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Sevoflurane preserves the endothelial glycocalyx against ischaemia–reperfusion injury

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Background. Healthy vascular endothelium is coated by the glycocalyx, important in multiple endothelial functions, but destroyed by ischaemia–reperfusion. The impact of volatile anaesthetics on this fragile structure has not been investigated. We evaluated the effect of cardiac pre- and post-conditioning with sevoflurane on integrity of the endothelial glycocalyx in conjunction with coronary vascular function.

Methods. Isolated guinea pig hearts perfused with Krebs–Henseleit buffer underwent 20 min stopped-flow ischaemia (37°C), either without or with 1 MAC sevoflurane. This was applied for 15 min before, for 20 min after, or both before and after ischaemia. Transudate was collected for assessing coronary net fluid extravasation and histamine release by mast cells. Coronary release of syndecan-1 and heparan sulphate was measured. In additional experiments with and without continuous sevoflurane, cathepsin B and tryptase β-like protease activity were measured in effluent. Hearts were perfusion-fixed to visualize the endothelial glycocalyx.

Results. Ischaemia led to a significant ($P<0.05$) increase by 70% in transudate formation during reperfusion only in hearts without sevoflurane. This was accompanied by significant ($P<0.05$) increases in heparan sulphate (four-fold) and syndecan release (6.5-fold), with electron microscopy revealing massive degradation of glycocalyx. After ischaemia, histamine was released into transudate, and cathepsin B activity increased in effluent ($P<0.05$). Sevoflurane application attenuated all these changes, except for histamine release.

Conclusions. Sevoflurane protects the endothelial glycocalyx from ischaemia–reperfusion-induced degradation, with both preconditioning and rapid post-conditioning being successful. The mechanism seems to involve attenuation of lysosomal cathepsin B release and to be independent from tissue mast cell degranulation.

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The potential of volatile anaesthetics to protect against myocardial ischaemia–reperfusion injury and to reduce infarct size is well established.[1–3] The mechanisms underlying the protective action are still incompletely understood, but both pre- and post-conditioning contribute.[4–7] There is both experimental and clinical evidence that other organs can also be effectively protected during ischaemia and reperfusion.[8–12]

The healthy vascular endothelium is coated by a glycocalyx. This consists of a highly sulphated layer of glycosaminoglycans bound to a variety of proteoglycans; 50–90% of those associated with endothelial cells are heparan sulphate proteoglycans. Together with solubilized glycosaminoglycans and plasma proteins, the endothelial glycocalyx forms the endothelial surface layer. With up to 1–3 μm thickness, this structure is, in some regions, even thicker than the endothelial cells themselves.[13 14] It plays a key role in regulation of vascular resistance, permeability,

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and leucocyte recruitment.[14–16] Recent clinical and experimental investigations show that the glycocalyx is severely affected after ischaemia–reperfusion.[17–19] Some protection of the glycocalyx against ischaemia and reperfusion-induced injuries has been afforded by ischaemic preconditioning and pre-ischaemic treatment with hydrocortisone and antithrombin.[20–22] However, the influence of commonly used anaesthetics on this fragile structure has not been investigated.

As anaesthetic and ischaemic preconditioning share similar pathways, we hypothesized that the volatile agent sevoflurane could also achieve a significant protection of the coronary endothelial glycocalyx. To test this hypothesis, we used an isolated perfused heart model designed to facilitate detection of the state of the glycocalyx. Particular attention was focused on the links to the functional state of the endothelial barrier and on the question whether pre- or post-conditioning of the heart with sevoflurane may be the preferable approach.

