The evaluation of constant coronary artery flow versus constant coronary perfusion pressure during normothermic ex situ heart perfusion

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BACKGROUND: Evidence suggests that hearts that are perfused under ex-situ conditions lose normal coronary vasomotor tone and experience contractile failure over a few hours. We aimed to evaluate the effect of different coronary perfusion strategies during ex situ heart perfusion on cardiac function and coronary vascular tone.

METHODS: Porcine hearts (n = 6 each group) were perfused in working mode for 6 hours with either constant aortic diastolic pressure (40 mmHg) or constant coronary flow rate (500 mL/min). Functional and metabolic parameters, cytokine profiles, cardiac and vascular injury, coronary artery function and oxidative stress were compared between groups.

RESULTS: Constant coronary flow perfusion demonstrated better functional preservation and less edema formation (Cardiac index: flow control = 8.33 vs pressure control = 6.46 mL/min/C0 kg, p = 0.016; edema formation: 7.92% vs 19.80%, p < 0.0001). Pro-inflammatory cytokines, platelet activation as well as endothelial activation were lower in the flow control group. Similarly, less cardiac and endothelial injury was observed in the constant coronary flow group. Evaluation of coronary artery function showed there was loss of coronary autoregulation in both groups. Oxidative stress was induced in the coronary arteries and was relatively lower in the flow control group.

CONCLUSIONS: A strategy of controlled coronary flow during ex situ heart perfusion provides superior functional preservation and less edema formation, together with less myocardial damage, leukocyte, platelet, endothelial activation, and oxidative stress. There was loss of coronary autoregulation and

KEYWORDS: ex situ heart perfusion; coronary artery flow; coronary autoregulation; leukocyte activation; oxidative stress

Abbreviation: ESHP, Ex Situ Heart Perfusion; dP/dt max, Maximum rates of pressure change; dP/dt min, Minimum rates of pressure change; CVR, Coronary vascular resistance; LVSW, Left ventricle stroke work; CBF, Coronary blood flow; IL-6, Interleukin-6; IL-1α, Interleukin-1α; IL-1β, Interleukin-1β; IL-10, Interleukin-10; TNF-α, Tumor necrosis factor-α; β-TG, β-Thromboglobulin; sCD-40L, Soluble CD-40 ligand; ELISA, Enzyme-linked immunosorbent assay; MPO, Myeloperoxidase; cTnI, Cardiac troponin I; VCAM-1, Vascular cell adhesion molecule 1; vWF, Von Willebrand factor; ETAR, Endothelin A receptor; ETBR, Endothelin B receptor; oxLDL, Oxidized low-density lipoprotein; MDA, Malondialdehyde; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; DAMP, Damage associate molecular pattern

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The clinical application of heart transplantation has expanded over time and outcomes have improved substantially. Meanwhile, recipient waiting lists continue to grow worldwide due to the discrepancy between the demand and supply of suitable organs. Ex situ heart perfusion (ESHP) allows for a shorter cold ischemic interval and provides the opportunity to assess cardiac function and viability, and metabolism. It also has the potential for reconditioning marginal donor hearts, thereby increasing the number and quality of donor organs available for transplant.

Maintenance of cardiac function relies on adequate myocardial blood supply through the coronary arteries. The regulation of coronary blood flow is an important issue in heart preservation. Thus, coronary artery function should be monitored and preserved. The ability of coronary resistance vessels to dilate in response to increase in myocardial oxygen demand, as illustrated by the tight correlation between myocardial oxygen consumption and coronary blood flow, is critical for maintaining an adequate, but not excessive supply of oxygen to the myocardium. Evidence indicates that perfused hearts may lose normal vasomotor tone and develop contractile dysfunction in a few hours. Our preliminary data also suggests that regulation of coronary artery function is disturbed during ESHP, leading to apparent excessive coronary blood flow over time.

