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Propofol Attenuates Hydrogen Peroxide-induced Mechanical and Metabolic Derangements in the Isolated Rat Heart

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Background: Oxygen-derived free radicals are involved in tissue damage during myocardial ischemia and reperfusion. Recent *in vitro* studies have demonstrated that a beneficial effect of propofol lies on its free radical scavenging properties. The current study, therefore, examined whether propofol is effective against the mechanical and metabolic damage induced by exogenously administered hydrogen peroxide in the isolated rat heart.

Methods: Rat hearts were perfused aerobically with Krebs-Henseleit bicarbonate buffer at a constant flow rate according to Langendorff's technique, while being paced electrically. Hearts were studied in control Krebs-Henseleit bicarbonate buffer, with Intralipid vehicle, with 25 μ M or 50 μ M propofol for 40 min, and with 50 μ M propofol for 30 min followed by Intralipid for 10 min. A similar set of hearts was treated with hydrogen peroxide for 4 min, either in the absence of or beginning 10 min after Intralipid or propofol infusion. Left ventricular pressure was recorded as an index of mechanical function. The tissue concentrations of adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, and creatine phosphate were measured as indices of energy metabolism. The tissue concentration of malondialdehyde was measured to evaluate lipid peroxidation.

Results: Hydrogen peroxide (600 μ M) significantly increased the left ventricular end-diastolic pressure, decreased the left ventricular developed pressure (*i.e.*, it produced mechanical dysfunction), and decreased tissue concentrations of adenosine triphosphate and creatine phosphate (*i.e.*, metabolic damage). Hydrogen peroxide also increased the tissue concentration of malondialdehyde. These mechanical and metabolic alterations induced by hydrogen peroxide were significantly attenuated by propofol (25 μ M or 50 μ M), while the

increase in malondialdehyde was completely suppressed by propofol.

Conclusions: The current study demonstrates that in the isolated heart, propofol attenuates both mechanical and metabolic changes induced by exogenously applied hydrogen peroxide. The beneficial action of propofol is probably correlated with reduction of the hydrogen peroxide-induced lipid peroxidation. (Key words: Anesthetics, intravenous: propofol. Heart: energy metabolism; mechanical function. Measurement technique: high-performance liquid chromatography; malondialdehyde. Metabolism: lipid peroxidation. Toxicity: hydrogen peroxide.)

REACTIVE oxygen species, such as superoxide anion, hydroxyl radical, and hydrogen peroxide (H_2O_2), have been shown to participate in tissue damage in various diseases.¹ For example, it is well known that oxygen free radicals are generated intracellularly and extracellularly in the myocardium and endothelium during ischemia and reperfusion.^{2,3} These reactive oxygen species cause lipid peroxidation of the cell membrane and intracellular Ca^{2+} overload, which are responsible for mechanical and metabolic damage.³⁻⁵ In fact, H_2O_2 induces intracellular Ca^{2+} accumulation in the cardiomyocyte.^{5,6} We have demonstrated that treatment with H_2O_2 produces mechanical dysfunction and metabolic changes, including lipid peroxidation in the isolated perfused heart.^{7,8}

Propofol has a chemical structure similar to phenol-based free radical scavengers such as butylated hydroxytoluene and the endogenous antioxidant vitamin E.¹ The phenolic structure is responsible for an antioxidant activity because the hydroxyl group can release hydrogen and thereafter be converted into a less active radical by the resonance of the aromatic ring.⁹ Recent *in vitro* studies with isolated organelles have demonstrated that propofol inhibits the lipid peroxidation induced by oxidative stress in the rat liver mitochondria,^{10,11} microsomes,¹¹ and brain synaptosomes.¹¹ Therefore, propofol exhibits the antioxidant action in some organelles.

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Nevertheless, it is unclear whether propofol attenuates the changes induced by reactive oxygen metabolites in the heart. In the current study, we produced myocardial damage by exogenous administration of H_2O_2 as a source of reactive oxygen species in the Langendorff rat heart. Hydrogen peroxide and ischemia-reperfusion are similar in that both produce myocardial damage and induce accumulation of lipid peroxides in the myocardium.⁷ We examined the effect of propofol on the H_2O_2 -induced myocardial damage in terms of both mechanical and metabolic dysfunctions. Furthermore, we measured the myocardial concentration of malondialdehyde (MDA), a product of lipid peroxidation, to delineate the protective effect of propofol against H_2O_2 -induced myocardial damage.

Materials and Methods

Heart Preparation

The protocol of animal experiments in the current study was approved by the Asahikawa Medical College Committee on Animal Research. Male Sprague-Dawley rats (weighing 300–350 g, aged 9–10 weeks) were used. The animals were anesthetized with $50 \text{ mg} \cdot \text{kg}^{-1}$ intraperitoneal sodium pentobarbital. After thoracotomy, the heart and aortic arch were rapidly excised and immersed in a cold Krebs-Henseleit bicarbonate (KHB) buffer (4°C). The composition of the KHB buffer was (in mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, MgSO_4 1.2, CaCl_2 2.5, NaHCO_3 25, and glucose 11. The heart was perfused *via* retrograde cannulation of the aorta with the KHB buffer at a constant pressure of 80 cmH_2O . The buffer was equilibrated with a gas mixture of 95% O_2 –5% CO_2 , which produced a P_{O_2} range of 500–550 mmHg, a P_{CO_2} range of 36–42 mmHg, and a pH value of 7.38–7.46. Temperature was maintained at 37°C throughout the experiment with a thermostatically controlled recirculating water bath. About 10 min after constant-pressure perfusion, the flow was switched to constant-flow perfusion ($10 \text{ ml} \cdot \text{min}^{-1}$) using a microtube pump (Eyela MP-3, Tokyo-Rikakikai Instruments, Tokyo, Japan), and this flow was maintained at a constant rate throughout the experiment. Constant-flow perfusion was regulated to maintain a constant supply of oxygen, H_2O_2 , propofol, and Intralipid (Kabi Pharmacia, Uppsala, Sweden) to the heart throughout the experiment. The heart rate was kept constant by pacing the heart at $300 \text{ beats} \cdot \text{min}^{-1}$ throughout the study *via* stimuli (3F46, San-Ei Instru-

ments, Tokyo, Japan) applied to the left ventricle (4 V, two time threshold; 2-ms duration).

