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Effect of Halothane and Isoflurane on Binding of ADP– and TRAP-6–activated Platelets to Leukocytes in Whole Blood

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Background: **Adhesion of activated platelets to neutrophils and monocytes has an important role in the regulation of inflammatory processes. This study investigates whether halothane and isoflurane affect binding of activated platelets to leukocytes in human whole blood.**

Methods: **Citrated whole blood was incubated for 60 min with either 1 or 2 minimum alveolar concentration (MAC) halothane or isoflurane. After stimulation with adenosine-5-diphosphate (ADP) or the thrombin receptor agonist protein TRAP-6, platelet–leukocyte adhesion and surface expression of CD62P on platelets were evaluated by flow cytometry.**

Results: **Halothane led to an inhibition of agonist-induced adhesion of activated platelets to neutrophils and monocytes. One MAC halothane reduced the formation of TRAP-6–induced platelet–monocyte conjugates. After exposure to 2 MAC halothane, agonist-induced platelet–monocyte and platelet–neutrophil adhesion were inhibited. Surface expression of CD62P on ADP– and TRAP-6–stimulated platelets were significantly reduced after 1 and 2 MAC halothane. After 2 MAC isoflurane, the authors observed an increase of the percentage of lymphocytes with bound platelets after activation with ADP. The percentage of neutrophils with bound platelets after activation with ADP or TRAP-6 was also increased in this group. Two MAC isoflurane led to an increase of the percentage of platelets expressing CD62P in the unstimulated and TRAP-6 stimulated samples, and of the amount of CD62P epitopes on the surface of platelets in the ADP-stimulated samples.**

Conclusion: **This study indicates that halothane inhibits, whereas isoflurane enhances, adhesion of agonist-activated platelets to leukocytes. Interaction of both anesthetics with the expression of CD62P on platelets contribute to theses effects.**

ADHESION of activated platelets to polymorphonuclear neutrophils and monocytes has an important role in the regulation of inflammatory processes and thrombosis. Increased platelet–neutrophil and platelet–monocyte conjugates have been shown in cardiopulmonary bypass,[1] myocardial infarction,[2] postischemic reperfusion damage,[3] thrombosis,[4] and sepsis.[5,6] An interaction between platelets and leukocytes may link these processes and contribute by intercellular communication pathways to the pathophysiology of these diseases.

It is well-established that activated platelets bind to neutrophils and monocytes *via* an interaction between CD62P on the platelet surface membrane and P-selectin ligand (PSGL-1) on the surface of leukocytes.[7,8] Binding of activated platelets to neutrophils induces respiratory burst[9] and mediates initial neutrophil attachment and rolling,[10] which may lead to neutrophil accumulation at sites of injury. Binding of activated platelets to monocytes is reported to induce secretion of different proinflammatory chemokines.[11,12] These results suggest that the tight interaction among platelets, neutrophils, and monocytes has an important part in the host defense system.

Halothane has been found to affect directly immune-competent cells. For example, during exposure to halothane, the respiratory burst activity of polymorphonuclear neutrophils is significantly reduced.[13] Furthermore, halothane is also known to inhibit human platelet aggregation by interaction with Ca^{2+}-dependent platelet activation processes.[14] Because halothane is rarely used during clinical situations associated with increased platelet–leukocyte formations, such as cardiopulmonary bypass, we also investigated the effect of isoflurane on platelet–leukocyte interaction.

In the current study, we attempted to clarify whether halothane and isoflurane influence adhesion of activated platelets to leukocytes to gain further insight into the mechanism of anesthetic-induced modulation of immune-competent cells and intercellular communication. Using activation-dependent monoclonal antibodies and two-color flow cytometry, we studied the effect of both anesthetics on platelet–leukocyte adhesion and expression of platelet adhesion membrane receptors in human whole blood.

