15-F_{2t}-isoprostane exacerbates myocardial ischemia-reperfusion injury of isolated rat hearts

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Xia, Zhengyuan, Kuo-Hsing Kuo, David V. Godin, Michael J. Walker, Michelle C. Y. Tao, and David M. Ansley. 15-F_{2t}-isoprostane exacerbates myocardial ischemia-reperfusion injury of isolated rat hearts. Am J Physiol Heart Circ Physiol 289: H1366–H1372, 2005.—Reactive oxygen species induce formation of 15-F_{2t}-isoprostane (15-F_{2t}-IsoP), a specific marker of in vivo lipid peroxidation, which is increased after myocardial ischemia and during the subsequent reperfusion. 15-F_{2t}-IsoP possesses potent bioactivity under pathophysiological conditions. However, it remains unknown whether 15-F_{2t}-IsoP, by itself, can influence myocardial ischemia-reperfusion injury (IRI). Adult rat hearts were perfused by the Langendorff technique with Krebs-Henseleit (KH) solution at a constant flow rate of 10 ml/min. 15-F_{2t}-IsoP was infused into the aorta at 10 ml/min. After 40 min of reperfusion. Using SQ-29548 (SQ), a thromboxane receptor antagonist that can abolish the vasoconstrictor effect of 15-F_{2t}-IsoP, 15-F_{2t}-IsoP + SQ in KH, or KH alone (vehicle control) was applied for 10 min before induction of 40 min of global ischemia followed by 60 min of reperfusion. Coronary effluent endothelin-1 concentrations were significantly higher in the group treated with 15-F_{2t}-IsoP than in the control group during ischemia and also in the later phase of reperfusion. Infusion of 15-F_{2t}-IsoP increased release of cardiac-specific creatine kinase, reduced cardiac contractility during reperfusion, and increased myocardial infarct size relative to the control group. SQ abolished the deleterious effects of 15-F_{2t}-IsoP. 15-F_{2t}-IsoP exacerbates myocardial IRI and may, therefore, act as a mediator of IRI. 15-F_{2t}-IsoP-induced endothelin-1 production during cardiac reperfusion may represent a mechanism underlying the deleterious actions of 15-F_{2t}-IsoP.

ischemia-reperfusion injury; SQ-29548; endothelin-1

MYOCARDIAL ISCHEMIA-REPERFUSION injury (IRI) and its sequelae, cardiac depression and arrhythmogenesis, have been shown experimentally to result, at least in part, from the disruptive action of reactive oxygen species (ROS) on membrane lipids and intracellular proteins required for cellular integrity and function (12, 15, 26). The release of high levels of ROS during ischemia-reperfusion can overwhelm endogenous antioxidant defenses, a crucial event determining the onset of irreversible cellular necrosis secondary to extensive lipid peroxidation (17).

A recent advance in free radical biology has been the discovery of isoprostanes, which are stable in vivo end products of arachidonic acid peroxidation (22). Of the variety of isoprostanes detected, 15-F_{2t}-isoprostane (15-F_{2t}-IsoP) (28) has been found to be a specific, reliable marker of oxidative stress. This has facilitated investigation of the role of ROS in a variety of disease states, most notably cardiovascular disease. 15-F_{2t}-IsoP possesses potent biological activity, including vasoconstriction and platelet activation, under pathophysiological conditions (21). 15-F_{2t}-IsoP at <250 nM has no effect on coronary flow in the absence of ischemia in the isolated rat heart, but 30 nM 15-F_{2t}-IsoP significantly reduces coronary flow in the hypoxic or postischemic reperfused rat heart (14).

Clinically, we identified an inverse correlation between the speed of decay of plasma 15-F_{2t}-IsoP concentrations during the early phase of reperfusion and postoperative cardiac functional recovery in patients undergoing coronary artery bypass surgery via cardiopulmonary bypass (CPB) (1). The characteristics of 15-F_{2t}-IsoP production and its effects under conditions of ischemia and reperfusion are similar to those of endothelin-1 (ET-1). ET-1 is one of the most potent vasoconstrictors known and has been postulated to contribute to postischemic myocardial dysfunction (25, 27). ET-1 release has been shown to increase during and after myocardial ischemia (3), and its vasoconstrictor effect appears to be potentiated during postischemic reperfusion in isolated hearts (23, 38).

