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Age-Dependent Basal Level and Induction Capacity of Copper-Zinc and Manganese Superoxide Dismutase and Other Scavenging Enzyme Activities in Leukocytes from Young and Elderly Adults

Yukie Niwa,* Osamu Iizawa,† Koichi Ishimoto,‡ Hirohiko Akamatsu,§ and Tadashi Kanoh||

From the Niwa Institute for Immunology Kochi-ken; Department of Dermatology,† Tohoku University School of Medicine, Sendai; Department of Pediatrics,‡ Faculty of Medicine, Juntendo University, Tokyo; Department of Dermatology,§ Kansai Medical University, Moriguchi; and Department of Internal Medicine,|| Faculty of Medicine, Kyoto University, Kyoto, Japan*

Several enzymes, including superoxide dismutase (SOD), catalase, glutathione peroxidase, and D-glucose-6-phosphate dehydrogenase are capable of scavenging reactive oxygen species in in vivo. We assessed both basal levels and the capacity of these enzyme activities to be induced in human leukocytes in response to a variety of agents. Basal activity of copper-zinc SOD, and manganese SOD showed little variation with age. In contrast, the basal activity of the three H_2O_2 scavenging enzymes, catalase, glutathione peroxidase, and D-glucose-6-phosphate dehydrogenase, was significantly higher in younger adults than in elderly individuals. Both manganese SOD and copper, zinc SOD activities were significantly induced by paraquat, interleukin-1, tumor necrosis factor, adriamycin, and bleomycin in lymphocytes and neutrophils from asymptomatic nonaged adults, whereas neither activity was induced in aged individuals. In contrast, glutathione peroxidase activity was significantly induced in both groups of subjects, whereas catalase and D-glucose-6-phosphate dehydrogenase were only slightly induced in either. Enzyme induction with paraquat, adriamycin, or bleomycin was inhibitable by neutralizing antibody to interleukin-1 and tumor necrosis factor, suggesting that the inductions observed with these three drugs are due to the distal mediators, interleukin-1 or tumor necrosis factor released from the cells. Finally, as observed in the regulation of genes in eukaryotes (Storz et al: Bacterial defenses against oxidative stress. Trends Genetics 1990, 6:363–368, ref. 1) O_2^- and H_2O_2 seem to differ in the rate of change with age in both basal levels and inducibility under oxygen stress. (Am J Pathol 1993, 143:312–320)

Two types of superoxide dismutase (SOD) exist in mammalian tissues: manganese SOD (Mn-SOD) in mitochondria and copper-zinc SOD (Cu-Zn-SOD) in the cytosol. SOD is a class of enzyme that effectively scavenges reactive oxygen species (ROS) and inhibits lipid peroxidation when O_2^- plays a role in the initiation of this reaction. Genetic polymorphism in man has been observed for both SOD isotypes, Cu-Zn-SOD (locus on chromosome 21) and Mn-SOD (locus on chromosome 6). It has recently been suggested that ROS are closely correlated with aging, carcinogenesis, and the pathogenesis of a variety of inflammatory disorders[2–8] and that basal SOD activity decreases with age.[9–11] However, we and others have not been able to confirm the decrease in the basal activity of SOD with aging.[12–15]

When exposed to the oxidative stress, a 10- to 20-fold increase in SOD activity occurs in some bacteria and plants.[16–18] We have shown that individuals with a capacity for strong SOD induction tend to respond more promptly to treatment of inflammatory diseases that involve ROS than do individuals whose SOD induction capacity is low.[14,19]

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Address reprint requests to Yukie Niwa, M.D. Ph.D. Niwa Institute for Immunology, 4-4 Asahimachi, Tosashimizu, Kochi-ken, 787-03, Japan.