Methods

Experiments were approved by local governmental authorities (No. 209.1/211-2531.3-3/99), and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, USA.[23]

Heart preparation

Hearts of male guinea pigs [body weight 300 (50 g) (mean (SD)] were isolated and perfused with a modified Krebs–Henseleit buffer [116 mM NaCl, 23 mM $NaHCO_3$, 3.6 mM KCl, 1.16 mM KH_2PO_4, 1.2 mM $CaCl_2$, 0.58 mM $MgSO_4$, 5.4 mM glucose, 0.3 mM pyruvate, and 2.8 U $litre^{-1}$ insulin, gassed with 94.6% oxygen and 5.4% carbon dioxide at 37°C, pH 7.40 (SD 0.05)] in a modified Langendorff model as described previously.[15 21 22] In brief, the animals were stunned by a blow to the neck/occipital area using a specially designed, spring-loaded instrument, before opening both carotid arteries. After opening the thorax, the hearts were immediately arrested with cold isotonic saline and the aorta quickly cannulated and retrogradely perfused at a constant perfusion pressure of 70 cm H_2O. Hearts were removed from the thorax and the pulmonary, caval, and azygos veins ligated. Cannulating the pulmonary artery allowed coronary venous effluent to be collected after draining from the coronary sinus via the right atrium and ventricle. Transudate, representing a mixture of interstitial and lymphatic fluids formed by net filtration and emerging at the epicardial surface, was collected over timed intervals as it dripped from the apex of the heart.

Experimental protocols

Immediately after explantation and preparation, an equilibration interval of 15 min was allowed to establish steady-state conditions before baseline measurements were performed on the spontaneously beating hearts. Six different protocols (n=7 hearts each) were then used: Group A (control; no sevoflurane) and Group B (control+sevoflurane 2%) hearts were perfused and oxygenated for 40 min. In Group C (ischaemia; no sevoflurane) warm (37°C), global stopped-flow ischaemia was induced for 20 min, followed by 20 min of reperfusion. In the intervention groups, the same protocol as in Group C was used, adding sevoflurane 2% either for 15 min before the induction of ischaemia (Group D), only during the reperfusion period (Group E), or during the whole duration of the experiment (Group F). The volatile sevoflurane was added at 2 vol% to the oxygen/carbon dioxide gas mixture used to equilibrate the Krebs–Henseleit perfusate using calibrated vaporizers (Vapor 19.3, Draeger, Germany). The concentration of 2 vol% was closely monitored in the gas phase using a piezo gas analyzer (Capnomax Ultima, Datex, Helsinki, Finland) and represents 1 MAC in humans.

Baseline measurements of coronary effluent and transudate were performed in the last 3 min before the induction of ischaemia. During reperfusion, effluent and transudate samples were collected over intervals at 0–10 and 10–20 min from the beating hearts. Development of coronary flow over time during reperfusion was normalized to the basal coronary flow rate of each individual animal and expressed as per cent change against the baseline value. As actual coronary flow may influence the filtration rate, net fluid filtration was expressed as a ratio between actual transudate formation and effluent flow rate. To allow for the slow time course of changes in coronary vascular leak, the total amount of transudate formed over the period of reperfusion (20 min) was entered into the calculation, together with the summed coronary flow during reperfusion.

Release of glycocalyx components, release of creatine kinase and histamine

Shedding of syndecan-1 (CD-138) and heparan sulphate was assessed in coronary effluent 0–10 min after reperfusion as described elsewhere.[21 22] Samples (4 ml) were first concentrated to 50–100 μl with 10 kDa cut-off ultra filters (Millipore, Billerica, MA, USA). Syndecan-1 concentrations were determined using an enzyme-linked immunosorbent assay (ELISA; Diaclone Research, Besancon, France), and heparan sulphate content was quantified with an ELISA purchased from Seikagaku Corporation, Tokyo, Japan. Results are given as total release during the first 10 min of reperfusion, related to dry heart weight. Creatine kinase and histamine were determined in transudate after ischaemia. The kits used were from Spibio, Montigny le Bretonneux, France, and BioAssay Systems, Hayward, CA, USA. The values were multiplied by the rates of transudate production to yield the rates of release from the interstitium.

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Determination of protease release in effluent

As proteases must be responsible for any changes in glycocalyx shedding, additional experiments (n=6 each) were performed in hearts with volume-controlled perfusion (7 ml min^{-1}) after 20 min of ischaemia. During 0–10 min of reperfusion, released cathepsin B- and tryptase β-like activity were measured in coronary effluent without (IR group) and with continuous sevoflurane application (IR+Sevo group). Infusion of exogenous cathepsin B into the coronary system was found to elicit shedding in preliminary experiments. Tryptase β is a major protease of mast cells.

