Sevoflurane Reduces Leukocyte and Platelet Adhesion after Ischemia-Reperfusion by Protecting the Endothelial Glycocalyx

Daniel Chappell, M.D.,* Bernhard Heindl, M.D., Ph.D.,† Matthias Jacob, M.D.,* Thorsten Annecke, M.D.,‡ Congcong Chen, M.D.,§ Markus Rehm, M.D.,† Peter Conzen, M.D.,‖ Bernhard F. Becker, M.D., Ph.D.#

ABSTRACT

Background: Adhesion of polymorphonuclear neutrophils and platelets to the vessel wall contributes to generating ischemia–reperfusion injury. Endothelial adhesion molecules are harbored within the glycocalyx, which covers every healthy vascular endothelium but is deteriorated by ischemia–reperfusion. Pretreating the heart with volatile anesthetics reduces myocardial infarct size and protects against ischemia–reperfusion injury. The authors analyzed a possible protective effect of sevoflurane on the glycocalyx and implications for posts ischemic cell adhesion.

Methods: Isolated guinea pig hearts were perfused with crystalloid buffer and subjected to 20 min of global warm ischemia and 10 min of reperfusion. An intracoronary bolus of 3 x 10⁶ polymorphonuclear neutrophilic leukocytes or 1 x 10⁹ platelets of human origin was applied after reperfusion, either with or without pretreating with 0.5 or 1 minimal alveolar concentration sevoflurane. The number of sequestered cells was calculated from the difference between coronary input and output. Coronary effluent was collected throughout reperfusion to measure shedding of the glycocalyx.

Results: Ischemia–reperfusion induced a significant increase in median (interquartile range) adhesion versus control nonischemic hearts of both leukocytes (38.9 (36.3–42.9)%) and platelets (25.0 (22.5–27.1)%) vs. 14.5 (13.1–16.0)%. Sevoflurane reduced cell adhesion to near basal at 1 minimal alveolar concentration (leukocytes: 21.2% (19.2–23.9%), platelets: 11.5% (10.4–12.0%). Shedding measurements and electron microscopy demonstrated that sevoflurane-treated hearts retained much of their 200 nm-thick glycocalyx.

Conclusions: Sevoflurane reduces glycocalyx shedding in the posts ischemic coronary bed, maintaining the natural cover for endothelial adhesion molecules and, thus, reducing cell adhesion. This may explain beneficial outcomes linked to clinical use of volatile anesthetics after ischemia–reperfusion.
heart. In clinical studies with patients undergoing open heart surgery volatile anesthetics have been shown to be cardioprotective.\textsuperscript{7\textendash}9 A trial in patients undergoing coronary artery bypass graft surgery demonstrated not only a decrease of postoperative myocardial dysfunction but also attenuation of renal damage, implying a possible multigorgan protection.\textsuperscript{9} In a sustained forearm ischemia model, endothelium was shown to be receptive to protection by sevoflurane even in lower concentrations (less than 1 vol%).\textsuperscript{10} Vascular endothelium is critically involved in many steps of tissue damage originating from I/R.\textsuperscript{11} So far, it has been shown experimentally that human endothelial cells exposed to volatile anesthetics developed a pronounced resistance against cytokine-induced toxicity, consistent with a preconditioning-like effect.\textsuperscript{12}

Every healthy vascular endothelium is coated by an endothelial glycocalyx that consists of a variety of transmembrane and membrane-bound molecules, predominantly syndecan-1 and heparan sulfate.\textsuperscript{13\textendash}16 Together with bound plasma proteins and solubilized glycosaminoglycans, the glycocalyx forms the endothelial surface layer with a functional thickness greater than 1 μm.\textsuperscript{17,18} Early manifestation of endothelial injury after I/R\textsuperscript{13} and tissue necrosis factor-α\textsuperscript{19} or lipopolysaccharide-induced\textsuperscript{20} inflammation consists of a disruption of the glycocalyx. Diminution of the glycocalyx increases capillary permeability and leads to tissue edema, suggesting that the glycocalyx acts as a competent permeability barrier.\textsuperscript{15,21} Adhesion molecules are normally harbored within and covered by the glycocalyx.\textsuperscript{22} Therefore, exposure and activation of adhesion molecules is presumed after diminution of this structure.\textsuperscript{14,18} Intravital microscopy has suggested that a reduction of the functional diameter of the endothelial surface layer leads to leukocyte\textsuperscript{23} and platelet\textsuperscript{24} adhesion. Treatment with volatile anesthetics has recently been shown to exert protective effects on the glycocalyx.\textsuperscript{25}

Our study aimed to show the extent to which adhesion of polymorphonuclear neutrophils (PMN) and platelets depends on damage of the glycocalyx in the face of I/R.

