinc aspartate can readily affect free-radical–mediated processes occurring in the central nervous system. The reasons for beneficial radioprotective effects of BN seem to be more complex. Besides its pronounced free-radical scavenging activity, BN appears to increase the organism potency to be adapted to oxidative stress, inducing glutathione synthesis and/or preventing glutathione oxidation and enhancing MnSOD activity in leukocytes, especially in patients. Probably, BN stimulates MnSOD indirectly, inducing α-TNF production by myelocytic leukocytes. Collectively, zinc aspartate and BN could be regarded as essential components of supportive care of cancer patients after intensive irradiation course.

TABLE IV.

CnZnSOD and MnSOD CONTENT IN THE BLOOD LEUKOCYTES (ng/mg protein)

Group	No. of patients	CuZnSOD	MnSOD
Control	39	89 ± 49	210 ± 148
ALL and AML	13	687 ± 222	619 ± 337
AML after chemotherapy	9	60 ± 15	37 ± 19
ALL after chemotherapy	13	111 ± 95	148 ± 118
AML after irradiation	5	95 ± 50	162 ± 105
ALL after irradiation	13	115 ± 65	119 ± 59
ALL after Unizinc administration	7	78 ± 49	56 ± 24
ALL after Bio-Normalizer administration	7	217 ± 75	74 ± 50
AML after Bio-Normalizer administration	5	199 ± 64	228 ± 67

REFERENCES

1. Pizzo PhA, Poplack DG. *Principles and practice of pediatric oncology*, 2nd ed. Philadelphia:Lippincott, 1993:290
2. DeAngelis LM, Shapiro WR. Drug/radiation interactions and central nervous system injury. In: Gutin PH, Leibel SA, Shehne GE, eds. *Radiation injury to the nervous system*, New York:Raven Press, 1991:361
3. Freeman JE, Johnston PGB, Voke JM. Somnolence after prophylactic cranial irradiation in children with acute lymphoblastic leukaemia. Br Med J 1973;4:523
4. Greenstock CL. Radiation-induced aging and induction and promotion of biological damage. In: *Free radicals, aging, and degenerative diseases*. New York:Liss, 1986:197
5. Petkau A. Protection of bone marrow progenitor cells by superoxide dismutase. Mol Cell Biochem 1988;84:133
6. Weiss L, Stem S, Reich S, Slavin S. Effect of recombinant human manganese superoxide dismutase on radiosensitivity of murine B cell leukemia (BCL1) cells. In: *Leukemia and lymphoma*. Philadelphia:Harwood Academic Press, 1993:477
7. Ramakrishnan N, Wolfe WW, Catravas GN. Radioprotection of hematopoietic tissues in mice by lipoic acid. Radiat Res 1992;130:360
8. De Flora S, Izzotti A, D'Agostini F, Cesarone CF. Antioxidant activity and other mechanisms of thiols involved in chemoprevention of mutation and cancer. Am J Med 1991;91(Suppl 3C):122S
9. Korkina LG, Afanas'ev IB, Diplock AT. Antioxidant therapy in children affected by irradiation from the Chernobyl nuclear accident. Biochem Soc Trans 1993;21:314S
10. Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. Free Radic Biol Med 1990;8:217
11. Floersheim GL, Bieri A. Further studies on selective radioprotection by organic zinc salts and synergism of zinc aspartate with WR 2721. Br J Radiol 1990;63:468
12. Wong GHW, McHugh Th, Weber R, Goeddel DV. Tumor necrosis factor-α selectively sensitizes human immunodeficiency virus-infected cells to heat and radiation. Proc Natl Acad Sci USA 1991;88:4372
13. Santiago LA, Osato JA, Hiramatsu M, Edamatsu R, Mori A. Free radical scavenging action of Bio-catalizer α*ρ NO. 11 (Bio-Normalizer) and its by-product. Free Radic Biol Med 1991;11:379
14. Santiago LA, Osato JA, Mori A. Stability of the hydroxyl radical scavenging components of the health food "Bio-Normalizer." Med Sci Res 1992;20:27
15. Santiago LA, Osato JA, Liu J, Mori A. Age-related increases in superoxide dismutase activity and thiobarbituric acid-reactive substances: effect of Bio-Catalizer in aged rat brain. Neurochem Res 1993;18:711
16. Santiago LA, Osato JA, Ogawa N, Mori A. Antioxidant protection of Bio-Normalizer in cerebral ischemia-reperfusion injury in the gerbil. NeuroReport 1993;4:1031
17. Osato JA, Korkina LG, Santiago LA, Afanas'ev IB. Effects of Bio-Normalizer (a food supplementation) on free radical production by human blood neutrophils, erythrocytes, and rat peritoneal macrophages. Int J Physic Chem Med 1995;2:65
18. Misra H, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for SOD. J Biol Chem 1972;247:3170
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265
20. Beutler E. *Red cell metabolism*. New York:Grune & Stratton, 1975:69 and 112
21. Conover WJ. *Practical nonparametric statistics*, 2nd ed. New York:Wiley, 1980:231
22. Afanas'ev IB, Suslova TB, Cheremisina ZP, Abramova NE, Korkina LG. Study of antioxidant properties of metal aspartates. Analyst 1995;120:859