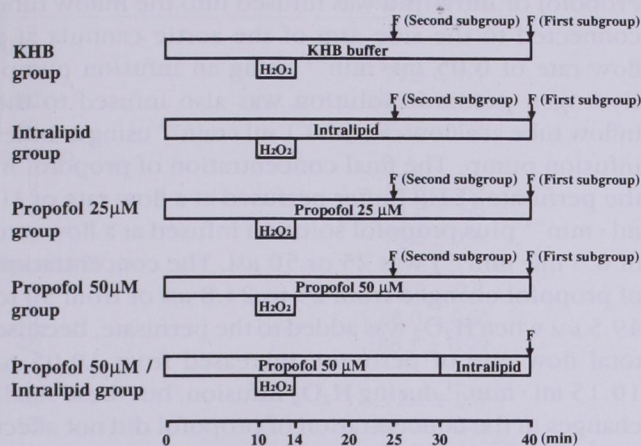
For measurement of left ventricular pressure, a saline-filled polyethylene cannula connected to a pressure transducer was inserted into the left ventricular cavity *via* the left atrium. The end of the polyethylene cannula in the left ventricle was open to the left ventricular cavity. The left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and left ventricular developed pressure (LVDP) were employed as indices of myocardial mechanical function. The values of LVSP, LVEDP, and LVDP were determined from the left ventricular pressure curves recorded continuously during the course of the study. The coronary perfusion pressure (CPP) was measured by a pressure transducer connected to the aortic cannula.

Experimental Protocol

The effects of propofol and/or vehicle solution on the mechanical function and energy metabolism in the H_2O_2 -untreated hearts (normal heart experiments) and the H_2O_2 -treated hearts (H_2O_2 -treated heart experiments) were investigated. The normal and H_2O_2 -treated heart groups were then divided into five groups: the KHB group, Intralipid group, 25- μM propofol group, 50- μM propofol group, and 50- μM propofol/Intralipid group. In these groups, all hearts were initially allowed to stabilize for 20 min under the condition of constant-flow perfusion before the observation period. The experimental protocol during the observation period is illustrated in figure 1.

H_2O_2 -treated Heart Experiments. Each of the KHB, Intralipid, 25- μM propofol, and 50- μM propofol groups was divided into two subgroups. In the first subgroup, KHB buffer, Intralipid, or propofol was infused for 40 min to evaluate the mechanical function and energy metabolism. In the second subgroup, KHB buffer, Intralipid, or propofol was infused for 25 min to evaluate lipid peroxidation. In the 50- μM propofol/Intralipid group, the hearts were infused with 50 μM propofol for 30 min followed by Intralipid for 10 min to evaluate the mechanical function and energy metabolism. KHB buffer, Intralipid, or propofol was infused into the aortic cannula at a constant flow rate of $0.05 \text{ ml} \cdot \text{min}^{-1}$. Hydrogen peroxide was infused for 4 min into the aortic cannula at a constant flow rate of $0.1 \text{ ml} \cdot \text{min}^{-1}$ from 10 min after the start of infusion of KHB buffer, Intralipid, or propofol.

Normal Heart Experiments. Each of the KHB, Intralipid, 25- μM propofol, and 50- μM propofol groups

PROPOFOL ON H_2O_2 -INDUCED MYOCARDIAL DAMAGE1. H_2O_2 -treated heart experiments

2. Normal heart experiments

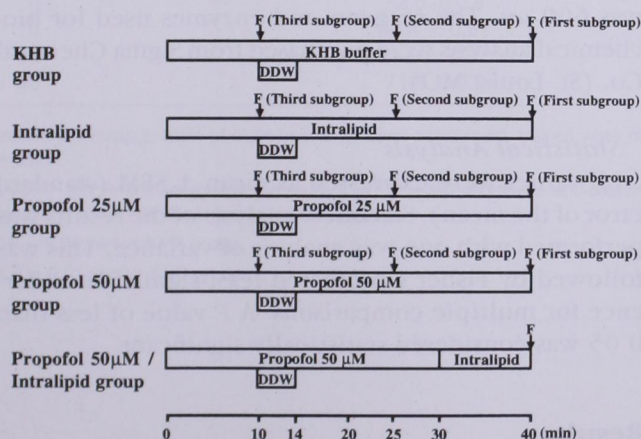


Fig. 1. Schematic representation of experimental protocol. Starting time of infusion with Krebs-Henseleit bicarbonate (KHB) buffer, Intralipid, or propofol was taken as 0 min. The effects of propofol on the mechanical function and energy metabolism were investigated in the H_2O_2 -treated and normal heart experiments. In the H_2O_2 -treated heart experiments, each of the Krebs-Henseleit bicarbonate, Intralipid, 25- μ M propofol, and 50- μ M propofol groups was divided into two subgroups; in the first subgroup the infusion was continued for 40 min, and in the second subgroup it was continued for 25 min. In the normal heart experiments, each of the Krebs-Henseleit bicarbonate, Intralipid, 25- μ M propofol, and 50- μ M propofol groups was divided into three subgroups; in the first, second, and third subgroups the infusion was continued for 40, 25, and 10 min, respectively. In the 50- μ M propofol/Intralipid group in both H_2O_2 -treated and normal heart experiments, propofol was infused for 30 min and then Intralipid was infused for 10 min. H_2O_2 (600 μ M as a final concentration in perfusate) or distilled deionized water (DDW) was infused from 10 min after to 14 min after the start of infusion with Krebs-Henseleit bicarbonate buffer, Intralipid, or propofol; H_2O_2 was infused for 4 min. All the hearts were frozen (F) for measurements of energy metabolites or malondialdehyde at the end of each infusion with Krebs-Henseleit bicarbonate buffer, Intralipid, or propofol.

was divided into three subgroups. In the first subgroup, KHB buffer, Intralipid, or propofol was infused for 40 min to evaluate the mechanical function and energy metabolism. In the second subgroup, KHB buffer, Intralipid, or propofol was infused for 25 min to evaluate lipid peroxidation. In the third subgroup, KHB buffer, Intralipid, or propofol was infused for 10 min to evaluate energy metabolism. In the 50- μ M propofol/Intralipid group, the hearts were infused with 50 μ M propofol for 30 min followed by Intralipid for 10 min to evaluate mechanical function and energy metabolism. The experimental conditions and protocol were almost the same as those in the H_2O_2 -treated heart experiments, except that distilled deionized water was infused instead of H_2O_2 .

In each experiment, LVSP, LVEDP, LVDP, and CPP were continuously recorded during a 40-min observation period. To measure the tissue concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), creatine phosphate (CrP), the hearts were frozen immediately before H_2O_2 infusion (the third subgroup in the normal heart experiment) or at the end of the observation period for 40 min (the first subgroup in the H_2O_2 -treated and normal heart experiments) with freezing clamps previously chilled in liquid nitrogen. Other hearts were frozen 11 min after the cessation of H_2O_2 infusion (the second subgroup in the H_2O_2 -treated and normal heart experiments) for measurements of the tissue concentrations of MDA.

Measurements of Metabolites

The frozen myocardial samples were stored in liquid nitrogen (at -173°C) until the biochemical analysis was performed. Each sample was pulverized in liquid nitrogen with a mortar and pestle. To measure the tissue water content and dry weight of the tissue, a part of the pulverized tissue powder was weighed and allowed to stand in a warm oven overnight. The remainder of the powder was used for determination of the tissue concentrations of ATP, ADP, AMP, CrP, and MDA.