### Materials and Methods

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. The study is approved by the officially installed independent ethics committee of the State of Bavaria (Munich, Bavaria, Germany). Licensure of the investigator has been granted by the Government of Upper Bavaria (file No 209.1/211–2531.3–3/99).

### Heart Preparation

Guinea pig hearts were isolated and perfused in a modified Langendorff mode.\textsuperscript{13,15} In brief, male animals (weight 250–300 g) were stunned by cervical dislocation. Immediately after median thoracotomy, the hearts were arrested with cold saline fluid. The aorta was then cannulated and the coronaries perfused in situ at constant aortic pressure (70 cm H₂O) with a modified Krebs-Henseleit buffer (in mM: 116 NaCl, 23 NaHCO₃, 3.6 KCl, 1.16 KH₂PO₄, 1.2 CaCl₂, 0.58 MgSO₄, 5.4 glucose, 0.3 pyruvate, and 2.8 U/l insulin) gassed with 94.6% O₂ and 5.4% CO₂ at 37°C, pH 7.40 ± 0.05. Hearts were removed from the thorax and prepared as previously described.\textsuperscript{26} The perfusate flow in the inflow aortic feed line, as well as in the outflow line from the pulmonary artery, was continuously recorded with small-animal flow meters (T106; Transonic Systems Inc., Ithaca, NY). Coronary venous effluent was collected from the cannulated pulmonary artery after draining from the coronary sinus into the right atrium and ventricle.

### Experimental Protocols

Immediately after explantation and preparation of the hearts, an equilibration interval of 15 min helped to establish steady-state conditions. Baseline measurements of coronary effluent for glycocalyx components were performed in the last 2 min before inducing 20 min of warm (37°C), global no-flow ischemia.

The experimental groups are characterized in table 1. Nonischemic time-control experiments were performed either without (group A) or with preconditioning of the hearts with either 0.5 (1 vol%, group B) or 1.0 (2 vol%, group C) minimum alveolar concentration (MAC) of sevoflurane (Abbott Laboratories, Abbott Park, IL). Freshly prepared human PMN (groups D, E, and F) or platelets (groups G, H, and I) were infused after 10 min of further perfusion. The infusions consisted of a 1-ml bolus of PMN (3 X 10⁹) or platelets (1 X 10¹⁰) suspended in Tyrode solution and were applied via infusion pump into the aortic canula over 1 min, passing on into the coronary system. I/R was induced after a 15-min

---

**Impact of Sevoflurane on Glycocalyx and Cell Adhesion**

**Table 1. Study Groups**

<table>
<thead>
<tr>
<th>Control</th>
<th>Control + PMN</th>
<th>Control + Plates</th>
<th>Ischemia</th>
<th>I/R + PMN</th>
<th>I/R + Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control + PMN</td>
<td>Control + Plates</td>
<td>Ischemia</td>
<td>I/R + PMN</td>
<td>I/R + Plates</td>
</tr>
</tbody>
</table>

Control: perfusion for 10 min after establishing a steady-state condition (15 min) without ischemia. I/R: 20 min warm (37°C) global no-flow ischemia followed by 10 min reperfusion. PMN: (3 X 10⁹) applied for 1 min after 10 min of reperfusion. Platelets: infusion of 1 X 10¹⁰ platelets for 1 min after 10 min of reperfusion.

I/R = ischemia-reperfusion; MAC = minimum alveolar concentration; PMN = polymorphonuclear neutrophils.
equilibration phase without pretreatment with sevoflurane and without (group J) or with infusion of PMN (group M) or platelets (group P) after 10 min of subsequent reperfusion. Other groups of heart received posts ischemic PMN or platelet application after pretreatment before ischemia with either 0.5 MAC (groups N and Q) or 1.0 MAC sevoflurane (groups O and R).