Constant coronary artery flow and constant coronary artery perfusion pressure are two strategies widely used in the field of ESHP. Controlled perfusion utilizing a low coronary perfusion pressure could potentially limit injury and preserve cardiac function. Although coronary perfusion pressure may affect the development of myocardial edema, inadequate flow may compromise myocardial oxygen delivery, while excessive pressure may damage endothelium. The optimal perfusion pressure in ESHP that minimizes microvascular fluid filtration and also ensures homogeneous myocardial perfusion to prevent ischemia has not been adequately determined. The TransMedics Organ Care System (OCS) targets the coronary flow in the 650 to 900 mL/min range, with a perfusion pressure of 60 to 80 mmHg. Our previous studies indicated that myocardial energy stores can be maintained with aortic pressure as low as 40 mmHg. However, the best perfusion parameters (e.g., continuous flow vs pulsatile flow, low perfusion pressure vs high perfusion pressure) needed to achieve ideal results remain unclear. Additionally, coronary flow results from the interplay of coronary perfusion pressure, myocardial work and coronary vascular tone. Determining the optimal coronary flow rate above which the oxygen demand of the heart is met is of critical importance to prevent myocardial ischemia and maintain organ preservation. The purpose of this study was to examine the effect of coronary artery perfusion strategy on preservation of myocardial contractile function and coronary vascular tone during ESHP.

Material and methods

All animal experimental procedures were approved and performed in accordance with the guidelines and regulations of the Institutional Animal Care and Use Committee of the University of Alberta. Female domestic pigs (n = 17) weighing 45 to 55 kg were used as heart and blood donors.

Experimental design

Normal hearts were procured as previously described, and normothermic ESHP was performed for six hours (Langendorff perfusion for one hour and 5 hours of working mode perfusion). The ex situ working heart apparatus, shown in Figure 1A, has been described in detail previously. Briefly, 2 centrifugal pumps connecting left atrium (LA) and aorta, providing preload and afterload, respectively. This arrangement allows the left ventricle to perform work with preload (LA pressure = 6 mmHg) and fixed afterload that permitted control of coronary perfusion. Automatic control of pump revolutions per minute (RPM) is achieved to maintain the pre-set diastolic and LA pressure by a custom designed software. A schematic of the experimental design is seen in Figure 1B.

**Coronary pressure control group (n = 6):** Diastolic pressure was set at 40 mmHg during working mode perfusion at 1h (T1), maintaining the same pressure for the rest of the experiment.

**Coronary flow control group (n = 6):** At one hour of perfusion, diastolic pressure was lowered to maintain coronary flow rate of 500 mL/min. For functional assessment, to ensure comparable conditions to the pressure control group, diastolic pressure was set to 40 mmHg only at 1 h (T1), 3 h (T3), and 5 h (T5).

**In situ heart control group (n = 5):** Freshly procured, unmanipulated hearts were obtained to serve as in situ control.

**Ex situ evaluation of myocardial function and coronary artery function**

Myocardial function was assessed at T1, T3, and T5 in working mode. Preload challenge was induced by increasing left atrial pressure from 6 to 12 mmHg. To correlate cardiac work with coronary flow and therefore estimate the integrity of coronary autoregulation, linear regression analyses were performed on coronary artery flow and LVSW at T1, T3, and T5 in each heart. The detailed methods are included in Supplementary Material.
Figure 1  A, a schematic view of the perfusion system and B, experimental study design. A, custom ESHP circuit. LA line connects one centrifugal pump and left atrium, providing the preload and Ao line connects another pump and the aorta, providing the afterload. PA line collects the effluent of the pulmonary to the reservoir. a, supportive membrane; b, reservoir; c, arterial filter; d and e, centrifugal pumps; f, oxygenator; g, heater; h, gas line; i, drug infusion line; k, flow probes. Ao, aorta; PA, pulmonary artery; LA, left atrium. B. Experimental design. Hearts were allocated to either of the following 2 groups (n = 6 each): (1) pressure-control group, diastolic pressure was adjusted to 40 mmHg or (2) Flow-control group, diastolic pressure was adjusted to make constant coronary blood flow of 500 mL/min. The hearts underwent 1 hour of Langendorff perfusion, followed by 5 hours working mode perfusion. Functional and metabolic evaluation, as well as ex-situ coronary artery function assessment were done at 1, 3, and 5 hours of working mode perfusion.
Leukocytes and platelet activation profiles

Proinflammatory cytokines and platelet activation marker were measured in the perfusate (R & D System Inc., USA; FineTest, China). MPO peroxidation activity was measure in the coronary artery tissue (Biovision, Milpitas, CA). The detailed methods are included in the Supplementary Material.