ATP, ADP, AMP, and CrP were extracted from the samples with 6% perchloric acid. The tissue extract was centrifuged at 10,000g for 10 min at 4°C . The supernatant was neutralized with 70% potassium hydroxide and centrifuged at 10,000g for 10 min at 4°C again. The resultant solution was used for determinations of ATP, ADP, AMP, and CrP. These metabolites were measured according to the standard enzymatic

procedures.¹² Values were expressed as micromoles per gram of dry weight.

Malondialdehyde, a product of lipid peroxidation, was measured using high-performance liquid chromatography instead of using thiobarbituric acid because the latter method may measure not only MDA but also other substances.¹³ High-performance liquid chromatographic procedure was performed according to the method described by Koller and Bergmann.¹⁴ Malondialdehyde was extracted from the pulverized tissue sample with 10 mM phosphate buffer (pH 8.0), and the tissue extract was centrifuged at 10,000g for 15 min at 4°C. The supernatant was filtered through a Millipore filter (UFC3LCC, Nihon Millipore Kogyo, Yonezawa, Japan), at 2,000g for 10 min. The filtered solution (20 μ l) was injected into the high-performance liquid chromatography system (LC-9A, Shimadzu, Kyoto, Japan), equipped with a guard column (G-ODS 0.4 \times 1 cm, Shimadzu) and an analytic column (CLC-ODS 0.46 \times 25 cm, Shimadzu). Malondialdehyde was separated with 15% acetonitrile (high-performance liquid chromatography grade; Nacalai tesque, Kyoto, Japan) containing 50 mM myristyltrimethylammonium bromide (Aldrich Chemical, Milwaukee, WI) and 1.0 mM Na₂HPO₄ adjusted to pH 6.8, at a flow rate of 1.0 ml \cdot min⁻¹. The effluent was monitored at 267 nm using a spectrophotometric detector (SPD-2AS, Shimadzu). The quantitative analysis was performed by comparison with standard curves. Malondialdehyde standards were prepared by acid hydrolysis of malondialdehyde bis(dimethyl acetal) (Aldrich Chemical, Milwaukee, WI). An additional experiment was performed to determine whether propofol itself interferes with MDA measurement. The mixture of various concentrations of MDA standards and 50 μ M propofol in the 10-mm phosphate buffer (pH 8.0) was incubated for 30 min. The concentration of MDA in the buffer in the presence or absence of propofol was measured by high-performance liquid chromatography according to the method described earlier.

The following drugs were used: propofol (2,6-diisopropylphenol, Tokyo Kasei Kogyo, Tokyo, Japan), 10% Intralipid (Kabi Pharmacia, Uppsala, Sweden), sodium pentobarbital (Nacalai tesque, Kyoto, Japan), and H₂O₂ (Santoku Chemical, Tokyo, Japan). The 10% Intralipid is a solvent of commercially available propofol, and contains 10% soy bean oil, 1.2% egg lecithin, and 2.25% glycerol. Propofol was dissolved in Intralipid and further diluted with distilled deionized water. The concentration of Intralipid in the drug solution was

2%. H₂O₂ was diluted with distilled deionized water. Propofol or Intralipid was infused into the inflow tube connected to the side arm of the aortic cannula at a flow rate of 0.05 ml \cdot min⁻¹ using an infusion pump. Hydrogen peroxide solution was also infused to the inflow tube at a flow rate of 0.1 ml \cdot min⁻¹ using another infusion pump. The final concentration of propofol in the perfusate (KHB buffer perfused at a flow rate of 10 ml \cdot min⁻¹ plus propofol solution infused at a flow rate of 0.5 ml \cdot min⁻¹) was 25 or 50 μ M. The concentration of propofol changed from 25 to 24.8 μ M or from 50 to 49.5 μ M when H₂O₂ was added to the perfusate, because total flow rate of perfusate increased from 10.05 to 10.15 ml \cdot min⁻¹ during H₂O₂ infusion, but these small changes in the concentration of propofol did not affect left ventricular pressure or CPP. The final concentration of H₂O₂ in the perfusate including infusion solution was 600 μ M. The reagents and enzymes used for biochemical analysis were purchased from Sigma Chemical Co. (St. Louis, MO).

Statistical Analysis

All values were expressed as mean \pm SEM (standard error of the mean). Statistical analysis of the results was performed with one-way analysis of variance. This was followed by Fisher's protected least-significant difference for multiple comparisons. A *P* value of less than 0.05 was considered statistically significant.

Results

Effects of Propofol on Mechanical Function and Energy Metabolism in Normal Heart

Experiments were performed to examine how propofol affects myocardial mechanical function and energy metabolism in normal hearts (hearts not treated with H₂O₂) during 40 min of perfusion with KHB, Intralipid, 25 μ M propofol, or 50 μ M propofol (tables 1 and 2). In the KHB and Intralipid groups (*n* = 8 for each), myocardial mechanical function and energy metabolism did not change significantly throughout the experimental period. Propofol significantly decreased LVSP, LVDP, and CPP in a dose-dependent fashion, whereas it did not affect LVEDP and energy metabolism. In the 50- μ M propofol/Intralipid group, LVSP, LVDP, and CPP decreased significantly during the period of infusion with 50 μ M propofol but returned to the initial level (0 min value) within 10 min after the cessation of propofol infusion.

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Table 1. Effect of Propofol and/or Intralipid on the Mechanical Function and Coronary Resistance in Normal Hearts

Min	LVSP (mmHg)	LVEDP (mmHg)	LVDP (mmHg)	CPP (mmHg)
KHB				
0	71.5 ± 1.1	0.6 ± 0.2	70.9 ± 1.2	61.0 ± 1.1
10	72.1 ± 1.0	0.8 ± 0.2	71.4 ± 1.1	62.5 ± 0.8
40	72.6 ± 1.2	0.8 ± 0.3	71.9 ± 1.3	63.0 ± 1.1
Intralipid				
0	72.8 ± 0.9	0.5 ± 0.2	72.3 ± 1.0	62.4 ± 0.7
10	74.8 ± 1.5	0.9 ± 0.1	73.9 ± 1.4	63.4 ± 1.1
40	76.3 ± 1.6	0.8 ± 0.3	75.5 ± 1.5	65.1 ± 1.2
Propofol 25 μM				
0	72.0 ± 0.6	0.5 ± 0.2	71.5 ± 0.6	60.9 ± 0.8
10	68.0 ± 0.6*	0.8 ± 0.2	67.3 ± 0.6*	58.1 ± 0.6†
40	66.1 ± 0.7†	0.9 ± 0.3	65.3 ± 0.8†	56.4 ± 0.5†
Propofol 50 μM				
0	72.1 ± 0.8	0.8 ± 0.2	71.4 ± 0.8	61.4 ± 1.1
10	62.0 ± 1.4†	0.5 ± 0.2	61.5 ± 1.3†	53.1 ± 0.5†
40	61.8 ± 1.5†	0.9 ± 0.2	60.9 ± 1.5†	52.9 ± 0.5†
Propofol 50 μM/Intralipid				
0	72.6 ± 0.4	0.8 ± 0.2	71.9 ± 0.5	61.5 ± 0.3
10	60.4 ± 0.5†	0.5 ± 0.2	60.0 ± 0.4†	52.4 ± 0.3†
30	59.9 ± 0.7†	0.5 ± 0.2	59.4 ± 0.9†	52.4 ± 0.5†
40	71.8 ± 0.9	0.8 ± 0.2	71.0 ± 1.0	59.6 ± 0.8

Values are mean ± SEM of eight hearts for each experiment. Values were obtained at 0, 10, 30 and 40 min after the start of KHB buffer, Intralipid, or propofol infusion.

LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure; LVDP = left ventricular developed pressure; CPP = coronary perfusion pressure; KHB = Krebs-Henseleit bicarbonate.

* $P < 0.05$ versus KHB group.

† $P < 0.01$ versus KHB group.

Table 2. Effect of Propofol and/or Intralipid on the Energy Metabolism in Normal Hearts

Min	CrP (μmol/g dry wt)	ATP (μmol/g dry wt)	ADP (μmol/g dry wt)	AMP (μmol/g dry wt)
KHB				
10	22.9 ± 0.7	19.4 ± 0.6	4.5 ± 0.2	1.1 ± 0.1
40	17.7 ± 0.7	18.9 ± 0.5	5.2 ± 0.2	1.2 ± 0.1
Intralipid				
10	21.1 ± 0.5	18.8 ± 0.6	4.6 ± 0.0	1.0 ± 0.0
40	17.9 ± 1.1	19.2 ± 0.7	5.4 ± 0.2	1.3 ± 0.1
Propofol 25 μM				
10	21.9 ± 1.9	19.7 ± 0.1	4.6 ± 0.1	1.0 ± 0.0
40	17.5 ± 0.5	19.9 ± 0.6	5.2 ± 0.1	1.2 ± 0.0
Propofol 50 μM				
10	21.7 ± 1.2	20.0 ± 0.3	4.9 ± 0.1	1.2 ± 0.0
40	19.4 ± 0.6	19.0 ± 0.8	4.8 ± 0.2	1.2 ± 0.1
Propofol 50 μM/Intralipid				
40	17.4 ± 0.6	18.5 ± 0.3	4.8 ± 0.1	1.2 ± 0.0

Values are mean ± SEM of eight hearts for each experiment. Values were obtained at 10 and 40 min after the start of KHB buffer, Intralipid, or propofol infusion. Values of "10 min" in the propofol 50 μM/Intralipid group were not determined because they correspond with values of "10 min" in the propofol 50 μM group. No significant differences were detected when compared with the value in the KHB group ($P > 0.05$).

CrP = creatine phosphate; ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate; KHB = Krebs-Henseleit bicarbonate.

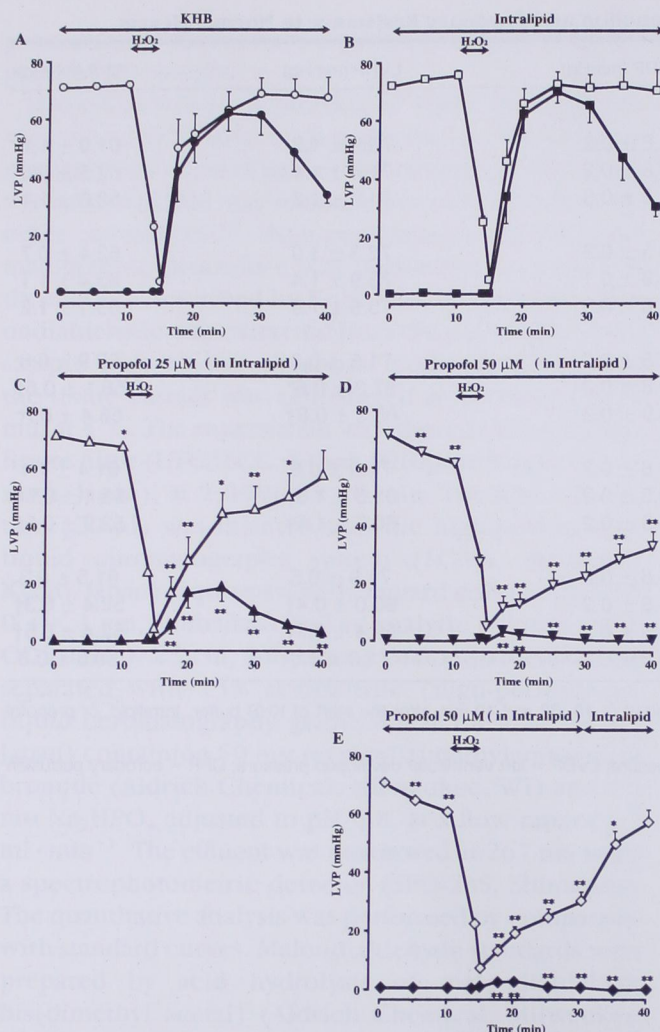


Fig. 2. Effect of propofol in Intralipid or Intralipid alone on mechanical function in hydrogen peroxide-treated heart. Changes of left ventricular pressure in Krebs-Henseleit bicarbonate (A), Intralipid (B), 25- μ M propofol (C), 50- μ M propofol (D), or 50- μ M propofol/Intralipid (E) groups are recorded. Open symbols indicate left ventricular systolic pressure, and solid symbols indicate left ventricular end-diastolic pressure. Each value represents mean \pm SEM ($n = 8$ in each group). * $P < 0.05$ and ** $P < 0.01$ when compared with value in the Krebs-Henseleit bicarbonate group.

Effects of Propofol on H_2O_2 -induced Changes of Mechanical Function and Coronary Resistance

Changes in left ventricular pressure induced by H_2O_2 are shown in figure 2. Before starting the H_2O_2 infusion, there were no significant differences in mechanical function between the normal and H_2O_2 -treated heart groups. In both KHB ($n = 8$) and Intralipid ($n = 8$) groups, H_2O_2 induced a temporary but severe decrease in LVSP (figs. 2A and 2B). Left ventricular end-diastolic

pressure also increased markedly after the H_2O_2 infusion, and the increase that was accompanied by elevation of the LVSP to the pre- H_2O_2 level, lasted throughout the observation period. Consequently, LVDP decreased significantly after the H_2O_2 infusion and did not recover to the pre- H_2O_2 level by the end of the experiment (fig. 3). These results indicate that H_2O_2 produced a long-lasting mechanical dysfunction, although LVSP returned to the control level within 15 min of the cessation of H_2O_2 infusion.

Before the start of H_2O_2 infusion, propofol induced a dose-dependent decrease in LVSP. In the presence of propofol, H_2O_2 was infused for 4 min. Hydrogen peroxide produced a temporary and severe decrease in LVSP in both the 25- and 50- μ M propofol groups ($n = 8$ for each group; figs. 2C and 2D). Increases in LVDP and LVSP after the end of H_2O_2 infusion, however, were significantly attenuated by propofol when compared with the KHB and the Intralipid groups. After 40 min of infusion, there was no significant difference in LVSP between the KHB and the 25- μ M propofol groups ($P > 0.05$), whereas LVSP in the 50- μ M propofol group was significantly lower than that in the KHB group ($P < 0.05$). Consequently, LVDP in the 25- μ M propofol group was significantly higher than that in the 50- μ M propofol and KHB groups (fig. 3).

