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ELEVATION OF IMMUNOREACTIVE SERUM Mn-SUPEROXIDE DISMUTASE IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Serum Mn-superoxide dismutase (Mn-SOD) levels were determined in patients with acute myocardial infarction by an enzyme-linked immunosorbent assay (ELISA). The serial determinations performed on 29 patients with acute myocardial infarction indicated that Mn-SOD appears in serum in a biphasic manner; an early small elevation was observed at approximately 16.2 (± 7.3) h and a high elevation at approximately 108 (± 20.6) h after the onset of symptoms. A negative correlation was found between the maxiumum value of the late elevation of Mn-SOD and left ventricular function.

Plasma levels of tumor necrosis factor-α (TNF-α) were also elevated in acute myocardial infarction. These facts suggest that TNF-α secretion from the infarcted area resulted in the production of Mn-SOD and that the induced Mn-SOD was released from myocytes into serum through damaged membranes, resulting in the late elevation.

KEY WORDS: Mn-superoxide dismutase, Enzyme-linked immunosorbent assay, acute mycocardial infarction, human serum.

INTRODUCTION

A great deal of interest has developed in the role of superoxide dismutase in modifying the toxic effects of superoxide anions arising in cardiac tissue during reperfusion following an ischemic episode.[1-3] Much of the interest in such enzymes centers on the role of the widely distributed manganese superoxide dismutase (Mn-SOD).[4-5] The intravenous administration of SOD appears to be effective in reducing infarct size when the infarct is induced experimentally.[4,6-8] The salvaging effects of SOD in the myocardium, however, are still contraversial.[3,9]

In this study, serial determination of serum Mn-SOD in patients with acute

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myocardial infarction revealed that its levels reflect the left ventricular ejection fraction (LVEF). Our interest has centered on the dynamics of cardiac Mn-SOD during acute myocardial infarction and the mechanism of its release into serum. The serum enzyme appears to originate from the cardiac tissue.

Recently TNF and interleukin-1 were found to selectively induce mRNA levels of Mn-SOD[10-11] in cultured cells and we also reported that TNF induces protein levels of Mn-SOD in culture.[12] In this study we have obtained evidence that Mn-SOD is induced in cardiac tissues by TNF and released into serum in patients with acute myocardial infarction.

MATERIALS AND METHODS

Serum Samples

Mn-SOD levels in the sera of 194 adult normal males and 207 normal females were determined. Serial serum samples were obtained from 29 patients with acute myocardial infarction admitted to the Coronary Care Unit of Sakurabahi Watanabe Hospital, Osaka, Japan within 12 h from the onset of infarction.

All research procedures were in accord with the ethical standards of the Helsinki Declaration of 1975. All sera were stored at −35°C until used. Patients included 22 men and 7 women, ranging in age from 40 to 78 years. Emergency coronary angiography was performed on 29 patients. The diagnostics of myocardial infarction was established by a history of typical chest pain, characteristic electrocardiographic abnormalities (such as ST segment elevation, abnormally large *Q* wave and negative T wave) and increases in serum enzymes. Of the 29 patients, one patient died of ventricular fibrillation on the 7th of hospitalization day.

The patients' age and gender population and their clinical diagnoses are summarized in Table I. None of these patients had elevations of ALT(L-alanine 2-oxoglutarate aminotransferase) or other indications of hepatic injury.

Cardiac Catheterization

This was performed on all 29 patients at admission and again about 1 month later except for the individual who had expired.

Out of 29 patients who had undergone coronary angiography at the acute stage, reperfusion of the related vessel was successful in 23 following intracoronary administration of urokinase (Green Cross Corp.) and/or use of percutaneous transluminal coronary angioplasty (PTCA).

Left Ventriculography (LVG)

During the convalescent stage (about 1 month after the onset of the infarction) LVG was performed on all patients except the one who had died on the 7th day. Left ventricular ejection fraction (LVEF) was calculated according to the method described[13] in 14 patients with anterior myocardial infarction without previous history.

