Propofol enhances ischemic tolerance of middle-aged rat hearts: effects on 15-F$_{2	au}$-isoprostane formation and tissue antioxidant capacity

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Abstract

Objective: Experimental study has shown that myocardial ischemic tolerance is reduced during middle-age. We investigated the effect of propofol on ischemic tolerance of middle-aged rat hearts. Methods: Hearts of young adult (10 weeks old, Y) and middle-aged rats (20 weeks old, M) were assigned to propofol (P-Y, P-M) and control (C-Y, C-M) groups ($n=6$ each). Hearts were perfused using a Langendorff preparation with Krebs–Henseleit solution (KH) at constant flow rates. We applied propofol (P-Y, P-M) for 10 min at 12 $\mu$g/ml before inducing 40 min global ischemia. During ischemia, saline (C-Y, C-M) or propofol (P-Y, P-M) in saline was perfused through the aorta at 60 $\mu$l/min. Propofol in KH was perfused at 12 $\mu$g/ml for the first 15 min of reperfusion and subsequently reduced to 5 $\mu$g/ml in propofol treatment groups. Coronary effluent was assayed for 15-F$_{2	au}$-isoprostane after equilibration, during ischemia ($T_i$) and at 0.5 ($T_1$) and 5 ($T_2$) min of reperfusion. After 90 min of reperfusion ($T_3$), hearts were harvested to assess tissue antioxidant capacity. Results: In P-Y, we observed an increased latency to ischemic-contracture and a significantly reduced contracture after 35 min ischemia compared to control groups. No ischemic contracture was observed in P-M. There were significantly lower 15-F$_{2	au}$-isoprostane levels in P-M and P-Y than in C-M and C-Y at $T_1$. At $T_3$, the recovery of left ventricular developed pressure in P-M was greater than in P-Y ($P<0.05$); both were greater than in C-M and C-Y. Conclusion: Propofol enhanced ischemic tolerance of middle-aged hearts, primarily by inhibiting lipid peroxidation.

Keywords: Ischemia; Free radicals; Aging; Reperfusion; Myocardium; Propofol

1. Introduction

Aging is known to be associated with biochemical and functional changes in the heart [1,2]. Animal studies have shown an age-related decrease in the recovery of cardiac function with post-ischemic reperfusion [3,4]. Clinical studies have shown that age is one of the best predictors for operative mortality in patients undergoing cardiac surgery using cardiopulmonary bypass (CPB) [5,6]. However, the post-operative outcomes in terms of long-term survival and freedom from angina were excellent in senescent as compared with those of middle-aged patients [7]. Thus, it is important to determine if the susceptibility of the myocardium to ischemia–reperfusion injury (IRI) varies with age, and to identify treatments that will effectively protect less tolerant myocardium.

Recent studies have shown that middle-aged rat hearts became more vulnerable to ischemic insult [8] and that rat coronary arteries became more sensitive to the vasoconstrictor endothelin-1 before and after ischemia–reperfusion, particularly during the period of maturation from young age to adulthood [9]. Oxidative stress may occur during myocardial ischemia–reperfusion and contribute to IRI secondary to lipid peroxidation of cell membranes. It
has been reported that reactive oxygen species (ROS) and products of oxidation increase with age [10–12], accompanied by a reduction in tissue antioxidant capacity.

Based on these observations, we postulate that antioxidant intervention could increase ischemic tolerance and enhance postischemic myocardial functional recovery of middle-aged rat hearts. Our previous work has demonstrated that the intravenous anesthetic propofol enhances red cell and tissue antioxidant capacity both in vitro and in vivo [13,14]. We have recently found that enhancing myocardial antioxidant capacity with propofol protects against the oxidant stress associated with ischemia–reperfusion damage in young rat hearts [15]. We hypothesize that propofol may provide effective protection against myocardial IRI of the more vulnerable middle-aged rat heart [8,9] and that this protection is related to the reduction of lipid peroxidation during ischemia–reperfusion. The hypothesis was tested in an isolated rat heart model, using 15-F₂,isoP (15-F₂,isoP, previous name 8-epi-PGF₃α), a specific and reliable index of lipid peroxidation [16,17], as a measure of oxidative injury.