There is a possibility that the depressed recovery of LVSP and LVDP in the 50- μ M propofol group is due to the cardiodepression induced by propofol itself. For

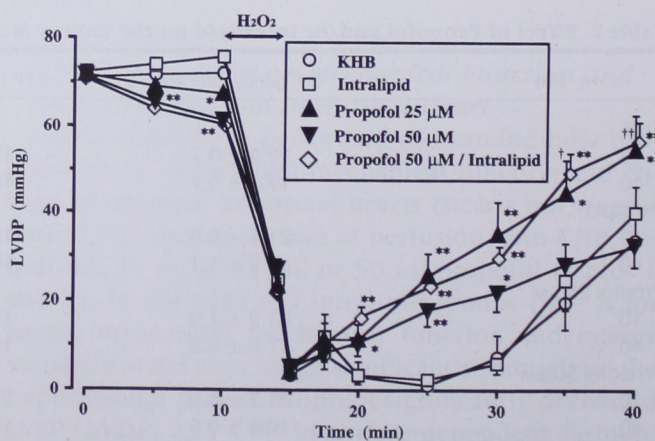


Fig. 3. Effect of propofol in Intralipid or Intralipid alone on left ventricular developed pressure in hydrogen peroxide-treated heart. Hearts are those in figure 2. Each value represents mean \pm SEM ($n = 8$ in each group). * $P < 0.05$ and ** $P < 0.01$ when compared with value in the Krebs-Henseleit bicarbonate group. † $P < 0.05$ and ‡ $P < 0.01$ when compared with value in the 50- μ M propofol group.

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this purpose, in the H₂O₂-treated heart experiments, we perfused the heart with 50 μ M propofol for 30 min and then with Intralipid for 10 min (50- μ M propofol/Intralipid group, $n = 8$), and compared the recovery of mechanical function between when the 50 μ M propofol infusion was performed during the whole course of the experiment (40 min) and when it was performed for 30 min and stopped. Changes in LVSP, LVEDP, and LVEDP during propofol infusion in the 50- μ M propofol/Intralipid group were similar to those in the 50- μ M propofol group. LVSP and LVDP increased greatly after the cessation of propofol infusion; these values at the end of experiment in the 50- μ M propofol/Intralipid group were significantly higher than those in the 50- μ M propofol group and similar to those in the 25- μ M propofol group (figs. 2 and 3). These results indicate that the cessation of propofol infusion does not reduce the recovery of mechanical function, or rather, it improves mechanical function (LVSP and LVDP) that has been suppressed by propofol.

Figure 4 shows changes in CPP throughout the experimental period. Hydrogen peroxide produced a transient decrease followed by a long-lasting increase in CPP in each of the five groups. Before the H₂O₂ infusion, there was a statistically significant decrease in CPP in both the 25- and 50- μ M propofol groups when compared with the KHB and the Intralipid groups. Increase in CPP after H₂O₂ infusion was significantly attenuated in either the 25- μ M propofol, the 50- μ M propofol, or the 50- μ M propofol/Intralipid group.

Effect of Propofol on H₂O₂-induced Changes of Myocardial Energy Metabolism

Figures 5 and 6 show the tissue concentrations of ATP, ADP, AMP, and CrP in the five groups 10 and 40 min after KHB buffer, Intralipid, or propofol infusions. There were no significant differences in the tissue concentrations of energy metabolites at 10 min (immediately before the H₂O₂ infusion) among the KHB, Intralipid, or propofol groups. The tissue concentrations of ATP, ADP, and CrP at the end of the experiment in the propofol 25- and 50- μ M groups were higher than in the KHB and the Intralipid groups (40 min in figs. 5 and 6). Decreases in ATP and ADP induced by H₂O₂ were significantly attenuated by propofol. In contrast, CrP did not change after H₂O₂ infusion in the 25- μ M propofol group, whereas it increased in the 50- μ M propofol group. The tissue concentrations of ATP and CrP at the end of experiment in the 50- μ M propofol/Intralipid group were higher than those in the KHB and

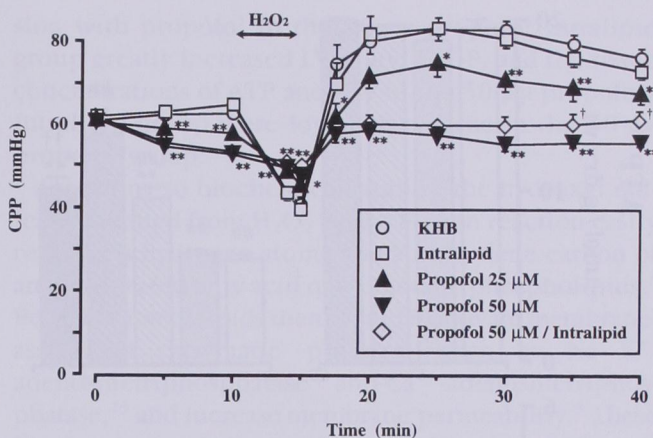


Fig. 4. Effect of propofol in Intralipid or Intralipid alone on coronary perfusion pressure in hydrogen peroxide-treated heart. Hearts are those in figure 2. Each value represents mean \pm SEM ($n = 8$ in each group). * $P < 0.05$ and ** $P < 0.01$ when compared with value in the Krebs-Henseleit bicarbonate group. † $P < 0.05$ and ‡ $P < 0.01$ when compared with value in the 50- μ M propofol group.

the Intralipid groups, but lower than those in the 50- μ M propofol group (40 min in figs. 5 and 6). The ratio of wet/dry tissue weight calculated for quantitative analysis of energy metabolites was not different among all groups in the both normal and H₂O₂-treated heart experiments.

Effect of Propofol on Lipid Peroxidation Induced by H₂O₂

To examine whether the beneficial effects of propofol on the H₂O₂-induced mechanical dysfunction and metabolic changes correlate with reduction of lipid peroxidation, the tissue concentration of MDA was measured 25 min after the start of infusion (*i.e.*, 11 min after the end of H₂O₂ infusion; fig. 7). The reason that a 25-min interval was chosen is that H₂O₂ produced the greatest increase in LVEDP in the KHB and the Intralipid groups around that time (fig. 2). In the KHB and Intralipid groups, H₂O₂ produced a significant increase in the MDA concentration by approximately 5 and 7 times their control values without H₂O₂, respectively. In contrast, the tissue concentration of MDA in both 25- and 50- μ M propofol groups did not increase even after H₂O₂ infusion. Propofol (25 and 50 μ M) also decreased the tissue MDA concentration in the normal heart (fig. 7). Nevertheless, propofol did not interfere with measurement of MDA (data not shown). These results suggest that propofol prevents the H₂O₂-induced lipid peroxidation.