Blood Sampling and Measurement of Serum Enzymes

Blood was drawn every hour for 6 h from the onset of the infarction, every 3 h for the

TABLE I
Summarized data for patients with acute myocardial infarction.

Patients	Age	Sex	Infarcted area		Related vessels	CK (peak) activity time		Mn-SOD (‡ early) content time		Mn-SOD († late) content time	
			past	present		(units/ml)	(h)	(ng/ml)	(h)	(ng/ml)	(h)
1.	65	M	A	I	RCA 1	2135	24	125	21	340	126
2.	47	M	-	A	LAD 6	1200	9	249	18	398	148
3.	56	M	A, I	I	RCA 1	1300	36	113	16	165	110
4.	68	M	A	I	RCA 1	1930	8	121	9	243	116
5.	72	M	-	I	RCA 1	2610	22	322	15	237	69
6.	55	M	-	I	RCA 3	2567	12	113	8	297	100
7.	62	M	-	A	LAD 6	695	32	N.D.	N.D.	145	138
8.	70	M	-	A	LAD 6	3745	11	151	17	411	105
9.	68	M	-	A	LAD 7	201	12	116	15	83	111
10.	53	M	-	P	LCX11	1670	18	113	24	N.D.	N.D.
11.	64	M	-	I	RCA 1	1080	11	142	12	309	102
12.	64	M	-	A	LAD 6	3110	9	190	8	242	96
13.	40	M	I	I	RCA 3	747	12	197	14	141	138
14.	41	F	-	I	RCA 1	531	21	N.D.	N.D.	188	99
15.	76	F	-	I	RCA 1	476	11	138	14	143	131
16.	55	M	-	I	RCA 4	279	18	83	18	N.D.	N.D.
17.	73	F	I	I	RCA 2	930	21	163	5	256	67
18.	69	M	-	A	LAD 6	3305	26	501	26	395	93
19.	68	F	-	I	RCA 1	344	12	125	18	383	112
20.	55	M	-	I	RCA 1	609	15	183	21	133	138
21.	77	F	-	A	LAD 7	690	21	120	30	205	132
22.	55	M	-	A	LAD 6	1700	12	204	21	295	91
23.	58	M	-	A	LAD 6	2760	20	160	21	200	95
24.	67	M	-	A	LAD 6	4455	12	115	9	125	93
25.	78	F	-	A	LAD 6	2695	12	112	4	263	115
26.	61	M	-	A	LAD 7	2615	12	167	18	426	93
27.	44	F	-	A	LAD 7	1259	18	110	12	132	100
28.	57	M	-	A	LAD 7	N.D.		135	34	169	95
29.	57	M	-	A	LAD 7	1680	20	165	9	376	92
Mean ± S.D.								164 ± 84	16.2 ± 7.3	248 ± 103	108 ± 21

N.D., not detected; M, male; F, female; A, anterior; I, inferior; P, posterior; RCA, right coronary artery; LAD, left anterior descending artery; LCX; left circumflex artery. † early and ‡ late indicate the maximum values at early and late elevation, respectively.

next 18 h, every 6 h for the next 24 h, twice a day during the next 2 days, and thereafter once a day. Blood was taken through the catheter sheath in the femoral artery during the first 48 h after admission to the hospital and thereafter from an antecubital vein.

Serum creatinine phosphokinase (CK), L-aspartate 2-oxoglutarate animotransferase (AST), L-alanine 2-oxogultarate aminotransferase (ALT), lactic dehydrogenase (LDH) and β-hydroxy butyric dehydrogenase (HBD) were simultaneously determined in all blood samples using a multichannel autoanalyzer (Hitachi 736-10).

