Pretreatment with a Single Bolus Injection of Polyoxyethylene-Modified Superoxide Dismutase Prevents Reperfusion Induced Arrhythmias in the Anesthetized Rat

Mario GALCIA-ALVES, Yoshihiro KADOWAKI, Yuji IWASHITA and Katsuhide NISHI*

Department of Pharmacology, Kumamoto University Medical School, 2-2-1 Honjo, Kumamoto 860, Japan
1 Central Research Laboratories of Ajinomoto Co., Inc., 1-1 Suzukicho, Kawasaki-ku, Kawasaki 210, Japan

Accepted June 15, 1989

Abstract—Effects of a newly introduced polyoxyethylene-modified superoxide dismutase (SOD-POE) on reperfusion induced arrhythmias were examined in the pentobarbital anesthetized rat. Reperfusion induced arrhythmias were elicited by occlusion of the left anterior descending coronary artery (LAD) for 15 min and subsequent release. The LAD occlusion was performed by compressing the artery using a suction cup of 2 mm in diameter placed on the LAD to which negative pressure was applied. The LAD occlusion and release was repeated at an interval of 30 min. SOD-POE or human SOD (h-SOD) (1000 U/kg) was injected intravenously 15 min prior to the occlusion at the second trial of the occlusion. In the control group, various types of arrhythmias including ventricular fibrillation (Vf), ventricular tachycardia (VT), premature ventricular contraction (PVC) and premature atrial contraction (PAC) were elicited immediately after release of the occlusion. In the SOD-POE-treated group, Vf and VT were completely prevented and the numbers of PVC and PAC significantly decreased, while pretreatment with h-SOD did not prevent the occurrence of reperfusion induced arrhythmias. The protective effects of SOD-POE lasted for more than 90-120 min. The plasma half life for SOD-POE was 10.8 hr, while that for h-SOD was 8.6 min. Results indicate that intravenous administration of SOD-POE would provide a new means of preventing reperfusion induced arrhythmias occurring in clinical situations.

A large body of evidence has been presented to indicate that oxygen free radicals are cytotoxic variations of oxygen molecules and might be, at least in part, responsible for ischemia and reperfusion-mediated injury of a variety of tissues including the brain (1), intestine (2, 3) and heart (4-12). Studies in a number of experimental preparations of ischemia and reperfusion in the heart have recently documented the beneficial effect on myocardial cell viability of superoxide dismutase (SOD); SOD administered intravenously before the onset of ischemia or 15 min before reperfusion induced reduction in experimental infarct size (13-16), significantly improved myocardial function (17-20), and prevented reperfusion induced arrhythmias (21-23). In a previous paper, we have shown that SOD to which polyoxyethylene was covalently attached, so as to prolong the circulating plasma-half life (polyoxyethylene-modified SOD: SOD-POE), continuously infused 15 min before the onset of ischemia can prevent reperfusion induced arrhythmias in the isolated rat heart and its effects are as potent as non-modified human SOD (h-SOD) (23).

In the present study, using a model in...
which reperfusion induced arrhythmias were consistently elicited in anesthetized rats (15), we tested whether SOD-POE, administered intravenously before a period of coronary occlusion, could prevent reperfusion induced arrhythmias, and if effective, how long its effect would last. We also measured changes in SOD activity in plasma after intravenous injections of SOD and SOD-POE in the rat to assess the relationship of the plasma level of exogenously applied SOD and its efficacy with respect to preventing reperfusion induced arrhythmias. Results show that an intravenous bolus injection of SOD-POE prevents occurrence of reperfusion induced arrhythmias in anesthetized rats, and its preventing effects last at least for 2 hr, suggesting that intravenous administration of SOD-POE would serve as an appropriate mode of administration for protecting against radical related injury in various tissues. A preliminary report of this work has been published elsewhere (24).