Pretreatment with sevoflurane was performed throughout the equilibration phase (15 min), allowing the applied concentrations to be present during the 20 min of no-flow ischemia. During the reperfusion phase, sevoflurane was washed out to avoid any direct effects on the human blood cells. 

At the end of each experiment the ventricles were weighed at once (wet weight) and again after 24 h at 60°C (dry weight) to establish a wet- to dry-weight ratio. This served as a quantitative measure for formation of edema.

**Preparation of PMN**

PMN were isolated from fresh venous blood of healthy volunteers, as described in detail previously. Briefly, the blood was anticoagulated with 0.1% EDTA and centrifuged at 380 g for 10 min. Plasma was discarded and the buffy coat carefully collected. The buffy coat was incubated with an iron-tagged monoclonal antibody against CD15 (Miltenyi, Bergisch Gladbach, Germany) and subsequently passed through a magnetized column. The column was flushed with phosphate-buffered saline (pH 7.4) to wash away unlabeled cells and, after removing the column from the magnetic field, PMN were eluted with phosphate-buffered saline. The eluent was centrifuged at 380 g for 10 min and the resulting cell pellet resuspended in Tyrode solution, counted, and adjusted to a final cell count of 3 x 10^6 cells/ml Tyrode solution. The total time for cell preparation was less than 60 min.

**Preparation of Platelets**

Blood from healthy volunteers who had not taken antiinflammatory drugs within the previous 10 days was drawn into polypropylene syringes containing EDTA 0.1% and loprost 50 ng/ml (final concentrations) for anticoagulation. The blood was centrifuged at 2,000 g for 15 min. The platelet-rich plasma was separated and centrifuged and the platelet pellet was twice resuspended and centrifuged at 10,000 g for 1 min in phosphate-buffered saline. The washing procedure served to remove plasma components, such as fibrinogen, that influence platelet aggregation and could, thereby, confound the interpretation of intracoronary sequestration data. Finally, washed platelets were resuspended in Tyrode solution and adjusted to a final concentration of 1 x 10^9 ml.

**Determination of Glycocalyx Components**

Samples of effluent were used for assessing shedding of heparan sulfate and syndecan-1 (CD-138) in all groups as described in detail elsewhere. Samples (4 ml) were concentrated to 50 μl with 10 kD cutoff ultrafilters (Millipore, Billerica, MA). Aliquots (20 μl) were used for an enzyme-linked immunosorbent assay based on two antibodies specific for heparan sulfate-related epitopes (Seikagaku Corporation, Tokyo, Japan). Syndecan-1 concentrations were determined in 20-μl aliquots using another enzyme-linked immunosorbent assay (Diaclone Research, Besançon, France).

**Adhesion Measurements**

Sequestration of PMN and platelets within the coronary system was determined from the arteriovenous difference in absolute cell count as described previously. Immediately before each intracoronary application of a cell bolus, a test bolus of equal volume and duration (1 ml in 1 min) was sampled to determine the number of cells actually leaving the application syringe (cell input). To quantify the number of cells leaving the coronary arteries (cell output), coronary effluent was collected continuously during the minute of bolus application and in the following 2 min, combined for a total of 3 min. Pilot studies had shown that only a negligible number of the applied cells (less than 1%) emerged after such a sampling period. A 10% formaldehyde solution was able to fix cells for counting and flow cytometric analysis. The percentage of PMN or platelets adherent to the endothelium was calculated as:

\[
\text{Adhesion [%]} = \left\{1 - \frac{\text{cell output/\text{cell input}}}{\text{cell input}}\right\} \times 100
\]

**Flow Cytometry**

Formalin-fixed cells of the test bolus and of the coronary effluent were analyzed in a FACScan flow cytometer (Becton Dickinson, San Jose, CA). PMN were analyzed by the expression of the integrin subunit CD11b using a fluorescent dye-labeled monoclonal antibody (Serotec, Oxford, United Kingdom) for PMN. Platelets were analyzed by glycoprotein expression, using labeled monoclonal antibodies against GPIIb/IIIa (Camon, Wiesbaden, Germany) and GPIb (SBA Incorporation, Birmingham, AL). Samples containing approximately 3 x 10^6 PMN or 1 x 10^9 platelets were prepared for measurement in the flow cytometer as described previously. To compensate for any day-to-day variation in instrument response, nonspecific relative mean particle fluorescence intensity of the corresponding negative control sample was subtracted from values measured with specific antibodies.