Cathepsin B- and tryptase β-like activity were determined by modifications of described methods.[24] In brief, 100 µl of effluent was incubated, respectively, with buffer made up of 50 mM sodium acetate, 2 mM EDTA, 2 mM DTT, 0.015% Brij (pH 5.5) and containing 50 µM of the cathepsin B substrate Z-Arg-Arg-AMC (Bachem, Weil am Rhein, Germany), or buffer made up of 50 mM Tris, 150 mM NaCl, 0.01% Triton, 50 µg ml^{-1} heparin (pH 7.6) and containing 20 µM of the substrate tos-Gly-Pro-Arg-AMC (Sigma-Aldrich, Munich, Germany) plus 35 µM antithrombin III (Kybernin; Behring, Marburg, Germany) to inhibit any residual thrombin. The hydrolysis of the substrates was followed spectrofluorometically using a HTS 7000 Bio Assay Reader (Perkin Elmer, Rodgau-Jügesheim, Germany) with excitation and emission wavelengths of 360 and 465 nm, respectively. Human cathepsin B (Calbiochem/Merck Biosciences, Schwalbach, Germany) and recombinant human tryptase β produced in-house served as positive controls. Activity was expressed as nM AMC released per minute and related to the total volume of effluent sampled during the first 10 min of reperfusion.

Light and electron microscopy

Additional hearts with protocols corresponding to those of Groups A (time control), C (ischaemia–reperfusion), and F (ischaemia–reperfusion with sevoflurane pre- and post-treatment) were perfusion fixed with either 4% formalin or lanthanium/glutaraldehyde mixture according to previously described procedures.[19 25 26] These hearts served for immunostaining and for electron microscopy of the glycocalyx, respectively. For immunostaining, paraffin sections were stained with monoclonal antibodies against syndecan-1 (Biosource) and heparan sulphate (Seikagaku Corp.). The primary antibodies, applied to generate an avidin-biotin horseradish peroxidase complex with the Vectastain kit (Vector, Burlingame, CA, USA), were diluted and handled as follows: anti-heparan sulphate 1:100, tissue preincubation with 0.2% trypsin at 37°C; antisyndecan 1:150, tissue pre-treatment by microwave irradiation. Controls, in which the primary antibody was replaced with buffer, were treated identically. Diaminobenzidine or aminoethylcarbazole served as chromogen.

Statistics

Previous experience in working with isolated heart models has shown sample sizes of about n=7 to suffice for statistical evaluation of biologically relevant effects. All data are given as median with inter-quartile range (IQR) and full range, unless indicated otherwise. Developments over time were analysed by the Friedman repeated-measures ANOVA on ranks. Multiple groups at a single time point were compared by the Kruskal–Wallis one-way ANOVA on ranks, followed by a Student–Newman–Keul or Dunn test, as appropriate. For comparing two measuring points within a single group, a Mann–Whitney rank-sum test was used. $P<0.05$ was considered statistically significant (SigmaStat, Systat Software, Richmond, VA, USA).

Results

Coronary flow

Median (1st/3rd quartile) basal coronary flow rate for all hearts was 9.4 (8.1/9.9) ml min^{-1}. Coronary flow rate did not change significantly during the experiment in the control group A. All the other groups, including the sevoflurane control (B), exhibited a significant decrease in coronary flow (*$P<0.05$, RM ANOVA on ranks), which was most pronounced in the ischaemia group C without sevoflurane (Fig. 1). However, an interruption of this general trend occurred during the first 5 min of reperfusion in the sevoflurane treatment groups (D, E, F): coronary flow was preserved, whereas it decreased significantly in the

