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

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Abstract

Background: 15-F_{2r}-isoprostane is a specific marker of \textit{in vivo} lipid peroxidation induced by reactive oxygen species (ROS) whose formation is increased after myocardial ischemia and during the subsequent reperfusion. 15-F_{2r}-isoprostane possesses potent bioactivity under pathophysiological conditions. However, it remains unknown whether 15-F_{2r}-isoprostane, by itself, can influence myocardial ischemia-reperfusion injury (IRI).

Methods: Adult rat hearts were perfused by the Langendorff technique with Krebs-Henseleit solution (KH) at a constant flow rate at 10 ml/min. 15-F_{2r}-isoprostane 100 nM (IsoP), SQ 29548 1µM (SQ), a thromboxane receptor antagonist that can abolish the vasoconstrictor effect of 15-F_{2r}-isoprostane, or their combination (IsoP+SQ) in KH or KH alone (vehicle control) were applied for 10 min before inducing 40 min global ischemia, followed by 60 min of reperfusion. During ischemia, saline (control), IsoP, IsoP+SQ or SQ in saline was perfused through the aorta at 60 µl/min. Either IsoP, IsoP+SQ or SQ in KH were infused during the first 15 min of reperfusion. Results: Coronary effluent endothelin-1 (ET-1) concentrations in the IsoP group were significantly higher than those in the control group during ischemia and also in the later phase of reperfusion (P<0.05). Infusion of 15-F_{2r}-isoprostane resulted in increased release of cardiac-specific creatine kinase (CK-MB) and reduced cardiac contractility during reperfusion and also increased myocardial infarct size relative to the control group. SQ 29548 abolished the deleterious effects of 15-F_{2r}-isoprostane. Conclusion: 15-F_{2r}-isoprostane exacerbates myocardial IRI, and may therefore act as a mediator of IRI. 15-F_{2r}-isoprostane induced ET-1 production during cardiac reperfusion may represent a mechanism underlying the deleterious actions of 15-F_{2r}-IsoP.

Key words: 15-F_{2r}-isoprostane; myocardial; ischemia-reperfusion injury
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. 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.

A recent advance in free radical biology has been the discovery of isoprostanes, which are stable in vivo end products of arachidonic acid peroxidation. Of the variety of isoprostanes detected, 15-F$_{2\alpha}$-isoprostane (15-F$_{2\alpha}$-IsoP) 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. Of interest, 15-F$_{2\alpha}$-IsoP possesses potent biological activity, including vasoconstriction and platelet activation under pathophysiological conditions. 15-F$_{2\alpha}$-IsoP has no effect on coronary flow in the absence of ischemia in the isolated rat hearts (up to a concentration of 256 nM), but significantly reduces coronary flow in the hypoxic or post-ischemic reperfused rat heart (at 30 nM).

Clinically we identified an inverse correlation between the speed of decay of plasma 15-F$_{2\alpha}$-IsoP concentrations during the early phase of reperfusion and post-operative cardiac functional recovery in patients undergoing coronary artery bypass surgery utilizing cardiopulmonary bypass (CPB). The characteristics of 15-F$_{2\alpha}$-IsoP production and its effects under conditions of ischemia and reperfusion are similar to those of endothelin-1 (ET-1). Endothelin-1 is one of the most potent vasoconstrictors known, and it has been postulated to contribute to post-ischemic myocardial dysfunction.
to increase during and after myocardial ischemia (3) and its vasoconstrictor effect appears to be potentiated during post-ischemic reperfusion in isolated hearts. (23;38)

We hypothesized that 15-F$_2$-IsoP can exacerbate myocardial ischemia-reperfusion injury and that the mechanism of 15-F$_2$-IsoP action may involve the release and/or enhancing the production of ET-1 during cardiac ischemia and reperfusion. Our hypothesis was tested in an isolated rat heart model, using SQ 29548, a thromboxane A$_2$ receptor (TXA$_2$) antagonist used to abolish the vasoconstrictive actions of 15-F$_2$-IsoP.(13)