ELISA Measurement

Serum levels of Mn-SOD were measured by an ELISA developed in our laboratories.[14] A monoclonal antibody, PG 11, to the human liver Mn-SOD was employed.[15]

Assay of Tumor Necrosis Factor-α

In a separate experiment plasma levels of tumor necrosis factor-α (TNF-α) were determined by radioimmunoassay according to the manufacturer's instructions (Medgenix. Co. Ltd). Whole blood was taken with Na-EDTA from 5 patients suffering with acute myocardial infarction and immediately centrifuged. The plasma samples were stored at $-80°C$ until use. In this experiment Mn-SOD levels were not simultaneously determined because of a difficulty of collecting serum from the same patients.

Statistical analysis

Statistical analysis was performed by Student's *t* test.

RESULTS

Mn-SOD Release Pattern in Acute Myocardial Infarction

Serum Mn-SOD levels of normal adults were 99.8 $\pm$ 24.8 (mean $\pm$ S.D.) for male and 88.8 $\pm$ 20.8 ng/ml for female as reported previously.[14] Figure 1 shows typical changes in serum Mn-SOD in two patients following acute myocardial infarction. Case (A) is an example of a successful reperfusion of the infarcted vessel at an acute stage and (B) is a case without reperfusion. In both instances, a biphasic elevation of Mn-SOD is noted. The small early rise is slightly higher than the levels seen in normal healthy controls and is similar to the pattern of CK. The later phase elevation is much larger in most cases and its peak occurs much later than those of other enzymes. The results of serial determinations of serum Mn-SOD for the 29 patients are shown in Figure 2. Figure 2-(A) shows results for 23 patients with reperfusion while Figure 2-(B) depicts 6 cases without reperfusion. In 4 of the latter patients either intracoronary thorombolysis or PTCA was unsuccessfully employed. In two cases reocclusion occurred after reperfusion. This was confirmed later by CAG during the convalescent stage. In most of these cases, irrespective of whether reperfusion was successful, two apparent elevations of Mn-SOD were observed. Table I summarizes that data for the 29 patients. The maximum levels of Mn-SOD for the early and late stage elevations were 164 ($\pm$ 84) (parenthesis, S.D.) and 248 ($\pm$ 103) ng/ml, respectively. The time of appearance of the early elevation was 16.2 ($\pm$ 7.3) h and the late elevation 108