Materials and Methods
Occlusion-and-release protocol: Male rats of the Wistar strain, weighing 250–300 g, were used. The animal was anesthetized with sodium pentobarbital (30 mg/kg, i.p.). The femoral vein was cannulated to allow drug administration, and the trachea cannulated for artificial respiration. Systemic blood pressure was monitored from the femoral artery by means of a pressure transducer. A standard lead I ECG was recorded with a polygraph recorder. The chest was opened through a left thoracotomy, and the heart was exposed. Positive pressure artificial ventilation was started immediately with room air, using a volume of 1.5 ml/100 g (body weight) and a rate of 20 strokes/min so as to maintain normal P\text{CO}_2, P\text{O}_2, and pH parameters. After incision of the pericardium, the left ventricular surface of the heart was exposed. Coronary occlusion was performed by compressing the left anterior descending coronary artery (LAD) close to its origin using a suction cup of 2 mm in diameter placed on the artery, and a negative pressure of about 200 mmHg was applied to the cup connected to an aspirator with a polyethylene tube, and thus, the coronary artery was occluded for 15 min and reperfused. The LAD occlusion-and-release was repeated at an interval of 30 min. SOD-POE or h-SOD was injected intravenously 15 min prior to the second trial of occlusion-and-release. ECGs and arterial blood pressure were continuously recorded on a pen-writing recorder (Nihon Kohden, Recticorder RJG 3024). Since the onset of reperfusion induced arrhythmias was rapid, ECGs were recorded at a fast chart speed from 30 sec prior to reperfusion to 5 min after reperfusion. Heart rate was counted from the ECG trace. The heart rate and arterial blood pressure were measured at 5-min intervals. All experiments were done at room temperatures of 25°C–28°C.

Arrhythmias occurring after the release of coronary occlusion were diagnosed following criteria described in the previous paper (23). Ventricular tachycardia (VT) was diagnosed as five or more premature ventricular contractions (PVC) which were clearly seen in the ECG-recording. Ventricular fibrillation (VF) was diagnosed when the ECG recording showed chaotic activity with an amplitude less than that of the normal ECG. Atrial premature contractions (PAC) were identified as ectopic beats with the same configuration as normal ECG, but smaller amplitude. The total PVC and PAC number were counted in a 3-min-period of reperfusion and expressed as beats/3 min in individual trial. The incidence of VF and VT were expressed in an all-or-none fashion in each trial.

Measurement of SOD activity in plasma: SOD activity in plasma was assayed by the nitrite assay method (25), because this method is suitable for measurements in the presence of other proteins such as albumin, and the sensitivity of the nitrite assay method was reported to be 8.5 times higher than that of the McCord and Fridovich method (26). Male rats of Wistar strain, weighing 250 g, were subjected for measurements of SOD activity in plasma. After a single bolus injection of SOD or SOD-POE (3,000 U dissolved in distilled water at 0.2%) into the jugular vein, 300–400 μl of blood samples were taken through the jugular vein with a heparin coated syringe. Samples were collected from each rat at 1, 2, 5, 15, 30 and 60 min and 2, 4, 16, 24 and 48 hr. The samples were centrifuged.
(3000 rpm for 15 min) at 4°C in heparin-coated tubes, and 100 µl of plasma fractions were separated.

Materials: Human superoxide dismutase (h-SOD) and polyoxyethylene-modified superoxide dismutase (SOD-POE) were prepared in the Central Laboratory of Ajinomoto Co. (23). In brief, crude Cu, Zn-SOD was isolated from human erythrocytes according to Tsuihishahi's method (27) and then subjected to DEAE-Sephadex chromatography. The crude Cu, Zn-SOD thus obtained was further purified by preparative HPLC using a packed column of TSK-GEL (DEAE-5PW) (Toyo Soda, Japan). a-Carboxymethyl-a-carboxymethoxy polyoxyethylene (POE) with a molecular weight of 3600 Da was purchased from Nippon Oil and Fats Co., Ltd. (Tokyo, Japan). "Activated POE" was obtained by coupling POE to N-hydroxysuccinimide using dicyclohexycarbodiimide. SOD was dialyzed against 0.1 M potassium phosphate buffer (pH 7.0-7.5) so that the SOD concentration was 1.4% and "activated POE" was added in a fifteen-fold molar excess. The mixture was stirred for 1 hr at 4°C. In order to eliminate unreacted activated ester, glycine was added in a fifty-fold molar excess. Unreacted POE and glycine were removed using an Amicon ultrafilter with a membrane (YM-30) (Amicon Co., U.S.A.). After the filtration with a membrane filter, MILLEX GV 0.22 µm (Millipore Co., Japan), the solution was freeze-dried and used as the preparation of SOD-POE. The number of POE attached to SOD was determined to be about 4 by the modified Sims's method after hydrolysis of SOD-POE (27). The molecular weight of SOD-POE was approximately 50,000 Da as determined using a light scattering photometer (Toyo Soda, Japan). Protein concentration of SOD or SOD-POE was determined by the measurement of absorption at 280 nm. The activity of unmodified SOD was about 3500 U/mg protein, and the residual activity of SOD-POE after modification was approximately 90%.

