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Elevated Superoxide Dismutase in Black Alcoholics

Abstract. *Superoxide dismutase concentrations in lysates of erythrocytes from black alcoholics were higher than those of white alcoholics and of nonalcoholics of both races. Higher concentrations of enzyme protein, as determined by competition radioimmunoassay, correspond to proportionately higher enzyme activity. Elevated superoxide dismutase levels were not related to any other clinical, historical, or demographic variables. Increased superoxide dismutase levels may delay or prevent some of the pathological sequelae of alcoholism and may be a useful biological marker for alcohol abuse.*

Superoxide dismutases (E.C.1.15.1.1) are enzymes that catalyze the conversion of potentially harmful superoxide free radicals to hydrogen peroxide and oxygen (*1*). Several distinct classes of superoxide dismutases have been described, and at least one such enzyme is found in almost every organism that can survive an environment in which oxygen is present. This led McCord *et al.* (*2*) to propose that the physiological function of superoxide dismutases is to protect against the toxic effects of oxygen metabolites.

There are two classes of superoxide dismutase (SOD) in humans (*1*): SOD-1, a copper-zinc enzyme present in the cytoplasm of all cells, and SOD-2, a manganese-containing enzyme found primarily in mitochondria and, therefore, not present in red blood cells (RBC's). These enzymes are the products of distinct genes on chromosomes 21 and 6, respectively (*3*), and have different molecular weights and unique immunologic specificities (*4*).

After determining that the gene for SOD-1 is located on chromosome 21, it was found that the levels of SOD-1 in cells from patients with trisomy 21 were approximately 50 percent greater than those of normal individuals—a phenomenon ascribed to the gene dosage (*5*). The level of SOD-1 in RBC's has been studied in order to evaluate the significance of the enzyme in human metabolism. Alterations of SOD-1 levels in a number of disorders were reported, but in many of these studies, essential supporting data were not given, making them difficult to evaluate (*6*).

Recently, we developed a competition radioimmunoassay for human SOD-1 (*7*) and observed that the SOD-1 level in the RBC's of a putative normal individual was markedly elevated. On discovering that this person was an alcoholic, we hypothesized that elevated SOD-1 in RBC's may be a biological marker for alcohol abuse. To test this hypothesis, we compared SOD-1 levels in RBC's from chronic alcoholics and nonalcoholic individuals. The Michigan alcoholism screening test (MAST) (*8*) and an interview concerning medical history, demographic data, and drinking habits were given to patients admitted to an alcoholism rehabilitation center and to putative normal subjects. Blood was obtained for automated multiple analyses, hematologic studies, and radioimmunoassay for SOD-1. Sixty-two subjects were classified as alcoholics based on their admission to an alcoholism rehabilitation program, a history of alcohol abuse, and a MAST score of 5 or higher. Twenty-seven nonalcoholics, including 18 social drinkers and 9 nondrinkers, served as controls.

Concentrations of SOD-1 in lysates of washed RBC's were determined by competition radioimmunoassay (*7*). Approximately 1 ng of ^{125}I-labeled SOD-1 was incubated with appropriate dilutions of an RBC lysate and antibody to SOD-1. The immune complexes were then precipitated with a *Staphylococcus aureus* immunosorbent. The concentration of SOD-1 was determined by comparing the displacement of ^{125}I-labeled SOD-1 from antibody to SOD-1 with that effected by known amounts of purified SOD-1 (*9*). Hemoglobin concentration was determined with Drabkin's solution (*10*).

The median SOD-1 level for the 27 controls was 815 ng per milligram of hemoglobin, and for the 62 alcoholics, 898 ng/mg (P = .007, Wilcoxon rank sum test). The relation between the amount of enzyme protein and each demographic, medical historical, and laboratory finding was evaluated by Wilcoxon rank sum test, chi-square test, and linear re-

Table 1. Activity of SOD-1 in RBC lysates from black alcoholics and nonalcoholics. Lysates were adjusted to 20 mg of hemoglobin per milliliter and then analyzed for SOD-1 by competition radioimmunoassay (*7*). Samples were extracted with chloroform and ethanol (*15*) and centrifuged; supernatants were assayed for SOD-1 activity by using the pyrogallol method (*11*) and then reassayed for SOD-1 in protein by competition radioimmunoassay. Differences among groups are not statistically significant.