Wong and Goeddel[20] and Asoh et al[21] demonstrated tumor necrosis factor (TNF)-induced mitochondrial Mn-SOD induction in various cell lines, including human cancer cells, as well as in murine tissues. The induction of mitochondrial Mn-SOD in human melanoma cells by interleukin-1 (IL-1)[22] and the induction of free-radical scavenging proteins by the action of cytokines[23,24] were also reported. However, the induction of Cu-Zn-SOD has not yet been reported in humans and animals although it has been demonstrated in yeast by Stevens and Autor.[25]

We have recently demonstrated[12] that total SOD activity in normal human leukocytes is significantly induced by the herbicide paraquat, a compound known to stimulate production of O_2^-,[26,27] with subsequent formation of OH·. Although the basal level of total SOD activity is comparable between young and old individuals,[13] the assay of SOD induction by paraquat identified both an age-dependent decline in the strength of the induction of total SOD activity and a direct correlation of the magnitude of SOD induction with longevity in elderly adults.[12]

In the present study, we examined the induction of Cu-Zn- and Mn-SOD, as well as of catalase, glutathione peroxidase (GSH-Px), and D-glucose-6-phosphate dehydrogenase (G-6-P-D) in normal human leukocytes; all these enzymes scavenge ROS, and SOD and catalase inhibit lipid peroxidation. The inducing agents studied were paraquat,[26,27] TNF,[28,29] IL-1,[30] and anti-cancer drugs[31–33] that are known to produce ROS or to induce the cells to produce ROS. In addition, it was tested whether the induction of these scavenging enzymes by paraquat and anti-cancer drugs is mediated through the cytokines IL-1 and TNF.

Materials and Methods

Human Subjects

Subjects consisted of 160 non-aged healthy volunteers (91 males, aged 18 to 64, 69 females aged 20 to 64) and 96 aged asymptomatic individuals (46 males, aged 65 to 90, 50 females, aged 65 to 92).

Enzyme Inducers

As inducers for the induction of each enzyme, paraquat (Wakojunyaku Kogyo Company, Osaka, Japan), TNF (specific activity 2×10^7 U/mg, Genzyme Co. Ltd., Boston, MA), IL-1 (specific activity 1×10^7 U/mg, Genzyme), adriamycin (Kyowa Hakko Co Ltd, Tokyo), and bleomycin (Kyowa Hakko) were used. Because of the large amount of blood required for the induction assays, blood was pooled from up to two or three donors in most experiments.

In preliminary experiments, optimal concentrations of each inducer were sought that had minimal effect on cell viability and maximal leukocyte SOD induction as described previously.[12] Preliminary time course experiments were also carried out to determine the optimal incubation time with each inducer for maximal leukocyte SOD induction, as previously described.[12] Optimal concentrations of each inducer thus obtained were as follows: paraquat: 4.9×10^{-3}, 2.45×10^{-2}, or 1.225×10^{-1} mmol/L; TNF: 0.4, 2, or 10 U/ml; IL-1: 1.6, 8, or 40 U/ml; adriamycin: 0.69, 3.45, or 17.25 μmol/L; bleomycin: 0.142, 0.71, or 3.55 μmol/L. The optimal incubation time was 18 hours. The wide range of inducer concentrations was necessary because of occasional variability in the response to a single concentration, possibly due to differing susceptibility to inducer toxicity.[12] For each individual subject, the percent induction reported in this study is the highest of the separate assays at different inducer concentrations.

To assess whether the induction by paraquat, bleomycin, and adriamycin is an indirect effect mediated by IL-1 or TNF, neutralizing antibody to IL-1 or TNF was added in the reaction mixture containing paraquat, bleomycin, or adriamycin. As control enzymes, aldolase, and the membrane phospholipid enzymes, ethanolaminephosphotransferase and cholinephosphotransferase, both of which mediate structural phospholipid production,[34–37] were tested for induction by the agents used in our study.

Cell Preparations

Peripheral blood neutrophils and mononuclear cells were carefully isolated by Ficoll-Hypaque gradient centrifugation and prepared under conditions previously shown.[12]

Cu-Zn- and Mn-SOD Activity Assay

0.2 ml of each supernatant was added to the xanthine-xanthine oxidase O_2^- generating system, which consisted of 16.5 μmol/L ferricytochrome c, 0.1 mmol/L hypoxanthine, and 1.25 mmol/L ethylenediaminetetraacetic acid in total volume of 2 ml of 125 mmol/L phosphate buffer. After the addition of cell supernatant, 0.006 U/ml of dialyzed xanthine oxidase was added to generate O_2^-. Under these assay conditions, the amount of SOD required to inhibit the rate of reduction of cytochrome c by 50%

(i.e., to a rate of 0.0125 absorbance at 550 nmol/L unit per minute) was defined as 1 U of activity. Because percent inhibition in leukocytes is not linear above approximately 1 unit, it was adjusted according to Asada's formula.[38]

To distinguish the activities of Mn-SOD and Cu-Zn-SOD, the assay mixture was made 1 mmol/L in KCN, and the remaining SOD activity (as assayed by inhibition of cytochrome c reduction) was referred to as Mn-SOD activity. Cu-Zn-SOD activity was estimated by subtraction of Mn-SOD activity from total SOD. Oberley and Spitz et al.[39,40] reported that 1 mmol/L cyanide is sufficient to completely eliminate Cu-Zn-SOD activity in normal cells.