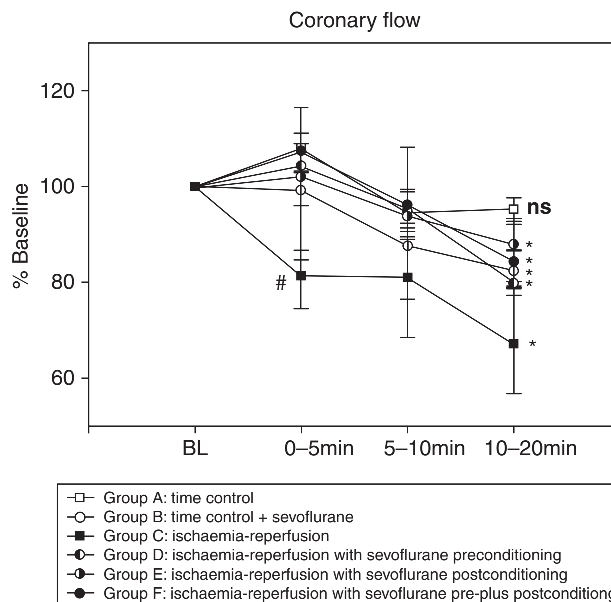

Fig 1 Coronary flow compared with the individual basal value during the experimental course. Data given as median and IQR (T-bars), n=7 each. *$P<0.05$ *vs* baseline; $^{\#}P<0.05$ C *vs* A, B, D, E, and F.

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ischaemic group without sevoflurane (C) ($P<0.05$). Flow between Groups D, E, and F did not differ (Fig. 1).

Fluid filtration

Median (1st/3rd quartile) basal transudate formation for all hearts was 0.36 (0.29/0.49) ml min^{-1}. This amounted to about 4.4% of the basal coronary venous effluent flow. Compared with baseline measurements, the transudate to effluent ratio increased only in Group C without sevoflurane treatment ($P<0.05$), whereas it remained stable in all sevoflurane treatment groups (Fig. 2). Sevoflurane groups showed no statistically significant difference in net fluid filtration, irrespective of whether pre-, post- or pre- plus post-conditioning had been performed.

Measurement of glycocalyx constituents

Heparan sulphate and CD-138 positive material (syndecan-1) were detected in the effluent of all hearts during 0–10 min of reperfusion. Values of both glycocalyx constituents were significantly increased compared with non-ischaemic hearts (Group A) in Group C without sevoflurane treatment ($P<0.05$, Table 1). The increase *vs* the time control group A was attenuated in all ischaemia groups receiving sevoflurane. There was no significant difference whatsoever between any groups receiving sevoflurane, that is, between the post-ischaemic rates of shedding of Groups D, E, F, and the sevoflurane control group B (Table 1).

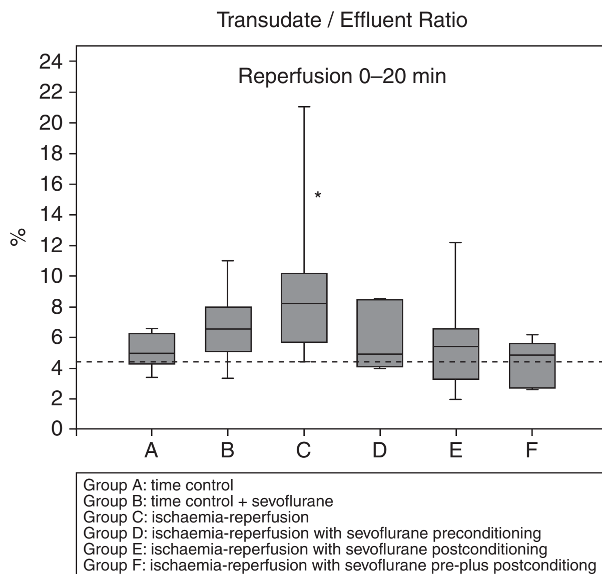

Fig 2 Net fluid filtration expressed as transudate/effluent ratio established throughout the first 20 min of reperfusion or corresponding time (controls A and B). Data given as median, IQR (boxes) and full range (T-bars), n=7 each. The dashed line represents the median basal ratio for all hearts (4.4%; n=42). *$P<0.05$ Group C reperfusion *vs* Group C baseline.