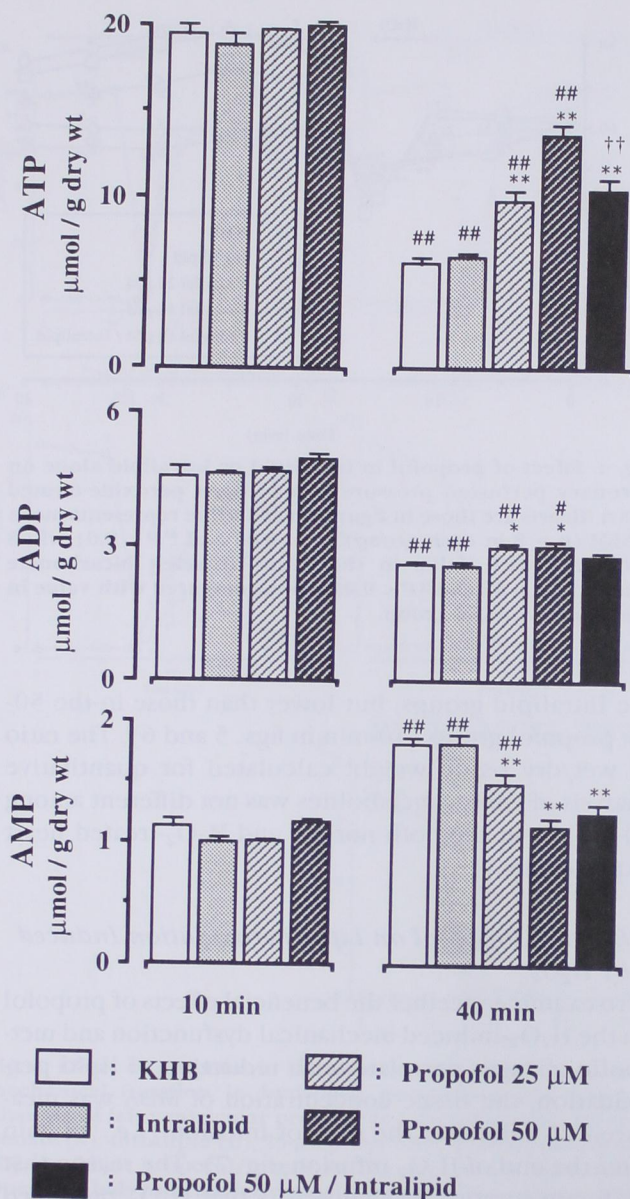


Fig. 5. Effect of propofol in Intralipid or Intralipid alone on hydrogen peroxide-induced changes in tissue concentrations of adenine nucleotides, which were measured immediately before hydrogen peroxide infusion (10 min after start of Krebs-Henseleit bicarbonate buffer, Intralipid, or propofol infusion; 10 min) and at end of experiment (40 min after start of Krebs-Henseleit bicarbonate buffer, Intralipid, or propofol infusion; 40 min). Values of "10 min" are those in table 2. Values of the metabolites measured are expressed as $\mu\text{moles/g dry weight}$ (mean \pm SEM; $n = 8$ in each group). ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate. * $P < 0.05$ and ** $P < 0.01$ when compared with value in the Krebs-Henseleit bicarbonate group. # $P < 0.05$ and ## $P < 0.01$ when compared with the respective value immediately before hydrogen peroxide infusion (10 min). † $P < 0.05$ and †† $P < 0.01$ when compared with value in the 50- μM propofol group.

Discussion

Many studies have addressed the actions of substances that protect the myocardium from reactive oxygen-induced damage. For example, nifedipine and propranolol inhibit the lipid peroxidation of the sarcolemmal membrane induced by oxidative stress.¹⁵ Catalase, lidocaine,⁷ and agonists for adenosine A_1 receptor¹⁶ attenuate the mechanical dysfunction and metabolic changes induced by H_2O_2 in the isolated perfused heart. In the current study, we examined the effect of propofol on mechanical dysfunction, metabolic changes, and lipid peroxidation induced by H_2O_2 in the isolated perfused rat heart.

The reasons we used H_2O_2 as a source of reactive oxygen metabolite can be summarized as follows: (1)

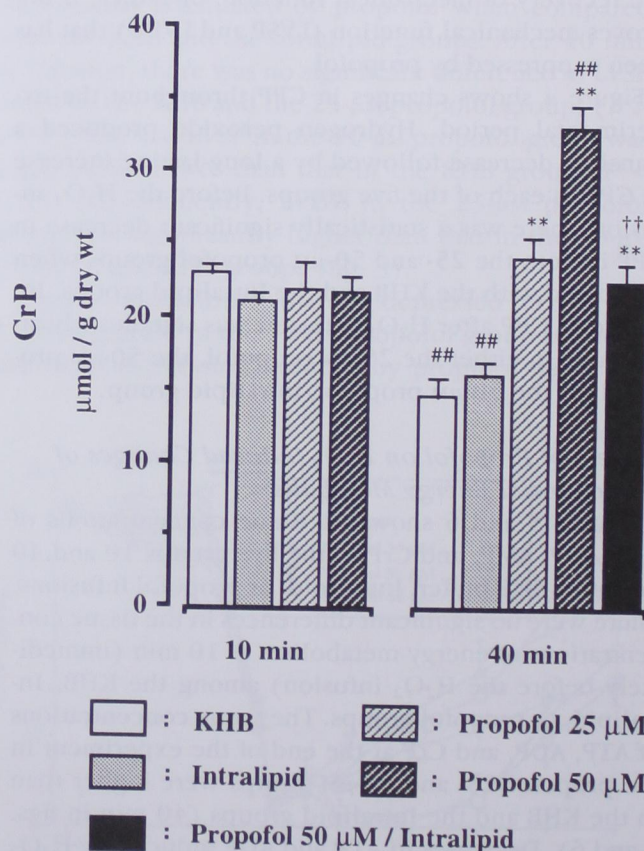


Fig. 6. Effect of propofol in Intralipid or Intralipid alone on hydrogen peroxide-induced changes in tissue concentrations of creatine phosphate (CrP). Hearts are those in figure 5. Each value represents mean \pm SEM ($n = 8$ in each group). ** $P < 0.01$ when compared with value in the Krebs-Henseleit bicarbonate group. ## $P < 0.01$ when compared with the respective value immediately before hydrogen peroxide infusion (10 min). # $P < 0.05$ and †† $P < 0.01$ when compared with value in the 50- μM propofol group.