Catalase Activity Assay

Catalase activity was determined by the reduction velocity of H_2O_2 in the presence of catalase containing leukocyte samples from 12 seconds to 30 seconds, using a spectrophotometer at 240 nm. The activity was expressed as follows[41]:

$$K = \frac{2.3}{18} \times \log\frac{A_1}{A_2}$$

A_1: OD (240 nm) at 12 seconds in the assay mixture consisting of 10 mmol/L H_2O_2 dissolved in 3 ml of 50 mmol/L phosphate buffer (pH 7.0) and 0.1 ml leukocyte sample. A_2: OD (240 nm) at 30 seconds in the above assay mixture.

Taking into consideration the possibility of the instability of H_2O_2 used as substrate in the catalase activity assay, we also substituted sodium perborate for the H_2O_2 substrate according to Thomson et al;[42] 0.002 to 0.05 ml samples were suspended in 2.8 ml of 0.05 mol/L potassium phosphate buffer (pH 7.4) and preincubated at 30 C for 5 minutes. Thereafter, the reaction was started in the cuvette by adding 0.2 N $NaBO_3$ solution (which was neutralized at pH 9.4 with 85% H_3PO_4), and then the data was recorded consecutively for 2 to 3 minutes at 220 nm.

GSH-Px Activity Assay

GSH-Px was measured by the method of Lawrence and Burk,[43] in which GSH-Px activity was coupled to the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) by glutathione reductase. The oxidation of NADPH was followed spectrophotometrically at 340 nm at 37 C. The reaction mixture consisted of 50 mmol/L potassium phosphate buffer (pH 7.0), 1 mmol/L ethylenediaminetetraacetic acid, 1 mmol/L NaN_3, 0.2 mmol/L NADPH, 1 mmol/L glutathione, 2 U of glutathione reductase, and 1.5 mmol/L cumene hydroperoxide, or 10 mmol/L tert-butyl hydroperoxide. Addition of the leukocyte samples brought the total volume to 2.0 ml. Enzymatic activity was expressed as nmol NADPH oxidized per minute. Protein was measured by the method of Lowry et al.[44] Specific activity was expressed as units per mg protein.

G-6-P-D Activity Assay

G-6-P-D activity was determined as described by Olive and Levy.[45] Briefly, assay mixtures contained the following components in a final volume of 3.0 ml: 33 mmol/L Tris-HCl buffer, pH 7.8; 3.3 mmol/L G-6-P-D; and either 0.16 mmol/L $NADP^+$ or 2.5 mmol/L NAD^+ (neutralized to pH 7). Substrates and co-enzymes were present routinely at a minimum of 10 times the concentration of the determined Km. Reactions were usually initiated either with enzyme or glucose-6-phosphate. The reaction was followed by noting the increase in absorbancy at 340 mμ with time. One unit is defined as the amount of enzyme required to catalyze the reduction of 1 μ mole of $NADP^+$/minute at 25 C. Km's for the reaction with $NADP^+$, or of glucose-6-phosphate in the presence of $NADP^+$, were determined by measuring $NADP^+$ reduction in a spectrofluorometer (Hitachi, U-3200, Japan) at 25 C using an excitation wavelength of 350 mμ and a fluorescence emission wavelength of 460 mμ.

Each enzyme induction capacity of each leukocyte supernatant was calculating from the following formula:

$$\text{\% each enzyme induction} = \left(\frac{b - a}{a}\right) \times 100$$

a: enzyme activity in the absence of inducer; b: enzyme activity in the presence of inducer.