**Electron Microscopy**

For electron microscopy the hearts were fixed with a solution composed of 2% glutaraldehyde, 2% sucrose, 0.1 M sodium cacodylate buffer (pH 7.3), and 2% lanthanium nitrate. The hearts were diced and three to four pieces of approximately 1 mm³ each were immersed in the fixation solution for 2 h and remained overnight in a solution without glutaraldehyde before being washed in alkaline (0.03 mole/l NaOH) saccharose (2%) solution. After contrast enhancement with a solution containing 2% osmium tetroxide and 2% lanthanum nitrate, embedding in araldite, and microtomic sectioning,
Results
Measurement of Constituents of the Glycocalyx
Efflux of components of the glycocalyx was measured in groups A, B, and C for time control hearts and in groups J, K, and L for hearts subjected to I/R without the infusion of PMN or platelets. Effluent samples were collected during the entire 10 min of reperfusion.

Small amounts of material positive for CD-138 (syndecan-1) were detected in the effluent of all perfused hearts (basal value 3.8 (3.0–4.0) ng/g/min). This value did not significantly change in the control hearts under sevoflurane treatment. As illustrated in figure 1A, I/R (group J) enhanced shedding of syndecan-1 approximately ninefold. Application of sevoflurane in either concentration significantly decreased shedding (group J vs. groups K and L, P < 0.01 each). Postischemic values after pretreatment with 1 MAC sevoflurane were significantly lower than after 0.5 MAC.

Heparan sulfate was also detected in the effluent of all hearts (basal value 52.0 (36.5–64.3) ng/g/min). I/R induced a sevenfold heightened rate of shedding of heparan sulfate from the glycocalyx. Although shedding was increased in the first minutes of reperfusion in comparison with basal values also in groups K and L with sevoflurane, this application of volatile anesthetic significantly lowered the mean release of heparan sulfate throughout the 10 min of reperfusion (P < 0.01). There were significant differences between the values obtained for the two concentrations of sevoflurane (P < 0.05, fig. 1B).

Electron Microscopy
Electron microscopic photographs illustrating the state of the endothelial glycocalyx of coronary vessels are depicted in figure 2. The control groups that did not undergo ischemia showed an intact glycocalyx with an average thickness of 390 nm (exemplary picture in fig. 2A). Only a rudimentary glycocalyx could be seen after ischemia in group J (fig. 2B). On the other hand, a mostly intact structure was seen in the other groups of more than two groups, ANOVA on ranks analysis was performed. Post hoc tests were performed using the Student-Newman-Keuls method at a Bonferroni correction adjusted significance level. P < 0.05 was considered to be significant. The statistical software used to conduct the analyses was SigmaStat 3.5 (Systat Software Inc., San Jose, CA).

PMN Adhesion
Intracoronary adhesion of PMN under nonischemic conditions (group D) amounted to 14.5% (13.1–16.0%) of the applied number (fig. 3). After I/R, the percentage of PMN becoming adherent significantly increased to 38.9% (36.3–42.9%) (group M, P < 0.01 vs. group D). Application of sevoflurane significantly reduced postischemic intracoronary sequestration of PMN, with no difference between the low and high concentration (group N: 22.8 [21.6–26.3] and O: 21.2 [19.2–23.9], both P < 0.05 vs. group D). Basal adhesion of PMN under nonischemic conditions is not significantly influenced by either concentration of sevoflurane (groups E and F).

Platelet Adhesion
Platelets applied to the nonischemic heart displayed an intracoronary adhesion of 9.4% (8.4–10.7%), which increased significantly after I/R (fig. 4). Pretreatment of hearts with low-dose sevoflurane showed no effect on platelet adhesion, whereas after high-dose application (1 MAC) the adhesion rate of platelets was significantly reduced to near-baseline values (groups K and L, fig. 2C).
values (fig. 4). As with PMN, sevoflurane had no influence on basal adhesion rates of platelets under nonischemic conditions (groups H and I).