We hypothesized that 15-F_{2t}-IsoP can exacerbate myocardial IRI and that the mechanism of 15-F_{2t}-IsoP action may involve the release and/or enhancement of ET-1 production during cardiac ischemia and reperfusion. Using SQ-29548 (SQ), a thromboxane A2 receptor (TxA2) antagonist used to abolish the vasoconstrictive actions of 15-F_{2t}-IsoP (13), we tested our hypothesis in an isolated rat heart model.

METHODS

Heart preparation. The study was approved by the Committee of Animal Care of the University of British Columbia. The animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. Male Sprague-Dawley rats (280–320 g) were anesthetized with pentobarbital sodium (70 mg/kg ip) and treated with heparin sodium (1,000 IU/kg ip). 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 prevent clot formation. Hearts were retrogradely perfused via the aorta in a nonworking “Langendorff” preparation at a constant flow rate of 10 ml/min by means of a peristaltic pump. The perfusion fluid (pH 7.4, 37°C) was KH solution that contained (in mM) 118 NaCl, 24 NaHCO₃, 4.63 KCl, 1.2 MgCl₂, 1.25 CaCl₂, 1.17 KH₂PO₄, and 11 glucose. The perfusate was bubbled with 95% O₂-5% CO₂. Temperature of the perfusate solution and the chamber in which the hearts were rested was maintained at 37°C with a thermostatically controlled water-circulating system. Coronary perfusion pressure (CPP) was measured via a sidearm of the perfusion cannula connected to a pressure transducer (Statham P23 ID, Gould Electronics, Cleveland, OH). A latex water-filled balloon fixed to a pressure transducer was inserted through the mitral valve into the left ventricle (LV) for determination of LV developed pressure (LVPD), which was calculated by subtracting LV end-diastolic pressure (LVEDP) from LV peak systolic pressure. LVEDP was adjusted to −5 mmHg before the start of the experiment by adjustment of the volume in the intraventricular balloon.

**Experimental protocol.** All hearts were initially equilibrated for 10 min (BS10). They were then randomly assigned to a sham group or one of the four experimental groups (n = 7 per group): ischemia-reperfusion untreated control (control), 15-F₂-t-IsoP (IsoP), 15-F₂-t-IsoP + SQ (IsoP + SQ), or SQ alone (SQ). After BS10, 15-F₂-t-IsoP (100 nM, IsoP), SQ (1 μM) or 15-F₂-t-IsoP + SQ (IsoP + SQ) was applied for 10 min in the corresponding groups before global ischemia (40 min) was induced by stopping perfusion. Control hearts underwent an additional 10 min of equilibration before global ischemia was induced. During ischemia, saline (control), 15-F₂-t-IsoP (100 nM, IsoP), SQ-29548 (1 μM, SQ), or 15-F₂-t-IsoP + SQ (IsoP + SQ) in saline was perfused through the aorta at 60 μl/min via a minipump. KH was perfused during 60 min of reperfusion in the control group. 15-F₂-t-IsoP, SQ, or 15-F₂-t-IsoP + SQ in KH was perfused for the first 15 min of reperfusion. Hearts were electrically paced at 300 beats/min before and after, but not during, the ischemic period, when hearts ceased to beat spontaneously.

The perfusion flow rate was based on the result of a pilot study, which showed that sham isolated hearts perfused at 10 ml/min with KH beat well and remain hemodynamically stable for 120 min (the duration of the experiment) in our experimental setup. The concentration of 15-F₂-t-IsoP was based on 1) a prior report that 100 nM 15-F₂-t-IsoP did not affect coronary flow in sham-perfused rat hearts but significantly reduced coronary flow in ischemic-reperfused rat hearts (13) and 2) our own pilot study, which showed that 30 nM 15-F₂-t-IsoP did not cause significant reduction in postischemic LVDP (n = 3) compared with control; however, when 300 nM 15-F₂-t-IsoP was given in our experimental setup, hearts were not able to resume beating during reperfusion (n = 2). Previous studies showed that 0.1 μM SQ, a TXA₂ receptor antagonist (24), abolished 15-F₂-t-IsoP (56 nM)-induced reduction in coronary flow in ischemic-reperfused rat hearts (13) and 1 μM SQ abolished 15-F₂-t-IsoP (>300 nM)-induced reduction in coronary flow in isolated perfused guinea pig hearts (20). Therefore, 1 μM SQ was applied to ensure blockade of 15-F₂-t-IsoP action in our model.

