Effects of Propofol on Ischemia and Reperfusion in the Isolated Rat Heart Compared with Thiamylal

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SUMMARY

The aim of the present study was to investigate whether clinical doses of propofol and thiamylal affect oxygen free radical production and intracellular calcium concentration ([Ca^{2+}]i) in the post-ischemic reperfused heart. Forty-eight rat hearts were perfused with a Langendorff system and loaded with Fura-2/AM as a [Ca^{2+}]i marker. The hearts were divided into 6 groups as follows (each group: n = 8); Group S (saline), Group TL (thiamylal 100 µM), Group TH (thiamylal 300 µM), Group I (Intralipid), Group PL (propofol 3 µM), and Group PH (propofol 10 µM). All hearts were initially perfused for 5 min as control aerobic perfusion. Afterwards, no-flow ischemia was induced for 15 min, followed by reperfusion for 20 min. The formation of hydroxyl radicals in the coronary effluent was measured with high performance liquid chromatography using salicylic acid. At the beginning of the ischemia and reperfusion periods, increases in systolic and diastolic [Ca^{2+}]i were observed in all groups except Group TH. The high dose of thiamylal significantly suppressed this initial increase in cytosolic [Ca^{2+}]i (Group S 1.30 ± 0.15; Group TL 0.99 ± 0.17; Group TH 0.70 ± 0.09, at 1 min after reperfusion; systolic [Ca^{2+}]i: p < 0.05). Total DHBAs in the coronary effluent of all groups increased significantly 1 min after reperfusion, however, there were no significant differences among the groups. Clinical doses of propofol had no significant effect on myocardial function and [Ca^{2+}]i before and after ischemia, whereas thiamylal suppressed the increase in [Ca^{2+}]i during ischemia and reperfusion. However, free radical formation during reperfusion was unaffected by thiamylal and propofol. (Jpn Heart J 2001; 42: 193-206)

Key words: Heart, Hydroxyl radical, Intracellular calcium concentration, Propofol, Thiamylal

PROPOFOL (2,6-diisopropylphenol) is widely used for the induction and maintenance of anesthesia because of its quick onset of action and its favorable pharmacokinetic profile for early emergence after discontinuation of an intravenous administration. The cardiovascular effects of propofol include decreases in arterial pressure as a result of reduced cardiac output and peripheral vasodilation.1) At
clinical doses, propofol depresses myocardial contractility less than ultra-short acting barbiturates that are also widely used as an induction agent.2-4) Thus, it is thought to be suitable for use as a total intravenous anesthetic agent. Recently, propofol has been shown to attenuate experimental reperfusion injury5,6) or hydrogen peroxide-induced alterations in the isolated rat heart.7) However, the precise mechanism has yet to be elucidated. Reperfusion injury is associated with a rise in the intracellular calcium concentration \([\text{Ca}^{2+}]\), after ischemia. We measured \([\text{Ca}^{2+}]\), as well as the concentration of the hydroxyl radical in the coronary effluent of a reperfused rat heart model. The aim of this study was to investigate whether propofol decreases oxygen free radical formation and \([\text{Ca}^{2+}]\), in the post-ischemic reperfused heart. We also compared this agent with thiamylal, which is used an induction agent in clinical situations.

**Methods**

The experiments were performed in accordance with the Guidelines for Animal Experiments of Yamanashi Medical University.

**Langendorff perfusion system:** Forty-eight Wistar-ST rats weighing 280-340 g were anesthetized by administering 6% sevoflurane. Hearts were excised and promptly immersed in the perfusion medium (modified Krebs Henseleit bicarbonate buffer [KHBB]) at 4°C. The aorta was cannulated and retrograde arterial perfusion was started at a constant flow of 16.2 ml/min by a perfusion pump (505S, Watson-Marlow, USA). The perfusion solution was modified KHBB containing (in mM) NaCl 118, NaHCO3 25, KCl 4.7, KH2PO4 1.2, MgSO4 1.2, CaCl2 2.0, di-NaEDTA 0.5, salicylic acid 1.0 and glucose 11. The KHBB solution was maintained at 37°C and gassed with 95% O2 and 5% CO2.