**Coronary Flow**

Hearts were perfused with a constant pressure of 70 cm H$_2$O. The amount of Krebs-Henseleit perfusate passing from the coronary system via the pulmonary artery was continuously recorded with a small-animal flow meter. Coronary flow values measured over 2 min immediately before inducing ischemia (preischemic) and after 10 min of perfusion (control) or directly before infusing PMN or platelets (10 min postischemic reperfusion) were used to assess changes in coronary vascular tone. As shown in figure 5, preischemic (basal) coronary flow showed no significant differences among the groups. After 10 min of (re)perfusion the coronary flow tended to decrease in all groups, reflecting an increase in coronary resistance. However, a significant reduction in flow only developed within the ischemic group J (preischemic vs. postischemic $P < 0.05$) and was significantly lower than that of the nonischemic group (J vs. A $P < 0.05$). Postischemic coronary flow was not significantly reduced versus preischemic flow after pretreatment with sevoflurane, both at 0.5 and 1 MAC. This was an “intermediate” effect, with no significant difference either versus the nonischemic time control group or the ischemic untreated group.

**Measured Tissue Edema**

Mean wet- to dry-weight ratios of isolated hearts after preparation and 25 min of perfusion without being exposed to only developed within the ischemic group J (preischemic vs. postischemic $P < 0.05$) and was significantly lower than that of the nonischemic group (J vs. A $P < 0.05$). Postischemic coronary flow was not significantly reduced versus preischemic flow after pretreatment with sevoflurane, both at 0.5 and 1 MAC. This was an “intermediate” effect, with no significant difference either versus the nonischemic time control group or the ischemic untreated group.

**Fig. 2.** Electron microscopic views of hearts stained to reveal the glyocalyx. (A) An intact glyocalyx after 25 min of nonischemic perfusion (group A). (B) A residual endothelial glyocalyx after 20 min of warm ischemia and 10 min consecutive reperfusion (group J). (C) The glyocalyx after pretreatment with 1 minimum alveolar concentration sevoflurane followed by 20 min of warm (37°C) no-flow ischemia and 10 min reperfusion (group L).

**Fig. 3.** Sequestration of polymorphonuclear neutrophils (PMN) within the coronary system. 20 min warm no-flow ischemia followed by 10 min reperfusion significantly increased the adhesion from control nonischemic conditions. Application of sevoflurane (Sevo) concentrations of 0.5 or 1 minimum alveolar concentration (MAC) significantly reduced adhesion of PMN in postischemic hearts to near the level obtained under control conditions. * $P < 0.01$ I/R (group M) versus Control groups (group D, E, and F). # $P < 0.05$ I/R + Sevo 0.5 (group N) and 1.0 MAC (group O) versus I/R (group M). I/R = ischemia-reperfusion.

**Fig. 4.** Adhesion of platelets within the coronary system. Undergoing 20 min warm no-flow ischemia followed by 10 min reperfusion significantly increased the adhesion from control nonischemic conditions. Application of sevoflurane (Sevo) in a concentration of 1 minimum alveolar concentration (MAC) significantly reduced adhesion of platelets in postischemic hearts to near the level obtained under control conditions. Application of 0.5 MAC had no significant effect on platelet adhesion. * $P < 0.05$ I/R (group P) and I/R + Sevo 0.5 MAC (group Q) versus Control groups (group G, H, and I) and sevoflurane 1.0 MAC (group R), respectively, I/R = ischemia-reperfusion.
glycocalyx integrity.14,17,22 Accessible to leukocytes and platelets upon disturbance of
Anesthesiology 2011; 115:483–91 Chappell
ure,

tion and organ failure involve microvascular perfusion fail-

tane. These results concur with previous studies of Heindl

reduced postischemic PMN sequestration to or near basal

10 min reflow at constant perfusion pressure, but sevoflurane

in hearts after 20 min of warm global no-flow ischemia and

adhesion of both PMN and platelets was more than doubled

with time controls (groups A, B, and C).

Discussion

Ischemia induced a significant shedding of constituents of

the coronary endothelial glycocalyx and destruction of this

structure as viewed by electron microscopy. These effects

were largely inhibited by pretreating hearts with sevoflurane

at concentrations of both 0.5 and 1 MAC. Intracoronary

adhesion of both PMN and platelets was more than doubled

in hearts after 20 min of warm global no-flow ischemia and

10 min reflow at constant perfusion pressure, but sevoflurane

reduced postischemic PMN sequestration to or near basal

levels. Platelet adhesion was reduced with 1 MAC sevoflurane.