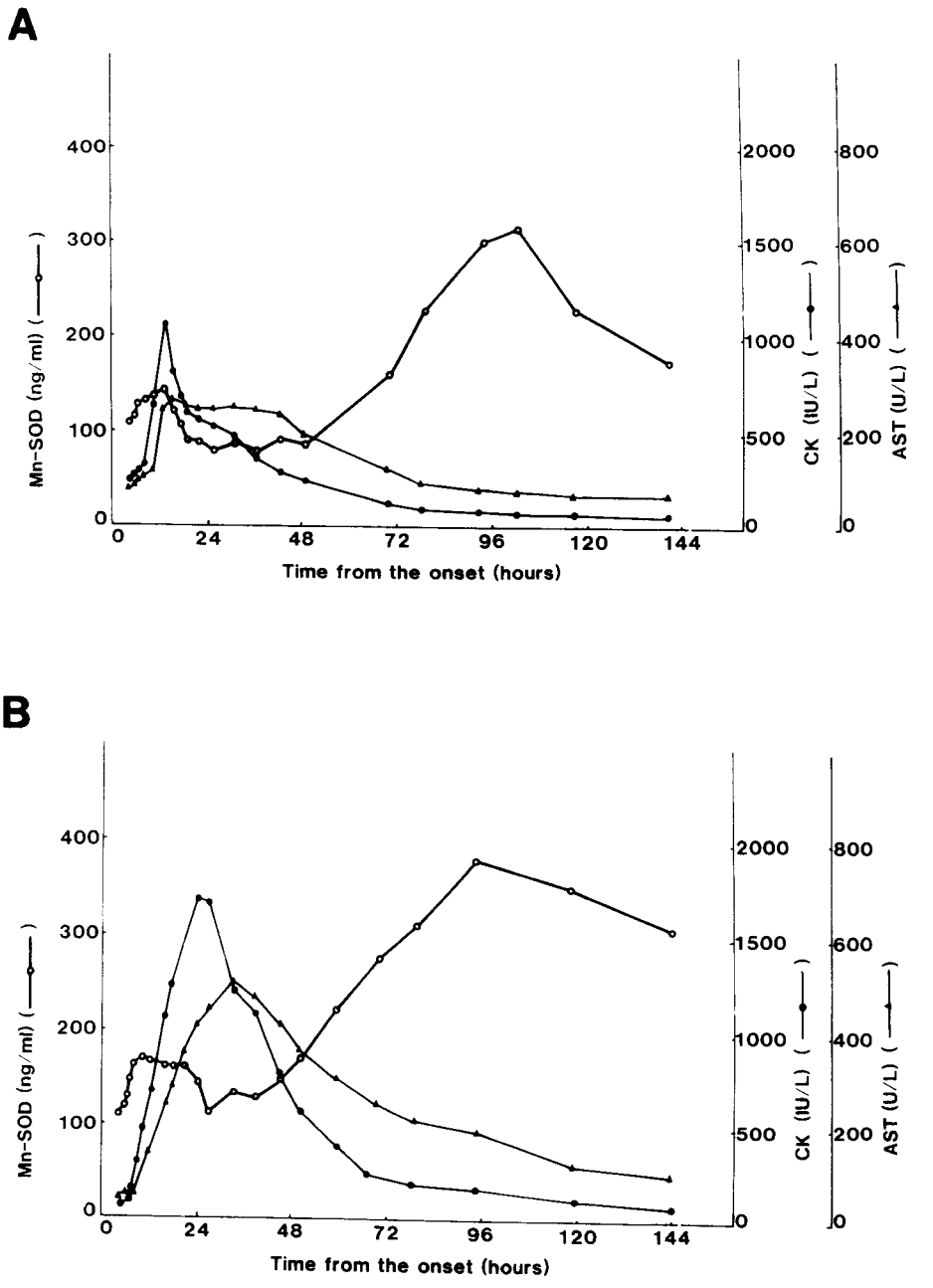

FIGURE 1 Typical patterns for Mn-SOD release into serum of 2 male patients with acute myocardial infraction. (A), A case (patient No. 11 in Table I) with successful reperfusion. (B), An unsuccessful case (patient No. 29).