Effluent perfusate was sampled at BS10, the first 30 min of ischemia, and 1 (Re-1), 5 (Re-5), 30 (Re-30), and 60 (Re-60) min of reperfusion in the four experimental groups or at the corresponding time points in the sham group. Aliquots of the effluent samples were immediately stored at −70°C until analysis for cardiac-specific creatine kinase (CK-MB) in all study groups and for 15-F₂-t-IsoP in the sham, control, and SQ groups. Another portion of the effluent sample was initially concentrated (see below) and then stored at −70°C for analysis of ET-1 concentration. At the end of the 60 min of reperfusion, 1%, 2.3,5-triphenyltetrazolium in buffer (0.1 M phosphate buffer adjusted to pH 7.4, 37°C) was pumped into the heart at 1 ml/g·min⁻¹ for 15 min until the epicardial surface became deep red. The hearts were then stored in 10% formaldehyde for later analysis of myocardial infarct size.

**Measurement of ET-1.** Enzyme immunoassays (EIA) of ET-1 concentrations in the coronary effluent were performed in duplicate according to the manufacturer’s instruction (human ET-1 EIA kit 900-020, Assay Designs, Ann Arbor, MI). The assay kit detects ET-1 levels in biological fluids of human, bovine, canine, murine, porcine, and rat (32) samples. On the basis of pilot studies, ET-1 concentrations in our samples were often below the ET-1 sensitivity of the assay (0.14 pg/ml). Therefore, effluent samples were concentrated fourfold by evaporation of solvent (i.e., KH) at room temperature under a stream of dry nitrogen. ET-1 concentration was calculated as one-fourth of the measured ET-1 level in the concentrated sample. The accuracy of this approach was confirmed by prior testing using known ET-1 standards. The assays plates were read at 450 nm, and the values of the unknowns were expressed as picograms of ET-1 per milliliter of effluent.

**Measurement of CK-MB.** Measurement of CK-MB was determined by EIA (catalog no. BC-1121, BioCheck, Burlingame, CA). The unknowns were expressed as nanograms of CK-MB per milliliter of effluent.

**Infarct size measurement.** The measurement of infarct size was essentially identical to that described by Downey (8), except for the method of quantification. After the 2,3,5-triphenyltetrazolium chloride reaction, the hearts were sectioned transaxially, and the infarct size was evaluated as a percentage of the sectional area of infarcted tissue relative to the sectional area of the whole heart in 1-mm layers (5 layers; LG scanner). Morphometric measurements of infarct size were performed with an LG scanner and 6.0 CE software. The histogram counts of the red (viable) and white (infracted) tissue were recorded. The percent infarction was calculated as white counts divided by the sum of the red and white counts.

**Statistical analysis.** Values are means ± SE. Cardiac variables and chemical assay parameters were compared by two-way ANOVA with repeated measures. One-way ANOVA was used to test for differences in infarct size between groups. The correlation between effluent ET-1 and 15-F₂-t-IsoP concentration was evaluated by Pearson’s test. P < 0.05 was considered statistically significant.

**RESULTS**

**ET-1 release and its relation with 15-F₂-t-IsoP.** Baseline effluent ET-1 concentrations did not differ among the experimental groups (Fig. 1A). Effluent ET-1 did not significantly change over time in the sham group (data not shown). ET-1 increased in the control group during ischemia (Fig. 1A; P < 0.001 vs. baseline) and increased further in the IsoP group compared with control (P < 0.05). ET-1 increased by ~20% at Re-1 and by 32.8 ± 26.9% at Re-30 compared with BS10 in the control group. These changes did not reach statistical significance (P > 0.1). Effluent ET-1 concentration was significantly higher in the IsoP than in the control group (P < 0.05) at Re-60. Effluent ET-1 concentrations in the IsoP + SQ and SQ groups did not differ from those in the control group during ischemia and reperfusion. A weak but statistically significant positive correlation (r = 0.77, P = 0.04; Fig. 1B) was noted between effluent concentrations of 15-F₂-t-IsoP and ET-1 during ischemia, but not during reperfusion, in the control (i.e., untreated) group.

**15-F₂-t-IsoP generation during ischemia-reperfusion.** Effluent 15-F₂-t-IsoP release in the sham group did not change over a 120-min perfusion period (Fig. 2). Effluent 15-F₂-t-IsoP levels
Increased during ischemia \((P < 0.001\) vs. BS10) and remained elevated at Re-1 \((P < 0.05\) or \(P < 0.01\) vs. BS10) in the control and SQ groups. Effluent 15-F2t-IsoP release during ischemia and reperfusion did not differ significantly between the control and SQ groups.