These results concur with previous studies of Heindl et al.

performing sevoflurane preconditioning and postcondi-
tioning and using a constant flow model.5,6 This coincidence

of phenomena, shedding and adhesion, supports the hypoth-

esis that the relatively "short" endothelial surface adhesion

molecules (extension approximately 10–20 nm) are har-
bored within the glycocalyx (extension more than 200 nm ex

vivo, up to 2,000 nm in situ) and are only exposed and made

accessible to leukocytes and platelets upon disturbance of

glycocalyx integrity.14,17,22

The pathomechanisms of I/R-associated tissue dysfunc-
tion and organ failure involve microvascular perfusion fail-

ure, i.e., no-reflow and tissue hypoxia despite restoration of

macrovascular perfusion.31,32 Postischemic reperfusion also

provokes an inflammatory response with activation and re-
cruitment of leukocytes as well as accumulation of platelets,

involving surface adhesion molecules such as intercellular

adhesion molecule-1 or P-selectin.33 The inflammatory
cells and activated platelets can produce cytokines, chemo-
kines, and oxygen radicals, in addition to activating coagula-
tory and complement systems, which all may contribute to

the manifestation of tissue injury.32 Although specific inhibi-
bition of single distinct molecules or mediators, such as tu-

mor necrosis factor-α, interleukins, oxygen radicals, or P-

selectin and intercellular adhesion molecule-1, have been

shown to be protective in the experimental setting, these

approaches have not found their way into clinical practice.32

Murry et al. first demonstrated that brief episodes of coro-
nary ischemia significantly reduce infarct size after subse-
quent prolonged myocardial ischemia.34 In the meantime

this phenomenon, known as preconditioning, has been in-
tensively studied and it is well known that volatile anesthetics

may also protect tissue from I/R injury if given before isch-

emia.35 Such protective effects include metabolic and func-
tional improvement3 after ischemia and have been demon-

strated for various inhalational anesthetics (e.g., sevoflurane,
isoflurane, or xenon) in several organs such as the heart,5,8,36

lung,35  kidney,37,38 and brain.39

Much of this pharmacologic preconditioning seems to be

everted via the endothelium. Endothelial function deterio-

rates in a wide range of conditions such as stress, pain, dia-
betes mellitus, and arterial hypertension, all associated with

increased perioperative cardiovascular morbidity and mor-
tality.10,40 In addition, the endothelial glycocalyx, present

on every healthy vascular endothelium,14 plays a role in clinical

situations and diseases such as I/R,41 sepsis,42 or diabetes43

and has been experimentally demonstrated to be deteriorated

by cytokines, inflammation, or proteases.19,20,24,42

The degradation of the glycocalyx occurs within already a

few minutes after administration of warm no-flow ischemia.10,44

In addition, events taking place within the first

moments of reperfusion are known to set the stage for later

time windows.45 To focus on the acute damage of the glyco-
calyx, we infused the human PMN or platelet suspensions

after 10 min of reperfusion. Of course, in vivo, there is an

ongoing interaction between PMN and endothelium, which

continues for hours and even days. However, this delayed

pathology may evolve from acute glycocalyx damage.

The potential of protecting the glycocalyx as a measure

benefiting organ function has been shown after I/R and in-

fusion of tissue necrosis factor-α in an isolated guinea pig

heart model using hydrocortisone and antithrombin III.15,19

Clinical trials indicated that the extent of glycocalyx dam-

age depends on the dimension of the ischemic insult,41 sug-

gesting causal correlations between the severity of disease and

glycocalyx integrity. Twenty minutes of warm no-flow ischemia

has been shown to cause severe damage to the vascular

endothelium in the isolated heart, especially the glycoca-
Sequelae are an increased coronary fluid leak, profound colloid extravasation, major tissue edema, and an increase of coronary resistance. Reduced endothelium-mediated dilator function during I/R may exacerbate vasospasm and, thus, be a critical early event of organ injury. Lucchinetti et al. suggested that sevoflurane may protect endothelium against I/R damage, thereby increasing postocclusive organ blood flow and tissue oxygenation.

The results of our study suggest that sevoflurane simultaneously reduces shedding and cell adhesion after I/R. The preservation of the glycocalyx should maintain the physiologic endothelial permeability barrier, thereby mitigating inflammation and tissue edema, and possibly alleviating the postischemic low-reflow phenomenon. Precisely these two actions were noted in hearts pretreated with sevoflurane. Most importantly, it mitigates PMN and platelet adhesion, known to mediate local and remote tissue damage after I/R. The adhesion and shedding rates from our study are in line with previous experiments in similar models.