**Hemodynamic measurements:** A thin latex balloon was inserted into the left ventricle through the mitral valve and connected to a pressure transducer (P10EZ, Gould, Oxnard, CA, USA) for continuous monitoring of left ventricular pressure (LVP). The balloon volume was adjusted with water to maintain the left ventricular end-diastolic pressure (LVEDP) at 5-10 mmHg. A catheter was inserted into the pulmonary artery to collect coronary venous return for measuring PO2 (Instrumentation Laboratory Model 1306, Lexington, MA, USA). Myocardial oxygen consumption (MVO2; \(\mu\)moles·min⁻¹·g⁻¹) was calculated as O2 solubility multiplied by coronary flow per gram heart tissue multiplied by the difference between inflow and outflow O2 tensions.

**Fluorescence recording apparatus:** Fluorescence was recorded using a fluorescence spectrometer (CAF-110, Japan Spectroscopic Co., Ltd, Tokyo). Fluorescence excitations were filtered from a xenon lamp and directed through a fiber-optic cable (FB-50, Japan Spectroscopic) the end of which was close to the ante-
rior epicardial surface of the left ventricle. Fluorescence emissions were collected with another fiber optics system and directed through a beam splitter into photomultipliers fitted with an optical band-pass filters. Fluorescence intensities at individual wavelengths and the ratio of fluorescence intensities were recorded simultaneously using an analogue-digital convertor (CA-200DP, Japan Spectroscopic) and polygraph recorder (Nihon Kohden, Tokyo).

**Measurement of intracellular calcium:** To measure $[\text{Ca}^{2+}]_i$, 1 mM Fura-2/AM (100 µl), (Molecular Probes, Inc., Oregon, USA), a calcium sensitive fluorescence dye, was solubilized in 25 µl of dimethyl sulfoxide (DMSO) containing cremophol (25 % wt/vol) and then mixed with 25 ml of KHBB to yield a final concentration of 4 µM. Hearts were loaded with this 4 µM Fura-2/AM solution by perfusion for 30 min at 25°C, after which they were washed out with KHBB for 15 min. During the washout period, the perfusate was slowly warmed to 37°C. The $[\text{Ca}^{2+}]_i$ was estimated by determining the ratio of the emission at 500 nm during excitation at 340 nm to the emission at 500 nm during excitation at 380 nm (the Fura-2/AM-dependent fluorescent ratio). Background fluorescence (auto-fluorescence of the heart before loading) was subtracted from total fluorescence. Preliminary studies indicated that there were no background fluorescence changes between the pre-ischemia and post-ischemia periods.

**Determination of dihydroxybenzoic acids (DHBAs):** Hydroxyl radicals add to the phenolic ring of salicylic acid to yield 2,3-, 2,4-, 2,5- and 3,4-DHBAs. The high pressure liquid chromatography apparatus with electrochemical detection (LCED) consisted of a Shimadzu Model LC-10AD pump and a detector module (C-R4A, CHROMATOPAC, Shimadzu, Tokyo). The column used was a Shim-Pack, CLC-ODS, 15 cm × 4.6 mm. Ninety percent of the mobile phase consisted of 20 mM sodium dihydrogenphosphate, 1 mM octanesulfonic acid sodium salt and 10 mM sodium sulphate, and 10% was acetonitrile. The flow rate was 0.5 ml/min. The mobile phase was kept anaerobic by N₂ (DEGASSER, DGU-3A, Shimadzu). 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-DHBAs and salicylic acid.

One ml of the perfusate from the coronary artery was treated with 1 ml of 1 M Tris buffer and extracted with 2 ml of methanol on a Vortex mixer for 10 min. Aluminium oxide (50 mg) was added to separate the methanol layer. The residue was dissolved in 200 µl of 0.2 M perchloric acid and 5µl of this solution was injected into the LCED unit. This method is a modification of that described by Floyd, et al.8,9)

**Experimental protocol (Figure 1):** After loading the perfused heart with fluorescence dye solution followed by washing out, the hearts were divided into 6
groups as follows; 1. Group S (control; n = 8): received saline. 2. Group TL (n = 8): received thiamylal 100 µM. 3. Group TH (n = 8): received thiamylal 300 µM. 4. Group I (control for propofol; n = 8): received fat emulsion (Intralipid®; Pharmacia AB, Stockholm, Sweden). 5. Group PL (n = 8): received propofol 3 µM. 6. Group PH (n = 8): received propofol 10 µM. These agents were administered during the initial perfusion and during 5 min reperfusion after ischemia. All hearts were perfused with KHBB at 37°C for 5 min as aerobic control perfusion. Afterwards, sustained normothermic no-flow ischemia and pacing at 333 bpm were then induced for 15 min by clamping the aortic cannula. The hearts were reperfused for 20 min at a constant flow of 16.2 ml·min⁻¹ and the KHBB was switched to normal KHBB (no drugs) during the last 15 min reperfusion. Measurements of [Ca²⁺], transients, LVP and heart rate were recorded before and at various times after the onset of ischemia. The coronary effluent was collected to measure DHBAs before and 1 min after ischemia.