Materials and Methods

In accordance with the human research standards of our institutional ethics committees (University Hospital, Rheinisch-Westfälische Technische Hochschule, Aachen, Germany) and informed consent, blood samples were taken from 38 healthy volunteers (18 women, 20 men) who had no history of smoking or infections and had not ingested nonsteroidal antirheumatics for at least 2 weeks before donation. Venous blood was carefully withdrawn without a tourniquet from an antecubital vein using a

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21-gauge butterfly into blood collection tubes (Sarstedt, Nümbrecht, Germany) containing a 1:10 volume of 3.2% sodium citrate. The first 3 ml of blood was used to perform a hemogram (complete blood count, differential leukocyte count). Blood samples of each donor were immediately diluted 1:1 with 37°C prewarmed Dulbecco's phosphate buffered saline without Ca^{2+} and Mg^{2+} (Sigma Chemicals, St. Louis, MO) in sterile polypropylene tissue culture dishes (Sarstedt). In a subset of experiments, one diluted blood sample was processed within 10 min after blood withdrawal for flow cytometric analysis to obtain baseline values. The remaining blood samples were incubated with either 1 or 2 minimum alveolar concentration (MAC) halothane or isoflurane for 60 min. The MAC value used for halothane in this study was 0.8%, and the value for isoflurane was 1.2%. Control samples were placed at the same time point into an incubator (Heraeus BB 16, Hanau, Germany) with an atmosphere of 21% oxygen and 5% carbon dioxide at 37°C. After incubation, blood samples were immediately processed for stimulation procedures and flow cytometric analysis.

For the incubation of the blood samples with halothane or isoflurane, we developed a small box that allows delivery of different volatile anesthetics at low gas flow rates in an atmosphere with 5% carbon dioxide at 37°C. To avoid artificial leukocyte and platelet activation, blood samples were not bubbled with fresh gas throughout the incubation time. Anesthetics were delivered as a volatile–air mixture (fraction of inspired oxygen [F_{IO_2}], 0.21) using a commercially available anesthetic machine (Cato; Dräger, Lübeck, Germany). Carbon dioxide (5%) was directly administered into the box using an external gas bottle. Initial fresh gas flow was 1 l/min, which was reduced to 250 ml/min after equilibration of the atmosphere inside the box. Oxygen, carbon dioxide, and anesthetic gas concentrations within the box were continuously monitored using a multigas analyzer (Datex Compact, Helsinki, Finland).

Flow Cytometric Analysis

Flow cytometric analysis was performed on a FACSCalibur flow cytometer and analyzed using CellQuest 3.1 software (Becton-Dickinson, San Jose, CA). Before each measurement, the flow cytometer was calibrated with fluorescence microbeads (Calibrite Beads; Becton-Dickinson). Antibodies (Mab) used were as follows: anti-CD45-FITC (clone HI30), Mab for leukocyte common antigen; anti-CD41a-PE (clone HIP8), Mab recognizing the platelet glycoprotein GPIIbIIIa; anti-CD62P-FITC (clone AK-4), Mab directed against CD62P expressed on platelet surface; and negative IgG_1-FITC and IgG_1-PE antibodies (clone MOPC-21) for nonspecific binding (all from Pharmingen, San Jose, CA).

Stimulation, immunofluorescence staining, and flow cytometric analysis were performed as previously described with minor modifications.[2] In brief, to determine the effect of halothane and isoflurane on CD62P expression and binding of activated platelets to leukocytes, blood samples were stimulated with either adenosine-5-diphosphate (ADP, final concentration 2 μM; Sigma Chemicals) or the thrombin receptor agonist peptide TRAP-6 (final concentration 6 μM; Bachem, Heidelberg, Germany) at room temperature. Stimulation was performed in closed Eppendorf tubes to prevent evaporation of the anesthetics. After 5 min, 100 μl unstimulated or stimulated citrated whole blood was added to polypropylene tubes containing saturating concentrations of fluorochrome-conjugated antibodies and then stained for 15 min at room temperature in the dark. The reaction was stopped by adding 2 ml lysing solution (Becton-Dickinson) for 10 min. After centrifugation (5 min, 350*g*, 4°C), the samples were washed with 2 ml phosphate buffered saline containing 1% bovine serum albumin and centrifugated, and the remaining pellet was resuspended in 500 μl phosphate buffered saline containing 1% bovine serum albumin and 1% paraformaldehyde. The cells were stored up to 1 h at 4°C until flow cytometric measurements were performed.

