J Anesth (1999) 13:23–28

Journal of Anesthesia

Effects of melatonin and superoxide dismutase on free radical formation in the postischemic reperfused heart

Satoshi Kashimoto, Masaki Kume, Kazumori Ikeya, Tadahiko Ishiyama, and Teruo Kumazawa

Department of Anesthesiology, Yamanashi Medical University, 1110 Shimokato, Tamaho-cho, Nakakoma-gun, Yamanashi 409-3821, Japan

Abstract:
Purpose. Melatonin has been reported to protect against oxygen free radicals. We investigated whether melatonin or superoxide dismutase (SOD) would decrease hydroxyl radical concentration in the postischemic reperfused heart.
Methods. An isolated rat heart-lung preparation was used. Eighty-one male Wistar rats were allocated into control (no drug), S1 (SOD 400 U·ml^{-1}), S2 (SOD 2000 U·ml^{-1}), M1 (melatonin 0.1 μg·ml^{-1}), M2 (melatonin 1.0 μg·ml^{-1}), M3 (melatonin 10 μg·ml^{-1}), SM (SOD 400 U·ml^{-1} and melatonin 1.0 μg·ml^{-1}) groups. The heart was perfused initially at the cardiac output of 30 ml·min^{-1} and the mean arterial pressure of 70 mmHg. Drugs were administered into the reservoir 7 min after the start of perfusion. Ten minutes after the start of perfusion, the heart was rendered globally ischemic for 10 min by reducing the preload and afterload to zero and then reperfused for 10 min. At the end of reperfusion, the heart was freeze-dried for 6 days. The perfusate blood was collected just before and after ischemia and at the end of reperfusion. The formation of hydroxyl radicals in perfusate blood and heart was measured with high-performance liquid chromatography using salicylic acid. Hydroxyl radicals react with salicylic acid, yielding 2,3-, 2,4-, 2,5-, and 3,4-dihydroxybenzoic acid (DHBA).
Results. Before and after ischemia, there were no significant differences among the groups in cardiac output, systolic pressure, heart rate, and right atrial pressure. The concentrations of DHBAs in the perfusate blood and heart after ischemia and reperfusion in all groups were significantly higher than those before ischemia. DHBAs in the heart of all drug-administered groups were significantly lower than those in the control group. In the perfusate blood, DHBAs in the S2 group were significantly lower than those in the control group.
Conclusions. SOD and melatonin decrease hydroxyl radical concentration in the postischemic reperfused heart.

Address correspondence to: S. Kashimoto
Received for publication on January 26, 1998; accepted on August 5, 1998

Key words: Free radical, Melatonin, SOD

Introduction

Oxygen-derived free radicals and their metabolites have been implicated in reperfusion injury following myocardial ischemia [1–4]. These radicals are thought to consist mainly of superoxide anion, hydrogen peroxide, and hydroxyl radical. To reduce cardiac damage from these radicals, there are some free radical scavengers [5,6]. As one of the free radical scavengers, melatonin has been reported to be a very efficient neutralizer of the hydroxyl radical [7–10]. However, little information is available about the effects of melatonin on postischemic reperfused hearts. Therefore, it is interesting to investigate whether melatonin decreases hydroxyl radical concentrations in the postischemic reperfused heart. We employed a sensitive method to detect the hydroxyl radical using high-pressure liquid chromatography with electrochemical detection (LCED) [11,12]. We also determined whether superoxide dismutase (SOD), a well-known free radical scavenger, decreased hydroxyl radical concentrations in the ischemic myocardium.