Group	N	Specific activity after extraction*	Recovery after extraction†
Black alcoholics (initial SOD-1 levels > 1000 ng/mg)	12	0.91 ± 0.18	0.85 ± 0.27
Black alcoholics (initial SOD-1 levels < 1000 ng/mg)	6	0.95 ± 0.18	0.88 ± 0.37
Black nonalcoholics (mean initial SOD-1 level, 843 ng/mg)	6	1.01 ± 0.17	0.88 ± 0.40

*Ratio of SOD-1 determined by pyrogallol assay to SOD-1 determined by radioimmunoassay. †Ratio of SOD-1 determined by radioimmunoassay before extraction to SOD-1 determined by radioimmunoassay after extraction.

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gression by least-squares methods using the Prophet computer system, and is discussed by Del Villano *et al.* (*11*). Of 37 parameters studied, the only statistically significant variable that differentiated alcoholics with SOD-1 levels above 1000 ng/mg from those with lower levels was race. Figure 1 shows the distribution of SOD-1 levels in RBC's from black and white alcoholics and controls. The median SOD-1 concentration for black alcoholics was 1033 ng/mg; 13 of 19 had levels above 1000 ng/mg. In contrast, the median SOD-1 concentration for white alcoholics was 874 ng/mg ($P = .0001$ when compared with black alcoholics, Wilcoxon rank sum test). Only 2 of 42 white alcoholics had SOD-1 levels above 1000 ng/mg. Two of the 27 controls (both white) had SOD-1 levels of 1030 and 1050 ng/mg. There was no statistically significant difference between the levels observed in black controls (843 ng/mg; $N = 9$) and white controls (794 ng/mg; $N = 15$).

The SOD activity in lysates from blacks was determined by pyrogallol assay (*12*) after chloroform and ethanol precipitation of hemoglobin, and the recovery of SOD-1 was monitored by radioimmunoassay. As shown in Table 1, the average ratio of enzyme activity to enzyme protein for black alcoholics with SOD-1 levels above 1000 ng/mg was not significantly different from that of black alcoholics with lower levels or from that of black normals. These results indicate that the higher levels of enzyme protein correspond to proportionately higher levels of enzyme activity.

The increased levels of SOD-1 observed in black alcoholics do not appear to be related to other blood disorders or to the degree of liver damage (*11*). The hemoglobin levels and hematocrit values of the alcoholic subjects were within normal limits, indicating that these subjects were not overtly anemic. The medians for the mean corpuscular hemoglobin and volume values for these alcoholics were somewhat elevated but did not account for the increased SOD-1. Further, the serum levels of aspartic aminotransferase (or glutamic-oxaloacetic transaminase, E.C. 2.6.1.1), lactate dehydrogenase, and alkaline phosphatase for alcoholics with high SOD-1 concentrations were not different from those of alcoholics with low SOD-1 concentrations.

The increased SOD-1 levels observed in some alcoholics raise the possibility that O_2^- could be involved in the pathogenesis of the sequelae of alcoholism. Alcohol metabolism takes place primarily in the liver, where ethanol is oxidized to acetaldehyde by alcohol dehydrogenase. Acetaldehyde is then oxidized to acetate by aldehyde dehydrogenase or aldehyde oxidase. Activity of the latter enzyme produces O_2^- (*13*). Thus consumption of large amounts of alcohol may produce corresponding amounts of O_2^-. In some individuals, this higher concentration of O_2^- may result in higher levels of SOD-1, possibly through a substrate induction mechanism. The reason that some alcoholics do not display increased levels of SOD-1 is not clear. The racial differences in SOD-1 levels suggest that genetic or environmental factors may be involved. Family studies and more extensive analyses of other racial groups from different environments are in order.