Supplement to *Nutrition* Vol. 11, No. 5, 1995

Novel Copper Superoxide Dismutase Mimics and Damage Mediated by $O_2^{\cdot-}$

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ABSTRACT

Metabolites of oxygen such as superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$) are potentially damaging to biological systems. Univalent reduction of oxygen produces $O_2^{\cdot-}$, which may be converted to H_2O_2 and $OH^{\cdot}$. The biological damage mediated by $O_2^{\cdot-}$ can be attenuated by a cytosolic copper- and zinc-containing enzyme known as superoxide dismutase (SOD). Certain transition metal complexes having properties similar to SOD may be useful in suppressing such damage. However, known complexes have either been ineffective in vivo or may have toxic side effects. We prepared mixed-ligand copper complexes of polyamine using biomolecules such as pyridine or imidazoles as secondary ligands. The choice of polyamines and biomolecules was made with the aim of producing products with low toxicity. Our studies suggest that these copper complexes act as mimics of SOD in a variety of $O_2^{\cdot-}$-generating systems and may be effective SOD mimics for their usage to abrogate such an injury in biological systems. This manuscript provides a brief state-of-the-art review on SOD mimics including our own studies. *Nutrition* 1995;11:559–563

Key words: copper complexes, superoxide dismutase mimics, superoxide anion, oxidative stress

INTRODUCTION

Oxygen is a vital component for living beings and constitutes about one-fifth of the atmospheric gases. Oxygen is a paramagnetic gas that is consumed continuously during respiration and is also regenerated by plants during photosynthesis.[1] Although it is essential for all aerobic biota, its excess or inappropriate metabolism can cause health hazards that can even result in death. The harmful effects of oxygen are mediated by reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), singlet oxygen (O_2), and hydroxyl radicals ($OH^{\cdot}$).[2–5]

Reports on oxygen toxicity were published as early as the end of the 1800s, when Smith[6] reported the toxic effects of oxygen on the pulmonary system. Oxygen and its progeny can be produced from molecular oxygen by step-wise reduction.

The pathway for the production of $O_2^{\cdot-}$ in biological systems includes both enzyme-catalyzed and nonenzymatic reactions. Examples of enzymatic reactions are NADPH oxidase, xanthine oxidase, phagocytic oxidative bursts, and mitochondrial electron chain leakage. Nonenzymatic reactions that lead to the generation of this oxidant molecule are the transfer of electrons from quinone-semiquinone to oxygen and certain photochemical reactions in which an electron is transferred from an excited state in a photoactive molecule to oxygen. The major events associated with the release of $O_2^{\cdot-}$ in the biological milieu are chemotaxis, oxidation of proteins, and the inactivation and degradation of proteins and lipids. The involvement of $O_2^{\cdot-}$ as a mediator in these damaging events has been shown in cases of inflammatory disorders, reperfusion injury, carcinogenesis, and various genetic disorders. Superoxide anion is also involved in photosensitization reactions such as those observed in cancer patients undergoing photodynamic therapy, after administration of hematoporphyrin derivative, a mixture of porphyrins used in this treatment for the detection and management of neoplasm.

Superoxide anion is not very reactive, with a short life span of only milliseconds at neutral pH. It is a relatively weak oxidant that can oxidize compounds such as ascorbate,[7] sulfite,[8] and certain catecholamines.[9] On the other hand, $O_2^{\cdot-}$ has been shown to be a potent reductant. It has a redox potential close to dithionite, and can reduce ferric ion and its complexes,[2] heme-proteins such as cytochrome *c*, methemoglobin, metmyoglobin, peroxidases,[9] and quinones.[10]

SUPEROXIDE DISMUTASE

Superoxide anion is unstable in an aqueous medium and gets dismutated spontaneously. However, this also occurs through an enzyme-catalyzed reaction. The enzyme responsible for this rapid dismutation is known as superoxide dismutase (SOD) . The rate constant for $O_2^{\cdot-}$ dismutation at pH 7.4 is 2.0×10^5 mol $\cdot$ $L^{-1} \cdot s^{-1}$. The rate of autodismutation is at least four times slower compared with the enzyme-catalyzed reaction.[2]

Three isozymes of SOD that perform dismutation reactions with a comparable efficiency have been reported to exist. The manganese-containing isozyme (MnSOD) is found in prokaryotes

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