SOD and SOD-POE were dissolved in saline solution at the appropriate concentration just before use.

Statistical methods: Results are presented as means±S.D. The x² test was used to compare the incidence of VT and VF in the control and drug treated groups. Statistical analysis of PVC and PAC numbers in the same preparation in the first and second trial was done using the paired t-test, and the PVC and PAC numbers in the control and drug-treated groups were also compared by Student's t-test. P<0.05 was considered to indicate a significant difference from the control values.

Results

Characteristics of arrhythmias induced by reperfusion: In the previous paper (23), we showed that the LAD occlusion-and-release using a suction cup placed on the artery consistently elicits reperfusion induced arrhythmias in the isolated rat heart. Therefore, in the present experiments, we employed the same procedure of occlusion-and-release in anesthetized rats in situ. Upon the LAD occlusion, the ST-segment started to elevate, and arrhythmias occurred within 2-5 min. In most cases, during an occlusion period, a sporadic of PVC was observed. VT was also observed and was often followed by Vf lasting for 3-5 min, which was not fatal in rats since spontaneous reversion to the sinus rhythm occurred. In other instances, akinesis of the whole left ventricular wall and a marked fall in arterial blood pressure occurred. In such cases, reperfusion did not restore the cardiac function and eventually the animals died. After a 15 min-period of occlusion, reperfusion elicited various types of arrhythmias within 3-10 sec which continued only for 1-3 min. Figure 1 shows typical ECG-traces upon release of the coronary occlusion. The incidences of VT and Vf were high: 12 (100%) and 9 out of 12 cases (75%), respectively, in the control group. The arrhythmias that occurred during an initial 1-2 min-period of reperfusion were more serious than those that occurred over the same period during ischemia alone. However, in preliminary experiments done in our laboratory, rats with a body weight of less than 250 g tended to have a low incidence of reperfusion induced arrhythmias when compared with aged rats. Therefore, in the present experiments, rats with body weights of more than 250 g were selected for use. In this model, the second trial of occlusion-and-release was performed.
which elicited reperfusion induced arrhythmias almost identical to the arrhythmias elicited by the first trial. In only 1 case out of 12 in the control group (8%), the second trial of occlusion-and-release performed 30 min after the first trial failed to induce arrhythmias after release. VT was observed in all remaining 11 cases upon the first trial of the occlusion-reperfusion procedure and in 10 cases (91%) in the second trial. The incidence of Vf was 45% in the second trial. Results were consistent with those obtained in situ by previous authors (28). The total number of PVCs counted in a 3 min-period of post occlusion (reperfusion) showed a considerable variation depending upon the individual case and were 28±16.9 and 17.5±11.5 beats/3 min (mean±S.D.) in the first and second trials, respectively. The numbers of PACs were 4.5±5.6 and 3.8±5.2 beats/3 min (mean±S.D.), respectively. In the same animal, there was no difference in the severity of the reperfusion induced arrhythmias between the first and second trials. No statistically significant difference in the incidence of Vf and VT and the total number of PVCs and PACs between the first and second trials. There was no difference in the mode of onset and severity of reperfusion induced arrhythmias in the present experiments and in the previous ones in vitro (see Fig. 4 in ref. 23).