**CK-MB release during ischemia-reperfusion.** Baseline CK-MB release was detectable in this model and did not differ among groups (Fig. 3). Effluent CK-MB release did not change significantly over time in the sham group. During ischemia, CK-MB increased significantly from baseline values only in the IsoP group \((P < 0.05)\).

During reperfusion, CK-MB increased gradually and was significantly higher than baseline at Re-30 in the control group \((P < 0.05)\). Effluent CK-MB concentration in the IsoP group increased more rapidly during reperfusion and was significantly higher at Re-5 than at baseline \((P < 0.05)\). This was also significantly greater than values measured in control hearts at the same time \((P < 0.05)\). CK-MB levels in the IsoP + SQ group were similar to values measured in untreated control hearts during ischemia and reperfusion. At Re-1, the CK-MB level in the SQ group was higher than in the control and IsoP groups \((P < 0.05)\) but then decreased quickly.

**Contracture development during ischemia.** LVEDP increased progressively during ischemia (Fig. 4A). LVEDP in the IsoP group increased more quickly. At 30 and 35 min of ischemia, LVEDP was significantly higher in the IsoP than in the control group \((P < 0.05)\). SQ attenuated the effect of 15-F2t-IsoP on ischemic contracture. LVEDP was significantly lower in the IsoP + SQ than in the IsoP group at 20 min of ischemia and thereafter. LVEDP in the IsoP + SQ and SQ groups did not differ from that measured in the control group during ischemia.

Time to the onset of ischemic contracture was significantly shorter in the IsoP than in the control group: 11.4 ± 1.9 vs. 17.4 ± 1.6 min \((P < 0.05\) Fig. 4B). The latency to ischemic contracture in the IsoP + SQ \((18.1 ± 1.5\) min) groups was significantly increased compared with that in the IsoP group \((P < 0.01\) or \(P < 0.05)\) but did not differ from that in the control group \((P > 0.05)\) Fig. 4B).

**Functional response to ischemia-reperfusion.** LVEDP in the control group was significantly higher during reperfusion than at baseline (Fig. 5A). 15-F2t-IsoP significantly augmented the increase of LVEDP during reperfusion at Re-30 and Re-60 \((P < 0.01)\). SQ attenuated the 15-F2t-IsoP-induced increase in LVEDP. LVEDP in the IsoP + SQ and SQ groups did not differ significantly from that in the control group during reperfusion.
LVDP in the sham group did not change significantly over time during the experimental period. LVDP in the control group recovered to a maximum of 87.0 ± 11.6% of its baseline value at Re-30 (P > 0.05 vs. BS10; Fig. 5B) and decreased thereafter. LVDP in the IsoP group recovered to a maximum of 56.5 ± 13.5% of its baseline value at Re-30 (P < 0.05 vs. BS10) and decreased quickly thereafter. At Re-60, LVDP was lower in the IsoP than in the control group. LVDP in the IsoP + SQ and SQ groups did not differ from that in the control group. SQ exacerbated 15-F2t-IsoP-induced reduction in LVDP relative to the control group at Re-10. Neither 15-F2t-IsoP, SQ, nor 15-F2t-IsoP-SQ affected CPP before ischemia. CPP did not increase significantly until Re-60 in the untreated control group: 80.4 ± 11.0 mmHg vs. 51.3 ± 1.1 mmHg at BS10 (P < 0.05). CPP increased more quickly during reperfusion in the IsoP than in the control group. At Re-30, CPP in the IsoP group (100.4 ± 13.9 mmHg) was higher (P < 0.05) than its baseline value (52.1 ± 3.9 mmHg) and higher (P < 0.05) than the corresponding value in the control group (65.6 ± 4.6 mmHg). SQ did not significantly affect CPP compared with the control group. At Re-60, CPP did not significantly differ among control, IsoP, IsoP + SQ, and SQ groups: 80.4 ± 11.0, 110.4 ± 14.9, 115.4 ± 14.9, and 84.8 ± 13.5 mmHg, respectively (P > 0.05).

**Myocardial infarct size.** Myocardial infarct was significantly larger in the IsoP than in the control (untreated) group (P < 0.05; Fig. 6). The myocardial infarcts were significantly smaller in the IsoP + SQ and SQ groups than in the IsoP group (P < 0.05 or P < 0.01). Infarcts were somewhat smaller in the SQ and IsoP + SQ groups than in the control group, but the differences did not attain statistical significance.