Effect of Antibody to IL-1 and TNF and Assay for Control Enzymes

Rabbit antisera against human IL-1α (specific activity 1×10^7 U/mg, Dainippon, Tokyo, Japan), IL-1β (specific activity 1×10^7 U/mg, Cistron, Pine Brood, NY) or TNF-α (specific activity 2×10^7 U/mg, Biogen, Geneva, Switzerland, and BASF/Knoll) was added into the reaction mixture including paraquat, adriamycin, or bleomycin, and its effect on each enzyme induction was examined. Control rabbit serum against mouse IL-1α or β was also tested in this experiment.

The activity of ethanolaminephosphotransferase and cholinephosphotransferase was assessed as described,[34,35,37] using cytidine-5′-diphosphate [^{14}C-methyl]choline ([^{14}C]CDP-choline) and cytidine-5′-diphosphate [1-^{3}H]ethanol-2-amine ([^{3}H]-CDP-ethanolamine), respectively. Aldolase was determined by routine clinical laboratory methods. The results were expressed as the mean ± SD of replicate assay. Statistical significance was determined by χ-square and by Student's *t*-test.

Results

The Basal Level of Four ROS Scavenging Enzyme Activities in Non-Aged and Aged Controls

The basal levels of Cu-Zn-and Mn-SOD activities in lymphocytes were 8.6 ± 1.3 and 2.8 ± 0.4 U/mg protein, respectively, and in neutrophils, 2.6 ± 0.4 and 0.6 ± 0.1 U/mg protein, respectively (Tables 1 and 2, data on Cu-Zn-SOD not shown). Thus, the ratio of the activity of Cu-Zn-SOD to Mn-SOD in lymphocytes was similar to that in neutrophils, being approximately 4:1. Neither Cu-Zn-SOD nor Mn-SOD activity showed significant variation with age (Tables 1 and 2, Cu-Zn-SOD not shown). This finding was consistent with our previous results on total SOD.[12,13]

The H_2O_2-scavengers showed different behaviors. The activity of catalase in both lymphocytes and neutrophils from young adults was significantly higher than that of elderly adults ($0.01 < P < 0.025$, Tables 1 and 2). Also, GSH-Px and G-6-P-D activities in lymphocytes and neutrophils, and G-6-P-D activity in lymphocytes, showed significant differences between non-aged and aged individuals, as shown in Tables 1 and 2 (GSH-Px in lymphocytes and neutrophils and G-6-P-D in lymphocytes: $0.01 < P < 0.025$, G-6-P-D in neutrophils: $P > 0.05$).

The Induction of Cu-Zn- and Mn-SOD Activities

Not only Mn-SOD but also Cu-Zn-SOD activity was significantly induced in both lymphocytes and neutrophils from young adults by each of the agents tested (Tables 1 and 2 and Figure 1, data on Cu-Zn-SOD in Table 1 and those on neutrophils in Figure 1 not shown). These results are consistent with our previous findings on total SOD activity using paraquat alone as an inducer.[12]

The induction of both Cu-Zn- and Mn-SOD activities in individuals over 70 years old was considerably lower than in those 60 and under (Tables 1 and 2, Figure 1, data on Cu-Zn-SOD in Tables 1 and 2 and those on neutrophils in Figure 1 not shown). As shown in Figure 1, the induction of not only Mn-but also Cu-Zn-SOD activity with each inducer tested declined with age, with a dramatic fall occurring between the ages 55 and 65 years (only data for TNF, Il-1, and bleomycin induction in lymphocytes are shown in Figure 1). This precipitous drop seems to correlate with the progression of the aging process

Table 1. *Induction of ROS Scavenging Enzyme Activities in Lymphocytes from Young (18 to 60 years) and Elderly (71 to 80 years) Adults, with Various Inducers*