Materials and methods

The experiment was performed in accordance with the Guidelines for Animal Experiments of Yamanashi Medical University. The techniques used were identical to those used in an earlier study [13]. Briefly, 81 male Wistar rats were randomly divided into eight groups, as follows: (1) control (C) group, which received no drugs during perfusion; (2) S1 group: SOD concentration in the perfusate was 400 U·ml^{-1}; (3) S2 group: SOD concentration in the perfusate was 2000 U·ml^{-1}; (4) M1

group: melatonin concentration in the perfusate was 0.1 μg·ml^{-1}; (5) M2 group: melatonin concentration in the perfusate was 1.0 μg·ml^{-1}; (6) M3 group: melatonin concentration in the perfusate was 10 μg·ml^{-1}; (7) SM group: SOD 400 U·ml^{-1} and melatonin 1.0 μg·ml^{-1}; (8) normal heart: normally perfused for 10 min. Melatonin and SOD (bovine; 3000 U·mg^{-1}) were obtained from Sigma Aldrich (St. Louis, WI, USA), and Boehringer Mannheim (Mannheim, Germany), respectively. All of these drugs were administered into the reservoir 7 min after the start of perfusion. At the beginning, all rats were anesthetized with sevoflurane only during preparation. Tracheostomy was performed, and intermittent positive-pressure ventilation was adjusted to maintain $PaCO_2$ at 30–36 mmHg with a 95% O_2 + 5% CO_2 gas mixture. The chest was opened and flooded with ice-cold saline and the heart was arrested. Cannulas were inserted into the aorta and the superior and inferior vena cava. The cannula in the superior vena cava was used to monitor right atrial pressure.

The heart-lung preparation was perfused with a solution (25 ml) containing red blood cells collected from another rat and Krebs Ringer bicarbonate buffer, with 25% hematocrit and pH 7.4. The concentrations (mM) of the buffer constituents were: NaCl 127, KCl 5.1, $CaCl_2$ 2.2, KH_2PO_4 1.3, $MgSO_4$ 2.6, $NaHCO_3$ 15, and glucose 5.5. In addition, 1 mM salicylic acid was mixed into the buffer. The perfusate blood was pumped from the aorta, passed through a pneumatic resistance, collected in a reservoir kept at 37°C, and then returned to the inferior vena cava. Thus, it was recirculated in this model. No other organs except the heart and lung were perfused, cardiac output was determined by the inflow, provided the heart did not fail, and systolic arterial pressure was regulated by the pneumatic resistance.

Heart rate was recorded with a bioelectric amplifier (AB-621G; Nihonkohden, Tokyo, Japan) and cardiac output was measured with an electromagnetic blood flow meter (MFV-1200; Nihonkohden). Arterial pressure and right atrial pressure were measured with transducers (TP101T and LPU-0.1A; Nihonkohden) and carrier amplifiers (AP-621G; Nihonkohden).

After the preparation was completed, the heart was perfused initially with a cardiac output of 30 ml·min^{-1} and a mean arterial pressure of 70 mmHg by regulating the venous return (inflow) and the pneumatic resistance, respectively. Each drug was administered into the reservoir 7 min after the start of perfusion. Ten minutes after the start of perfusion, all hearts were made globally ischemic for 10 min by clamping the venous return and reducing the pneumatic resistance to zero. Subsequently, the preparations were reperfused for 10 min by regulating the venous return and the pneumatic resistance.

Ten minutes after the start of reperfusion, the hearts were freeze-clamped and freeze-dried for 6 days. The tissue was minced into small pieces and then homogenized in a polytron. An aliquot was extracted with perchloric acid and centrifuged at 3000 *g*. A small amount of perfusate blood was collected from the reservoir just before ischemia, at recovery from ischemia, and 10 min after reperfusion. The perfusate blood was also centrifuged at 3000 *g* and the supernatant was used for the detection of dihydroxybenzoic acids.

Determination of dihydroxybenzoic acids

Hydroxyl radicals react with salicylic acid yielding 2,3-, 2,4-, 2,5-, and 3,4-dihydroxybenzoic acid (DHBA). The liquid chromatography apparatus consisted of a Shimazu Model LC-10AD pump and a detector module (C-R4A, Chromatopac, Shimazu, Tokyo, Japan). The column used was a Shim-Pack, CLC-ODS, 15 cm × 4.6 mm. The mobile phase was 90% 20 mM sodium dihydrogen phosphate, 1 mM octanesulfonic acid sodium salt, 10 mM sodium sulfate, and 10% acetonitrile at a flow rate of 0.5 ml·min^{-1}. The mobile phase was kept anaerobic by N_2 (Degasser, DGU-3A, Shimazu). The detector was set at a detector voltage of 0.6 V. Preliminary experiments determined these settings to yield maximal detection with minimal interference from other cellular constituents. All samples were measured against external standards of 2,3-, 2,4-, 2,5-, and 3,4-DHBA and salicylic acid.