**Statistical analysis:** Hemodynamic data were analyzed by two-way analysis of variance with repeated measures and the paired t-tests with Bonferroni correction. Other data were analyzed by one-way analysis of variance followed by
**Figure 2.** Heart rate changes. *p < 0.05.
Figure 3. Changes in left ventricular dP/dt maximum. * p < 0.05.
Figure 4. Myocardial oxygen consumption.
Figure 5. Systolic [Ca\textsuperscript{2+}]\textsubscript{i} changes. * $p < 0.05$. 

- Saline, Thiamylal, Intralipid, or Propofol
- Aerobic
- Ischemic
- Reperfusion
Figure 6. Diastolic $[\text{Ca}^{2+}]_i$ changes. * $p < 0.05$. 
Figure 7. Total DHBA\textsubscript{s} in the buffer.

\[ p<0.05 \text{ vs pre-ischemia} \]
Fisher's PLSD test for multiple comparisons. A probability of $p < 0.05$ was regarded as statistically significant. The data are presented as mean ± SD.

**RESULTS**

The heart rate changes are shown in Figure 2. Heart rate in Group TH was significantly lower than those in Groups S and TL at 5 and 25 min. Left ventricular $dP / dt$ maximum (LV max) in Group TH was also significantly lower than those in Groups S and TL (Figure 3). However, there were no significant differences in the heart rate and LV max among groups S, PL and PH. There were no significant differences in MVO$_2$ among the groups (Figure 4).

Systolic [Ca$^{2+}$], in all groups except Group TH increased at the beginning of ischemia and during the reperfusion periods. Systolic [Ca$^{2+}$], in Group TH was significantly lower than in Groups S and TL from 6 to 25 min. Systolic [Ca$^{2+}$], in Group TL was also lower than that in Group S at 6, 21 and 22 min. However, there were no significant differences among Groups I, PL and PH (Figure 4). Diastolic [Ca$^{2+}$], in all groups changed in the same manner as the systolic [Ca$^{2+}$], (Figure 6).

Total DHBAs (sum of 2,3-, 2,4-, 2,5- and 3,4-DHBAs) in the coronary effluent are presented in Figure 7. Total DHBAs in all groups increased significantly 1 min after reperfusion. However, there were no significant differences in any of the groups before or after ischemia.

**DISCUSSION**

These results indicate that propofol 3 and 10 µM did not affect cardiac function, intracellular [Ca$^{2+}$], MVO$_2$ or hydroxyl radical production in the ischemic reperfused heart. On the other hand, thiamylal 300 µM depressed cardiac function before ischemia, reduced increasing intracellular [Ca$^{2+}$], at the beginning of both ischemia and reperfusion, but did not affect MVO$_2$ and hydroxyl radical formation before or after ischemia.

The concentrations of propofol under anesthesia are reported to be 5-10 µM. At this dosage in the present study, propofol did not show any significant negative inotropic effect on the isolated heart. This is consistent with previous studies which indicated that propofol does not inhibit myocardial contractility at clinical dosages. However, propofol shows negative inotropic effects in a dose-dependent manner at higher concentrations. This effect is mainly due to the inhibition of cardiac L-type calcium currents. In contrast, thiopental at doses greater than 100 µM has been reported to reduce the L-type calcium current and thiamylal 300 µM showed a negative inotropic effect in our model.
The plasma concentration of thiopental at a clinical dosage, which resembles thiamylal, reaches approximately 380 µM.\textsuperscript{17} Therefore, depressant effects of thiamylal seemed to be stronger than that of propofol at clinical concentrations. This was confirmed by Azuma, \textit{et al.}\textsuperscript{18} who reported that propofol did not produce any depression of cardiac contractile force, whereas thiamylal produced a concentration-dependent negative inotropic effect in rat papillary muscles.