**Methods**

**Heart preparation**

This study was approved by the Committee of Animal Care of the University of British Columbia. Animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. Male Sprague-Dawley rats (280–320g) were anesthetized with pentobarbital (70mg/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 to prevent clot formation. Hearts were retrogradely perfused via the aorta in a non-working "Langendorff" preparation at a constant flow rate of 10 ml/min using a peristaltic pump. The perfusion fluid (pH 7.4; temperature, 37°C) was KH solution that contained (in mM): NaCl 118; NaHCO$_3$ 24; KCl 4.63; MgCl$_2$ 1.2; CaCl$_2$ 1.25; KH$_2$ PO$_4$ 1.17; glucose 11. The perfusate was bubbled with a mixture of 95% O$_2$ and 5% CO$_2$. Temperatures of the perfusate solution and of the chamber in which the hearts were rested were maintained at 37°C using a thermostatically controlled water circulating
system. 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.

**Experimental Protocol**

All hearts were initially equilibrated for 10 min (BS10). They then were randomly assigned to a sham group or one of the four experimental groups (n=7 per group): ischemia-reperfusion untreated control (control), 15-F2t-IsoP (IsoP), 15-F2t-IsoP plus SQ 29548 (IsoP-SQ) or SQ 29548 (SQ) alone. After BS10, 15-F2t-IsoP 100 nM/L (IsoP), SQ 29548 1µM/L (SQ) or 15-F2t-IsoP 100 nM/L plus SQ 29548 1µM/L (IsoP+SQ) were applied for 10 min respectively in the corresponding groups, before global ischemia (40 min) was induced by stopping perfusion. Control hearts underwent an additional 10 min period of equilibration before global ischemia was induced. During ischemia, saline (control), 15-F2t-IsoP 100 nM/L (IsoP), SQ 29548 1µM/L (SQ) or 15-F2t-IsoP 100 nM/L plus SQ 29548 1µM/L (IsoP+SQ) in saline was perfused through the aorta at 60 µl/min using a mini-pump. KH was perfused during 60 min of reperfusion in the control group. Either 15-F2t-IsoP, SQ 29548 or 15-F2t-IsoP plus SQ 29548 in KH was perfused for the first 15 min of reperfusion. Hearts were electrically paced at a rate of 300 beats/min, prior to and following, 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 set-up. The concentration of 15-F_{2t}-IsoP applied in the current study was based on: 1) prior reporting that 15-F_{2t}-IsoP 100 nM 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 15-F_{2t}-IsoP at 30 nM did not cause significant reduction in post-ischemic LVDP (n=3) compared to control, but when 300 nM 15-F_{2t}-IsoP was given in our experimental set-up, hearts were not able to resume beating during reperfusion (n=2). Studies have shown that SQ 29548, a thromboxane A2 (TXA2) receptor antagonist (24), abolished 15-F_{2t}-IsoP (56 nM)-induced reduction in coronary flow in ischemic-reperfused rat hearts at a concentration of 0.1µM (13) and abolished 15-F_{2t}-IsoP (>300 nM) -induced reduction in coronary flow in isolated perfused guinea pig heart at 1µM.(20) Therefore, 1µM SQ 29548 was applied in the current study to ensure blockade of 15-F_{2t}-IsoP action in our model.

Effluent perfusate was sampled at BS10, the first 30 min of ischemia (isch) and at 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-F_{2t}-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, 37°C 1% 2,3,5-triphenyltetrazolium in buffer (0.1 M phosphate buffer adjusted to pH 7.4) was pumped into
the heart at 1 ml/gm/min 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 Endothelin-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, Inc. Ann Arbor). The Assay kit detects ET-1 levels in biological fluids of human, bovine, canine, murine, porcine and rat (32) samples. Based on pilot studies, ET-1 concentrations in our samples were often below the ET-1 sensitivity of the assay (0.14pg/ml). Therefore effluent samples were concentrated 4-fold by evaporation of solvent (i.e. the KH solution) at room temperature under a stream of dry nitrogen. ET-1 concentration was calculated as 1/4 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 ET-1 per milliliter effluent.