Effects of SOD-POE on reperfusion induced arrhythmias: In the control experiments, it has been shown that in the rats in which the first trial of occlusion-and-release had elicited reperfusion induced arrhythmias, the second trial consistently provoked arrhythmias with the same degree of severity as the previous ones. Therefore, in order to assess the effects of SOD-POE on the reperfusion induced arrhythmias in this model, SOD-POE was applied 15 min before the second trial of occlusion-and-release in rats in which the first trial had induced the arrhythmias. Since previous authors described that protective doses of SOD against reperfusion injury in the myocardium in situ were 1,000–30,000 U/kg, when given intravenously (20), and preliminary experiments conducted in our laboratory revealed that the minimal effective dose of human-SOD (h-SOD) was 200–500 U/kg, in the present series of experiments, we selected a single dose of SOD-POE of 1,000 U/kg for evaluating drug effects. Examples of the results are shown in Fig. 2, in which in the first trial of occlusion-and-release without drug, severe reperfusion arrhythmias including VT and Vf were induced. Fifteen minutes after the release, SOD-POE was injected in the same animal, and then the second trial of occlusion-and-release was performed, but this time severe arrhythmias, like those observed in the first trial, were not induced; the number of PVCs were markedly reduced, and neither Vf nor VT occurred. Thus, in the second trial, SOD-POE markedly reduced the incidence of occurrence of reperfusion arrhythmias. The results of a total of 12 experiments are illustrated in Fig. 3.

As the next step of evaluation of the effectiveness of SOD-POE, we examined how long its effect would last by repeating the same occlusion-and-release procedure at an interval of 30 min. In 6 animals in which the first trial elicited the arrhythmias, after an injection of SOD-POE, the procedure was repeated up to 120 min. In 5 cases, reperfusion induced arrhythmias were not elicited 120
In Vivo-Effect of Polyoxyethylene-SOD

Fig. 2. Effects of SOD-POE on reperfusion induced arrhythmias. Upper ECG-record was taken 15 sec after release of coronary occlusion without any treatment (1st trial without SOD-POE) and the lower one, at 15 sec after release of coronary occlusion (2nd trial of occlusion-and-release) in the same animal pretreated with SOD-POE (10,000 U/kg, i.v.) (2nd trial with SOD-POE).

Fig. 3. Effects of SOD-POE on various types of reperfusion induced arrhythmias. Upper graphs illustrate the incidences of ventricular fibrillation (VF) and ventricular tachycardia (VT) expressed in % and lower ones, the numbers of premature ventricular contractions (PVC) and atrial premature contractions (PAC) counted in a 3-min period after release of coronary occlusion. Empty columns represent results of the first trial of occlusion-and-release and hatched columns, the second trial. Cont., control group (n=12); SOD-POE, SOD-POE treated group (n=12). Vertical bar of each column indicates S.D. *P<0.05, when compared with the control group. †P<0.05, when compared with the control in the same animal.
The table summarizes results obtained in 6 different animals. Coronary occlusion-and-release was repeated at a time interval of 30 min, and the numbers of PACs and PVCs occurring in a 3 min-period after release of coronary occlusion were counted and presented. The incidence of VT and Vf was expressed as (+) and (-) in an all-or-none fashion. Empty space indicates that at the time indicated, the occlusion-and-release was not performed. Control: the first trial of the occlusion-and-release. 30 min (2nd): 15 min after an intravenous injection of SOD-POE, the second trial of the occlusion-and-release lasting for 15 min was done, and thus, the upper column indicates the time of release of the coronary occlusion after the injection of SOD-POE. No.: animal number, PAC: premature atrial contraction, PVC: premature ventricular contraction, VT: ventricular tachycardia, Vf: ventricular fibrillation.

| No. | Control (1st) | 30 min (2nd) | 60 min (3rd) | 90 min (4th) | 120 min (5th) |
|-----|---------------|--------------|--------------|--------------|--------------|
|     | PAC | PVC | VT | Vf | PAC | PVC | VT | Vf | PAC | PVC | VT | Vf | PAC | PVC | VT | Vf |
| 1   | 1   | 8   | +  | -  | 0   | 0   | -  | -  | 0   | 0   | -  | -  | 0   | 0   | -  | -  |
| 2   | 4   | 45  | +  | +  | 0   | 0   | -  | -  | 0   | 0   | -  | -  | 0   | 0   | -  | -  |
| 3   | 2   | 7   | +  | -  | 0   | 1   | -  | -  | 0   | 0   | -  | -  | 0   | 1   | -  | -  |
| 4   | 6   | 73  | +  | +  | 0   | 0   | -  | -  | 0   | 0   | -  | -  | 0   | 0   | -  | -  |
| 5   | 1   | 25  | +  | +  | 0   | 7   | -  | -  | 0   | 0   | -  | -  | 0   | 1   | -  | -  |
| 6   | 2   | 12  | +  | -  | 0   | 0   | -  | -  | 0   | 1   | -  | -  | 0   | 1   | -  | -  |
min after the injection of SOD-POE. However, a further trial of occlusion-and-release was not done, because the repeated trials induced myocardial damage which would lead to the production of arrhythmias different from the reperfusion induced arrhythmias or cardiac failure. Results are summarized in Table 1 in which the incidences of Vf, VT and the number of PVCs PACs counted at each trial of the occlusion-and-release are presented. The heart rate in the individual cases did not change throughout the experimental procedures.