Table 1 Soluble markers of glycocalyx shedding. Total heparan sulphate and syndecan-1 (CD-138) release per gram dry heart weight in coronary effluent during the first 10 min of reperfusion or corresponding control time. Data given as median (1st/3rd quartile). *$P<0.05$ C *vs* A

Group	Heparan sulphate release (μg g^{-1})	CD-138 release (ng g^{-1})
Control (A)	0.5 (0.4/0.7)	33.5 (0.0/131.6)
Sevo-Control (B)	1.1 (0.7/1.4)	129.3 (113.5/222.3)
Ischaemia (C)	2.1* (1.8/2.9)	217.8* (150.1/476.4)
Sevo-Pre (D)	0.9 (0.8/1.2)	109.6 (105.2/142.0)
Sevo-Post (E)	1.1 (0.7/1.3)	153.5 (133.3/201.7)
Sevo-All (F)	0.8 (0.7/0.9)	176.4 (137.9/345.5)

Light and electron microscopy

Light microscopy after immunohistochemical staining revealed heparan sulphate and CD-138 positive material as components of the endothelial lining. Intense staining could be achieved in Group A without ischaemia. In Group C, ischaemia and reperfusion caused a dramatic loss of staining intensity, but brought clear signs of tissue oedema. The continuous presence of sevoflurane decreased the shedding (Fig. 3). Electron microscopy of Group A hearts showed a well-structured endothelial glycocalyx of about 200 nm thickness. This layer was nearly completely absent after ischaemia and reperfusion, especially in the microvasculature (Group C). Furthermore, leakage of the fixation solution from the perfused vessels was evidenced by staining of extravascular cell surfaces. However, with sevoflurane (Group F), an almost intact glycocalyx could be visualized (Fig. 4).

Creatine kinase activity and histamine release

Creatine kinase activity was not detectable in time-control hearts. Activity became present in transudate immediately after reperfusion in all hearts subjected to ischaemia. Activity was significantly higher in Groups C (no sevoflurane) and D (preconditioning with sevoflurane) than in Groups E and F, both of which received sevoflurane immediately upon reperfusion. Also histamine could be detected in transudate of post-ischaemic hearts, a sign of degranulation of resident mast cells. Pertinently, there was no significant difference in washout of histamine between groups without and with sevoflurane (Table 2).

Activity of proteases

Activity of the lysosomal protease cathepsin B increased significantly in coronary effluent after ischaemia (Fig. 5). Pre- plus post-ischaemic application of sevoflurane attenuated this effect. On the other hand, tryptase β-like activity in coronary effluent did not change consistently between baseline and post-ischaemia reperfusion conditions and there was no significant difference between the groups, regardless of whether sevoflurane was applied or not (data not shown).

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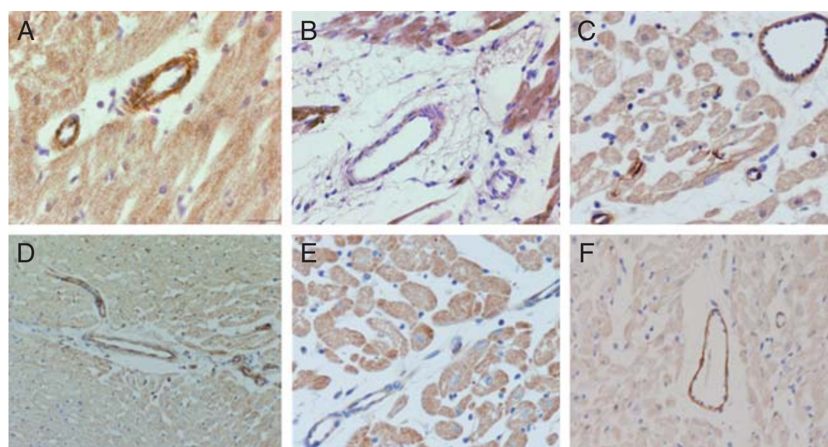

Fig 3 Light microscopy of exemplary hearts after immunohistochemical staining of heparan sulphate and syndecan-1. (A) (magnification ×1000) and (D) (magnification ×300) represent hearts without ischaemia and an intact glycocalyx. Loss of staining is evident in (B) (magnification ×1000) and (E) (magnification ×600), indicating destruction of the endothelial glycocalyx after 20 min ischaemia and reperfusion. In (C) (magnification ×600) and (F) (magnification ×300) glycocalyx staining is still present after ischaemia and reperfusion (20 min) with sevoflurane pre-plus post-treatment. Each panel is taken from a heart representative of one of the three experimental groups mentioned above.