Myocardial and vascular injury

Myocardial injury was estimated by measuring cardiac troponin I in the perfusate using ELISA (Life Diagnostics, West Chester, PA). Endothelial activation factor VCAM-1 (MyBioSources Inc., San Diego, CA) and vascular injury marker vWF (FineTest, China) were evaluated in the perfusate as well. Coronary artery expression of ETAR, ETBR were detected by standard western blot techniques. Full details of methods used are described in the Supplementary Material.

Determination of oxidative stress related modification

Lipid peroxidation was determined by measuring of oxidized oxLDL in the perfusate by ELISA (MyBioSource Inc.) and MDA in the coronary artery tissues (R & D System Inc., MN). Oxidative protein modification was measured in the coronary arteries tissue by assessment of protein carbonyl formation (Abcam, Cambridge, UK). Detailed methods are described in the Supplementary Material.

Statistical analysis

The SPSS 25.0 (SPSS Inc., IL) was used for statistical analysis. The data (mean ± standard error of the mean) were compared using the independent sample t-test or analysis of variance (ANOVA). Repeated measures ANOVA with paired t-test for comparison across time within group was used to identify change to the baseline. Correlation between LVSW and CBF was assessed by linear regression analysis, and $R^2$ was reported for the regression analyses.

Results

Physiologic parameters and blood analysis

Physiologic parameters at T1, T3, and T5 were comparable between the two groups (Supplemental Table). To achieve a coronary flow rate of 500 mL/min in the flow control group, the diastolic pressure was maintained at approximately 20 mmHg, which was half of that compared to the pressure control group (Figure 1B). The lactate level remained well below 2.5 mmol/L during perfusion in both groups. The calculated red blood cell hemolysis in the perfusate was increased from less than 0.5% at baseline to approximately 1.5% at the end of perfusion in both groups (Supplemental Figure 1).

Reduced flow improves cardiac function and decreases edema formation

Typical hemodynamic changes during the 6 hours of pressure-controlled ex situ heart perfusion are depicted in Figure 2. Cardiac function was better preserved in the flow control group at T5, indicated by higher cardiac index, stroke work (Figure 3A, B). Contractility was better in the flow control group at T5, indicated by $dP/dt_{max}$ (Figure 3C).

![Figure 2](Image)

Figure 2  Typical hemodynamic changes during 6 hours pressure control ex situ heart perfusion in Langendorff and working mode. Left ventricle stroke work (yellow) and coronary blood flow rate (blue) are depicted. The blue arrows indicate the workload challenge induced by manually increasing the left atrium pressure from 6 to 12 mmHg. After switching to working mode at 60 minutes, left ventricle stroke work and coronary blood flow rate reach a plateau after approximately 30 minutes. LVSW, left ventricle stroke work; CBF, coronary blood flow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
At the end of perfusion, there was significantly lower edema formation in the flow control group (7.9 ± 0.7% vs 19.8 ± 0.6%, \( p < 0.0001 \); Figure 3D). Macroscopically, the myocardium in the pressure control group had evidence of epicardial hemorrhage and edema formation. However, the hearts in the flow control group showed higher gross similarity to the in situ appearance of the heart (Supplemental Figure 2).