One milliliter of effluent was treated with 1 ml of 1 M Tris buffer and extracted with 2 ml of methanol on a Vortex mixer for 10 min. Aluminum oxide 50 mg was added to this and the methanol layer was separated. The residue was dissolved in 200 μl of 0.2 M perchloric acid, and 5 μl of this solution was injected into the LCED unit. These methods were modified as described by Floyd et al. [11,12].

Hemodynamic and metabolic data within groups were analyzed by two-way analysis of variance with repeated measures. The other data were analyzed by one-way analysis of variance followed by the Newman-Keuls test for multiple comparisons. A probability of $P < 0.05$ was regarded as statistically significant. The data are given as means ± standard deviation.

Results

There were no significant differences in cardiac output, heart rate, systolic blood pressure, and right atrial pressure among the groups before ischemia and after reperfusion (Table 1). Table 2 shows the concentrations of 2,3-, 2,4-, 2,5-, and 3,4-DHBA in the heart at the conclusion of reperfusion. The concentrations of 2,3-,

Table 1. Hemodynamic changes before and after ischemia

Groups	C (10)	S1 (10)	S2 (10)	M1 (10)	M2 (11)	M3 (10)	SM (11)
Cardiac output ($ml \cdot min^{-1}$)							
5 (min)	30.7 ± 1.5	30.2 ± 0.9	30.1 ± 1.0	30.1 ± 0.9	30.3 ± 0.6	29.8 ± 1.1	30.1 ± 0.9
10	30.0 ± 1.3	30.6 ± 1.1	30.2 ± 1.4	29.8 ± 0.8	30.4 ± 0.5	30.2 ± 1.1	30.3 ± 0.8
R	30.7 ± 0.8	30.4 ± 2.5	32.9 ± 1.8	29.5 ± 0.7	29.0 ± 1.3	28.8 ± 0.8	28.2 ± 1.4
R5	30.9 ± 2.2	30.1 ± 1.4	31.9 ± 2.1	29.4 ± 1.4	29.5 ± 1.4	29.7 ± 1.3	29.6 ± 1.1
R10	30.5 ± 1.8	30.5 ± 1.3	32.3 ± 2.5	29.8 ± 1.1	30.0 ± 1.3	30.1 ± 1.2	30.0 ± 1.0
Heart rate ($beats \cdot min^{-1}$)							
5 (min)	253 ± 37	242 ± 20	234 ± 31	236 ± 29	257 ± 24	251 ± 39	258 ± 33
10	256 ± 35	258 ± 41	252 ± 26	236 ± 28	264 ± 24	268 ± 33	268 ± 33
R	224 ± 51	239 ± 44	251 ± 27	227 ± 29	214 ± 58	231 ± 31	235 ± 20
R5	247 ± 29	249 ± 33	245 ± 14	226 ± 24	264 ± 35	257 ± 21	255 ± 20
R10	259 ± 26	241 ± 20	251 ± 13	233 ± 30	272 ± 31	257 ± 24	259 ± 20
Systolic blood pressure (mmHg)							
5 (min)	111 ± 20	110 ± 17	111 ± 14	102 ± 5	107 ± 9	109 ± 8	100 ± 6
10	107 ± 16	107 ± 8	112 ± 11	100 ± 5	106 ± 6	105 ± 8	101 ± 8
R	130 ± 18	113 ± 12	117 ± 10	112 ± 17	126 ± 24	106 ± 13	99 ± 7
R5	101 ± 7	98 ± 7	101 ± 12	96 ± 6	98 ± 4	97 ± 5	95 ± 4
R10	106 ± 12	107 ± 11	106 ± 13	99 ± 7	100 ± 5	99 ± 7	98 ± 5
Right atrial pressure (mmH_2O)							
5 (min)	54 ± 12	54 ± 12	57 ± 11	54 ± 7	52 ± 10	57 ± 13	51 ± 8
10	52 ± 13	51 ± 12	54 ± 10	52 ± 7	50 ± 10	55 ± 14	49 ± 5
R	61 ± 18	51 ± 14	55 ± 9	54 ± 7	57 ± 11	59 ± 12	62 ± 11
R5	59 ± 16	54 ± 13	54 ± 11	53 ± 8	51 ± 9	56 ± 13	56 ± 9
R10	53 ± 12	51 ± 11	51 ± 9	52 ± 8	49 ± 9	54 ± 12	52 ± 9