2. Methods

2.1. Heart perfusion

The study was approved by the Committee of Animal Care of the University of British Columbia. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-25, 1996). Young (Y, 10 weeks, weighing 250–300 g) or middle-aged (M, 20 weeks, weighing 550–620 g) male Sprague–Dawley rats were anesthetized with pentobarbital (70 mg/kg, intraperitoneally) and heparinized with sodium heparin (1000 IU/kg, intraperitoneally). After median thoracotomy, hearts were quickly excised and immersed in ice-cold Krebs–Henseleit (KH) solution to stop contractions. Hearts were gently squeezed to remove residual blood and thereby prevent clot formation. Hearts were retrogradely perfused via the aorta as non-working ‘Langendorff’ preparations at a constant flow rate using a peristaltic pump. The perfusion flow rate was 10 ml/min in young and 15 ml/min in middle-aged rat hearts. The choice of different perfusion flow rates was based on our pilot study results showing: (1) these flow rates yielded a comparable initial coronary perfusion pressure of about 50 mmHg in hearts we used; (2) hearts beat well and remain hemodynamically stable for a duration of 150 min (the duration of our study) when sham-perfused without ischemia and reperfusion (n=3 each for young and middle-aged rat hearts). The flow rate for the young rat heart was the same as that used by Mathur et al. [21], whereas the flow rate for the middle-aged rat hearts was comparable to that achieved by Goodwin et al. [9]. The perfusion fluid (pH 7.4; temperature, 37 °C) was KH solution that contained: 120 mM NaCl; 20 mM NaHCO₃; 1.43 mM KH₂PO₄; 1.25 mM CaCl₂; 1.17 mM KH₂PO₄ and 8 mM glucose. The perfusate was bubbled with a mixture of 95% O₂ and 5% CO₂. The perfusate solution and the bath temperature were maintained at 37 °C using a thermostatically controlled water circulating system. During the experiment, the heart was in a chamber with circulating water in its jacket thermostatically controlled at 37 °C. The chamber was properly sealed during the experiment, and the inside chamber environmental temperature was continuously monitored with a thermometer and maintained between 36.9 and 37.1 °C during ischemia. Coronary perfusion pressure (CPP) was measured via a side arm of the perfusion cannula connected to a pressure transducer (Statham p23 ID, Gould Electronics, Cleveland). A latex water-filled balloon fixed to a pressure transducer was inserted through the mitral valve into the left ventricle for the determination of left ventricular (LV) developed pressure (LVDP), which was calculated by subtracting end-diastolic pressure (LVEDP) from LV peak systolic pressure (LVSP). LVEDP was adjusted to approximately 5 mmHg before the start of the experiment by adjusting the volume in the intraventricular balloon. Hearts were perfused within 30–40 s after excision. Exclusion criteria included heart preparation times longer than 60 s and/or LVSP lower than 70 mmHg after 10 min equilibration.

2.2. Experimental protocol

All hearts were initially equilibrated for 10 min (baseline, BS), and they were then randomly divided into one of four groups (n=6 each): ischemia–reperfusion untreated control groups of young (C-Y group) and middle-aged (C-M group) rat hearts, propofol treatment groups of young (P-Y group) and middle-aged (P-M group) rat hearts. Previous studies in our laboratory have shown that the carrier vehicle for propofol was devoid of antioxidant activity, and therefore it was deemed unnecessary to include vehicle controls into an experimental protocol [31].

After the initial equilibration, propofol was applied for 10 min at 12 μg/ml (67 μM) in the P-Y and P-M groups prior to inducing global ischemia by stopping the perfusion flow for 40 min. Control (non-propofol-treated) hearts were equilibrated for another 10 min prior to inducing global ischemia for 40 min. During ischemia, saline (controls) or propofol (P-Y and P-M) in saline (12 μg/ml) was perfused through the aorta at 60 μl/min using a mini-pump. KH was perfused during the 90 min of reperfusion in the control groups. Propofol 12 μg/ml in KH was perfused for the first 15 min of reperfusion, followed by propofol 5 μg/ml in KH for 75 min in both propofol treatment groups. This reduction in propofol concentration during reperfusion was effected to avoid or reduce the possible direct negative effects of propofol on
myocytes [30]. Hearts were electrically paced at a rate of 300 beats/min, prior to and following, but not during the ischemic period. LV function was continuously monitored using a polygraph. At the end of the 90-min of reperfusion period, hearts were immediately removed from the cannula, precooled in liquid nitrogen and stored at −70°C. Hearts were assayed for formation of tissue thiobarbituric acid reactive substances (TBARS) within 48 h of storage.