Effects of SOD on reperfusion induced arrhythmias: Effects of human SOD (h-SOD) (1,000 U/kg, i.v.) on reperfusion induced arrhythmias were examined in the same manner as the previous section. Results are shown in Fig. 4. In the first trial of occlusion-and-release without SOD, Vf and VT occurred in 5 (83%) and 4 (66%) of 6 animals examined, respectively, and the numbers of PVC and PAC were 33±32.2 and 4.7±7.9 beats/3 min, respectively. Pretreatment with SOD did not prevent occurrence of reperfusion induced arrhythmias; the incidences of Vf and VT were 5 (83%) and 5 (83%) out of 6 cases, respectively; and there was no statistically significant difference in the numbers of PVC and PAC between the first and second trials. Thus, non-modified h-SOD, when administered 15 min prior to coronary occlusion, has no prophylactic action on the reperfusion induced arrhythmias in situ; and these results were clearly different from those obtained in animals pre-treated with SOD-POE.

The circulating life of SOD-POE and SOD: Two group of rats were injected in the tail vein with 1 mg of SOD-POE or h-SOD. Blood was obtained from 6 rats in each group at the specified times. The kinetics of serum SOD-POE and h-SOD activity in rats following the intravenous injection is shown in Fig. 5. Following the injection, h-SOD was virtually gone after 10–20 min; at 10 min, only 20–30% of 0 hr h-SOD activity remained. Calculated T1/2 for h-SOD was 5–10 min, which varied among the individual cases. Thus, non-modified h-SOD rapidly disappeared from the circulation. SOD-POE had a greatly prolonged circulating life in rats given a single bolus injection of the enzyme. Approximately 10% of 0-hr SOD-POE activity was still present in the serum at 24 hr after the injection. The T1/2 varied in individual rats, but was greatly prolonged (mean: 10.8 hr).

Discussion

The present study demonstrated that arrhythmias can be consistently elicited upon
release of occlusion of the coronary artery in anesthetized rats, and these arrhythmias are characterized by their rapid onset and short duration. These results are consistent with those described by previous authors using a coronary ligation method in the rat heart in vivo (15). The distinct features of arrhythmias observed in the present model are similar to reperfusion induced arrhythmias in the isolated rat heart as shown in our previous paper (23). Therefore, we think that the method employed in the present experiments for obtaining reperfusion induced arrhythmias and estimation of drug effects are valid.

SOD-POE, when administered intravenously 15 min prior to coronary occlusion, prevented the occurrence of reperfusion induced arrhythmias in anesthetized rats and its effects lasted, at least, for 120 min. The results are consistent with the previous ones in isolated rat hearts in which SOD was continuously infused 15 min prior to coronary occlusion (8, 21–23). These results confirmed the previous notion that oxygen-derived free radicals are involved in the genesis of reperfusion induced arrhythmias (8, 21–23). SOD acts only by catalyzing the dismutation of \( \text{O}_2^- \) to \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \), while catalase accelerates the conversion of \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \) (16, 29, 30). \( \text{H}_2\text{O}_2 \) has been shown in several experimental preparations to be cytotoxic, either by a direct effect (31) or through the superoxide-mediated generation of more reactive hydroxyl radicals (\(-\text{OH}\)) by the Haber-Weiss and Fenton reactions (30, 32, 33). Therefore, several studies have indicated beneficial effects using the combination of SOD and catalase on the recovery of myocardial contractilities as well as the extent of myocardial necrosis (4, 13, 14, 19, 20). However, the fact that SOD alone was effective in preventing reperfusion induced arrhythmias (23) and also produced the same reduction in infarct size as with the combination of SOD plus catalase (16–19), would suggest that endogenous levels of catalase and/or glutathione peroxidase are sufficient to catabolize hydrogen peroxide as it is formed by dismutation of superoxide radicals catalyzed by SOD. The present result that h-SOD injected intravenously 15 min prior to coronary occlusion did not prevent reperfusion induced arrhythmias indicates that the quantity of SOD administered exogenously would not be enough to scavenge the superoxide radicals as fast as they were generated during ischemia and/or at the time of re-oxygenation. On the other hand, in the animals pretreated with SOD-
In Vivo-Effect of Polyoxymethylene-SOD