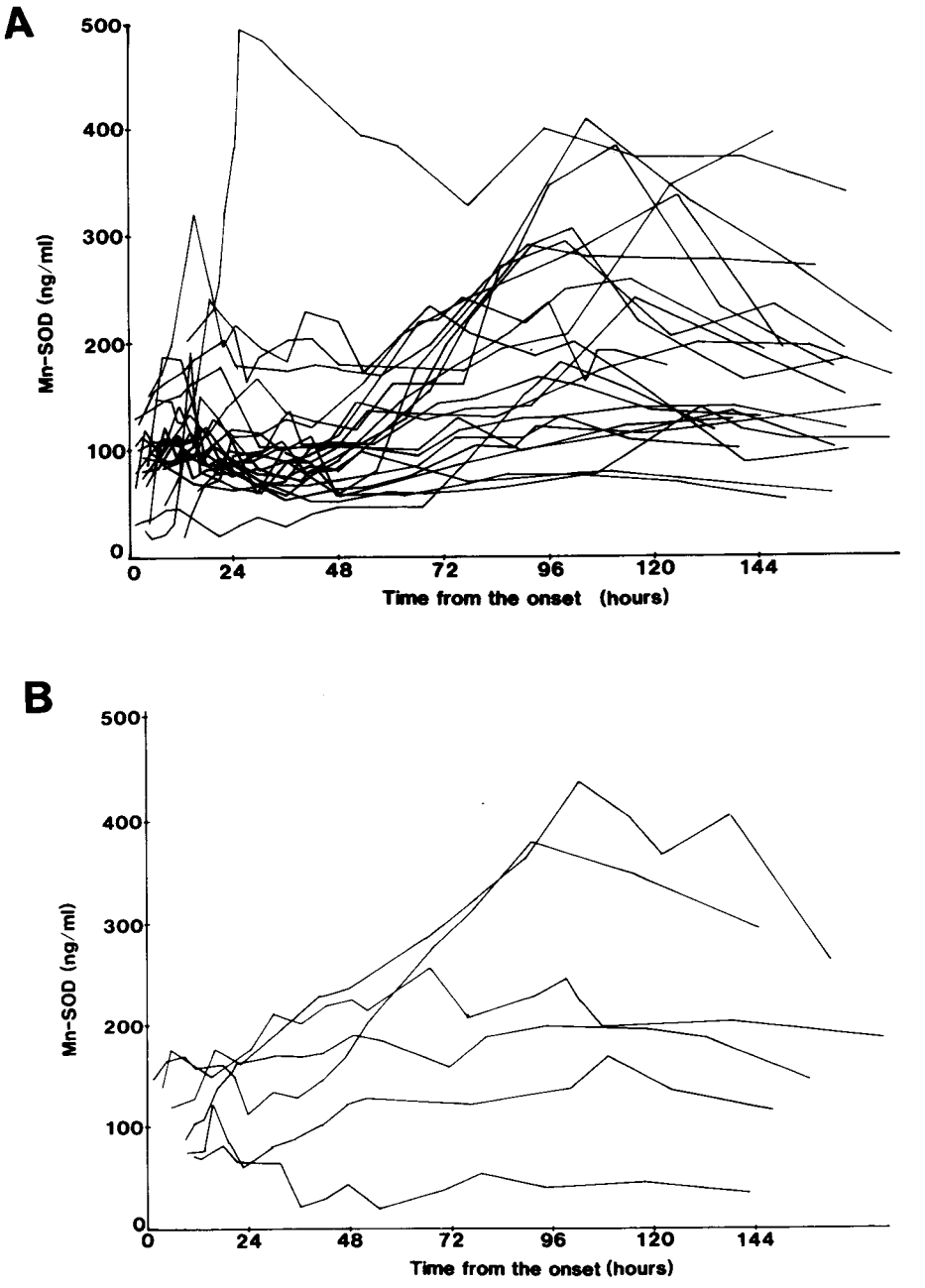

FIGURE 2 Serial determinations of serum Mn-SOD in 29 patients with acute myocardial infarction. (A), 23 cases with successful and (B), 6 cases with unsuccessful reperfusion.

($\pm$ 20.6) h. No significant correlation was found between the peak level of CK and the maximum level of late elevation of Mn-SOD ($r = 0.26$). This indicates that different mechanisms are operating for the release of these two enzymes.

Reperfusion did not affect the time required for the late elevation to occur, but shortened the time for appearance of the early rise. The appearance of the early elevation correlated with the time at which reperfusion was carried out.

The difference in serum Mn-SOD level was examined between samples taken from the ascending portion of the aorta and the coronary sinus immediately following reperfusion in 4 patients with anterior myocardial infarction. In all cases the Mn-SOD levels were higher in the coronary sinus (228 $\pm$ 61, mean $\pm$ S.D) than in the aorta samples (121 $\pm$ 54). This suggests that the noted elevations of serum Mn-SOD originate from cardiac tissue.

In order to verify that the Mn-SOD was not released as a result of coronary angiography or heart failure, serum levels of Mn-SOD were determined in 7 patients with congestive heart failure and 8 patients with angina pectoris who had undergone coronary angiography. No such elevation was found, which indicates that Mn-SOD release is not an effect of angiography.