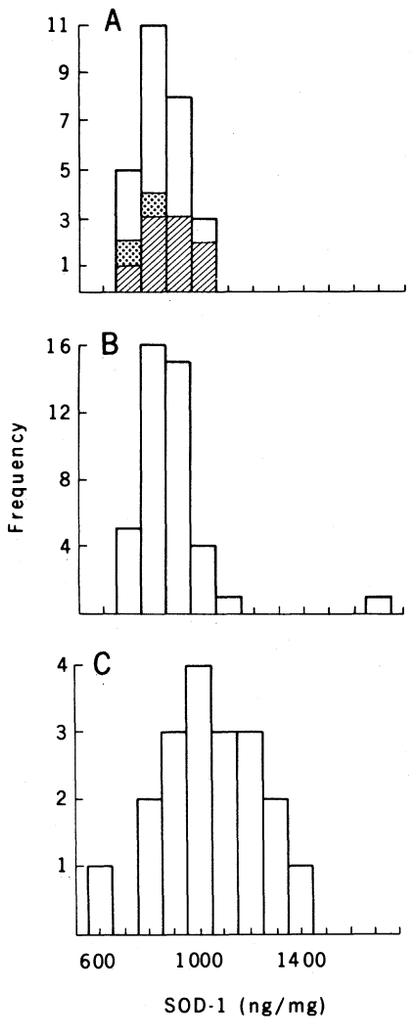

Fig. 1. Frequency distribution of SOD-1 in RBC's. Lysates of washed RBC's were analyzed for SOD-1 concentration by competition radioimmunoassay (*7, 9*) and for hemoglobin by Drabkin's method (*10*). (A) Nonalcoholic individuals [16 whites (unshaded), 9 blacks (crosshatched), 2 Asians (dotted)]; (B) white alcoholics ($N = 42$); and (C) black alcoholics ($N = 19$). Results shown in (A) and (B) are not significantly different; results in (C) are significantly different from those in both (A) and (B) at $P = .0001$. One alcoholic was an American Indian (SOD-1 = 922 ng/mg) and is not included in this figure.

Since O_2^- interacts with DNA, lipids, and proteins to disrupt cellular metabolism (*1*), increased SOD-1 levels may protect against alcohol-induced pathogenesis by quickly dismutating O_2^-. Such a protective effect, however, could be overcome by continued excessive use of alcohol. It may be significant that cirrhosis of the liver was not evident in 11 of the 13 black alcoholics with the highest SOD-1 levels, although all of these patients had elevated levels of liver enzymes.

Between 100,000 and 200,000 deaths per year are attributed to the direct and indirect effects of alcoholism, and the cost of this disease to the United States in 1975 was estimated to be $43 billion (*14*). Numbers, of course, do not reflect the magnitude of suffering caused by alcohol abuse. The key to successful treatment, early diagnosis, has depended primarily on subjective criteria. Thus an objective biochemical test for alcohol abuse would be of great benefit.

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Tooth Induction in Chick Epithelium: Expression of Quiescent Genes for Enamel Synthesis

Abstract. *Intraocular grafts of chick epithelium combined with mouse molar mesenchyme produced a variety of dental structures including perfectly formed crowns with differentiated ameloblasts depositing enamel matrix. The results suggest that the loss of teeth in Aves did not result from a loss of genetic coding for enamel synthesis in the oral epithelium but from an alteration in the tissue interactions requisite for odontogenesis.*

Embryonic induction has been shown experimentally to operate normally between tissues of different phylogenetic origins. Both neural induction (*1*) and ectodermal-mesenchymal interactions (*2*) have been examined in xenoplastic tissue combinations. Thus we may conclude that inductive signals are general and can be interpreted by interacting tissues despite genetic disparities. Further, once the inductive influence has been exerted, the responding tissue differentiates as expected from its genetic repertoire. Thus undifferentiated corneal epithelium of the chick responds to mouse dermis from embryonic back skin by producing feathers (*3*).