Time: after the start of perfusion. R: at recovery time; R5: 5 min after reperfusion; R10: 10 min after reperfusion. Values are means ± SD. Numbers are in parentheses.
1. C: Control group. 2. S1 group: SOD concentration in perfusate was $400\,U \cdot ml^{-1}$. 3. S2 group: SOD concentration in perfusate was $2000\,U \cdot ml^{-1}$. 4. M1 group: melatonin concentration in perfusate was $0.1\,\mu g \cdot ml^{-1}$. 5. M2 group: melatonin concentration in perfusate was $1.0\,\mu g \cdot ml^{-1}$. 6. M3 group: melatonin concentration in perfusate was $10\,\mu g \cdot ml^{-1}$. 7. SM group: SOD $400\,U \cdot ml^{-1}$ and melatonin $1.0\,\mu g \cdot ml^{-1}$.

Table 2. Concentrations of 2,3-DHBA, 2,4-DHBA, 2,5-DHBA, and 3,4-DHBA in the heart

$\mu mol \cdot g^{-1}$	2,3-DHBA	2,4-DHBA	2,5-DHBA	3,4-DHBA
Normal heart (9)	117 ± 24	28.6 ± 6.9	27.4 ± 8.1	2.98 ± 0.69
C (10)	209 ± 27*	58.7 ± 15.9*	44.6 ± 15.0*	4.84 ± 0.86*
S1 (10)	176 ± 28*†	48.1 ± 13.3*†	35.6 ± 11.5	3.56 ± 1.19†
S2 (10)	114 ± 29†	26.8 ± 6.9†	16.8 ± 4.3*†	2.15 ± 0.88†
M1 (10)	160 ± 37*†	40.7 ± 12.3*†	39.8 ± 11.1*	3.61 ± 1.30†
M2 (11)	148 ± 31*†	35.6 ± 8.6†	34.1 ± 7.2†	3.00 ± 1.03†
M3 (10)	151 ± 18*†	38.7 ± 5.6*†	44.7 ± 10.4*	3.86 ± 1.48
SM (11)	161 ± 17*†	43.0 ± 11.8*†	48.0 ± 10.0*	3.31 ± 1.15†

*$P < 0.05$ vs. normal heart.
†$P < 0.05$ vs. C group. Values are means ± SD. Numbers are in parentheses.
1. C: Control group. 2. S1 group: SOD concentration in perfusate was $400\,U \cdot ml^{-1}$. 3. S2 group: SOD concentration in perfusate was $2000\,U \cdot ml^{-1}$. 4. M1 group: melatonin concentration in perfusate was $0.1\,\mu g \cdot ml^{-1}$. 5. M2 group: melatonin concentration in perfusate was $1.0\,\mu g \cdot ml^{-1}$. 6. M3 group: melatonin concentration in perfusate was $10\,\mu g \cdot ml^{-1}$. 7. SM group: SOD $400\,U \cdot ml^{-1}$ and melatonin $1.0\,\mu g \cdot ml^{-1}$. 8. Normal heart: normally perfused for 10 min.