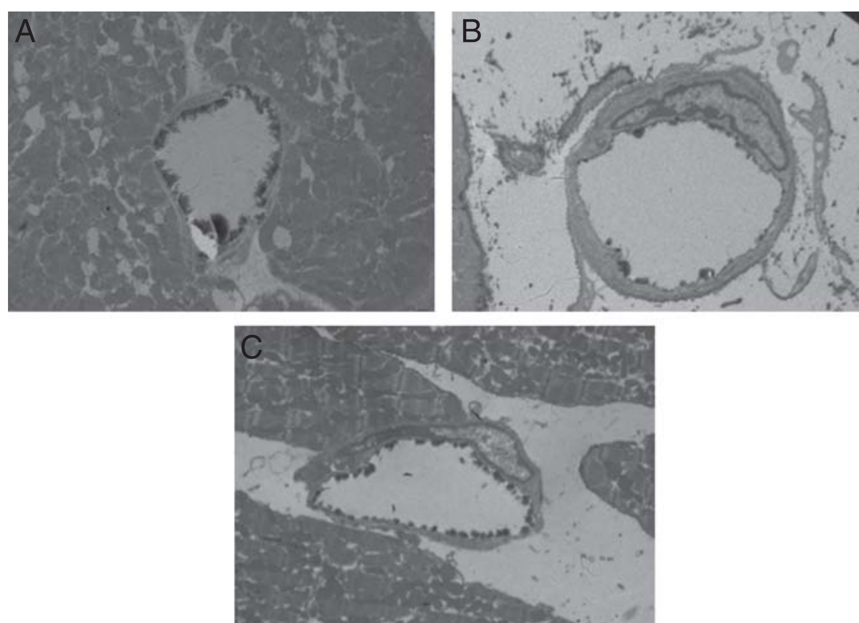

Fig 4 Electron microscopy of vascular endothelial glycocalyx without ischaemia (A), after 20 min of ischaemia and reperfusion without (B), and with sevoflurane pre- plus post-treatment (C). Each panel is taken from a heart representative of one of the three experimental groups mentioned above. Original magnifications all ×3640.

Table 2 Markers of mast cell degranulation and myocardial cell injury. Histamine and creatine kinase release in coronary transudate during 0–5 min of reperfusion. Data given as median (1st/3rd quartile). $^{*}P<0.05$ C *vs* E; $^{\#}P<0.05$ C *vs* F; $^{\sim}P<0.05$ D *vs* E, $^{+}P<0.05$ D *vs* F

Group	Histamine release (nmol min^{-1} g^{-1})	CK release (mU min^{-1} g^{-1})
Control (A)	Not detectable	Not detectable
Sevo-Control (B)	Not detectable	Not detectable
Ischaemia (C)	620 (349/1226)	$130^{*,\#}$ (66/526)
Sevo-Pre (D)	771 (645/1282)	$84^{\sim,+}$ (58/101)
Sevo-Post (E)	798 (432/997)	59 (39/87)
Sevo-All (F)	576 (461/612)	43 (33/62)

Discussion

We have shown that myocardial ischaemia–reperfusion increased coronary net fluid extravasation and reduced coronary flow in unprotected hearts. These changes were accompanied by a significant increase in the concentration of soluble glycocalyx components in coronary effluent. Electron microscopy, supported by immunohistochemistry, revealed a massive destruction of the endothelial glycocalyx. A direct casual link between these functional and structural changes has been established before.[21 22] We now demonstrate that sevoflurane application attenuates

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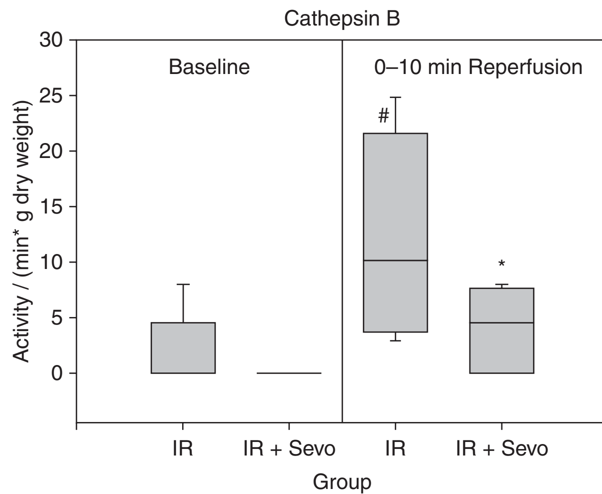