In contrast, in chorioallantoic membrane grafts of chick epithelium recombined with mouse molar dental papilla (*4*), the chick epithelium responded in an unanticipated manner (*5*). After 1 week's growth the chick epithelium showed an enamel organ-like arrangement and the dental papilla had begun to secrete dentin. Enamel matrix protein was not deposited, however, and epithelial cells adjacent to the dentin matrix were undifferentiated. One week was too short a time to permit development of a fully formed tooth. Nonetheless, the epithelium had assumed a mammalian character, as evidenced by its invasion into the mesenchyme. In addition, dentin secretion by differentiated mouse odontoblasts indicated that the epithelium was acting as an enamel organ; the initiation of dentin synthesis requires permissive tissue interaction with an enamel organ (*6*). Thus it was hypothesized that if a longer culture period were provided, complete odontogenesis might be achieved. The results reported here show that chick epithelium can secrete enamel matrix proteins and suggest that the genetic information for this synthesis was not lost during evolution.

We separated and then recombined the epithelium of the first and second pharyngeal arches of 5-day-old chick embryos with the mesenchyme from first mandibular molars of 16- to 18-day-old CD-1 mouse embryos. The isolations, separations, recombination, and grafting procedures have been described in detail elsewhere (*5*). The tissues were dissected in Hanks balanced salt solution containing 10 percent fetal calf serum and were separated into epithelial and mesenchymal components after digestion with 1 percent trypsin (Bacto-Difco) in the salt solution (1:250) for 2 hours at 4°C. Recombinations were incubated overnight on agar-solidified medium in a humidified atmosphere (37°C) to ensure that the recombined tissues would adhere to each other during grafting into the anterior chambers of the eyes of adult nude athymic mice. After 1, 2, or 4 weeks the grafts were harvested, decalcified, histologically processed, and stained with hematoxylin and Biebrich Scarlet Red. Fifty-five chimeric recombinants were examined.

A range of tissue responses was seen. The chick epithelium made some unusual invasions into the mouse mesenchyme, suggesting an attempt at enamel organ histogenesis (Fig. 1A). [These epithelial patterns resembled our earlier finding (*4*), and variations appeared in young and old grafts.] We found ten aberrant structures consisting of dentin and odontoblasts in molar-like configurations. The odontoblasts of these structures appeared normal, and dentinal tubules were visible (Fig. 1B). Dentinal tubules and well-defined odontoblastic layers clearly distinguished these structures from the ubiquitous spongy bone that was distributed randomly in these grafts. Spongy bone is the only hard tissue found in control grafts of dental mesenchyme alone. In one case, the aberrant dentinal structure was clearly developing in close association with keratinizing epithelium (Fig. 1C). Enamel matrix was not seen next to the dentin of these structures (the normal relationship).

Complete teeth developed in four grafts. This occurred when the chick epithelium also developed well, consisting of keratinizing epithelium and the mucus-secreting, ciliated epithelium (Fig. 1, D and E) characteristic of avian esophageal glands (*7*). In one case (Fig. 1, F and G), the tooth could be seen with transmitted light in the graft at the time of harvesting, permitting serial longitudinal sectioning. The tooth epithelium was contiguous with the epithelium of the keratinizing cyst and of the glands. The entire tooth structure was well formed, with root development in proper relation to the crown, but the latter did not have the typical first-molar morphology, since it lacked the cusp pattern usually present in intraocular grafts of first-molar rudiments. In other cases there were similar alterations of the enamel matrix deposition and crown morphology.

Several criteria were used to confirm the avian source of the epithelium tissue. First, control grafts of isolated mouse dental papilla were examined for possible contaminating remnants of mouse epithelium. These control grafts never

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