Mn-SOD Release and Left Ventricular Function

The maximum value of the late elevation of Mn-SOD and the LVEF at the later convalescent stage for fourteen patients suffering from their first anterior myocardial infarction were found to be roughly correlated ($r = -0.68, p < 0.05$). However, the peak of CK activity and the LVEF at the convalescent stage showed a slightly lower correlation ($r = 0.54$).

TNF Release in Acute Myocardial Infarction

Plasma levels of TNF-α in patients with acute myocardial infarction are shown in Figure 3. The dotted line shows the upper limit of plasma TNF-α levels of 30 normal healthy volunteers.

These data show that plasma levels of TNF-α were elevated in patients with acute myocardial infarction and that elevation was maximum 2–4 days after the onset of infarction, which is slight earlier than the peak of late elevation of Mn-SOD. This elevation pattern was similar to that previously report by Maury and Teppo.[16]

DISCUSSION

In mammalian tissues, three kinds of SOD occur.[15,17–19] These isozymes, designated Cu, Zn-SOD, Mn-SOD and extracellular-SOD, are immunochemically distinct and are encoded at different gene loci.

In previous studies a monoclonal antibody that recognized the COOH terminal peptide of human liver Mn-SOD was prepared. Its use demonstrated that the enzyme is localized in the mitochondrial matrix of the liver.[15] This antibody was employed in an ELISA method to demonstrate elevated serum levels of Mn-SOD in patients with hepatoma, acute myeloid leukemia, ovarian carcinoma and acute myocardial infarction.[14–20]

The present study indicated that patients with acute myocardical infarction have

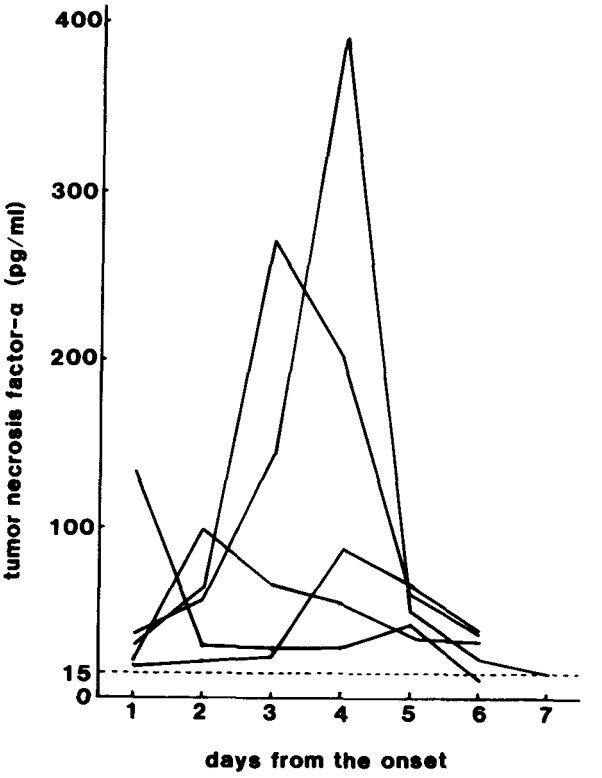

FIGURE 3 Serial determination of plasma TNF-α in 5 patients with acute myocardial infarction. Dotted line shows upper limit of plasma TNF-α in 30 normal healthy volunteers.

elevated serum levels of Mn-SOD at approximately 16 and 108 h after onset. Care must be taken, however, to determine the source of the serum Mn-SOD. The possible role of the liver must be considered since it is relative rich in this enzyme.[19,21] Patients with acute hepatitis that is accompanied by significant necrosis show increased serum levels of Mn-SOD (13). These elevations, however, are in proportion to increases in ALT and AST. None of the 29 patients with acute myocardial infarction showed increased levels of ALT. Furthermore, serum levels of Mn-SOD in the coronary sinus were higher than those in the ascending aorta. These results suggest than Mn-SOD is released from myocardial tissues.