Inducers/Enzymes	Mn-SOD (U/mg protein)	Catalase (K/mg protein)	GSH-Px (U/mg protein)	G-6-P-D (U/mg protein)
Paraquat				
(4.9×10^{-3}	3.5 ± 0.7§	0.110 ± 0.0187‡	0.101 ± 0.0203‡	0.307 ± 0.062‡
2.45×10^{-2} or	(3.1 ± 0.3)	(0.0784 ± 0.014)†	(0.0839 ± 0.0194)‡	(0.297 ± 0.048)†
1.225×10^{-1} mmol/L)				
TNF	3.6 ± 0.5§	0.114 ± 0.0212‡	0.115 ± 0.0224§	0.311 ± 0.068‡
(0.4, 2, or 10 U/ml)	(3.0 ± 0.3)	(0.0797 ± 0.018)†	(0.0933 ± 0.0201)§	(0.299 ± 0.043)†
IL-1	3.5 ± 0.4‡	0.126 ± 0.0227§	0.106 ± 0.0199‡	0.259 ± 0.057
(1.6, 8, or 40 U/ml)	(2.9 ± 0.3)	(0.0824 ± 0.015)	(0.0842 ± 0.0199)	(0.222 ± 0.049)
Adriamycin	3.3 ± 0.4‡	0.1107 ± 0.0175‡	0.128 ± 0.0264‖	0.319 ± 0.056‡
(0.69, 3.45, or 17.25 μmol/L)	(2.9 ± 0.3)	(0.0758 ± 0.0094)	(0.0991 ± 0.0190)‖	(0.232 ± 0.045)†
Bleomycin	3.2 ± 0.5§	0.1104 ± 0.0156‡	0.131 ± 0.0236‖	0.265 ± 0.064
(0.142, 0.71, or 3.55 μmol/L)	(2.9 ± 0.3)	(0.0751 ± 0.0089)	(0.0993 ± 0.0180)‖	(0.220 ± 0.051)
Control*	2.8 ± 0.4	0.0868 ± 0.017	0.0828 ± 0.011	0.244 ± 0.038
	(2.4 ± 0.3)	(0.0671 ± 0.012)	(0.0645 ± 0.010)	(0.188 ± 0.031)

The data on 40 asymptomatic young adult volunteers (aged 18 to 60) were presented; the parentheses ( ) denote those on 14 healthy aged individuals (aged 71 to 80).

* Control denotes each enzyme activity in the absence of each inducer.

† $0.025 < P < 0.05$ vs control.

‡ $0.01 < P < 0.025$.

§ $P < 0.01$.

‖ $P < 0.001$.

Table 2. *Induction of ROS Scavenging Enzyme Activities in Neutrophils from Young (18 to 60 years) and Elderly (71 to 80 years) Adults, with Various Inducers*

Inducers/Enzymes	Mn-SOD (U/mg protein)	Catalase (K/mg protein)	GSH-Px (U/mg protein)	G-6-P-D (U/mg protein)
Paraquat (4.9×10^{-3}	0.8 ± 0.1‡	0.349 ± 0.069‡	0.0304 ± 0.0056‡	1.439 ± 0.32†
2.45×10^{-2} or 1.225×10^{-1} mmol/L)	(0.6 ± 0.08)	(0.252 ± 0.042)†	(0.0230 ± 0.0053)‡	(1.329 ± 0.34)†
TNF	0.9 ± 0.1‡	0.358 ± 0.067‡	0.0326 ± 0.0056§	1.386 ± 0.38
(0.4, 2, or 10 U/ml)	(0.7 ± 0.09)	(0.257 ± 0.040)†	(0.0259 ± 0.0049)§	(1.205 ± 0.32)
IL-1	0.8 ± 0.1†	0.328 ± 0.059†	0.0301 ± 0.0054‡	1.345 ± 0.42
(1.6, 8, or 40 U/ml)	(0.7 ± 0.09)	(0.246 ± 0.038)†	(0.0232 ± 0.0047)‡	(1.241 ± 0.30)
Adriamycin	0.8 ± 0.1†	0.322 ± 0.051†	0.0297 ± 0.0060‡	1.383 ± 0.40
(0.69, 3.45, or 17.25 μmol/L)	(0.6 ± 0.09)	(0.235 ± 0.039)	(0.0227 ± 0.0043)‡	(1.216 ± 0.35)
Bleomycin	0.9 ± 0.09‡	0.317 ± 0.048†	0.0295 ± 0.0059‡	1.338 ± 0.37
(0.142, 0.71, or 3.55 μmol/L)	(0.6 ± 0.08)	(0.231 ± 0.037)	(0.0226 ± 0.0038)‡	(1.256 ± 0.35)
Control*	0.6 ± 0.1	0.269 ± 0.041	0.0231 ± 0.0043	1.218 ± 0.19
	(0.5 ± 0.08)	(0.210 ± 0.032)	(0.0182 ± 0.0031)	(1.101 ± 0.14)

The data on 40 asymptomatic young adult volunteers (aged 18 to 60) were presented; the parenthesis ( ) denotes those on 14 healthy aged individuals (aged 71 to 80).