2,4-, 2,5-, and 3,4-DHBA in group C were significantly higher than those in the nonischemic hearts, which were normally perfused for 10 min. In addition, the concentrations of 2,3-, 2,4-, and 3,4-DHBA in all groups administered drugs were significantly lower than those in group C. Moreover, the DHBA levels of group S2 were almost same as or even lower than those of normal heart. As the perfusate blood was recirculated in this preparation, the concentrations of 2,3-, 2,4-, 2,5-, and 3,4-DHBA in the perfusate blood after ischemia and

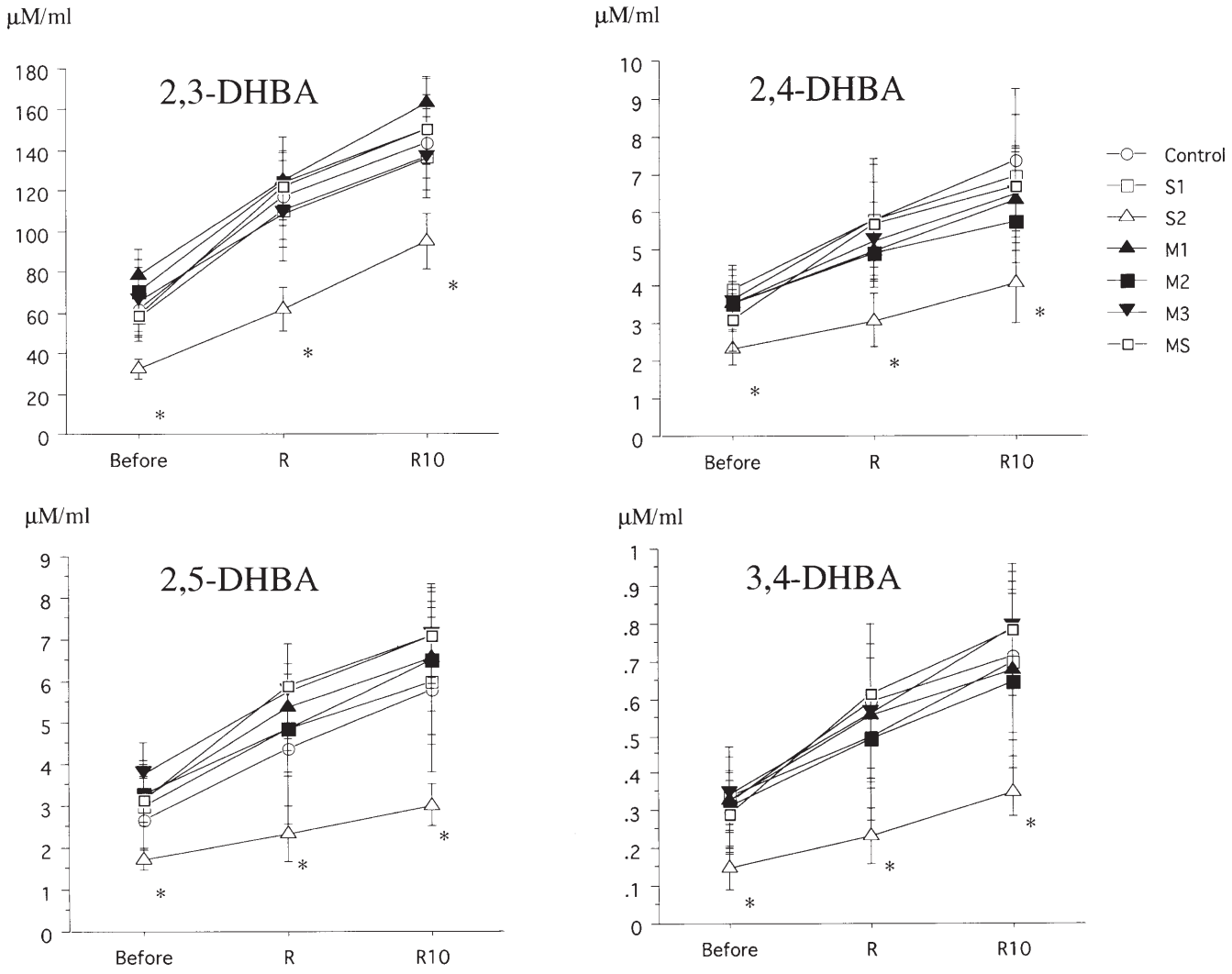

Fig. 1. 2,3-DHBA, 2,4-DHBA, 2,5-DHBA, and 3,4-DHBA levels in the perfusate blood. *Before*: just before ischemia; *R*: at time of recovery from ischemia; *R10*: 10 min after reperfusion. 1. C: Control group. 2. S1 group: SOD concentration in perfusate was 400 U·ml^{-1}. 3. S2 group: SOD concentration in perfusate was 2000 U·ml^{-1}. 4. M1 group: melatonin concentration in perfusate was 0.1 μg·ml^{-1}. 5. M2 group: melatonin concentration in perfusate was 1.0 μg·ml^{-1}. 6. M3 group: melatonin concentration in perfusate was 10 μg·ml^{-1}. 7. SM group: SOD 400 U·ml^{-1} and melatonin 1.0 μg·ml^{-1}. *$P < 0.05$ vs. control group