Neutrophils, monocytes, and lymphocytes were differentiated by anti-CD45-FITC fluorescence, and cell size and granularity in the forward and side scatter. Platelet adhesion to leukocytes was defined as cell particles positive for CD41a-PE in the leukocyte subgroups. The percentage of leukocytes with bound platelets and the CD41a-PE mean fluorescence intensity of the positive leukocytes were measured. The CD41a-PE mean fluorescence intensity reflects the number of platelets bound per leukocyte.[8] For each sample, 40,000 leukocytes were measured.

To determine CD62P expression on the surface of platelets, single platelets were identified by size (forward scatter) and CD41a-PE immunofluorescence in a logarithmic scaled dot plot. Results are expressed as percentage of platelets positive for CD62P and mean fluorescence intensity of CD62P-FITC. The CD62P-FITC mean fluorescence intensity reflects the number of epitopes expressed on the surface membrane of single platelets. For each sample, 10,000 platelets were collected.

Gas Chromatography and Mass Spectrometry

In a subset of experiments, concentrations of halothane and isoflurane were determined in the gas and fluid phases using gas chromatography and mass spectrometry on a HP 6890/MSD 5973 Series instrument (Hewlett-Packard, Wilmington, DE) equipped with a head space injector system (Model 7050; Tekmar-Dohrmann, Cincinnati, OH) as previously described.[13] Equilibration between the gas–fluid phase was completed within 15 min for both anesthetics. The following concentrations and diluted blood/gas partition coefficient of halothane and isoflurane were determined for 1 MAC at

Table 1. Spontaneous and Agonist-induced Platelet–Leukocyte Adhesion after Exposure to Halothane

	Control (60 min)	1 MAC Halothane (60 min)	Control (60 min)	2 MAC Halothane (60 min)
Platelet–lymphocyte				
% Positive lymphocytes	2.1 (1.8–3.0)	2.2 (1.8–3.2)	2.3 (2.1–2.6)	2.5 (2.2–2.6)
MFI CD41a on lymphocyte	109 (74–117)	97 (81–99)	123 (102–154)	135 (124–151)
Platelet–lymphocyte (2 μM ADP)				
% Positive lymphocytes	2.5 (2.0–2.7)	2.5 (2.1–2.8)	3.7 (2.7–4.7)	3.2 (2.3–4.0)
MFI CD41a on lymphocyte	283 (220–342)	291 (251–302)	394 (258–532)	244 (189–299)*
Platelet–lymphocyte (6 μM TRAP-6)				
% Positive lymphocytes	2.5 (2.1–3.5)	2.6 (1.8–3.3)	3.2 (2.9–3.7)	2.5 (2.3–3.1)*
MFI CD41a on lymphocyte	314 (248–562)	258 (187–326)	369 (245–459)	297 (222–400)
Platelet–neutrophil				
% Positive neutrophils	2.3 (1.4–6.1)	2.7 (2.1–6.5)	3.2 (2.7–3.8)	2.5 (2.0–3.3)
MFI CD41a on neutrophil	180 (157–220)	181 (159–207)	144 (124–159)	135 (124–151)
Platelet–neutrophil (2 μM ADP)				
% Positive neutrophils	10.9 (5.4–16.3)	12.9 (3.9–15.6)	8.6 (5.0–21.2)	6.2 (4.3–12.1)*
MFI CD41a on neutrophil	532 (434–635)	534 (375–638)	625 (406–681)	341 (295–392)*
Platelet–neutrophil (6 μM TRAP-6)				
% Positive neutrophils	46.5 (27.8–65.1)	36.2 (14.1–48.1)*	17.6 (11.1–42.7)	20.3 (14.9–33.6)
MFI CD41a on neutrophil	1,553 (373–2,126)	1,698 (402–1,962)	842 (612–1,966)	662 (357–949)*
Platelet–monocyte				
% Positive monocytes	6.1 (4.0–12.7)	5.7 (3.7–14.8)	7.0 (4.8–11.0)	6.8 (2.9–9.9)
MFI CD41a on monocyte	222 (189–271)	229 (204–250)	188 (146–228)	158 (139–203)
Platelet–monocyte (2 μM ADP)				
% Positive monocytes	41.9 (28.9–55.9)	40.1 (34.3–52.9)	30.1 (20.9–38.4)	25.7 (15.5–29.0)
MFI CD41a on monocyte	701 (527–810)	715 (494–773)	545 (424–625)	346 (285–476)*
Platelet–monocyte (6 μM TRAP-6)				
% Positive monocytes	87.8 (65.0–91.4)	75.1 (51.1–84.8)*	65.3 (47.1–82.7)	54.7 (44.9–74.9)
MFI CD41a on monocyte	2,459 (610–2,977)	1,377 (574–2,516)	1,321 (858–2,154)	723 (424–866)*