* Control denotes each enzyme activity in the absence of each inducer.

† $0.025 < P < 0.05$ vs control.

‡ $0.01 < P < 0.025$.

§ $P < 0.01$.

during this interval. These results are similar to those reported with total SOD activity induced by paraquat.[12]

The Induction of H_2O_2 Scavenging Enzyme Activities

As shown in Tables 1 and 2, and Figure 2, catalase activity was induced in both lymphocytes and neutrophils from asymptomatic young adults with all of the inducers tested, though not so markedly as observed for SOD activity (data on catalase and GSH-Px of neutrophils in Figure 2 not shown). In contrast to SOD, catalase was slightly induced in the leukocytes of subjects above 71 years old in the presence of paraquat, TNF, and IL-1 (Tables 1 and 2, Figure 2). The decline in catalase induction with age was considerably less dramatic than the decline in SOD induction (compare Figure 2 with Figure 1; data on neutrophils in age brackets not shown in Figure 2).

Figure 2 and Tables 1 and 2 show that GSH-Px activity in both lymphocytes and neutrophils from healthy young adults was significantly induced with every agent tested, as was also observed in the induction of SOD activity. However, in contrast to SOD and catalase activities, GSH-Px induction showed no decline with age (Tables 1 and 2, Figure 1).

Although the data are not shown in Figure 1 and 2 in which the changes in each enzyme induction in every age bracket are presented, G-6-P-D activity was slightly induced in lymphocytes from both age groups in the presence of adriamycin, paraquat, and TNF ($0.01 < P < 0.025$, Table 1). Neutrophils from either group showed no significant G-6-P-D induction, except with paraquat alone (paraquat: $0.025 < P < 0.05$, others: $P > 0.05$, Table 2).

Comparison of the Basal Activity and Inducibility of Scavenging Enzymes between Lymphocytes and Neutrophils

Tables 1 and 2 show that the basal activity of SOD and GSH-Px was higher in lymphocytes than in neutrophils, whereas catalase and G-6-P-D activities in neutrophils were higher than in lymphocytes. On the other hand, the levels of induction of each enzyme tested were higher in lymphocytes than in neutrophils (Tables 1 and 2), a phenomenon observed previously in the induction of total SOD in leukocytes by paraquat.[12]

Effect of Neutralizing Antibody against IL-1 or TNF on Each Enzymatic Induction

Antisera to IL-1 α, IL-1β, and TNF-α significantly decreased the induction percentage of each enzyme induced with paraquat, adriamycin, or bleomycin in dose-dependent fashion, respectively (data not shown). Control rabbit serum did not show any effect on enzyme induction. These results suggested that induction of these enzymes is not a direct effect of the three drugs, but rather is mediated through IL-1 or TNF.

Control enzymes (aldolase, ethanolaminephosphotransferase, and cholinephosphotransferase)