reperfusion in all groups gradually increased following ischemia. Here, DHBA levels in the perfusate blood of group S2 were significantly lower than those in group C (Fig. 1).

Discussion

Although oxygen free radicals are chemical species generated in normal cells, they are normally equipped with endogenous scavenging systems. However, large amounts of oxygen free radicals generated during reperfusion of postischemic tissues can overwhelm cellular defenses and induce tissue damage [1–4]. The hydroxyl radical is highly reactive and one of the most damaging species. The measurement of free radical formation is important to understand their role in the pathogenesis of postischemic reperfusion injury. Although the existence of free radicals has been demonstrated with electron spin resonance spectroscopy, this technique requires expensive equipment. Floyd et al. [11,12] have shown that hydroxyl radicals react with salicylic acids, producing DHBAs, especially 2,3-DHBA. These reaction products can be detected by LCED. In this study, we modified Floyd's method and measured the concentrations of 2,3-, 2,4-, 2,5-, and 3,4-DHBA. In the present study, ischemia and reperfusion enhanced the production of DHBAs in the

heart, because DHBA concentrations in postischemic reperfused hearts were significantly higher than those in normally perfused hearts. DHBAs were also detected in the perfusate blood before ischemia and increased after ischemia and reperfusion. This may imply that the production of hydroxyl radicals always occurs in this preparation under high oxygen pressure, even before ischemia. Freeman and Crapo [14] suggested that hyperoxia increased the pulmonary production of oxygen radicals and that the mitochondria contributed significantly to this phenomenon.

The hormone melatonin participates in many important physiological functions, including the control of seasonal reproduction, as well as influencing the immune system [15]. Recently, melatonin was shown to be a very efficient scavenger of the hydroxyl radical [7–10]. The results of our study indicated that all three doses of melatonin decreased hydroxyl radical concentrations in the postischemic reperfused heart, though it could not decrease hydroxyl radical concentrations in the perfusate blood. In addition, this reducing effect was not dose-dependent. Since the human melatonin level is less than 100 $pg \cdot ml^{-1}$ [16], even the lowest dose (0.1 $\mu g \cdot ml^{-1}$) may be nonphysiological and may have had maximum effects on the hearts. We did not measure DHBA levels in the heart before ischemia. Therefore, there is a possibility that SOD and melatonin decreased hydroxyl radical concentrations before ischemia, although the production of hydroxyl radical may mainly occur during the reperfusion period.

Reiter et al. [10] have suggested that melatonin is remarkably potent in protecting against free radical damage induced by a variety of means. For example, melatonin is more effective than glutathione in neutralizing the hydroxyl radical [17] and more effective than vitamin E in inactivating the peroxyl radical [18]. Besides these direct actions of melatonin, it stimulates glutathione peroxidase activity [19] and inhibits nitric oxide synthase [20]. Glutathione peroxidase is an important antioxidative enzyme, and melatonin decreases the formation of the free radical nitric oxide by inhibiting nitric oxide synthase. Therefore, melatonin either decreases the generation of free radicals or neutralizes them. We do not know why melatonin could not decrease the hydroxyl radical in the perfusate blood. It is likely that a higher dose of SOD (2000 $U \cdot ml^{-1}$) could reduce hydroxyl radical concentrations by decreasing superoxide, but the effect of melatonin would be weaker than that of SOD.