In the present study no significant difference in the degree of elevation of Mn-SOD was found between groups with successful and unsuccessful reperfusion. If unsuccessful, however, the early elevation occurred slightly later than in the successful cases. This indicates that the early elevation of Mn-SOD is affected slightly by reperfusion as is observed for CK. The time of appearance of the late elevation of Mn-SOD in these patients did not change. This indicates that the secondary elevation of Mn-SOD is not affected by reperfusion and therefore is a more reliable marker for the assessment of ischemic myocardial damage.

It is possible that increased synthesis is responsible for the late elevation of Mn-SOD.

Recently two groups have independently reported that interleukin-1 and tumor necrosis factor specifically induce the mRNA of Mn-SOD.[10-11] And we also reported that TNF-α increased protein levels of Mn-SOD in cultured cells.[12] In myocardial infarction, neutrophiles and macrophages could move to the necrotized tissues following the elevation, and release the above cytokines. This could induce Mn-SOD synthesis in mitochondria followed by release of the enzyme from damaged cells at the later phase. In such an induction hypothesis ischemic tissues would be thought of as inflammatory foci. Indeed the ischemic myocardium, neutrophiles adhere to the endothelial cells, which is typical of initiation of an inflammatory process. Very recently Visner *et al.* reported that induction of Mn-SOD by interleukin-1, tumor necrosis factor and lipopolysaccharide occurred in pulmonary epithelial cells.[22] In this study we found that the TNF-α levels were elevated in acute myocardial infarction. Probably TNF-α levels are much higher in ischemic regions than in plasma. These results suggested that a cytokine, such as TNF-α or IL-1 was released from macrophages and neutrophiles on the 2nd or 3rd day after ischemia and induced Mn-SOD in cardiac tissued. The increase Mn-SOD was then released from myocytes whose membranes were damaged by the ischemia.

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References

1. B.A. Freeman and J.E. Crapo (1982) Biology of disease: free radicals and tissue injury. *Laboratory Investigation,* **47,** 412–426.
2. I. Fridovich (1987) The biology of oxygen radicals. *Science,* **201,** 875–880.
3. K.P. Gallagher, A.J. Buda, D. Pace, R.A. Green and M. Schlafer (1986) Failure of superoxide dismutase and catalase to alter size of infarction in conscious dogs after 3 hours of occlusion followed by reperfusion. *Circulation,* **73,** 1065–1076.
4. G. Ambrosio, L.C. Becker, G.M. Hutchinson, H.F. Weisman and M.L. Weisfeldt (1986) Reduction in experimental infarct size by recombinant human superoxide dismutase: insights into pathophysiology of reperfusion injury. *Circulation,* **74,** 1424–1433.
5. J.M. McCord and I. Fridovich (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *The Journal of Biological Chemistry,* **244,** 6049–6055.
6. K.P. Burton, (1985) Superoxide dismutase enhances recovery following myocardial ischemia. *American Journal of Physiology,* **248,** H637–H643.
7. S.R. Jolly, W.J. Kane, M.B. Bailie, G.D. Abrams and B.R. Luccchessi, (1984) Canine myocardial reperfusion injury: its reduction by the combined adminstration of superoxide dismutase and catalase. *Circulation Research,* **54,** 227–285.
8. S.W. Werns, M.J. Shea, E.N. Driscoll, C. Cohen, G.D. Agrams, B. Pitt and B.R. Lucchesi (1985) The independent effects of oxygen radical scavengers on canine infarct size reduction by superoxide dismutase but not catalase. *Circulation Research,* **56,** 895–898.
9. A. Uraizee, K.A. Reimer, C.E. Murray and R.B. Jennings (1987) Failure of superoxide dismutase to limit size of myocardial infarction after 40 minutes of ischemia and 4 days of reperfusion in dogs. *Circulation,* **75,** 1237–1248.
10. A. Masuda, D.L. Longo, Y. Kobayashi, Y.E. Appelia, J.J. Oppenheim and K. Matsushima (1988) Induction of mitochondrial manganese superoxide dismutase by interleukin 1. *FASEB Journal,* **2,** 3087–3090.
11. G.H.W. Wong and D.V. Goeddel, (1988) Induction of manganous superoxide dismutase by tumor necrosis factor: Possible protective mechanism. *Science,* **242,** 941–944.
12. T. Kawaguchi, A. Takeyasu, K. Matsunobu, T. Uda, M. Ishizawa, K. Suzuki, T. Nishiura, M.