**Measurement of CK-MB**

Measurement of CK-MB was determined by enzyme immunoassay (Catalog number: BC-1121, BioCheck, Inc, Burlingame, CA). The unknowns were expressed as nanograms CK-MB per milliliter effluent.
**15-F_{2t}-IsoP Assays**

Enzyme immunoassay of free 15-F_{2t}-IsoP was performed according to the methods provided by the manufacturer (Cayman Chemical, Ann Arbor) as previously described (33,34). The values of the unknowns were expressed as picograms 15-F2t-IsoP per milliliter 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-triphenyl-tetrazolium chloride (TTC) reaction, the hearts were sectioned transaxially, and size of infarct was evaluated as percentage of sectional area of infarcted tissue to the sectional area of the whole heart in 1 mm layers (five layers, LG scanner). Morphometric measurements of infarct size were performed with a LG scanner and 6.0 CE software. The histogram counts of the red (viable tissue) and white (infracted tissue) were recorded. The percent infarction was calculated as white counts divided by the sum of the red plus white counts.

**Statistical analysis**

All data are presented as means ± SEM. Cardiac variables and chemical assay parameters were compared by two-way analysis of variance (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-F_{2t}-IsoP concentration was evaluated by the Pearson test. P<0.05 was considered statistically significant.
Results

Endothelin-1 release and its relation with 15-F_{2\alpha}-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 Iso-P group compared to control (P<0.05). ET-1 increased by approximately 20% at Re-1 and by 32.8±26.9% at Re-30 compared to baseline (BS10) in the control group. These changes did not reach statistical significance (P>0.1). Effluent ET-1 concentration in the IsoP group was significantly higher than that in the control group (P<0.05) at reperfusion 60min (Re-60). Effluent ET-1 concentrations in both the IsoP-SQ and the SQ groups did not differ from those found in the control 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-F_{2\alpha}-IsoP and ET-1 during ischemia, but not during reperfusion, in the control (i.e., untreated) group.

15-F_{2\alpha}-IsoP generation during ischemia-reperfusion

Effluent 15-F_{2\alpha}-IsoP release in the sham group did not change over a 120 min perfusion period (Fig 2). As shown in Fig 2, effluent 15-F_{2\alpha}-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-F_{2\alpha}-IsoP release during ischemia and reperfusion did not significantly differ 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 significantly change over time in the sham group. During ischemia, CK-MB increased significantly from baseline values only in group iso-P (p<0.05).

During reperfusion, CK-MB increased gradually and was significantly higher than baseline at Re-30 in the control group (P<0.01). Effluent CK-MB concentration in the IsoP group increased more rapidly during reperfusion and was significantly higher at Re-5 than its baseline value (P<0.05). This was also significantly greater than values measured in control hearts at the same time interval (p<0.05). CK-MB levels in IsoP-SQ were similar to values measured in untreated control hearts during ischemia and reperfusion. At Re-1, CK-MB level in SQ was higher than in the control group and the IsoP group (<0.05) but then decreased quickly thereafter.

**Contracture development during ischemia**

The LVEDP increased progressively during ischemia in the control group (Fig 4A). LVEDP in the IsoP group increased more quickly. At 30 and 35 min of ischemia, the magnitude of LVEDP in the IsoP group was significantly higher than that in the control group (P<0.05). SQ 29548 attenuated the effect of 15-F2t-IsoP on ischemic contracture. LVEDP was significantly lower in the IsoP-SQ group than the IsoP group at ischemia 30 min and onwards. LVEDP in the IsoP-SQ and the SQ group 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 group (11.4±1.9 min) compared to control (17.4±1.6 min, P <0.05, Fig 4B). The latency to ischemic
contracture in the IsoP-SQ (20.0±1.5 min) and the SQ (18.1±1.5 min) groups was significantly increased compared to 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**

During reperfusion, LVEDP in the control group was significantly higher than that at baseline (Fig 5A). 15-F$_2$-IsoP significantly augmented the increase of LVEDP during reperfusion at Re-30 and Re-60 (P<0.01). SQ 29548 attenuated the 15-F$_2$-IsoP-induced increase in LVEDP. The magnitude of LVEDP in the IsoP-SQ and the SQ groups did not significantly differ from that in the control group during reperfusion.

The LVDP in the sham group did not change significantly over time during the experimental period. The 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. The 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 in the IsoP group was lower than that in the control group. The LVDP values in the IsoP-SQ and the SQ group did not differ from those in the control group at Re-60. SQ 29548 exacerbated 15-F$_2$-IsoP induced reduction in LVDP relative to control group at Re-10.