There were no significant differences in cardiac output, heart rate, systolic blood pressure, and right atrial pressure among the groups before ischemia and after reperfusion. Melatonin exerts its hemodynamic effects by decreasing serotonin release, resulting in sympathetic inhibition or parasympathetic stimulation, which leads to hypotension and bradycardia in rats [21]. Since our preparation is free from neural influences and the peripheral circulatory resistance is constant, the hemodynamic effects of melatonin might be difficult to detect in our model.

A higher dose of SOD (2000 $U \cdot ml^{-1}$) decreased hydroxyl radical concentrations in both the perfusate blood and the heart. However, a lower dose of SOD (400 $U \cdot ml^{-1}$) decreased it only in the heart. This reducing effect seems to be dose-dependent. SOD dismutases superoxide radical to hydrogen peroxide, which, in the presence of iron, produces hydoxyl radical via the Fenton reaction. Therefore, in spite of the fact that SOD decreases ischemia-reperfusion injury [22–24], some reports have demonstrated a virtual lack of any protective effect of SOD alone [5,25,26]. However, SOD combined with catalase has been reported to be very effective against oxygen toxicity [27] and reperfusion injury [28,29]. In this study, although we did not administer catalase, SOD alone decreased hydroxyl radical concentrations in the reperfused heart. In addition, the combination of SOD 400 $U \cdot ml^{-1}$ and melatonin 1.0 $\mu g \cdot ml^{-1}$ decreased hydroxyl radical concentrations in the heart but not in the perfusate blood. This implies that a synergistic effect between SOD and melatonin was not seen.

In summary, SOD and melatonin decreased hydroxyl radical concentrations in the postischemic reperfused heart. Although it may be inappropriate to apply the results of this study in animals directly to humans, further studies are necessary to elucidate the efficacy of melatonin during anesthesia of patients with ischemic heart disease or undergoing cardiopulmonary bypass surgery.

Acknowledgments. The authors are indebted to Mr. Koshimizu and Miss Amemiya for their valuable technical assistance.

References

1. Marklund SL (1988) Role of toxic effects of oxygen in reperfusion damage. J Mol Cell Cardiol 20 (Suppl II):23–30
2. Hearse DJ, Tosaki A (1988) Free radicals and calcium: simultaneous interacting triggers as determinants of vulnerability to reperfusion-induced arrhythmias in the rat heart. J Mol Cell Cardiol 20:213–223
3. Ambrosio G, Zweier JL, Flaherty JT (1991) The relationship between oxygen radical generation and impairment of myocardial energy metabolism following post-ischemic reperfusion. J Mol Cell Cardiol 23:1359–1374
4. Takemura G, Onodera T, Ashraf M (1994) Characterization of exogenous hydroxyl radical effects on myocardial function, metabolism and ultrastructure. J Mol Cell Cardiol 26:441–454
5. Myers CL, Weiss SJ, Kirsh MM, Shlafer M (1985) Involvement of hydrogen peroxide and hydroxyl radical in the 'oxygen paradox': reduction of creatine kinase release by catalase, allopurinol or