POE, the enzymatic activity of SOD in plasma was still high at the time of reperfusion, thereby preventing the formation of hydroxyl radicals and reperfusion induced arrhythmias. The results indicate that despite the chemical modification of SOD, enzymatic activity of SOD-POE exogenously applied in situ is not lost but remains intact in plasma. Thus, SOD-POE has been proved to have the same biochemical and pharmacological properties in situ as native SOD.

It was our objective to evaluate the potential therapeutic efficacy of SOD-POE in clinically relevant experimental preparations of reperfusion induced arrhythmias or reperfusion-related injury in various tissues. However, an important issue raised by our findings and others (18, 22, 23, 28) relates to the sources of oxygen radicals and the ability of the interventions to reach them. Although SOD-POE has been shown to prevent reperfusion induced arrhythmias, the concept that a macromolecule crossing an intact sarcolemmal membrane to detoxify cytoplasmic free radicals has been difficult to accept, and hence the present results led to a question of whether a molecule with a large molecular weight like SOD-POE (approximately 50,000 Da) could have access to the sarcolemma, where it might degrade superoxide or cross through the sarcolemma to the sarcoplasm. The nature of our experimental preparations precludes clarification of the question, and further investigation will be required to address this issue. Nevertheless, the beneficial effect of SOD-POE may be explained by free-radical scavenging within the vasculature rather than within the myocytes, as suggested by Ambrosio et al. (18); the endothelium appears to represent a primary source of free-radical production in the heart, and perfusion with an enzymatic system that generates free radicals in vascular lumen is capable of inducing myocardial ultrastructural and functional abnormalities (14). Another interesting feature of reperfusion induced arrhythmias in the present model and others is that not only SOD but also drugs that reduce the intensity of the fast inward sodium current in mammalian myocardium inhibit the development of reperfusion induced arrhythmias in anesthetized rats (28) and isolated rat hearts (34–36), although in the dog, conflicting results concerning their effectiveness have been obtained (35, 37). However, calcium antagonists were ineffective in rat hearts (28). We have not examined effects of Class I antiarrhythmic drugs such as lidocaine on reperfusion induced arrhythmias in the present model, but previous results indicate a possible involvement of the sodium channel in the genesis of this type of arrhythmia.

In experiments done by previous researchers, because of the short duration of the enzyme activity of SOD in the circulation, the enzyme was administered with a continuous infusion method either before coronary occlusion, 15 min before reperfusion, or at the moment of reflow (6, 14, 18, 22, 23). In the present experiments, SOD-POE has exhibited a highly extended circulating life when injected intravenously in rats, and prophylactic administration of SOD-POE with a single bolus injection has been shown to be effective for preventing the occurrence of reperfusion induced arrhythmias in an animal experimental model. Thus, the present results would offer a new approach for the development of better methods for protecting purified enzymes from degradation and for supplementing enzyme therapy in various organs and tissues in which the quantity of the enzymes are deficient. The approach is still very much in evolution. Although the effects of SOD per se are experimental, and SOD may prove useful only in selective circumstances, on the basis of the results of present experiments with SOD-POE, we believe that the prospects for effective treatment of reperfusion induced arrhythmias or oxygen-radical related injury in various tissues in the clinical setting would be promising.

In conclusion, the present experiments clearly demonstrate that SOD-POE shows a potent protecting action reperfusion induced arrhythmias in situ, and it will serve as an effective mode of administration in preventing reperfusion arrhythmias occurring in clinical situations. This issue is becoming increasingly important as thrombolytic therapy has become routine treatment in many hospitals for patients presenting early after the onset of symptoms (38).
References

1 Kontos, H.A., Wei, E.P., Ellis, E.F., Dietrich, W.D. and Povlishock, J.T.: Prostaglandins in physiological and in certain pathologic responses of the cerebral circulation. Fed. Proc. 40, 2326–2330 (1981)