**Coronary perfusion pressure (CPP)**

Neither 15-F$_2$-IsoP, SQ 29548, nor their combination affected CPP before ischemia. CPP did not increase significantly until after 60 min of reperfusion in the untreated control group (80.4±11.0 mmHg at Re-60 vs 51.3±1.1 mmHg at BS10, P<0.05). CPP in the IsoP group increased more quickly during reperfusion relative to the control group. At Re-30, the
CPP value 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 29548 did not significantly affect CPP as compared to the control group. At Re-60, CPP values did not significantly differ among the control (80.4±11.0 mmHg), the IsoP (110.4±14.9 mmHg), the IsoP+SQ (115.4±14.9 mmHg) and the SQ (84.8±13.5 mmHg) groups (P>0.05).

**Myocardial infarct size**

As shown in Figure 6, myocardial infarct size in the IsoP group is significantly larger than that of the control (untreated) group (P<0.05). The myocardial infarct sizes in the IsoP-SQ and SQ groups are significantly smaller than those in the IsoP group (P<0.05 or P<0.01). Infarct sizes in the SQ group and IsoP-SQ groups were somewhat smaller than those in the control group, but the differences did not attain statistical significance.

**Discussion**

This is the first study providing evidence that 15-F$_{2t}$-IsoP exacerbates myocardial IRI in isolated perfused rat hearts. Our findings include the following: (1) 15-F$_{2t}$-IsoP did not affect pre-ischemic cardiac mechanics and coronary perfusion pressure but did reduce cardiac tolerance to ischemic insult, as manifested by an early onset and higher magnitude of ischemic contracture; (2) 15-F$_{2t}$-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-F$_{2t}$-IsoP increased myocardial infarct size and
exacerbated post-ischemic myocardial dysfunction, which may be attributable, in part, to stimulation of ET-1 production and/or release during reperfusion.

Endothelin-1 has potent vasoconstrictor properties and is known to reduce myocardial contractility and contribute to the progression of the heart failure.(27) Plasma levels of ET-1 increase during cardiac operations requiring cardiopulmonary bypass (CPB)(2;31). A high plasma ET-1 level during the early postoperative period has been associated with prolonged pharmacologic management (i.e., inotropic support), longer intensive care unit stay, and complicated recovery.(2;7). The present study clearly demonstrates that 15-F$_{2t}$-IsoP, whose formation increased in the myocardium and coronary artery during CPB surgery,(18) can increase the release and/or production of ET-1 during myocardial ischemia-reperfusion. This might be a mechanism whereby 15-F$_{2t}$-IsoP exacerbates myocardial IRI. The positive correlation between effluent concentrations of 15-F$_{2t}$-IsoP and ET-1 during ischemia in the control (untreated) group suggests that endogenous 15-F$_{2t}$-IsoP may act to stimulate increased ET-1 release during ischemia.

We observed a reduction in ET-1 concentration at 30 min of reperfusion (Re-30), but a statistically significant increase by 60 min of reperfusion (Re-60) in the IsoP group compared to control (Fig 1A). 15-F$_{2t}$-IsoP may have triggered an increased formation of ET-1 during late reperfusion. In the IsoP group, the infusion of 15-F$_{2t}$-IsoP was terminated at 15 min of reperfusion. Sequestration of a significant amount 15-F$_{2t}$-IsoP in the heart tissue 45 min after the termination of exogenous 15-F$_{2t}$-IsoP infusion is unlikely in this study, since the 15-F$_{2t}$-IsoP decay half-life in this model is about 4 min (data not presented). 15-F$_{2t}$-IsoP stimulation of ET-1 formation during late reperfusion could represent an important mechanism responsible for post-ischemic myocardial dysfunction in the clinical setting. Whereas we
previously found that plasma free 15-F_{2t}-IsoP levels increased during ischemia-reperfusion for approximately 30 min during cardiac surgery, the 15-F_{2t}-IsoP decay pattern during early reperfusion correlated with early postoperative cardiac recovery.\(^{(1)}\) Plasma ET-1 levels may remain elevated at least 24 hours after cardiac surgery.\(^{(35)}\) Recent study conducted in isolated rat cardiomyocytes has shown that 30 min 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-F_{2t}-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.