Fig 5 Cathepsin B-like activity in coronary effluent during baseline conditions and the first 10 min of reperfusion. Data given as median, IQR (boxes), and full range (T-bars), n=6 each. #$P<0.05$ 0–10 min reperfusion IR *vs* baseline IR and baseline IR + Sevo; *$P<0.05$ 0–10 min reperfusion IR + Sevo *vs* reperfusion IR.

the negative effects of ischaemia–reperfusion on coronary leak and glycocalyx shedding. Sevoflurane application before ischaemia or rapid application solely during reperfusion seems to be as effective as providing sevoflurane throughout the procedure regarding the prevention of endothelial glycocalyx damage. However, preconditioning alone was not sufficient to induce a significantly different release of creatine kinase compared with ischaemia without sevoflurane. Subgroup analysis revealed that post-ischaemic release of the lysosomal protease cathepsin B was attenuated by continuous application of sevoflurane.

The endothelial glycocalyx has recently come into focus as a major player in many pathophysiologic vascular states such as inflammation and atherosclerosis.[13 14 17 27 28] Both endothelial cells and glycocalyx are affected by ischaemia–reperfusion.[12 21 22] Together, they constitute a double barrier, with both components necessary to control coronary extravasation and oedema formation.[15] As a consequence, extensive tissue oedema, increases in transudate production and colloid permeability, and also endothelial dysfunction are frequently found after destruction of this large and important structure.[12 20–22] The glycocalyx is also an important mediator of the biochemical response to fluid shear stress.[13 14] Consistent with our observations, extensive destruction of the endothelial glycocalyx has been described after myocardial and intestinal ischaemia–reperfusion injury.[17 18 20–22]

Increased glycocalyx shedding can also be triggered by atrial natriuretic peptide and inflammatory stimuli like tumour-necrosis factor alpha (TNFα).[27–29] In fact, the actual composition of the glycocalyx seems to result from the balance between biosynthesis of glycosaminoglycans and shedding. The mechanisms underlying shedding are incompletely understood, but may involve membrane-bound proteases and are, at least in part, activated by G-protein signalling.[17] Also, activated inflammatory cells and resident macrophages may contribute to an increased shedding by generating reactive oxygen radicals.[18]

Some protection of the endothelial glycocalyx against ischaemia–reperfusion-induced injuries has been achieved by ischaemic preconditioning, and by treatment with hydrocortisone and antithrombin III.[20–22] Interestingly, halogenated anaesthetics like sevoflurane protect against ischaemia–reperfusion injury in different organ systems.[4 8 9 12] Anaesthetic-induced protection presumably relies on multiple mechanisms, in part sharing pathways with 'classic' ischaemic preconditioning.[1 4] Also, during reperfusion, beneficial effects like a reduction of endothelial–leucocyte interaction have been shown for volatile anaesthetics in different experimental settings.[5 6] In clinical practice, a combination of 'pre' and 'post' conditioning seems to be especially effective.[7] However, no studies have directly considered the effects of anaesthetics on the glycocalyx before.

In a clinical investigation, Bruegger and colleagues[30] reported that sevoflurane attenuated oedema formation compared with propofol. Lucchinetti and colleagues[12] recently reported an improved endothelium-mediated hyperaemic reaction with sevoflurane in human volunteers after forearm ischaemia–reperfusion injury. These findings are consistent with the present investigation showing, for the first time, improved coronary flow, attenuation of transudate formation, and alleviation of coronary glycocalyx shedding with sevoflurane. Preserving the endothelial glycocalyx will restrict microvascular fluid extravasation and prevent oedema formation, and contribute to better maintained endothelium-dependent vasodilatation, reducing the no-reflow phenomenon. Any longer-term effects of sevoflurane, for instance, of endothelial cell replacement or regeneration,[31] cannot be assessed on the basis of the present study.