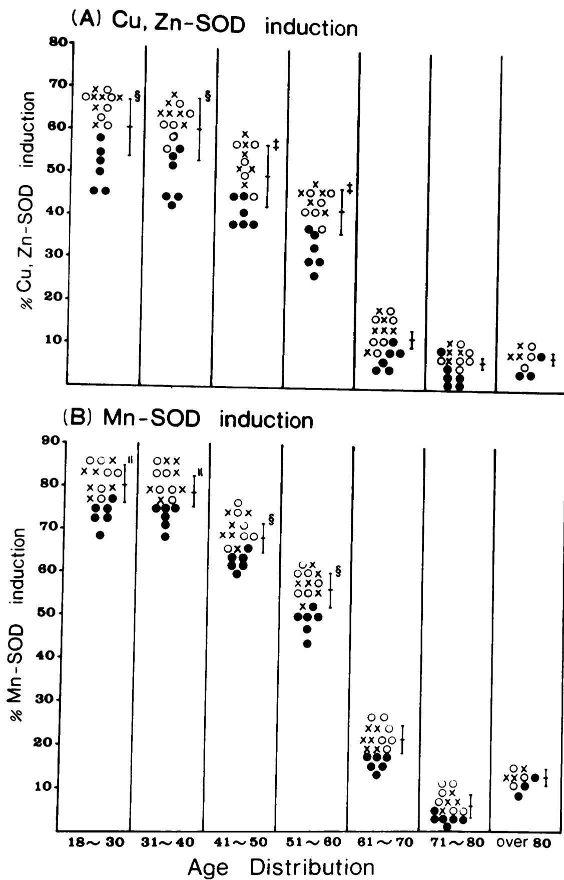

Figure 1. A: *Percent Cu-Zn-SOD induction of lymphocytes with TNF, bleomycin, and IL-1 in each age bracket.* B: *Percent Mn-SOD induction of lymphocytes with TNF, adriamycin, and paraquat in each age bracket. Lymphocytes were incubated for 18 hours at 37 C in the presence of 2U/ml TNF, 0.71 μmol/L bleomycin, 8 U/ml IL-1, 3.45 μmol/L adriamycin, and 2.45×10^{-2} mmol/L paraquat, respectively. Cu-Zn-SOD activity was assessed and presented in the figure. (○) TNF, (•) bleomycin, (x) IL-1. † $0.025 < P < 0.05$ vs control (without addition of any inducer), ‡ $0.01 < P < 0.025$, § $P < 0.01$, ‖ $P < 0.001$.*

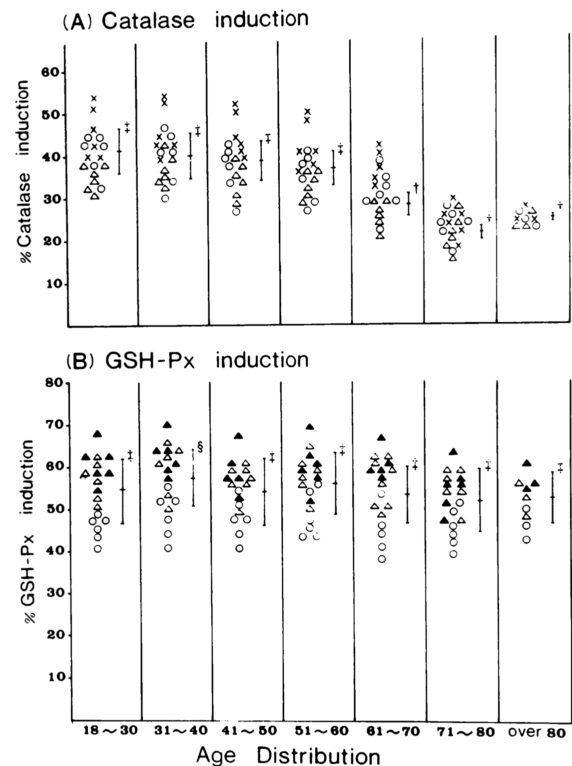

Figure 2. A: *Percent catalase induction of lymphocytes with TNF, paraquat, and IL-1 in each age bracket.* B: *Percent GSH-Px induction of lymphocytes with paraquat, TNF, and bleomycin in each age bracket. (△) adriamycin, (▲) paraquat, (○), (x), †, ‡, §: see legend for Figure 1.*

were not induced with any of the agents in our induction study (not shown). This helps exclude the possibility that the observed induction of ROS scavenger enzymes is nonspecific.

To assess the possibility that the assay of catalase activity may be compromised by the instability of H_2O_2, in some experiments, sodium perborate rather than H_2O_2 was used as the substrate for catalase activity. No significant difference in catalase activity was seen when assayed in this manner (not shown).

Discussion

Previously, we had found comparable basal levels of leukocyte SOD activity in young and elderly adults.[13] However, in the present study, the basal levels of three other H_2O_2 scavenging enzyme activities were found to be decreased in leukocytes of elderly adults in comparison with young adults. In contrast, the inducibility of the two SOD activities was more dramatically reduced than that of the other H_2O_2 scavenging enzymes in the leukocytes of elderly adults, in comparison with young adults.

It has been previously reported[20-22,24] that Mn-SOD but not Cu-Zn-SOD activity is induced by TNF or IL-1 in tumor cell lines and murine tissues. However, our study has demonstrated that both Mn-SOD and Cu-Zn-SOD activities are inducible in leukocytes from asymptomatic young adults by a variety of inducing agents.