### Assessment of coronary vascular resistance

In the pressure control group, coronary blood flow increased from less than 250 mL/min/100g to over 500 mL/min/100g within a few minutes after switching from Langendorff mode to working mode perfusion (Figure 2). CBF in the flow control group at 3 (T3) and 5 (T5) hours was lower compared to the pressure control group (\( p = 0.012 \) at

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**Figure 3**  Functional assessment and edema formation. Cardiac function was better preserved in the flow control group, indicated by cardiac index (A), stroke work (B). Contractility was better in the flow control group at T5, indicated by dP/dt\(_{\text{max}}\) (C). After perfusion, less edema formation was observed in the flow control group (D). (Comparison between groups, independent t-test, \*\( p < 0.05 \), **\( p < 0.0001 \); comparison across time within group, repeated measure ANOVA, \#\( p < 0.05 \), ##\( p < 0.001 \), n = 6 each group).
T3, \( p = 0.027 \) at T5, Figure 4A). CVR gradually declined during 6 hours of perfusion in both group \( (p < 0.05, \text{Figure 4B}) \). However, in the flow control group, CVR was higher than that in the pressure control group at both T3 and T5 \( (p = 0.009 \text{ at T3 and } p = 0.008 \text{ at T5}) \). Consistent with decreased CVR, oxygen consumption and percentage of oxygen extraction decreased during perfusion in both groups as well \( (p < 0.05, \text{Figure 4C, D}) \). Oxygen consumption was higher in the flow control group at T3 and T5 \( (p = 0.006 \text{ at T3, } p = 0.01 \text{ at T5}) \), similar to oxygen extraction \( (p = 0.004 \text{ at T3, } p = 0.047 \text{ at T5}) \).

**Figure 4** Metabolic evaluation of ex situ heart perfusion. During 6 hours perfusion, there was a significant increase of coronary blood flow \( \text{(A)} \) over time in the pressure control group. Coronary vascular resistance \( \text{(B)} \) was gradually decreasing in both groups, with relative higher in the flow control group. Oxygen consumption \( \text{(C)} \) and oxygen extraction \( \text{(D)} \) both decreased significantly, with both values higher in the flow control group. (comparison between groups, independent t-test, \(*p < 0.05, **p < 0.01; \) comparison across time within group, repeated measure ANOVA, \( *p < 0.05, **p < 0.01, n = 6 \text{ each group} \)).

**Leukocyte and platelet activation were induced for both groups**

There was a significant increase of leukocyte and platelet activation compared to the baseline in both groups (evaluated by concentration of proinflammatory cytokines and platelet activation factors in the perfusate collected from the coronary sinus effluent, \( p < 0.001, \text{Figure 5A}) \). The flow control group had less pro-inflammatory cytokines compared to the pressure control group \( (p < 0.05) \). Both groups had increased platelet activation, indicated by the level of \( \beta \)-TG and sCD40L \( (\text{Figure 5B}) \). The \( \beta \)-TG level was significantly lower in the flow control group at T5 compared with the pressure control group \( (6.0 \pm 0.4 \text{ vs } 7.3 \pm 0.3 \text{ ng/mL, } p = 0.038) \). In the coronary tissue, there was significantly higher MPO peroxidation activity in the pressure control group than both flow control group and in situ group \( (p = 0.0001 \text{ vs in situ and } p = 0.0003 \text{ vs flow control group, Figure 5C}) \).

**Cardiac and endothelial injury**

Both perfusate cTnI and hyaluronan levels increased significantly compared to baseline \( (p < 0.001, \text{Figure 6A and B}) \). The flow control group had a lower degree of cardiac damage markers than the pressure control group starting from T3 (cTnI, 17.8 ± 4.5 vs 37.5 ± 4.7 ng/mL, \( p = 0.017 \text{ at T3, } 31.8 ± 9.5 \text{ vs } 48.6 ± 3.3 \text{ ng/mL, } p = 0.006 \text{ at T5; hyaluronan, } 75.8 ± 10.3 \text{ vs } 330.8 ± 75.2 \text{ ng/mL, } p = 0.01 \text{ at T3, } 115.1 ± 34.6 \text{ vs } 501.0 ± 75.5 \text{ ng/mL, } p = 0.002 \text{ at T5}) \).
Figure 5  Leukocyte and platelet activation markers. Pro-inflammatory and anti-inflammatory cytokines (A) were increased in both groups. The flow control group had less leukocyte activation than the pressure control group. Both groups had significantly increased platelet activation, indicated by $\beta$-Thromboglobulin and sCD40L (B). In coronary tissue, myeloperoxidase (MPO) peroxidation activity was higher in the pressure control group than both the flow control group and in situ group (C). MPO, myeloperoxidase; (Comparison between groups, independent t-test, $^*p < 0.05$, $^{**}p < 0.001$. comparison across time within group, repeated measure ANOVA, $^{\#}p < 0.01$, $^{##}p < 0.001$, pressure group vs. in situ: $^{\&}p < 0.001$, n = 6 for cytokines and n = 3 for MPO activity).
the pressure control group, VCAM-1 increased starting from T1 and was higher than that in the flow control group at T1 and T3 (*p = 0.002 and **p = 0.003, separately). However, there was no significant difference in perfusate VCAM-1 value over time in the flow control group. The pressure control group had significantly higher values of vWF than those in flow control group at both T3 and T5 (117.4 ± 8.0 vs 157.4 ± 11.7 ng/mL, *p = 0.018 at T3 and 170.3 ± 9.2 vs 130.6 ± 5.2 ng/mL, **p = 0.004, Figure 6D, E). There were no significant differences in ET₄R/ET₃R ratio expression between groups (Figure 6F-H).