Based on our results we postulate that 15-F_{2t}-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 \textit{versus} secretion to coronary effluent is about 6.6 at baseline in isolated perfused rat hearts\(^{(3)}\). This ratio is reduced to about 2.5 during the period of low-flow ischemia and the first 30 min of reperfusion.\(^{(3)}\) The relative reduction of ET-1 concentration observed in the IsoP group at 30 min of reperfusion, 15 min after the termination of 15-F_{2t}-IsoP infusion, indicates 15-F_{2t}-IsoP may have primarily stimulated ET-1 release rather than its production during ischemia and early reperfusion.

Despite 15-F_{2t}-IsoP’s bioactivity as a vasoconstrictor,\(^{(21,29)}\) reduction of coronary flow is not likely a major mechanism of 15-F_{2t}-IsoP action during myocardial IRI, at least in this model. In the current study, hearts were perfused at a constant flow rate. In addition, CPP at Re-60 did not differ significantly among experimental groups although LVDP in the IsoP group was significantly lower than that in the control, IsoP-SQ and the 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 the CPP value at Re-30 was higher in the IsoP group relative to control irrespective of the similar effluent levels of ET-1 at this time point.

Despite abolishing the deleterious effects of high concentration exogenous 15-F₂t-IsoP, SQ 29548 did not confer any beneficial effect in attenuating myocardial IRI compared to 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, since the infarct size of the SQ group was comparable to that in controls. The inability of SQ 29548 to attenuate myocardial ischemia-reperfusion injury in the isolated perfused heart model may suggest the following: 1) endogenous 15-F₂t-IsoP production in the myocardium during ischemia and reperfusion is relatively low under the current experimental condition, and it is mainly a marker (i.e., the result of lipid peroxidation) rather than a mediator of oxidative damage; 2) TXA₂ may play little role in myocardial IRI in rat, a finding similar to that found in gene knock-out mice. (37) Given that TXA₂ may stimulate rat heart smooth to generate ET-1(6), we can not exclude the possibility that SQ 29548 blockade of endogenous TXA₂ action may have contributed to the decrease in ET-1 release at Re-60 seen in the isoP-SQ group. It seems apparent that, SQ 29548 blockade of 15-F₂t-IsoP action rather than the blockade of TXA₂ action represents a major mechanism of myocardial protection seen in the current 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 ischemia 40 min (Fig. 4A), the magnitude of LVEDP in group IsoP-SQ was not only significantly lower than that in group IsoP, but was also about 40% lower than the corresponding values in the control or SQ groups (P=NS). The effluent level of ET-1 during ischemia in the IsoP-SQ group was about 20% lower than the corresponding values in the control or SQ groups. It is possible that this slight 20% difference in ET-1 concentration during ischemia caused the 40% difference in the magnitude of LVEDP mentioned above. Study has shown that ischemia may cause time-dependent externalization of ET-1 receptor binding sites in rat cardiac membranes (16), which may sensitise 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.

It is should be noted that ET-1 has been shown to exert a cardioprotective or preconditioning-like effect in both *in vivo* (10) and *in vitro* (5) models of myocardial ischemia-reperfusion in the rat, when applied prior to ischemia. Of interest, our most recent study suggests that ET-1 may confer post-preconditioning-like effect as well in the isolated ischemic-reperfused rat hearts (36). We found that the ET-1 A and B receptor antagonist bosentan, when applied during the first 15 min of reperfusion worsened post-ischemic 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 later phase of reperfusion, post-ischemic myocardial infarct size was reduced. Hence, as observed in the current study, an 15-F2t-IsoP induced ET-1 increase during ischemia, and
especially during later reperfusion, may represent a major mechanism underlying the deleterious actions of 15-F$_2$-IsoP.

In conclusion, our finding that 15-F$_2$-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-F$_2$-IsoP has been observed to dramatically increase shortly after the start of CPB.(30) These high levels of 15-F$_2$-IsoP could enter the heart either 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 current study combined with our previous work on the effect of antioxidant supplementation with propofol suggest that combined therapy with antioxidant and 15-F$_2$-IsoP antagonism during ischemia and early reperfusion could offer a promising approach to attenuate myocardial IRI.