**DISCUSSION**

This is the first study providing evidence that 15-F2t-IsoP exacerbates myocardial IRI in isolated perfused rat hearts. Our findings are as follows: 1) 15-F2t-IsoP did not affect preischemic cardiac mechanics and CPP but did reduce cardiac tolerance to ischemic insult, as manifested by an early onset and higher magnitude of ischemic contracture. 2) 15-F2t-IsoP stimulated the release and/or production of ET-1 during ischemia, which was accompanied by an increased severity of myocardial cellular damage, as evidenced by increased CK-MB release. 3) 15-F2t-IsoP increased myocardial infarct size and exacerbated postischemic myocardial dysfunction, which may be attributable, in part, to stimulation of ET-1 production and/or release during reperfusion.

ET-1 has potent vasoconstrictor properties and is known to reduce myocardial contractility and contribute to the progression of heart failure (27). Plasma levels of ET-1 increase during cardiac operations requiring CPB (2, 31). A high plasma ET-1 level during the early postoperative period has been associated with prolonged pharmacological management (i.e., inotropic support), longer intensive care unit stay, and compli-
In the IsoP group, infusion of 15-F2t-IsoP was terminated at 15 min of reperfusion, which resulted in an increased formation of ET-1 during late reperfusion compared with control (Fig. 1). 15-F2t-IsoP may have triggered a statistically significant increase by Re-60 in the IsoP group as compared with the control (untreated) group suggesting that endogenous 15-F2t-IsoP may act to stimulate increased ET-1 release during ischemia.

We observed a reduction in ET-1 concentration at Re-30 but a statistically significant increase by Re-60 in the IsoP group compared with control (Fig. 1A). 15-F2t-IsoP may have triggered an increased formation of ET-1 during late reperfusion. In the IsoP group, infusion of 15-F2t-IsoP was terminated at 15 min of reperfusion. Sequestration of a significant amount of 15-F2t-IsoP in the heart tissue 45 min after termination of exogenous 15-F2t-IsoP infusion is unlikely in this study, because the 15-F2t-IsoP decay half-life in this model is ~4 min (data not shown). 15-F2t-IsoP stimulation of ET-1 formation during late reperfusion could represent an important mechanism responsible for postischemic myocardial dysfunction in the clinical setting. Whereas we previously found that plasma-free 15-F2t-IsoP levels increased during ischemia-reperfusion for ~30 min during cardiac surgery, the 15-F2t-IsoP decay pattern during early reperfusion correlated with early postoperative cardiac recovery (1). Plasma ET-1 levels may remain elevated ≥24 h after cardiac surgery (35). A recent study conducted in isolated rat cardiomyocytes has shown that a 30-min period of ischemia with or without 30 min of reperfusion is sufficient to rapidly stimulate the gene expression of myocyte ET-1 as well as the ET-1 receptors (9). It is possible that high levels of 15-F2t-IsoP during ischemia and/or early reperfusion may have induced ET-1 gene expression, resulting in increased ET-1 production during late reperfusion. Further study is merited to address the underlying mechanism.

On the basis of our results, we postulate that 15-F2t-IsoP may increase ET-1 release into the coronary circulation relative to the myocardial tissue during ischemia. It has been previously shown that the ratio of ET-1 secretion to the interstitial transudates to ET-1 secretion to coronary effluent is ~6.6 at baseline in isolated perfused rat hearts (3). This ratio is reduced to ~2.5 during low-flow ischemia and the first 30 min of reperfusion (3). The relative reduction of ET-1 concentration in the IsoP group at 30 min of reperfusion, 15 min after the termination of 15-F2t-IsoP infusion, indicates that 15-F2t-IsoP may have primarily stimulated ET-1 release, rather than its production, during ischemia and early reperfusion.

Despite the bioactivity of 15-F2t-IsoP as a vasoconstrictor (21, 29), reduction of coronary flow is not likely a major mechanism of 15-F2t-IsoP action during myocardial IRI, at least in this model. In the present study, hearts were perfused at a constant flow rate. In addition, CPP at Re-60 did not differ significantly among experimental groups, although LVDP was significantly lower in the IsoP group than in the control, IsoP + SQ, and SQ groups. It is possible that 15-F2t-IsoP aggravates myocardial IRI by a complex mechanism involving the activation of Na+/H+ exchange indirectly through ET-1 (4, 11). Alternatively, 15-F2t-IsoP may act by reducing the intrinsic activity of nitric oxide (19), an endogenous vasodilator. This may explain why CPP at Re-30 was higher in the IsoP than in the control group, irrespective of the similar effluent levels of ET-1 at this time point.