deferoxamine, but not by superoxide dismutase. J Mol Cell Cardiol 17:675–684
6. Ku K, Kin S, Hashimoto M, Saitoh Y, Hosaka S, Iwasaki S, Alam MS, Nakayama K (1996) The role of a hydroxyl radical scavenger (nicaraven) in recovery of cardiac function following preservation and reperfusion. Transplantation 27:1090–1095
7. Pierrefiche G, Topall G, Courboin G, Henriet I, Laborit H (1993) Antioxidant activity of melatonin in mice. Res Commun Chem Pathol Pharmacol 80:211–223
8. Reiter RJ, Tan DX, Poeggeler B, Menendez-Pelaez A, Chen LD, Saarela S (1994) Melatonin as a free radical scavenger: implications for aging and age-related disease. Ann NY Acad Sci 72:1–12
9. Hardeland R, Reiter RJ, Poeggeler B, Tan DX (1993) The significance of the metabolism of the neurohormone melatonin: antioxidative protection and formation of bioactive substances. Neurosci Biohav Rev 17:347–357
10. Reiter RJ, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden L, Chuang J, Ortiz GG, Acuña-Castroviejo D (1995) A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res 18:1–11
11. Floyd RA, Watson JJ, Wong PK (1984) Sensitive assay of hydroxyl free radical formation utilizing high pressure liquid chromatography with electrochemical detection of phenol and salicylate hydroxylation products. J Biochem Biophys Meth 10: 221–235
12. Floyd RA, Henderson R, Watson JJ, Wong PF (1986) Use of salicylate with high pressure liquid chromatography and electrochemical detection (LCED) as a sensitive measure of hydroxyl free radicals in adriamycin treated rats. J Free Radicals Biol Med 2:13–18
13. Kashimoto S, Kumazawa T (1987) Protective effects of superoxide dismutase against oxygen toxicity in rat's heart lung preparation. Jpn Circ J 51:1022–1026
14. Freeman BA, Crapo JD (1981) Hyperoxia increases oxygen radical production in rat lungs and lung mitochondria. J Biol Chem 256:10986–10992
15. Reiter RJ (1991) Melatonin: that ubiquitously acting pineal hormone. News Physiol Sci 6:223–227
16. Nagtegaal E, Smits M, Swart W, van der Meer G, Kerkhof G (1995) Melatonin secretion and coronary disease. Lancet 346:1299
17. Meister A (1992) On the antioxidant effect of ascorbic acid and glutathione. Biochem Pharmacol 44:1905–1915
18. Pieri C, Marra M, Moroni F, Recchioni R, Marcheselli F (1994) Melatonin: a peroxyl radical scavenger more effective than vitamin E. Life Sci 55:PL271–276
19. Barlow-Walden L, Reiter RJ, Abe M, Pablos MI, Menendez-Pelaez A, Chen LD, Poeggeler B (1995) Melatonin stimulates brain glutathione peroxidase activity. Neurochem Int 26:497–502
20. Pozo D, Reiter RJ, Calvo JP, Guerrero JM (1994) Physiological concentrations of melatonin inhibit nitric oxide synthase in rat cerebellum. Life Sci 55:PL455–460
21. Chuang JI, Chen SS, Lin MT (1993) Melatonin decreases brain serotonin release, arterial pressure and heart rate in rats. Pharmacology 47:91–97
22. Woodward B, Zakaria MNM (1985) Effect of some free radical scavengers on reperfusion induced arrhythmias in the isolated rat heart. J Mol Cell Cardiol 17:485–493
23. Yamakawa T, Kadowaki Y, Garcia-Alves M, Yokoyama M, Iwashita Y, Nishi K (1989) Effects of polyoxyethylene-modified superoxide dismutase on reperfusion induced arrhythmias in isolated rat and guinea-pig hearts. J Mol Cell Cardiol 21:441–452
24. Kalra J, Prasad K (1994) Oxygen free radicals and cardiac depression. Clin Biochem 27:163–168
25. Shirato C, Miura T, Ooiwa H, Toyofuku T, Wilborn WH, Downey JM (1989) Tetrazolium artifactually indicates superoxide dismutase-induced salvage in reperfused rabbit heart. J Mol Cell Cardiol 21:1187–1193
26. Matsuda M, Fujiwara H, Kawamura A, Ishida M, Takemura G, Kida M, Uegaito T, Fujiwara Y, Fujiwara T, Kawai C (1991) Failure to reduce infarct size by intracoronary infusion of recombinant human superoxide dismutase at reperfusion in the porcine heart: immunohistochemical and histological analysis. J Mol Cell Cardiol 23:1287–1296
27. Turrense JF, Crapo JD, Freeman BA (1984) Protection against oxygen toxicity by intravenous injection of liposome-entrapped catalase and superoxide dismutase. J Clin Invest 73:87–95
28. Näslund U, Häggmark S, Johansson G, Marklund SL, Reiz S, Öberg A (1986) Superoxide dismutase and catalase reduce infarct size in a porcine myocardial occlusion-reperfusion model. J Mol Cell Cardiol 18:1077–1084
29. Mao GD, Thomas PD, Lopaschuk GD, Poznansky MJ (1993) Superoxide dismutase (SOD)-catalase conjugates. Role of hydrogen peroxide and the Fenton reaction in SOD toxicity. J Biol Chem 268:416–420