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Figure legends

Fig. 1. A. Effluent Endothelin-1 (ET-1) concentrations during myocardial ischemia-reperfusion. BS10 and isch indicate 10 min after stabilization and 30 min during global myocardial ischemia, respectively; Re-1, Re-5, Re-30 and Re-60 indicate 1, 5, 30 and 60 min after reperfusion, respectively. * P<0.001 vs BS10; # P<0.05 or P<0.01 vs control. (n=7 for each group). B. Relationship between 15-F_{2t}-isoprostane (15-F_{2t}-isoP) and ET-1 concentration during the first 30 min of ischemia in the control group. ET-1 release is positively correlated with 15-F_{2t}-isoP concentration (r = 0.7695, 95% CI: 0.0389 – 0.9640, n=7, P (two-tailed) = 0.043).

Fig. 2. Effect of SQ 29548 (SQ) on 15-F_{2t}-isoprostane (15-F_{2t}-isoP) release during myocardial ischemia-reperfusion. BS10 and isch indicate 10 min after stabilization and 30 min during global myocardial ischemia, respectively; Re-1, Re-5, Re-30 and Re-60 indicate 1, 5, 30 and 60 min after reperfusion, respectively. * P<0.001 or P<0.05 vs BS10. # P<0.001 vs control. P >0.05 SQ vs control, n=7 per group.

Fig. 3. Effluent CK-MB concentration during myocardial ischemia-reperfusion. BS10 and isch indicate 10 min after stabilization and 30 min during global myocardial ischemia, respectively; Re-1, Re-5, Re-30 and Re-60 indicate 1, 5, 30 and 60 min after reperfusion, respectively. *P<0.05 vs BS10; #P<0.05 vs control; ^P<0.05 vs isoP group. (n=7 for each group)
Fig. 4. A: Development of left ventricular end-diastolic pressure (LVEDP), reflecting myocardial contracture, during ischemia. B: Ischemic contracture onset time. # P<0.05 vs control; † P<0.05 or P<0.01 vs IsoP (15-F₂-isoprostane) group; *P<0.05 or P<0.01 vs ischemia 30 min within the same group (n=7 per group).

Fig. 5. A: Variations of left ventricular end-diastolic pressure (LVEDP), reflecting myocardial stiffness, during reperfusion. BS10 and Pre-isch indicate 10 min after stabilization and the time immediately prior to ischemia, respectively; Re-30 and Re-60 indicate 30 and 60 min after reperfusion, respectively. *P<0.05 or P<0.01 vs BS10; † P<0.05 or P<0.01 vs control; ‡ P<0.05 or P<0.01 vs IsoP (15-F₂-isoprostane) group. B: Recovery of left ventricular developed pressure (LVDP), reflecting effective myocardial contractility, during reperfusion. BS10 and Pre-isch indicate 10 min after stabilization and the time immediately prior to ischemia, respectively; Re-30 and Re-60 indicate 30 and 60 min after reperfusion, respectively. *P<0.05 vs BS10; † P<0.05 vs control; ‡ P<0.05 or P<0.01 vs IsoP (15-F₂-isoprostane) group (n=7 per group).

Fig. 6. Myocardial infarct size. Top: representative images showing myocardial infarction (white) in the control (A), 15-F₂-isoprostane plus SQ 29548 (IsoP+SQ, C) and SQ 29548 (SQ, D) groups. Bottom: Percentage infarction (Mean ± SEM, n=7 per group): *P<0.05 vs control; † P<0.05 or P<0.01 vs IsoP group.
Fig. 1.
Time during ischemia-reperfusion

Fig. 2.
Fig. 3.

**CK-MB concentration (ng/ml)**

- **Control**
- **IsoP**
- **IsoP-SQ**
- **SQ**

**Time during ischemia-reperfusion**

BS10, isch, Re-1, Re-5, Re-30, Re-60
Fig. 4.

A

LVEDP (mm Hg)

Duration of ischemia (min)

Control IsoP IsoP-SQ SQ

B

Contracture onset time (min)

Group

Control IsoP IsoP-SQ SQ

Fig. 4.
Fig. 5.
Fig 6.
Reference List


8. Downey, J. M. Measuring infarct size by the tetrazolium method. *Available at:*