2.3. 15-F₂-isoprostane assays

Effluent perfusate was sampled at baseline (BS), during the first 30 min of ischemia (I-30) and at 0.5 (Re-0.5), 5 (Re-5) and 30 (Re-30) min of reperfusion in control and propofol-treated groups. Effluent perfusate samples at baseline and after reperfusion were collected over a 10-s period, while the samples during ischemia were collected over a period of 30 min. The effluent samples were stored at −70°C until analysis for free 15-F₂-isoprostane.

Enzyme-linked immunoassay (EIA) was used to measure free 15-F₂-isoprostane levels according to the methods provided by the manufacturer (Cayman Chemical, Ann Arbor). In brief, effluent samples were removed from −70°C storage and thawed on ice. Fifty-μl standards and samples (thawed effluent) were added in duplicate to the 96-well plate provided in the kit, followed by addition of 15-F₂-isoprostane acetylcholinesterase tracer and antobody. The prepared plates were then incubated overnight at room temperature. The next day, the plates were washed 5 times with the wash buffer, followed by addition of Ellman’s reagent. After optimal development, which normally took 80–100 min under our conditions, the plates were read at 405 nm, and the values of the unknowns were expressed as pg/ml effluent. The samples were coded and the investigator responsible for 15-F₂-isoprostane assays was blinded until the completion of the assay.

2.4. Heart tissue antioxidant capacity determination

Tissue antioxidant capacity was determined by exposure of tissue homogenates to the peroxidizing agent tert-butylhydroperoxide (t-BHP). The oxidation of tissues by t-BHP results in the formation of numerous lipid byproducts, which are collectively termed TBARS. The levels of TBARS in the sample were estimated from the absorbance at 532 nm [18]. Heart tissue samples (300 mg) were thawed and homogenized on ice in 3 ml Tris–EDTA buffer using a Polytron (PT-10, Brinkman Instruments, Canada) homogenizer for 30 s at 25% power. The resulting homogenates were used for in vitro forced peroxidation and subsequent determination of TBARS as previously described [19]. In brief, 400 μl of tissue homogenate were combined with 400 μl t-BHP (in 0.9% saline–2 mM sodium azide to produce t-BHP concentrations ranging from 0.5 to 10 mM). These suspensions were incubated for 30 min at 37°C, then 400 μl of cold 28% (w/v) TCA–0.1 M sodium arsenite were added. The mixture was centrifuged at 12,000×g for 5 min at 4°C, and 800 μl of supernatant were removed and added to 400 μl of thiobarbituric acid (0.5% in 25 mM NaOH). The samples were boiled for 15 min, and the absorbance at 532 nm was measured spectrophotometrically.

2.5. Data analysis

All data are presented as mean±S.E.M. Effluent 15-F₂-isoprostane concentration and hemodynamic data were compared by two-way ANOVA with Bonferroni’s correction (GraphPad Prism). One-way repeated measures ANOVA and Tukey’s multiple comparison test were applied for within-group comparison. The correlation relationships were evaluated by the Pearson test. P<0.05 (two-tailed) was considered significant.

3. Results

3.1. 15-F₂-isoprostane generation during ischemia–reperfusion

Baseline 15-F₂-isoprostane values were low (5.0±0.5, 6.1±1.0, 4.5±0.4 and 4.0±0.1 pg/ml in C-Y, P-Y, C-M and P-M groups, respectively) and did not differ among the various experimental groups (P>0.1). Since the perfusion flow rates were different between young and middle-aged rat hearts, 15-F₂-isoprostane production at baseline and during reperfusion was converted according to the flow rates and presented as the amount of 15-F₂-isoprostane produced per minute (pg/min) in the effluent. 15-F₂-isoprostane production during ischemia was, however, presented as pg/ml.