Values are presented as percentage of leukocytes with bound platelets and mean fluorescence intensity (MFI) in arbitrary units of CD41a on each leukocyte, representing the number of bound platelets (median [25–75 percentile] of nine independent experiments for each concentration of halothane).

* $P < 0.05$ *versus* control in the absence of halothane.

MAC = minimum alveolar concentration.

37°C: halothane, 0.73 ± 0.05 mM (partition coefficient, 2.01); isoflurane, 0.62 ± 0.04 mM (partition coefficient, 1.15).

Statistical Analysis

The Kolmogorov-Smirnov test showed that the flow cytometric data were not normally distributed. Therefore, results are expressed as median (25–75 percentile) unless otherwise indicated. Differences between the anesthetic exposed samples and control samples were tested by the Wilcoxon test. A value of $P < 0.05$ was regarded as significant.

Results

Hemogram

The average hemoglobin concentration of all of the volunteers was 14.0 ± 1.0 g/dl (mean ± SD), leukocyte count average was 6,200 ± 1,900/μl, and platelet count average was 224 ± 54 x 10^3/μl. Differential leukocyte counts were 60.3 ± 7.5% neutrophils, 27.9 ± 7.2% lymphocytes, 7.0 ± 2.9% monocytes, 3.6 ± 2.0% eosinophils, and 0.8 ± 0.6% basophils.

Effect of Incubation Time on Platelet Activation and Platelet–Leukocyte Adhesion

To exclude artificial activation during the incubation time of the control blood samples, we compared baseline and control values of unstimulated and agonist-induced platelet–leukocyte binding in a subset of experiments. The 60-min treatment in the incubator had no effect on either basal or agonist-induced CD62P expression on platelets, nor was there an increase in leukocytes with bound platelets (data not shown).

Effect of Halothane on Platelet–Leukocyte Adhesion

The influence of halothane at 1 and 2 MAC on platelet–leukocyte adhesion is summarized in table 1. Halothane had no effect on binding of unstimulated platelets to the three investigated leukocyte subpopulations. Exposure of blood samples to 1 MAC halothane decreased the percentage of neutrophils and monocytes with bound platelets after stimulation with 6 μM TRAP-6 compared with control samples.

In the 2 MAC halothane group, we observed a reduction of the percentage of lymphocytes with bound platelets after activation with ADP. After stimulation with TRAP-6, the amount of bound platelets on lymphocytes was lower in comparison with the control values ($P < 0.05$).