**Correlation for coronary blood flow versus left ventricle stroke work**

At T1, T3 and T5, CBF and LVSW were recorded while increasing left atrial pressure from 6 to 12 mmHg, as depicted in Figure 7A and B. The slope of the correlation line for coronary blood flow versus left ventricle stroke work was higher in T1 than both T3 and T5 in both groups. At later time points, the change of CBF during the workload challenge decreased (Figure 7C). Within each group, both T3 and T5 had significantly less change in CBF in response to increased work than at T1 (pressure control group, ***p < 0.001 at T3 and T5; flow control group, **p = 0.0244 at T3, **p = 0.001 at T5). Altogether, these data suggest loss of coronary autoregulation during perfusion.

**Oxidative stress related modification in the coronary arteries**

There was a significant increase in oxLDL in the perfusate in both groups, compared with baseline (p < 0.01, Figure 8A), although it was lower in the flow control group.
at T5 compared to the pressure control group (76.4 ± 27.0 vs 143.1 ± 37.5 nmol/mL, \( p = 0.005 \)). The pressure control group had significantly higher MDA in coronary tissue compared to both the in situ group and the flow control group (\( p < 0.01 \)), whereas there was a nonsignificant increase in the flow control group (Figure 8B). These data suggest lower lipid peroxidation was induced in the flow control group. Moreover, the pressure control group had significantly higher protein carbonylation compared to both in situ group (\( p = 0.0005 \)), and the flow control group (\( p = 0.046 \), Figure 8C).

**Discussion**

In this study, we have shown that controlled coronary flow during working mode ESHP provides superior functional preservation and lower edema formation compared to pressure-controlled coronary perfusion. The most striking finding was the gradual loss of coronary vascular resistance and coronary autoregulation during ESHP irrespective of the two strategies. This study focused on the coronary arteries in order to evaluate the benefits of the controlled coronary flow strategy over the pressure-controlled strategy. We have reported here the potential advantage of using low coronary flow perfusion in the ex situ setting, including better ventricular functional preservation and attenuated leukocyte and platelet activation, less myocardial damage and endothelial activation and less oxidative stress in the vasculature. The benefits of utilizing the low flow perfusion strategy in organ preservation has also been suggested before, where targeting a low flow perfusion resulted in better function and structure of cardiomyocytes and endothelium.

### Controlled coronary blood flow ex situ heart perfusion provides superior functional preservation and less edema formation

In this study, the pressure controlled perfusion was performed with a target diastolic pressure of 40 mmHg based on the physiological arterial pressure of juvenile pigs.  