Despite abolishing the deleterious effects of a high concentration of exogenous 15-F2t-IsoP, SQ did not confer any beneficial effect in attenuating myocardial IRI compared with the control in this model. This is in keeping with previous findings describing the effect of exogenous 15-F2t-IsoP on the isolated guinea pig heart (20). The relatively high concentration of CK-MB at Re-1 in the SQ group is likely due to rapid release of CK-MB from the ischemic tissue, rather than the result of more intense tissue damage, because the infarct size was comparable in the SQ and control groups. The inability of SQ to attenuate myocardial IRI in the isolated perfused heart model may suggest that 1) endogenous 15-F2t-IsoP production in the myocardium during ischemia and reperfusion is relatively low under the present experimental condition, and it is mainly a marker (i.e., the result of lipid peroxidation), rather than a mediator, of oxidative damage; and 2) TxA2 may play a more intense role in myocardial IRI in the rat, a finding similar to that found in gene-knockout mice (37). Given that TxA2 may stimulate rat heart smooth muscle to generate ET-1 (6), we cannot exclude the possibility that SQ blockade of endogenous TxA2 action may have contributed to the decrease in ET-1 release at Re-60 in our IsoP + SQ group. It seems apparent that SQ blockade of 15-F2t-IsoP action, rather than blockade of TxA2 action, represents a major mechanism of myocardial protection seen in the present study.
It is intriguing that SQ blocked the effects of 15-F2t-IsoP on LVEDP during ischemia but not during reperfusion. It appears that the concentration of 15-F2t-IsoP may be a determinant of the effectiveness of SQ. At 40 min of ischemia (Fig. 4A), LVEDP was not only significantly lower in the IsoP + SQ group than in the IsoP group, it was also ~40% lower than the corresponding values in the control or SQ group ($P = $ not significant). The effluent level of ET-1 during ischemia in the IsoP + SQ group was ~20% lower than the corresponding values in the control or SQ group. It is possible that this slight 20% difference in ET-1 concentration during ischemia caused the 40% difference in LVEDP mentioned above. A previous study showed that ischemia may cause time-dependent externalization of ET-1 receptor binding sites in rat cardiac membranes (16), which may sensitize and exacerbate ET-1 deleterious effects, such as the exacerbation of ischemic contracture (4). Further study is merited to address why SQ could act differently, during myocardial ischemia, in the presence or absence of 15-F2t-IsoP.

ET-1 has been shown to exert a cardioprotective or preconditioning-like effect in vivo (10) and in vitro (5) models of myocardial ischemia-reperfusion in the rat when applied before ischemia. Our most recent study suggests that ET-1 may confer a preconditioning-like effect as well in the isolated ischemic-reperfused rat hearts (36). We found that the ET-1 type A and B receptor antagonist bosentan, when applied during the first 15 min of reperfusion, worsened postischemic myocardial dysfunction in the rat heart and unmasked any potential beneficial effects of ET-1 blockade during ischemia (36). However, when ET-1 receptor blockade was applied only during the later phase of reperfusion, postischemic myocardial infarct size was reduced. Hence, as observed in the present study, a 15-F2t-IsoP-induced ET-1 increase during ischemia, and especially during the later phase of reperfusion, may represent a major mechanism underlying the deleterious actions of 15-F2t-IsoP.

In conclusion, our finding that 15-F2t-IsoP can increase myocardial infarct size and exacerbate myocardial IRI may have important clinical implications. During cardiac surgery, systemic production of ROS occurs during CPB and may exceed production arising from reperfusion of the ischemic heart. The plasma level of 15-F2t-IsoP has been observed to dramatically increase shortly after the start of CPB (30). These high levels of 15-F2t-IsoP could enter the heart before aortic cross-clamping (the beginning of global myocardial ischemia) or at the time of aortic declamping, triggering and/or exacerbating myocardial IRI. The findings of the present study combined with our previous work on the effect of antioxidant supplementation with propofol suggest that combined therapy with antioxidant and 15-F2t-IsoP antagonism during ischemia and early reperfusion could offer a promising approach to attenuate myocardial IRI.

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