2 Granger, D.N., Ruttili, G. and McCord, J.M.: Superoxide radicals in feline intestinal ischemia. Gastroenterology 81, 22–29 (1981)

3 Parks, D.A., Bulkley, C.B., Granger, D.N., Hamilton, S.R. and McCord, J.M.: Ischemic injury in the cat small intestine. Role of superoxide radicals. Gastroenterology 82, 9–15 (1982)

4 Schlafer, M., Kane, P.F., Wiggins, V.Y. and Kirsh, M.M.: Possible role for cytotoxic oxygen metabolites in the pathogenesis of cardiac ischemic injury. Circulation 66, Supp. I, 1–85 (1982)

5 Hess, M.J., Mason, N.H. and Okabe, E.: Involvement of free radicals in the pathophysiology of ischemic heart disease. Can. J. Physiol. Pharmacol. 60, 1382–1389 (1982)

6 Rao, P.S., Cohen, M.V. and Mueller, H.S.: Production of free radicals and lipid peroxidase in early experimental myocardial ischemia. J. Mol. Cell. Cardiol. 15, 713–716 (1983)

7 Corr, P.B. and Witowski, F.X.: Potential electrophysiologic mechanisms responsible for dysrhythmias associated with reperfusion of ischemic myocardium. Circulation 66, Supp. I, 1–24 (1983)

8 Manning, A.S. and Hearse, D.J.: Reperfusion-induced arrhythmias: Mechanisms and prevention. J. Mol. Cell. Cardiol. 16, 497–518 (1984)

9 Burton, K.P., McCord, T.M. and Ghai, G.: Myocardial alterations due to free radical generation. Am. J. Physiol. 246, H776–H783 (1984)

10 McCord, J.M.: Oxygen derived free radicals in post-ischemic tissue injury. N. Engl. J. Med. 312, 159–163 (1985)

11 McCord, J.M.: Oxygen derived free radicals in post-ischemic tissue injury. N. Engl. J. Med. 312, 159–163 (1985)

12 Chambers, D.E., Parks, D.A., Patterson, G., Roy, R., McCord, J., Yoshida, S., Parmely, L.F. and Downey, J.M.: Xanthine oxidase as a source of free radicals in myocardial ischemia. J. Mol. Cell. Cardiol. 17, 145–152 (1986)

13 Schlafer, M., Kane, P.F. and Kirsh, M.M.: Superoxide dismutase plus catalase enhances the efficacy of hypothermic cardioplegia to protect the globally ischemic, reperfused heart. J. Thorac. Cardiovasc. Surg. 83, 830–839 (1982)

14 Casale, A.S., Bulkley, G.B., Bulkley, B.H., Flahery, J.T., Gott, V.L. and Gardner, T.J.: Oxygen free-radical scavengers protect the arrested, global ischemic heart upon reperfusion. Surg. Forum 34, 313–316 (1983)

15 McCord, J.M.: Are free radicals on major culprit? In Therapeutic Approaches to Myocardial Infarct Size Limitation, Edited by Hearse, D.J. and Yellon, D.M., p. 209–218. Raven Press, New York (1984)

16 Jolly, S.R., Kane, W.J., Bailie, M.B., Abrams, G.D. and Lucchesi, B.R.: Canine myocardial reperfusion injury: Its reducton by the combined administration of superoxide dismutase and catalase. Circ. Res. 54, 277–285 (1984)

17 Werns, S.W., Shea, M.J., Driscoll, E.M., Cohen, C., Abrams, G.D., Pitt, B. and Lucchesi, B.R.: The independent effects of oxygen radical scavengers on canine infarct size: reduction by superoxide dismutase but not catalase. Circ. Res. 56, 885–898 (1985)

18 Ambrosio, G., Weisfeldt, M.L., Jacobus, W.E. and Flahery, J.T.: Evidence for a reversible oxygen radical-mediated component of reperfusion injury: reduction by recombinant human superoxide dismutase administered at the reflow. Circulation 75, 282–291 (1987)

19 Downey, J.M., Hearse, D.J. and Yellon, M.: The role of xanthine oxidase during myocardial ischemia in several species including man. J. Mol. Cell. Cardiol. 20, Supp. II, 55–63 (1988)

20 Przyklenk, K. and Kloner, R.A.: Superoxide dismutase plus catalase improve contractile function in the canine model of the “stunned myocardium”. Circ. Res. 58, 148–156 (1986)