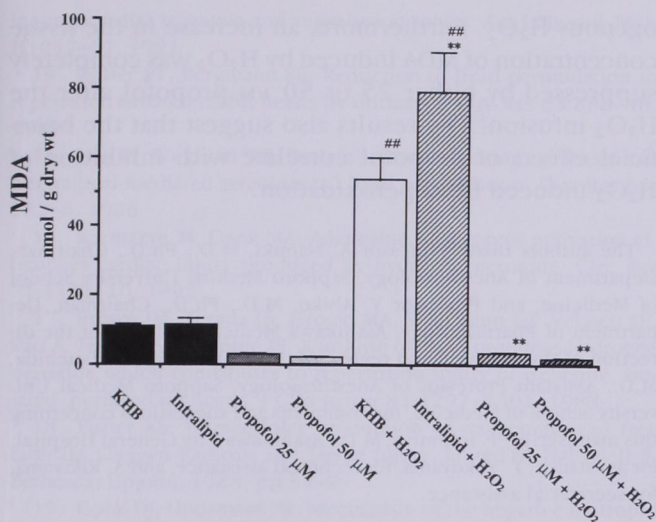
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Fig. 7. Effect of propofol in Intralipid or Intralipid alone on hydrogen peroxide-induced changes of the tissue concentration of malondialdehyde, which was measured 25 min after start of Krebs-Henseleit bicarbonate buffer, Intralipid, or propofol infusion (11 min after hydrogen peroxide infusion). Each value represents means \pm SEM ($n = 8$ in each group). ** $P < 0.01$ when compared with value in the hydrogen peroxide-treated Krebs-Henseleit bicarbonate group (KHB + H₂O₂). ## $P < 0.01$ when compared with the respective value in hydrogen peroxide-untreated (normal) heart.

H₂O₂ and its metabolite, the hydroxyl radical, are known to be important in the pathogenesis of myocardial damage induced by ischemia-reperfusion¹⁷; and (2) H₂O₂ penetrates the cell membrane, reaching intracellular sites,¹⁸ and may therefore induce severe damage to the myocardial cell. The current study demonstrated that H₂O₂ produced mechanical dysfunction (as evidenced by an increase in LVEDP and a decrease in LVDP), an increase in coronary resistance (as evidenced by an increase in CPP), and metabolic changes (as evidenced by a decrease in the tissue ATP and CrP concentrations). These alterations induced by H₂O₂ were attenuated by 25 μ M and 50 μ M propofol, suggesting that propofol has a beneficial effect on the H₂O₂-induced mechanical dysfunction and metabolic derangements.

We found that the recovery of LVDP after H₂O₂ infusion was better in the 25- μ M propofol group than that in the 50- μ M propofol group. However, high-energy phosphate after the H₂O₂ infusion was better preserved in the 50- μ M propofol group than that in the 25- μ M propofol group. This is probably because propofol itself induced dose-dependent decrease in mechanical function,¹⁹⁻²¹ which may preserve energy in the H₂O₂-treated hearts. In fact, the cessation of infu-

sion with propofol in the 50- μ M propofol/Intralipid group greatly increased LVSP and LVDP, and the tissue concentrations of ATP and CrP in the 50- μ M propofol/Intralipid group were lower than those in the 50- μ M propofol group.

According to biochemical studies, the hydroxyl radical generated from H₂O₂ by the Fenton reaction easily removes a hydrogen atom from a methylene carbon of an unsaturated fatty acid of membrane phospholipids.³ Peroxidation of lipids then would inactivate membrane-associated enzymatic proteins, such as Na⁺-K⁺-adenosinetriphosphatase²² and Ca²⁺-adenosinetriphosphatase,²³ and increase membrane permeability.³ These biochemical and physicochemical alterations of the cell membrane may be responsible for mechanical and metabolic derangements.

To test the hypothesis that the beneficial effects of propofol on the H₂O₂-induced mechanical dysfunction and metabolic changes correlate with reduction of lipid peroxidation induced by H₂O₂, we measured the tissue MDA concentration 11 min after the H₂O₂ infusion, when H₂O₂ produced the greatest increase in LVEDP in the KHB or Intralipid groups. In the KHB and the Intralipid groups, H₂O₂ increased the tissue concentration of MDA significantly. The increase in the tissue MDA concentration in the Intralipid group was greater than that in the KHB group. There may be some interaction between H₂O₂ and Intralipid, because Intralipid contains egg lecithin (phosphatidylcholine). Nevertheless, both 25 and 50 μ M propofol completely suppressed the H₂O₂-induced increase in tissue MDA. Our findings suggest that the beneficial effects of propofol on H₂O₂-induced myocardial damage correlate with inhibition of lipid peroxidation. According to reports on propofol's antioxidant effect,⁹⁻¹¹ it is assumed that propofol behaves in the same way as phenol-based antioxidants, which scavenge free radical species (e.g., hydroxyl radical and lipid peroxy radical) by a process of hydrogen abstraction and thereby themselves become a less reactive phenoxyl radical.

It should be noted, however, that propofol did not attenuate completely the H₂O₂-induced mechanical dysfunction (an increase in LVEDP and a decrease in LVDP) and metabolic derangements (a decrease in the tissue ATP and CrP concentrations), in spite of the complete inhibition of lipid peroxidation. Recently, Kong *et al.*²⁴ reported that prevention of lipid peroxidation does not prevent myocardial contractile dysfunction induced by reactive oxygen in isolated rabbit hearts. Therefore, factors other than lipid peroxidation

may play an important role in myocardial damage induced by reactive oxygen. In fact, oxidation of sulfhydryl groups in protein or nonprotein has been demonstrated to contribute to myocardial damage induced by reactive oxygen.²⁵ These biochemical alterations, which are not attributed to lipid peroxidation of the cell membrane, may account for the incomplete action of propofol on H₂O₂-induced mechanical and metabolic derangements.

Propofol inhibited preferentially the H₂O₂-induced increase in LVEDP rather than the H₂O₂-induced decrease in LVDP. The beneficial action of propofol on the increase in LVEDP may be due to inhibition of intracellular Ca²⁺ accumulation, because myocardial contracture induced by H₂O₂ is, at least in part, mediated by an increase in intracellular Ca²⁺.²⁶ Cook and Housmans¹⁹ suggested that propofol inhibits the slow inward L-type Ca²⁺ current across the sarcolemma. Although the beneficial role of nisoldipine and flunarazine against H₂O₂-induced mechanical dysfunction has been demonstrated,[‡] there is no direct evidence to show that the beneficial effect of propofol to attenuate the H₂O₂-induced mechanical dysfunction and metabolic derangements is caused by inhibition of the Ca²⁺ current. Further studies are needed to determine the detailed mechanism of the protective action of propofol on the H₂O₂-induced changes.

Peak plasma concentration of propofol after an intravenous induction dose increases to as much as 44 μ M,²⁷ whereas stable plasma concentrations of approximately 10–20 μ M are observed during a maintenance period.²⁸ Furthermore, more than 90% of propofol is bound to plasma protein.²⁹ The concentration range tested in this study therefore is considerably greater than that likely to be encountered during clinical practice. However, even trace plasma concentrations of propofol may play a crucial role in protecting the cell membrane against oxidant stress as well as vitamin E.^{9,30} It may also be that propofol that is bound to proteins or that which is present in membranes may still be able to perform its antioxidant role in these settings, so that free drug concentration is less important than in receptor or partitioning studies.