The inhibitory effect of volatile anesthetics on PMN and platelet adhesion could be mediated by "nonspecific" effects on the myocardium or the microvasculature, or by "specific" effects on the integrin-mediated adhesion event. As an example of the former, sevoflurane might influence the severity of ischemic alterations and, thereby, indirectly protect the myocardium in the reperfusion phase. However, in previous studies sevoflurane has been shown to have no effect on the production of lactate by isolated hearts after ischemia, nor was an increased recovery of heart function detectable, as would be expected for an ischemia-alleviating effect.

The relatively high basal adhesion rates in the nonischemic control hearts might be the consequence of mechanical trapping in the coronary system. However, coronary flow showed no alteration after PMN application, unlike what has been observed after capillary plugging. Thus, formation of cell aggregates and any substantial capillary plugging can be excluded in our experimental setting. For these reasons most PMNs, especially under postischemic conditions, probably become adherent as the result of specific, integrin-mediated interaction with the coronary endothelium. Moreover, the presence of sevoflurane has been shown not to alter the expression of major platelet adhesion molecules (e.g., GPIIb/IIIa). Glycoproteins of the platelet surface are known to bind directly to ligands on the surface of the intact endothelium. Therefore, the preservation of the endothelial glycocalyx could underlie the observed inhibition of adhesion. It should be mentioned that glycocalyx is expressed not only throughout all blood vessels but also by all parenchymal body cells. Thus, application of sevoflurane may exert whole body protection.

As recently shown, there are to be large differences between the thickness of the glycocalyx in vivo and on cultured cells, these having no relevant glycocalyx. In vivo experiments on endothelial cell cultures, Möbert et al. found that the pretreatment of endothelial cells with volatile anesthetics could not reduce PMN adhesion. In light of the current findings, this may be readily explained by the absence of a glycocalyx on the cultured cells: sevoflurane lacking a major point of action. In addition, the direct inhibitory effect of sevoflurane on PMN noted by Möbert et al. played no part in our work because PMN were not applied until 10 min after initiating washout of the volatile anesthetic.

The current results cannot be directly transferred to the clinical situation. For instance, in our xenogenic model we infused human PMN and human platelets, not those of guinea pigs. However, Zahler et al. previously demonstrated that PMN from guinea pigs and humans show a quantitatively similar degree of adhesion in our model under both preischemic and postischemic conditions. PMN of both humans and guinea pigs provoke a postischemic increase in coronary leak, which is readily accessible in isolated heart preparations. Another issue is that the hearts we used were not of human origin and that they were perfused with a crystalloid buffer, not with whole blood. The latter induces a certain degree of edema that may affect cardiac performance. The significant basal adhesion rate of PMN probably reflects discrete cell activation following separation and preparation. Therefore, we chose not to artificially activate PMN further in vitro with lipid mediators. The late addition of cells after 10 min of reperfusion is not what normally occurs in vivo, where PMN and platelets are known to interact with the endothelium and glycocalyx right from the start. Hence, no firm conclusions can be drawn concerning cell-glycocalyx interrelation. Furthermore, a possible modulation of PMN and platelet reactivity by other blood constituents or plasma components cannot be assessed. Finally, shear rates in the coronary system may essentially influence PMN adhesion and may be different during reperfusion in the presence of erythrocytes. We expect, at the utmost, quantitative but no qualitative consequences from such deviations.

In conclusion, shedding of the endothelial glycocalyx, edema, coronary resistance and adhesion of PMN and platelets were significantly increased in isolated heart preparations in the face of I/R. The presence of sevoflurane in the perfusate before ischemia significantly attenuated all of these adverse changes, suggesting protection of the glycocalyx to be a further facet of beneficial action of sevoflurane. Such an action should help reduce complications associated with I/R and supports clinical evidence of volatile anesthetics possessing cardioprotective properties.

References


17. Möbert J, Zahler S, Becker BF, Conzen PF: Inhibition of neutrophil activation by volatile anesthetics decreases adhesion to cultured human endothelial cells. ANESTHESIOLOGY 1999; 90:1572–81