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**Figure 7** Correlation for coronary blood flow versus stroke work. Each dot in A and B is a different level of workload induced by increasing of left atrium pressure (LAP) from 6 to 12 mmHg. The corresponding values of left ventricle stroke work (LVSW) are on the X axis and coronary blood flow (CBF) is on the Y axis. Correlation was evaluated and a regression line was generated. The slope (correlation coefficient) of generated linear regression line was higher in T1 than T3 and T5 (A and B). CBF change (C) illustrates the difference in coronary blood flow from the low workload (left atrial pressure = 6 mmHg) to the high workload (left atrial pressure = 12 mmHg). At later timepoints, each group had a significant decrease in CBF change (C) in response to increased workload. (Comparison within groups, paired t-test, T3 vs T1 and T5 vs T1 "\( p < 0.05 \), ""\( p < 0.001 \), n = 6).
This strategy is widely used in ESHP studies. However, in this study, we compared this approach with constant coronary flow perfusion, targeting a coronary flow rate of 500 mL/min, approximately 200 mL/min/100g heart weight. To keep this flow rate, the diastolic pressure was reduced to 20 mmHg. The 500 mL/min coronary flow control perfusion has been reported in a previous study.\(^\text{15}\) In our pilot study, this flow rate would keep the coronary sinus oxygen saturation below 80% till end of perfusion. While in the pressure control group, coronary sinus oxygen saturation is over 90%, suggesting coronary vasodilation. However, the value 500 mL/min flow rate is still higher than the physiological value, in which coronary flow is 0.5 to 1.0 mL/min/g myocardium in a typical adult heart.\(^\text{30}\) Usually the baseline/resting coronary blood flow accounts for 5% of cardiac output.\(^\text{31}\)

In the pressure-controlled strategy, we have observed a gradual increase in CBF, concurrently with decreasing ventricle stroke work during 6 hours of perfusion. CBF increased to over 1400 mL/min, which was almost 3 times of that in the coronary flow-controlled group. By comparison, we have demonstrated that controlled coronary blood flow perfusion provides superior preservation of cardiac function and less edema formation. Qazi et al.\(^\text{27}\) demonstrated that higher flow can cause a higher degree of cardiac edema and related diastolic dysfunction, and general impairment of contractility of the heart. Cardiac edema can result from endothelial damage that leads to increased vascular permeability due to the loss of intracellular junctions. Lower cTnI and hyaluronan in the flow control group suggests less cardiac damage and endothelial injury in this group. The accumulation of Hyaluronan in the perfusate suggest endothelial glycocalyx shedding which may lead to increased vessel permeability.\(^\text{32}\) The glycocalyx damage could also greatly enhance the adherence of leukocytes to the endothelium.\(^\text{33}\) Plasma hyaluronan level has also been proposed as a biomarker of myocardial damage.\(^\text{34}\) In our study, the flow control group had lower hyaluronan and correspondingly less edema formation.

![Figure 8](image)

**Figure 8** Less oxidative stress and related modification in the flow control group. A, oxLDL (oxidized low-density lipoprotein). B, MDA (Malondialdehyde). C, Protein carbonyl content. The pressure control group had significantly higher lipid peroxidation than the pressure control group (A and B) and higher oxidative stress related modification of proteins in the coronary arteries (C). (Comparison between groups, independent t-test, pressure vs. flow group: *\(p < 0.05\), **\(p < 0.01\); Pressure group vs. in situ: ¥\(p < 0.001\); comparison across time within group, repeated measure ANOVA, †\(p < 0.001\), n = 6).
Leukocyte, platelet and endothelial activation

Leukocyte, platelet and endothelial activation process are interrelated. The endothelial lining of the coronary vasculature forms the physical barrier between the blood and underlying myocardial tissues. The vasculature is also pivotal for a range of other homeostatic functions relating to the circulation such as hemostasis, lipid transport, and immune surveillance. During ESHP the coronary vasculature resides in directly contacting with the circulating mediators in the perfusate, so it is prone to the direct attack. We have reported here that leukocyte, platelet and endothelial activation were all significantly lower in the flow control group than the pressure control group. The up-regulation of the expression of adhesion molecules in the vascular endothelium is the initiating event and allows leukocytes and monocytes to adhere to the endothelial cell surface. Leukocytes and monocytes penetrate into the sub-endothelial environment, where TNF-α, IL-6 and other cytokines are released, resulting in recruitment of additional circulating cells. The membrane-spanning protein CD40 is also up-regulated in leukocyte and endothelial activation and, after engagement with its natural ligand CD40L, amplify these events by further promoting cytokines release and adhesion of circulating cells to the endothelium. VWF plays a pivotal role in platelet adhesion and aggregation at sites of high shear rates. Plasma levels of VWF are increased in different states of endothelial damage and have been proposed as markers of endothelial dysfunction. Our results indicated that lower coronary flow reduces leukocyte and platelet activation and therefore may maintain a healthier regulation of the vasculature and better endothelial protection during ESHP.