Relatively little is known about mechanisms of glycocalyx shedding. In conjunction with protection by sevoflurane, our attention focused on two major proteases, tryptase β and cathepsin B. Tryptase β is stored in resident myocardial mast cells, known to degranulate upon ischaemia–reperfusion.[32] Their release of histamine was used in this study to document activation. The amounts released were larger than in previous studies of our group, a result of greater maturation of the heart donor animals used in the present study. However, treatment of hearts with sevoflurane had no effect on the elevated washout of histamine during post-ischaemic reperfusion. Similarly, though tryptase β-like activity tended to be elevated in coronary effluent during reperfusion, pre- plus post-conditioning with sevoflurane elicited no decrease. Quite in contrast, cathepsin B-like activity, was profoundly lower in the sevoflurane-treated hearts during reperfusion. This is an extremely exciting finding, because it offers an explanation for the protective action of sevoflurane on the glycocalyx.

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Cathepsin B is a ubiquitously expressed protease, found in all cell types and especially in the endothelium.[33] It is stored in lysosomes, but can be easily released by stressed cells and, as such, is associated with degradation of the extracellular matrix, tumour progression, and inflammatory diseases. Stabilization of lysosomal membranes by a lipophilic agent such as sevoflurane could easily account for a lower rate of liberation into the reperfused heart tissue. This concept is supported by the fact that also the release of creatine kinase was attenuated by pre- and post-treatment of hearts with sevoflurane. The intracellularly localized enzyme creatine kinase is only released from cells sustaining membrane damage, induced exocytosis, or both. As an alternative to membrane effects, one may speculate that suppressed expression of proinflammatory cytokines like TNFα, as shown for sevoflurane in an isolated cell model,[34] may indirectly reduce release of cathepsin B. We have no direct data to this end, but it should be called to attention that TNFα acutely released during myocardial reperfusion stems from mast cells.[32] Sevoflurane was unable to attenuate degranulation of mast cells, at least at 1 MAC in the present model.

The finding that early post-ischaemic application of sevoflurane proved to be protective against shedding of the glycocalyx is both surprising and important. Shedding induced by ischaemia takes place in the early course of reperfusion and must be ascribed to the activation of hitherto unidentified protease(s). This role may be filled by cathepsin B, following its enhanced release into the coronary system. It now seems that immediate post-conditioning supplies the volatile anaesthetic with sufficient rapidity to halt such liberation processes.

There are some potential shortcomings in the present investigation. Hearts were perfused with an artificial, colloid-free medium, leading to tissue oedema after some 30 min of perfusion, as noted in previous experiments.[15] This was evident even in control groups, although an intact glycocalyx could be visualized (Fig. 4). However, apart from the addition of sevoflurane, perfusate composition and the duration of the experiments did not differ between the groups here. Neutrophilic granulocytes, platelets, and plasma-borne factors (complement, enzymes, etc.) are important contributors to reperfusion-induced injuries. These effects could not be studied in our blood-free preparation. Further investigations, preferably *in vivo*, will be necessary to study the impact of sevoflurane via a better preserved endothelial glycocalyx on leucocyte–endothelial interactions. Protection of the glycocalyx should decrease adherence, simply because the glycocalyx extends far beyond the reach of the cell adhesion molecules. This would be consistent with previous experiments in our laboratory, in which sevoflurane and other volatile anaesthetics mitigated post-ischaemic adhesion of granulocytes and platelets in the coronary vasculature.[5 6 35] Also, experiments with a cathepsin B inhibitor should substantiate whether the mechanism proposed here is valid. However, a specific agent without untoward myocardial effects has yet to be identified for this.

Conclusion

We demonstrated for the first time that sevoflurane protects the post-ischaemic guinea pig myocardium against increased coronary leakage and endothelial dysfunction by reducing shedding of the endothelial glycocalyx. Inhibition of shedding may be ascribed to attenuated release of the protease cathepsin B during reperfusion in hearts stabilized by sevoflurane. Preconditioning seems to be as effective as immediate post-conditioning or application of sevoflurane throughout the procedure. However, this result may perhaps only apply to the short time-span of follow-up inherent to the present model. It should prove rewarding to investigate links between clinical outcomes and protection of the fragile glycocalyx by volatile anaesthetics.

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