As shown in Fig. 1, 15-F₂-isoprostane levels increased significantly at Re-0.5 (P<0.05 or P<0.01 versus baseline) in all experimental groups. It decreased to close to baseline levels in P-Y and P-M groups (P>0.05 versus baseline) at Re-5 and in C-Y and C-M groups at Re-30 (Fig. 1A). During early reperfusion, 15-F₂-isoprostane levels fell more rapidly in the C-Y than in the C-M group. At Re-5 min, a significant decrease of 15-F₂-isoprostane from Re-0.5 was seen in the C-Y, but not in the C-M group. Propofol significantly reduced 15-F₂-isoprostane production at 0.5 min of reperfusion in young (P<0.05, P-Y versus C-Y) and at 5 min of reperfusion in the middle-aged rat hearts (P<0.05, P-M versus C-M). 15-F₂-isoprostane was produced in substantial amounts during ischemia in the C-Y and C-M groups (Fig. 1B). Propofol significantly reduced 15-F₂-isoprostane production during ischemia in both young and middle-aged rat hearts.

3.2. Tissue antioxidant capacity

As shown in Fig. 2, tissue TBARS formation following in vitro peroxide challenge (as reflected by absorbance at 532 nm) was significantly higher in control groups than in propofol treatment groups at t-BHP concentrations higher
the P-Y (0.222 ± 0.014) group, suggesting that propofol could more effectively preserve tissue antioxidant capacity during ischemia–reperfusion in middle-aged rat hearts than in young rat hearts.

3.3. Effects of propofol on contracture development during ischemia and reperfusion

The LVEDP increased progressively during ischemia in the two control groups (Fig. 3). Maximum ischemic contracture (reflected as peak LVEDP) in the C-M group (38.3 ± 3.8 mmHg) was slightly higher than, but not significantly different from, that in the C-Y (37.8 ± 3.0 mmHg) group (Table 1). There was no significant difference in the onset time of ischemic contracture and time to peak LVEDP between C-Y and C-M groups. LVEDP increased more rapidly in the C-M group after 25 min of ischemia. LVEDP at ischemia 30 min (and onwards) was significantly higher than that at ischemia 25 min (P = 0.03) in the C-M group (Fig. 3). The gradual increase in LVEDP in the C-Y group only became significant after 40 min of ischemia. Propofol significantly reduced LVEDP in young rat hearts after 35 min of ischemia, and completely abolished ischemic contracture in middle-aged rat hearts during the 40 min period of ischemia (Fig. 3).

Reperfusion was associated with a generalized elevation in LVEDP in both C-Y and C-M groups (Fig. 4) when compared to the LVEDP of approximately 5 mmHg before ischemia. The LVEDP further increased over time after 30 min of reperfusion in the C-Y and C-M groups. The LVEDP at 5 min of reperfusion was 7.8 ± 1.2 mmHg in P-Y and 5.8 ± 0.5 mmHg in the P-M group and was significantly lower than the corresponding values in C-Y and C-M (Fig. 4). LVEDP did not increase over time during the 90-min period of reperfusion in either the P-Y or the P-M group.

3.4. Coronary perfusion pressure

As shown in Table 1, initial CPP values were compar-
able in C-Y, P-Y, C-M and P-M groups. CPP values increased gradually after reperfusion in the C-Y and C-M groups and were significantly higher than baseline after 30 min of reperfusion in the C-M group and after 60 min of reperfusion in the C-Y group. Administration of propofol for 10 min before ischemia significantly reduced CPP in young but not in the middle-aged rat hearts. Propofol prevented the increase of CPP after reperfusion that was seen in the control groups. The CPP was significantly lower in P-Y and P-M than in C-Y and C-M, respectively, after 90 min of reperfusion.