The percentage of neutrophils that were positive for the platelet marker CD41a after activation with either ADP or TRAP-6 was decreased after incubation with 2 MAC halothane. Furthermore, halothane reduced the

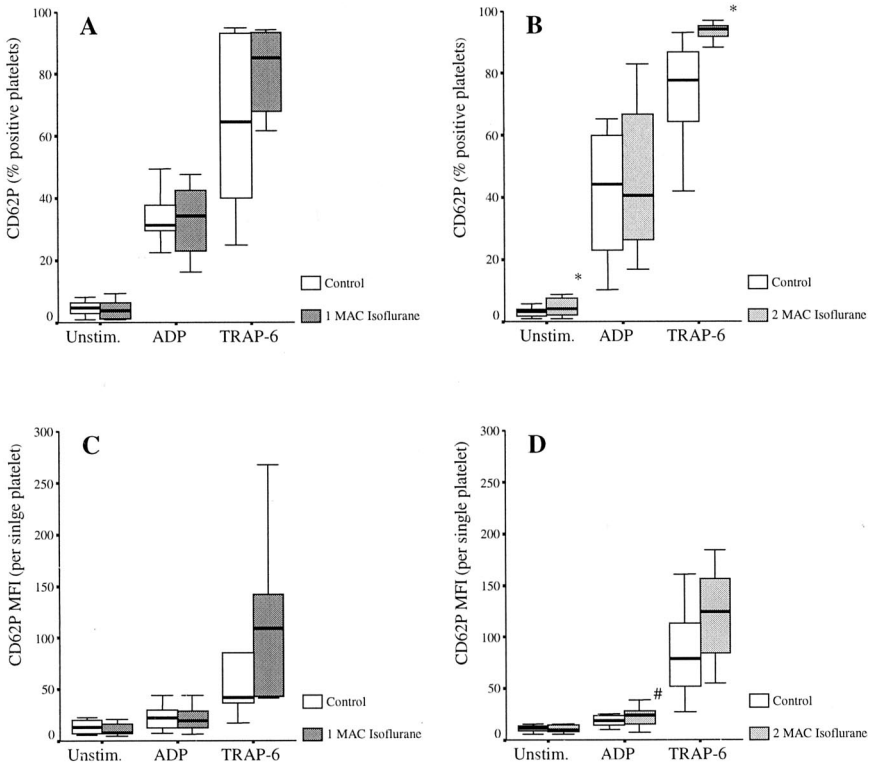

Fig. 1. Dose-dependent effect of halothane on unstimulated and agonist-induced (2 μM ADP; 6 μM TRAP-6) expression of CD62P on the platelet surface membrane. Data are presented as percentage of platelets positive for CD62P (*A* and *B*) and the mean fluorescence intensity (MFI) of expressed CD62P in arbitrary units (*C* and *D*). CD62P MFI represents the amount of CD62P epitopes expressed on the surface membrane per single platelet. Box plots show 25th and 75th percentiles, median, and range of nine independent experiments for each concentration of halothane. **P* < 0.05 compared with control in the absence of halothane.

number of bound platelets per neutrophil associated with ADP stimulation.

The percentage of monocytes with adherent agonist-activated platelets remained unchanged. However, CD41a mean fluorescence intensity, reflecting the number of adherent platelets on each single monocyte, was reduced significantly after stimulation with ADP and TRAP-6 in comparison with controls.

Effect of Halothane on Platelet Surface CD62P Expression

The effect of halothane on basal and agonist-induced platelet surface CD62P expression is shown in figure 1. Halothane *per se* had no effect on basal platelet CD62P expression. Both tested halothane concentrations significantly ($P < 0.05$) reduced the percentage of ADP- and TRAP-6–activated platelets positive for CD62P and the amount of expressed CD62P epitopes (mean fluorescence intensity CP62P) in comparison with controls.

Effect of Isoflurane on Platelet–Leukocyte Adhesion

The effect of isoflurane on platelet–leukocyte adhesion is summarized in table 2. After exposure to 1 MAC isoflurane, binding of unstimulated and stimulated platelets to leukocytes was not altered in comparison with untreated control samples.