In conclusion, the results of the current study demonstrate that propofol attenuates both mechanical dysfunction and metabolic derangements induced by ex-

ogenous H₂O₂. Furthermore, an increase in the tissue concentration of MDA induced by H₂O₂ was completely suppressed by either 25 or 50 μ M propofol after the H₂O₂ infusion. The results also suggest that the beneficial effects of propofol correlate with inhibition of H₂O₂-induced lipid peroxidation.

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References

1. Halliwell B, Gutteridge JMC: Free Radicals in Biology and Medicine. 2nd edition. Oxford, Clarendon, 1989, pp 22–81, 237–45
2. Lucchesia BR: Myocardial ischemia, reperfusion and free radical injury. *Am J Cardiol* 65:141–231, 1990
3. Hess ML, Manson NH: Molecular oxygen: Friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. *J Mol Cell Cardiol* 16:969–85, 1984
4. Nakaya H, Tohse N, Kanno M: Electrophysiological derangements induced by lipid peroxidation in cardiac tissue. *Am J Physiol* 253:H1089–97, 1987
5. Josephson RA, Silverman HS, Lakatta EG, Stern MD, Zweier JL: Study of the mechanisms of hydrogen peroxide and hydroxyl free radical-induced cellular injury and calcium overload in cardiac myocytes. *J Biol Chem* 266:2354–61, 1991
6. Hayashi H, Miyata H, Watanabe H, Kobayashi A, Yamazaki N: Effects of hydrogen peroxide on action potentials and intracellular Ca²⁺ concentration of guinea pig heart. *Cardiovasc Res* 23:767–73, 1989
7. Hara A, Matsumura H, Abiko Y: Lidocaine attenuates both mechanical and metabolic changes induced by hydrogen peroxide in the rat heart. *Am J Physiol* 265:H1478–85, 1993
8. Hara A, Abiko Y: Protective effect of hypoxia on mechanical and metabolic changes induced by hydrogen peroxide in rat hearts. *Am J Physiol* 268:H614–20, 1995
9. Murphy PG, Myers DS, Davies MJ, Webster NR, Jones JG: The antioxidant potential of propofol (2,6-diisopropylphenol). *Br J Anaesth* 68:613–8, 1992
10. Eriksson O, Pollesello P, Saris N-EL: Inhibition of lipid peroxidation in isolated rat liver mitochondria by the general anaesthetic propofol. *Biochem Pharmacol* 44:391–3, 1992
11. Musacchio E, Rizzoli V, Bianchi M, Bindoli A, Galzigna L: Antioxidant action of propofol on liver microsomes, mitochondria and brain synaptosomes in the rat. *Pharmacol Toxicol* 69:75–7, 1991
12. Bergmeyer HU: Methods of Enzymatic Analysis. 2nd edition. New York, Academic, 1974, pp 1777–81, 2101–10, 2127–31
13. Ceconi C, Cargnoni A, Pasini E, Condorelli E, Curello S, Ferrari R: Evaluation of phospholipid peroxidation as malondialdehyde dur-

‡ Britnell SL, Nayler WG: The protective effect of calcium antagonists against oxidative injury (abstract). *J Mol Cell Cardiol* 25:1, 1993.

PROPOFOL ON H₂O₂-INDUCED MYOCARDIAL DAMAGE

ing myocardial ischemia and reperfusion injury. *Am J Physiol* 260: H1057-61, 1991

14. Koller PT, Bergmann SR: Reduction of lipid peroxidation in reperfused isolated rabbit hearts by diltiazem. *Circ Res* 65:838-46, 1989

15. Mak IT, Weglicki WB: Protection by β -blocking agents against free radical-mediated sarcolemmal lipid peroxidation. *Circ Res* 63: 262-6, 1988

16. Karmazyn M, Cook MA: Adenosine A₁ receptor activation attenuates cardiac injury produced by hydrogen peroxide. *Circ Res* 71:1101-10, 1992

17. Brown JM, Terada LS, Grosso MA, Whitmann GJ, Velasco SE, Patt A, Harken AH, Repine JE: Xanthine oxidase produces hydrogen peroxide which contributes to reperfusion injury of ischemic, isolated, perfused rat hearts. *J Clin Invest* 81:1297-1301, 1988

18. Fisher AB: Intracellular production of oxygen-derived free radicals, Oxygen Radicals and Tissue Injury. Edited by Halliwell B. Bethesda, Upjohn, 1988, pp 34-9

19. Cook DJ, Housmans PR: Mechanism of the negative inotropic effect of propofol in isolated ferret ventricular myocardium. *ANESTHESIOLOGY* 80:859-71, 1994

20. Mouren S, Baron J-F, Albo C, Szekely B, Arthaud M, Viars P: Effects of propofol and thiopental on coronary blood flow and myocardial performance in an isolated rabbit heart. *ANESTHESIOLOGY* 80: 634-41, 1994

21. Stowe DF, Bosnjak ZJ, Kampine JP: Comparison of etomidate, ketamine, midazolam, propofol, and thiopental on function and metabolism of isolated hearts. *Anesth Analg* 74:547-58, 1992

22. Kramer JH, Mak IT, Weglicki WB: Differential sensitivity of canine cardiac sarcolemmal and microsomal enzymes to inhibition by free radical-induced lipid peroxidation. *Circ Res* 55: 120-4, 1984

23. Kaneko M, Beamish RE, Dhalla NS: Depression of heart sarcolemmal Ca²⁺ pump activity by oxygen free radicals. *Am J Physiol* 256:H368-74, 1989

24. Kong Y, Lesnefsky EJ, Ye J, Horwitz LD: Prevention of lipid peroxidation does not prevent oxidant-induced myocardial contractile dysfunction. *Am J Physiol* 267:H2371-7, 1994

25. Lesnefsky EJ, Dauber IM, Horwitz LD: Myocardial sulfhydryl pool alterations occur during reperfusion after brief and prolonged myocardial ischemia in vivo. *Circ Res* 68:605-13, 1991

26. Corretti MC, Koretsune Y, Kusuoka H, Chacko VP, Zweier JL, Marban E: Glycolytic inhibition and calcium overload as consequences of endogenously generated free radicals in rabbit hearts. *J Clin Invest* 88:1014-25, 1991

27. Cockshott ID: Propofol (Diprivan) pharmacokinetics and metabolism: An overview. *Postgrad Med J* 61(suppl 3):45-50, 1985

28. Servin F, Desmonts JM, Haberer JP, Cockshott ID, Plummer GF, Farinotti R: Pharmacokinetics and protein binding of propofol in patients with cirrhosis. *ANESTHESIOLOGY* 69:887-91, 1988

29. Morgan DJ, Campbell GA, Crankshaw DP: Pharmacokinetics of propofol when given by intravenous infusion. *Br J Clin Pharmacol* 30:144-8, 1990

30. Burton GW, Ingold KU: Vitamin E as an *in vitro* and *in vivo* antioxidant. *Ann NY Acad Sci* 570:7-22, 1989