In the present study, propofol did not cause a change in intracellular \([\text{Ca}^{2+}]\), when compared with control (Intralipid). This result disagrees with a previous report showing that 0.5-2.0 µg·m\textsuperscript{-1} (2.8-11.2 µM ) propofol resulted in a significant and dose-dependent decrease in \([\text{Ca}^{2+}]\), in rat myocardial cells.\textsuperscript{19} However, Kanaya, \textit{et al.}\textsuperscript{20} have suggested a direct negative inotropic effect for supraclinical concentrations of propofol mediated by a decrease in \([\text{Ca}^{2+}]\). In contrast, a higher dose of thiamylal reduced intracellular \([\text{Ca}^{2+}]\) at the beginning of both ischemia and reperfusion when compared with control (saline). Kanaya, \textit{et al.}\textsuperscript{21} also reported that thiopental has a direct negative inotropic effect on cardiac excitation-contraction coupling at the cellular level, which is mediated by a decrease in \([\text{Ca}^{2+}]\). However, this cannot explain the fact that thiamylal showed a direct negative inotropic effect before ischemia without decreasing \([\text{Ca}^{2+}]\). This may result from the fact that thiamylal would decrease myofilament \(\text{Ca}^{2+}\) sensitivity. Intracellular \([\text{Ca}^{2+}]\) regulation should at least consist of the transmembrane \(\text{Ca}^{2+}\) influx through voltage-activated \(\text{Ca}^{2+}\) channels, the release of \(\text{Ca}^{2+}\) from intracellular stores such as mitochondria or sarcoplasmic reticulum, and the clearance of cytosolic \(\text{Ca}^{2+}\) by reuptake in the intracellular stores or extrusion into the extracellular medium. Therefore, it is likely that there would be differences resulting from different drugs, dosage and experiment models. Sakai, \textit{et al.}\textsuperscript{22} have pointed out that the distinct cardiodepressant effects of propofol and thiopental may result, at least in part, from their different actions on membrane currents during the repolarization phase of action potentials. The important observation in our findings is that thiamylal may have a protective effect during ischemia and reperfusion because it reduces \([\text{Ca}^{2+}]\). If we had used higher doses of propofol in this study, propofol would have demonstrated its reduction of \([\text{Ca}^{2+}]\) via the L-type calcium current.

Oxygen free radicals have been implicated in various types of myocardial injury, especially reperfusion injury.\textsuperscript{23-26} These radicals mainly consist of superoxide anion, hydrogen peroxide and hydroxyl radical. Hydroxyl radicals are highly reactive, and thus, damaging. Measurement of free radical formation is important for understanding their role in the pathogenesis of post-ischemic reperfusion injury. Although the existence of free radicals has been demonstrated with electron spin resonance spectroscopy, this technique requires expensive equipment. Floyd, \textit{et al.}\textsuperscript{8,9} have shown that hydroxyl radicals react with salicylic acids producing DHBAs. These reaction products can be detected by LCED. In the
present study, we modified Floyd’s method and were able to measure the concentrations of DHBAs. Our results indicated that propofol and thiamylal did not affect the formation or elimination of hydroxyl radical in the reperfused heart. Propofol has been shown to attenuate experimental reperfusion injury\textsuperscript{5,6}) or hydrogen peroxide-induced alterations in the isolated rat heart.\textsuperscript{7}) However, these effects were apparent only at higher concentrations. Ross, \textit{et al.}\textsuperscript{27}) have suggested that with clinical concentrations propofol had no protective effect against myocardial stunning. As for thiamylal, there is a report showing that thiopental 1000 µM, but not 100 µM, prevented lipid peroxidation in rat brain tissue.\textsuperscript{28}) A free radical scavenging effect of thiamylal is not likely at clinical concentrations.

In summary, clinical doses of propofol had no significant effects on myocardial function or [Ca\textsuperscript{2+}] before and after ischemia. Thiamylal had a direct negative inotropic effect under aerobic conditions, which was not mediated by a decrease in [Ca\textsuperscript{2+}]. Thiamylal suppressed the increase in [Ca\textsuperscript{2+}] during ischemia and reperfusion, suggesting the possibility that thiamylal may attenuate reperfusion injury. However, free radical formation during reperfusion was unaffected with thiamylal and propofol.

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