21 Bernier, M., Hearse, D.J. and Manning, A.S.: Reperfusion-induced arrhythmias and oxygen-derived free radicals: Studies with “anti-free-radical” interventions and a free radical-generating system in the isolated perfused rat heart. Circ. Res. 58, 331–340 (1986)

22 Woodward, B. and Zakaria, M.V.M.: Effects of some free radical scavengers on reperfusion induced arrhythmias in the isolated rat heart. J. Mol. Cell. Cardiol. 17, 485–493 (1985)

23 Yamakawa, T., Kadowaki, Y., Galcia-Alves, M., Yokoyama, M., Iwashita, Y. and Nishi, K.: Effects of polyoxyethylene-modified superoxide dismutase on reperfusion induced arrhythmias in the isolated rat heart. J. Mol. Cell. Cardiol. 24, 441–452 (1989)

24 Galcia-Alves, M., Kadowaki, Y., Nakamura, S. and Nishi, K.: Effects of a newly introduced conjugated superoxide dismutase on reperfusion arrhythmias in rats in vivo. Japan. J. Pharmacol. 43, Supp. 100P (1987)

25 Oyanagui, Y.: Reevaluation of assay methods and establishment of kit for superoxide dismutase
activity. Anal. Biochem. 142, 290–296 (1984)

26 McCord, J.M. and Fridovich, I.: Superoxide dismutase and enzymatic function. J. Biol. Chem. 244, 6049–6055 (1969)

27 Bannister, J.V. and Bannister, W.H.: Isolation and characterization of superoxide dismutase. Methods Enzymol. 105, 88–93 (1984)

28 Kane, K.A., Parratt, J.R. and Williams, F.M.: An investigation into the characteristics of reperfusion-induced arrhythmias in the anesthetized rat and their susceptibility to antiarrhythmic agents. Br. J. Pharmacol. 82, 349–357 (1984)

29 Fridovich, I.: The biology of oxygen radicals. Science 201, 875–880 (1978)

30 Gutteridge, J.M.C.: Superoxide dismutase inhibits the superoxide-driven Fenton reaction at two different levels: implication for a wider protective role. FEBS Lett. 185, 19–23 (1985)

31 Rubin, R. and Farber, J.L.: Mechanisms of the killing of cultured hepatocytes by hydrogen peroxide. Arch. Biochem. Biophys. 228, 450–459 (1984)

32 Halliwell, B.: Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts is a feasible source of hydroxyl radicals in vivo. Biochem. J. 205, 461–462 (1982)

33 Starke, P.E. and Farber, J.L.: Ferric iron and superoxide ions are required for the killing of cultured hepatocytes by hydrogen peroxide; evidence for the participation of hydroxyl radicals formed by an iron-catalyzed Haber-Weiss reaction. J. Biol. Chem. 260, 10099–10104 (1985)

34 Lubbe, W.F., Daries, F.S. and Opie, L.H.: Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the isolated perfused rat heart; a model for assessment of antifibrillatory action of antiarrhythmic agents. Cardiovasc. Res. 12, 212–220 (1978)

35 Bergey, J.L., Nocella, K. and McCallum, J.D.: Acute coronary artery occlusion-reperfusion-induced arrhythmias in rats, dogs and pigs: anti-arrhythmic evaluation of quinidine, procainamide and lidocaine. Eur. J. Pharmacol. 81, 205–216 (1982)

36 Iwasaki, S., Araki, H., Yamakawa, T., Nishi, K. and Miyauchi, Y.: Effects of aprindine and disopyramide on reperfusion induced arrhythmias and cardiac function in isolated rat hearts. Arch. Int. Pharmacodyn. Ther. (1989) (in press)

37 Naito, M., Michelson, E.L., Kmetzo, J.J., Kaplinsky, E. and Dreifus, L.S.: Failure of antiarrhythmic drugs to prevent experimental reperfusion ventricular fibrillation. Circulation 58, 1023–1035 (1981)

38 Goldberg, S., Greenspon, A.J., Urban, P.L., Muza, B., Berger, B., Walinsky, P. and Maroko, P.R.: Reperfusion arrhythmia; a marker of restoration of antegrade flow during intracoronary thrombolysis for acute myocardial infarction. Am. Heart J. 105, 26–32 (1983)