In the 2 MAC isoflurane group, we observed an increase of the percentage of lymphocytes with bound platelets after activation with ADP ($P < 0.01$). Furthermore, the percentage of neutrophils that were positive for the platelet marker CD41a after activation with either ADP or TRAP-6 was significantly increased after incubation with 2 MAC isoflurane ($P < 0.01$). Platelet–monocyte adhesion was not affected after incubation with 2 MAC isoflurane.

Effect of Isoflurane on Platelet Surface CD62P Expression

The effect of isoflurane on basal and agonist-induced platelet surface CD62P expression is shown in figure 2. At 2 MAC, isoflurane increased the percentage of platelets positive for CD62P in the unstimulated and TRAP-6–stimulated samples in comparison with control samples ($P < 0.05$). Furthermore, in the ADP-stimulated samples, isoflurane lead to an increase of the CD62P mean fluorescence intensity, reflecting the amount of CD62P epitopes of the surface of platelets ($P < 0.01$).

Discussion

In the current study, we investigated the effect of halothane and isoflurane on adhesion of unstimulated and ADP- or TRAP-6–activated platelets to leukocytes in human whole blood *in vitro*. The major findings are as follows.[1] One MAC halothane inhibits the percentage of neutrophils and monocytes with bound platelets after stimulation with TRAP-6.[2] Two MAC halothane reduces binding of ADP- and TRAP-6–activated platelets to lymphocytes, neutrophils, and monocytes.[3] Expression of platelet surface CD62P, which has a major role in the

Table 2. Spontaneous and Agonist-induced Platelet–Leukocyte Adhesion after Exposure to Isoflurane

	Control (60 min)	1 MAC Isoflurane (60 min)	Control (60 min)	2 MAC Isoflurane (60 min)
Platelet–lymphocyte				
% Positive lymphocytes	4.1 (3.3–4.6)	4.5 (3.1–4.9)	2.8 (2.4–3.2)	2.9 (2.4–3.9)
MFI CD41a on lymphocyte	139 (102–175)	155 (119–193)	148 (116–168)	147 (125–183)
Platelet–lymphocyte (2 μM ADP)				
% Positive lymphocytes	4.5 (3.7–5.0)	4.5 (4.4–5.2)	3.0 (2.4–3.3)	3.5 (2.6–3.8)*
MFI CD41a on lymphocyte	277 (157–503)	256 (181–458)	246 (219–295)	257 (205–300)
Platelet–lymphocyte (6 μM TRAP-6)				
% Positive lymphocytes	3.6 (3.2–4.8)	4.5 (3.6–5.0)	3.2 (2.8–3.5)	4.0 (3.1–4.5)†
MFI CD41a on lymphocyte	225 (166–306)	322 (217–392)	233 (176–338)	300 (224–428)
Platelet–neutrophil				
% Positive neutrophils	3.5 (2.5–5.7)	4.2 (3.7–7.1)	3.0 (1.9–4.4)	3.7 (2.9–4.8)
MFI CD41a on neutrophil	209 (171–289)	185 (131–269)	180 (165–231)	172 (136–226)
Platelet–neutrophil (2 μM ADP)				
% Positive neutrophils	9.4 (6.3–11.3)	10.2 (8.0–16.0)	10.9 (7.6–18.1)	21.0 (14.8–40.9)*
MFI CD41a on neutrophil	598 (321–674)	516 (303–674)	346 (297–493)	501 (383–539)
Platelet–neutrophil (6 μM TRAP-6)				
% Positive neutrophils	59.0 (20.8–66.7)	62.5 (50.1–73.6)	40.0 (18.4–51.0)	61.9 (42.4–75.0)†
MFI CD41a on neutrophil	764 (451–2,260)	1,124 (743–2,127)	1,156 (500–1,459)	1,065 (674–1,769)
Platelet–monocyte				
% Positive monocytes	7.9 (4.6–16.5)	9.6 (7.4–20.8)	9.7 (4.3–17.7)	11.6 (6.3–19.1)
MFI CD41a on monocyte	248 (160–352)	227 (186–301)	265 (221–396)	220 (181–315)
Platelet–monocyte (2 μM ADP)				
% Positive monocytes	49.7 (36.4–59.4)	45.2 (36.6–563.9)	48.0 (32.4–74.4)	67.0 (38.8–87.0)
MFI CD41a on monocyte	775 (503–974)	748 (405–861)	628 (453–765)	745 (401–948)
Platelet–monocyte (6 μM TRAP-6)				
% Positive monocytes	93.1 (76.4–95.0)	95.0 (92.3–96.5)	85.8 (74.5–92.6)	94.2 (90.0–96.0)
MFI CD41a on monocyte	1,225 (743–3,326)	1,842 (1,232–3,617)	915 (797–1,579)	1,317 (1,090–2,122)