Ishikawa and N. Taniguchi, (1990) Stimulation of Mn-superoxide dismutase expression by tumor necrosis factor-α: Quantitative determination of Mn-SOD protein levels in TNF-resistant and sensitive cells by ELISA. *Biochemical and Biophysical Research Communication,* **171,** 1378–1386.

13. J.W. Kennedy, S.E. Treholme and I.S. Kasser, (1970) Left ventricular volume and mass from singl-plane cineangiocardiogram: A comparison of anteroposterior and right anterior oblique methods. *American Heart Journal,* **80,** 343–352.
14. T. Kawaguchi, K. Suzuki, Y. Matsuda, T. Nishiura, T. Uda, M. Ono, C. Sekiya, M. Ishikawa, S. Iino, Y. Endo and N. Taniguchi (1990) Serum manganese-superoxide dismutase: normal values and increased levels in patients with acute myocardial infarction and several malignant diseases determined by an enzyme-linked immunosorbent assay using a monoclonal antibody. *Journal of Immunological Methods,* **127,** 249–254.
15. T. Kawaguchi, S. Noji, T. Uda, Y. Nakashima, A. Takeyasu, Y. Kawai, H. Takagi, N. Thoyama and N. Taniguchi, (1989) A monoclonal antibody against COOH-terminal peptide of human liver manganese superoxide dismutase. *The Journal of Bilogical Chemistry,* **284,** 5762–5767.
16. C.P.J. Maury and A.-M. Teppo (1989) Circulating tumor necrosis factor-α (cachectin) in myocardial infarction. *Journal of Internal Medicine,* **225,** 333–336.
17. S.L. Marklund, (1982) Human copper-containing superoxide dismutase of high molecular weight. *Proceedings of the National Academy of Sciences of the United States of America,* **79,** 7634–7638.
18. R.A. Weisiger and I. Fridovich (1983) Superoxide dismutase; organelle specificity. *The Journal of Biological Chemistry,* **248,** 3582–3592.
19. R.A. Weisiger and I. Fridovich (1973) Mitochondrial superoxide dismutase: site of synthesis and intramitochondrial localization. *The Journal of Biological Chemistry,* **248,** 4793–4796.
20. M. Ishikawa, Y. Yaginuma, H. Hayashi, T. Shimizu, Y. Endo and N. Taniguchi (1990) Reactivity of a monoclonal antibody to manganese superoxide dismutase with human ovarian carcinoma. *Cancer Research,* **50,** 2538–2542.
21. Y. Matsuda, S. Higashiyama, Y. Kijima, K. Suzuki, K. Kawano, M. Akiyama, S. Kawata, S. Tarui, H.F. Deutsch and N. Taniguchi, (1990) Human liver manganese superoxide dismutase: Purification and crystallization, subunit association and sulfhydryl reactivity. *European Journal of Biochemistry,* **194,** 713–720.
22. G.A. Visner, W.C. Dougall, J.M. Wilson, I.A. Burr and H.S. Nick, (1990) Regulation of manganese superoxide dismutase by lipopolysaccharide, interleukin-1, and tumor necrosis factor. *The Journal of Biological Chemistry,* **256,** 2856–2864.

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