3.5. Left ventricular mechanics

Fig. 5 depicts the pre- and post-ischemic values of LVDP, a measure of myocardial contractile function. Baseline LVDP values did not differ among the four experimental groups. Propofol administration for 10 min before ischemia reduced LVDP by 43.2% in P-Y and 36.5% in P-M groups. The pre-ischemia values of LVDP in P-Y and P-M were significantly lower than the corresponding values at baseline (P<0.01) prior to inducing ischemia. The LVDP in the C-Y and C-M groups recovered to 82.0±8.6 and 87.2±9.0% of the corresponding baseline values, respectively, at the 30 min (Re-30) reperfusion time-point and decreased progressively thereafter. At 90 min of reperfusion (Re-90), LVDP in the C-Y and C-M groups was significantly lower than their baseline values (P<0.05). Propofol treatment resulted in better recovery of LVDP in middle-aged than in young rat hearts following reperfusion. LVDP in the P-M group was significantly higher than that in the P-Y group after 60 min (Re-60) and 90 min of reperfusion. At Re-90, LVDP in the P-M was significantly higher than that in the C-M group (P<0.05).

Since one of the major goals of this study was to optimize the long-term protective effects of propofol, administration of propofol was not discontinued during reperfusion. For a better comparison among groups, the percentage change of LVDP from Re-60 to Re-90 was calculated. The percentage decrease of LVDP from Re-60 to Re-90 in the C-M group (~27.8±1.8%) was significantly higher than that in the C-Y group (~17.9±3.9%) (P<0.05), both being significantly higher than that in the corresponding propofol treatment groups. The percentage decrease of LVDP from Re-60 to Re-90 did not differ between P-Y (~3.6±2.7%) and P-M (~6.7±2.9%) groups.

3.6. Correlation analysis

Fig. 6 depicts the relationship between heart tissue antioxidant capacity (as reflected in peroxide-induced TBARS generation) at the end of 90 min of reperfusion and coronary effluent 15-F_{2α}-isoP production during ischemia. Tissue TBARS formation was significantly positively correlated with effluent 15-F_{2α}-isoP production during ischemia (R=0.68, P=0.0003), indicating an inverse correlation between tissue antioxidant capacity preservation and 15-F_{2α}-isoP production during ischemia. Tissue...
Changes of LVDP from Re-60 to Re-90 were inversely correlated with changes of CPP from Re-60 to Re-90 in ischemic–reperfused middle-aged rat hearts (R = -0.68, P = 0.015, n = 12). Such a relationship did not exist in the young rat hearts (P = 0.8). This extends the finding of Goodwin et al. [9] that middle-aged (5-month old) rat hearts became more sensitive to vasoconstriction induced by endothelin-1, whose release was increased in isolated ischemic–reperfused rat hearts [29].

### 4. Discussion

Previous laboratory studies investigating the effect of propofol on myocardial IRI have focused primarily on young animal hearts [14,15,20–23]. To our knowledge, this is the first study to compare the cardioprotective effects of propofol on isolated hearts from young and middle-aged rats and to use 15-F_{2α}-isoP as an index of lipid peroxidation to explore the mechanism of the protection. This approach is important to understanding the effects of age, which could identify patient populations that could benefit most from antioxidant intervention during clinical settings of myocardial IRI.

The principal findings of this study include the following: (1) lipid peroxidation occurs during global ischemia and reperfusion in both young and middle-aged rat hearts; (2) myocardial tissue antioxidant capacity preservation following IRI is highly negatively correlated with the extent of lipid peroxidation that occurs during prolonged global ischemia rather than during reperfusion; (3) 15-F_{2α}-isoP generation during ischemia is highly negatively correlated with post-ischemic myocardial functional recovery; (4) propofol applied before and during ischemia as well as during early reperfusion significantly reduced 15-F_{2α}-isoP generation during ischemia and abolished ischemic contracture in middle-aged rat hearts.

In attempts to clarify the contribution of ROS to myocardial IRI, experiments using a wide array of antioxidants have yielded conflicting results. Certain pathophysiological mechanisms may predominate depending on the conditions of the experiment, such as the duration of ischemia, the timing of the antioxidant intervention as well as the nature and dosage of the antioxidant used. If significant cellular necrosis has occurred during the preceding ischemic event, antioxidant interventions during reperfusion may have little effect. Cardiac mitochondria, critical to the energy status and function of the heart, are a source of ROS during ischemia [24] and reperfusion [25,26], and exhibit increased rates of ROS production with age [10–12]. Based on previous studies from our laboratory [13,14], we postulate that the cardio-
protective effects of propofol on IRI are mostly attributable to its antioxidant properties.