Potential mechanism of decreased coronary vascular resistance and loss of coronary autoregulation

The decreasing LVSW alongside increasing CBF indicates uncoupling between cardiac function and myocardial perfusion. At the initiation of working mode perfusion, as expected, CBF increased appropriately with increased LVSW, consistent with normal coronary autoregulation. However, over extended perfusion, LVSW and CBF trended in opposite directions: while LVSW decreased over 5 hours of working mode perfusion, CBF increased continuously. The uncoupling of these two parameters indicates the loss of coronary autoregulation during perfusion. Here we showed that with the preload challenge at T1, T3, and T5, there was further increase of CBF together with increasing of the LVSW, suggesting that the coronary autoregulation phenomenon was not completely lost. However, the LVSW-related changes of CBF at T3 and T5 were lower than that at T1. These observations suggest that at 6 hours of ESHP, CBF may approach its maximum capacity, and that coronary artery vasomotor response to increased myocardial demand was decreasing. The exact mechanism underlying these observations remains unclear. However, we evaluated inflammation as well as oxidative stress state in the coronary artery tissue. ROS and RNS play a role in altered vascular reactivity and breakdown of the vascular barrier and promote cellular injury during various pathophysiological events. For example, ROS can cause fragmentation of the glycocalyx. We have evaluated ROS in the perfusate, as well as in the coronary vasculature. The flow control group had a relatively lower level of oxLDL in the perfusate, and MDA in the vasculature, suggesting less oxidative stress and lipid peroxidation in this group. There was also lower protein carbonyl content in the flow control group, indicating less ROS modification on the protein.

Our results suggest that the oxidative stress environment of the perfusate may play an important part in the activation of the coronary endothelial cells and facilitate protein modification, affecting normal endothelial function, leading to coronary artery vasodilation, increased permeability, and loss of coronary autoregulation. Furthermore, increased flow in the coronary arteries may increase the shear-stress on the vascular wall, which may facilitate more ROS production in the vasculature as a feed-forward mechanism, leading to supraphysiological dilation of the vessel through flow-mediated mechanism. Leukocytes are another potential source of ROS during ESHP. We have previously reported the infiltration of neutrophils in the myocardium. Here we have shown that controlled coronary flow leads to lower MPO activity. This may potentially contribute to the lower level of oxidative stress in this group. It is also known that the mitochondrial respiratory chain is a significant source of ROS in the myocardium. Thus, myocardial ROS production may be the predominant source of oxidative stress during ESHP, leading to coronary vascular dysfunction in addition to myocardial contractile dysfunction.

Conclusion

Herein we have demonstrated that coronary flow-controlled perfusion during ESHP is superior compared to pressure control with respect to myocardial contractile function and edema formation. Flow-controlled ESHP is associated with lower myocardial tissue injury and leukocyte, platelet and endothelial activation as well as lower induction of oxidative stress. The loss of coronary autoregulation and physiologic coronary vascular resistance during normothermic ESHP may be attributed to the oxidative stress state developed in the coronary vasculature and may be at least partially prevented with flow-controlled perfusion.

Author contributions

XQ and DF designed the research. XQ, SH, SB, MB, KF, MK and CO conducted the experiments. XQ, SH, XW, CO and DF analyzed the data and interpreted the results. XQ...
drafted the manuscript. SH, CO, DN, JA, JN and DF edited the manuscript.

Disclosure statement

DF and JN are founders of Tevosol, Inc, and provide consulting services to Bridge to Life. This work was presented at the 41st International Society of Heart and Lung transplantation Annual Meeting, April 27, 2021.

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Supplementary materials

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