Maral et al,[46] who investigated the O_2^- and H_2O_2 scavenging systems in the erythrocytes of several animal species and observed two major patterns, speculated regarding the O_2^- scavenging system that SOD may be constitutively present only at low levels in most species, but is highly inducible under oxidative stress, whereas, in regard to the H_2O_2 scavenging system, catalase or GSH-Px are normally abundant in most species and therefore have not required the evolution of a potent induction

mechanism. In this respect, they hypothesized that SOD is the rate-limiting activity for scavenging ROS. However, this hypothesis is not entirely supported by our results, in which the H_2O_2 scavengers (catalase, GSH-Px, and G-6-P-D) were also induced in human leukocytes with some of the same agents that could induce SOD activity, although SOD activity was induced to a higher degree than the other three H_2O_2 scavenging enzymes.

In comparing the O_2^- and H_2O_2 scavenging systems, Storz et al[1] recently speculated from investigation of ROS-inducible genes in *Escherichia coli* and *Salmonella typhimurium* that the induction of O_2^- and H_2O_2 scavenging systems is regulated by two different types of genes. The expression of the proteins that are induced with O_2^- is regulated by sox R, whereas H_2O_2-inducible genes are controlled by oxy R. Although it is impossible to compare directly the observed results in human leukocytes with bacteria because co-regulated genes in eukaryotes are not organized in operons, it seems interesting that the changes in the basal levels and inducibility of enzyme activities under oxygen stress are also different between O_2^- and H_2O_2 scavengers, as observed in the regulation of genes in eukaryotes.

However, the behavior of G-6-P-D activity in neutrophils differed somewhat from the other two H_2O_2 scavengers, catalase and GSH-Px, in showing only slight induction of activity with paraquat alone in both young and elderly adults, despite showing comparable levels of basal activity between these two groups. G-6-P-D is considered important in the metabolism of peroxides by providing a source of NADPH through the oxidation of G-6-P. NADPH in turn, through the action of glutathione reductase, maintains reduced glutathione, which reduces lipid and other peroxides through the action of glutathione peroxidase. In this communication, G-6-P-D is, if anything, the enzyme that collaborates with the action of H_2O_2 scavenging system. On the other hand, NADPH generated by G-6-P-D in neutrophil membranes stimulates O_2^- generation. These two reciprocal mechanisms of action observed in G-6-P-D seem to have produced different results from other H_2O_2 scavenging enzymes.

All of the agents tested for induction of enzyme activities in this study are known to generate ROS in tissues and/or phagocytes;[26–33] paraquat, bleomycin, and anthracycline analogs including adriamycin directly generate ROS; paraquat at a much higher rate than the others. Meanwhile, IL-1 and TNF prime nonadherent neutrophils and monocytes to make O_2^- at a higher rate if subsequently activated by an appropriate stimulus, and there have been many reports[20–22,24] that IL-1 and TNF induce Mn-SOD activity. The production of IL-1 and TNF was potentiated in paraquat-stimulated human mononuclear cells[47] or in cases of bleomycin-induced pneumopathy and fibrosis.[48] The finding obtained in our study that antibody against IL-1 and TNF decreased SOD induction with these three drugs suggests that the induction is not due to direct effect of paraquat, bleomycin, or adriamycin but occurs through distal mediators, IL-1 and TNF.

Kimball et al[49] demonstrated marked induction of Mn-SOD, GSH-Px, and G-6-P-D activities in young rat lung maintained for 1 week in 1 standard atmosphere of 90% O_2. The induction of SOD, catalase, and GSH-Px under oxygen toxicity in rat lung and the possible protective role of IL-1 in this toxicity were also reported.[50] Their data are consistent with the present study. Significant induction of catalase and GSH-Px activities have also been reported in neonatal mouse lung[51] and in bacteria.[25]

However, we have found that the induction of ROS scavenging activity of these four enzymes is quite complex, with each enzyme showing varying behaviors from species to species and possibly from organ to organ.[52–54] Accordingly, for a more coherent picture of the regulation of ROS scavenging enzymes to appear, further investigation will be needed to study not only the induction of these enzymes in leukocyte subpopulations, but also other cells, organs, and tissues in humans and other species.

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