15-F₂,isoP is a chemically stable end product of lipid peroxidation, which is detectable in rat heart and plasma under control conditions [27]. EIA provides a sensitive measure for 15-F₂,isoP in biological specimens. Baseline 15-F₂,isoP values in our model of myocardial IRI were within the limits of detection of the assay. The baseline generation of 15-F₂,isoP in our model likely represents release from normal myocardial tissue rather than an ischemic insult during heart isolation, because of careful organ harvesting and reperfusion within 40 s after excision. Since large quantities of isoprostanes can be generated ex vivo [17], it was important to ensure that the measured 15-F₂,isoP was generated by the heart during ischemia, and not produced during collection or storage. We found that the values did not change when effluent was stored overnight at room temperature (data not shown). This is similar to a report by Morrow et al. [28], who found that urinary F₂-isoprostane levels did not increase when urine was incubated at 37 °C for 5 days.

Forty minutes of ischemia induced a profound ischemic insult, as evidenced by the magnitude of the ischemic contracture in the two control groups. It is noteworthy that the magnitude of the ischemic contracture in the C-M group significantly increased every 5 min when the duration of ischemia exceeded 25 min, which was not the case in the C-Y group. This indicates that middle-aged rat hearts are more vulnerable to ischemic insult after prolonged ischemia. Interestingly, propofol abolished ischemic contracture in middle-aged but not in young rat hearts. The precise mechanism underlying this effect is uncertain. It seems reasonable to postulate, however, that antioxidant therapy would be more effective in hearts with decreased endogenous antioxidant capacity. This will attenuate or prevent subsequent cellular damage resulting from ROS-mediated membrane lipid peroxidation. On the other hand, propofol may inhibit rat cardiomyocyte calcium channels at a concentration as low as 6 μM [32]. This inhibition increases in a concentration-dependent fashion when propofol concentration exceeds 50 μM [32]. This property of propofol could have contributed, in part, to the reduction or abolition of ischemic contracture in the propofol treatment groups. It has been shown that contracture development in rat cardiomyocyte is potentiated by a rise in intracellular calcium concentration [33,34]. In addition, propofol may attenuate contracture development by enhancing energy preservation during myocardial ischemia–reperfusion [20]. Energy depletion may play an important role in the acceleration of contracture development [35], when the contracture has been initiated in the presence [33,34] or absence [35] of a rise in intracellular calcium. The strong negative correlation between 15-F₂,isoP generation during ischemia and post-ischemic myocardial function recovery highlights the role of ROS in mediating cellular injury early during ischemia. Susceptibility of heart tissue to ex vivo TBARS formation in the presence of the peroxidizing agent r-BHP provided a functional measure of tissue antioxidant capacity. The significant correlation between tissue TBARS formation and 15-F₂,isoP generation during ischemia but not during reperfusion indicates that the reduction in tissue antioxidant capacity occurred primarily during the ischemic phase in this experimental model. This would suggest that antioxidant interventions aimed at protecting against myocardial IRI are likely to be most effective if undertaken immediately prior to the ischemic insult.

The propofol concentration (67 μM or 12 μg/ml) used in the present study is high, but still clinically achievable, based on our previous study [13]. A recent study from our laboratory has shown that the cardiac protective effect of propofol was concentration-dependent, being greater at 12 μg/ml, when used primarily before and during ischemia as well as during the early phase of reperfusion, than at 5 μg/ml in this experimental model [15]. Therefore, only the highest concentration of propofol was used in the current study before and during ischemia and in the early phase of reperfusion. Propofol significantly reduced 15-F₂,isoP generation during ischemia and reperfusion in both young and middle-aged rat hearts. It is noteworthy that, during early reperfusion, 15-F₂,isoP levels decreased more rapidly in the C-Y than in the C-M group (Fig. 1A). This is coincident with a more profound percentage decrease of LVDP from Re-60 to Re-90 in the C-M group. This is of clinical significance. We have recently shown that the percentage changes of plasma free 15-F₂,isoP concentration during the early phase of myocardial reperfusion inversely correlates with post-operative cardiac index [48].

A recently published study has shown that propofol may offer myocardial protection by inhibiting the mitochondrial permeability transition (MPT) [36], another major cause of reperfusion injury [37], at concentrations low as 2–4 μg/ml (11–22 μM) in ischemic–reperfused rat hearts [36]. Inhibition of the MPT by propofol, when applied at low concentrations, is likely attributable to the well-known membrane stabilizing action of lipophilic anesthetic molecules [47] and the antioxidant properties of propofol. Propofol 2 μg/ml did not inhibit MPT when applied directly to isolated de-energised mitochondria [36]. Indeed, accumulating evidence strongly suggests oxidative stress as the link between excessive mitochondrial calcium overload and MPT (for a review, see Ref. [38]). It has been proposed that MPT is not a consequence of the opening of a pre-formed pore, but the consequence of oxidative damage to pre-existing membrane proteins [38]. Propofol, however, may directly inhibit rat heart MPT at concentrations ≥50 μM [39]. This is of importance. Clinically, propofol failed to offer appreciable protection against myocardial IRI [40] when administered using a target-controlled infusion system with propofol plasma concentrations between 2 and 4 μg/ml (concentration provided by author in response to letter-to-editor by...
Anstew and Xia). Interestingly, propofol, when used at a high concentration of 67 μM before and during ischemia as well as during the early phase of reperfusion, enhanced myocardial function recovery in middle-aged rat hearts 90 min after reperfusion compared to untreated control and young rat hearts. Clinically, the application of high-concentrations of propofol (average 11 μg/ml) before and during myocardial ischemia in a patient population 35 years of age or older resulting in better myocardial function recovery 12–24 h post-operatively compared to application of propofol at lower concentrations [13]. Improved cardiac functional recovery by propofol 90 min after reperfusion in the middle-aged rat hearts and 12–24 h after cardiac surgery in patients is similar in effect to the ‘first window’ and ‘second window’ of protection phenomena observed after ischemic or pharmacological preconditioning [43].

The proposed ‘preconditioned state’ in the heart induced by propofol, when applied at high concentrations as aforementioned, is unlikely to be mediated by the activation of mitochondrial \(K_{ATP}\) (m\(K_{ATP}\)) channels, a mechanism by which volatile anesthetics are claimed to mimic cardiac preconditioning [41]. Propofol, at concentrations between 10 and 200 μM, did not affect m\(K_{ATP}\) channel activity in cultured myocytes isolated from male Sprague–Dawley rat hearts [42]. We propose that propofol, primarily at high concentrations, may ‘precondition’ the heart via mechanism(s) downstream of m\(K_{ATP}\) channel activation. Indeed, accumulating evidence supports m\(K_{ATP}\) as a trigger and/or a mediator rather than an end-effector in myocardial preconditioning and PKC is likely one of the kinases downstream from m\(K_{ATP}\) that may be involved (for a review, see Ref. [43]). Propofol stimulated purified rat brain PKC activity in vitro [44], and attenuated isoproterenol-stimulated increases in intracellular calcium via activation of PKC activation in rat cardiomyocytes [45]. Therefore, activation of PKC could be a potential signal pathway through which propofol may ‘precondition’ the heart. Also, it is important to note that inhibition of the MPT in early reperfusion could represent a distal effector mechanism of myocardial ‘preconditioning’ with m\(K_{ATP}\) activation acting as a trigger or an intermediate step [46]. Taken together, propofol cardiac protection might involve the activation of protein kinase C (PKC) activity before ischemia, and the inhibition of the MPT, directly [39] or indirectly through reducing oxidative stress [38,46], during reperfusion.

In summary, our present study suggests adequate antioxidant therapy with propofol as a potentially useful means to reduce myocardial IRI under clinical conditions (e.g., coronary revascularization and heart transplantation), especially in the more vulnerable population of the middle-aged patients [7]. Propofol may mediate cardiac protection through a variety of mechanisms or signal pathways, with inhibition of ROS-mediated lipid peroxidation likely being one of the major mechanisms of protection.

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