Values are presented as percentage of leukocytes with bound platelets and mean fluorescence intensity (MFI) in arbitrary units of CD41a on each leukocyte, representing the number of bound platelets (median [25–75 percentile] of 10 independent experiments for each concentration of isoflurane.

* $P < 0.01$ *versus* control in the absence of isoflurane. † $P < 0.05$ *versus* control.

MAC = minimum alveolar concentration.

mechanism of platelet–leukocyte adhesion, associated with ADP or TRAP-6 stimulation is suppressed by halothane.[4] Two MAC isoflurane increases the percentage of neutrophils with bound platelets after stimulation with ADP or TRAP-6 as well as the percentage of lymphocyte–platelet formations in the ADP-stimulated samples.[5] After exposure to 2 MAC isoflurane, the percentage of platelets expressing CD62P is increased after stimulation with TRAP-6, whereas ADP-induced platelet activation results in an enhanced expression of CD62P epitopes on the surface of platelets.

In contrast to previous studies using isolated leukocyte populations or platelet-rich plasma, we used a whole blood system and a previously described two-color flow cytometry assay to study platelet–leukocyte adhesion. The advantage of a whole blood system is that cells are not artificially activated by isolation processes, and cells are studied in an almost-natural environment, with many intercellular mechanisms still intact.[15] However, the value of this system is limited by the lack of endothelial cells.

It is well-established that activated platelets bind to leukocytes[8] and modulate their immunologic function.[11,12] Furthermore, adhesion of platelets to leukocyte seems not to be an *in vitro* phenomena because several studies showed increased platelet–leukocyte conjugates in cardiopulmonary bypass,[1] myocardial infarction,[2] postischemic reperfusion damage,[3] thrombosis,[4] and sepsis.[5,6] Therefore, we were interested to evaluate whether halothane or isoflurane may alter platelet–leukocyte adhesion *in vitro*. Halothane is known to inhibit the function of both leukocytes[13,16] and platelets,[14] whereas isoflurane has only minor or negligible impact on the function of platelets.[17,18] However, in contrast to halothane, isoflurane is commonly used in clinical situations, in which increased platelet–leukocyte adhesion has been reported.

CD62P is a glycoprotein located in the membranes of α granules, which become externalized on the surface membrane on activation of platelets.[19] CD62P has a prominent role in mediating cellular interactions among platelets, leukocytes,[20] and endothelial cells.[21] Accordingly, after stimulation with either ADP or TRAP-6, activated platelets bind rapidly to monocytes in human whole blood *via* an interaction between CD62P on platelet surface and PSGL-1 on the surface of monocytes.[7,8] TRAP-6, as a strong platelet agonist, is more effective in generating platelet–monocyte adhesion and CD62P expression than the weak agonist ADP. In our study, 1 MAC halothane inhibited the percentage of monocytes with bound platelets only after stimulation with TRAP-6, but ADP-induced binding of platelets and

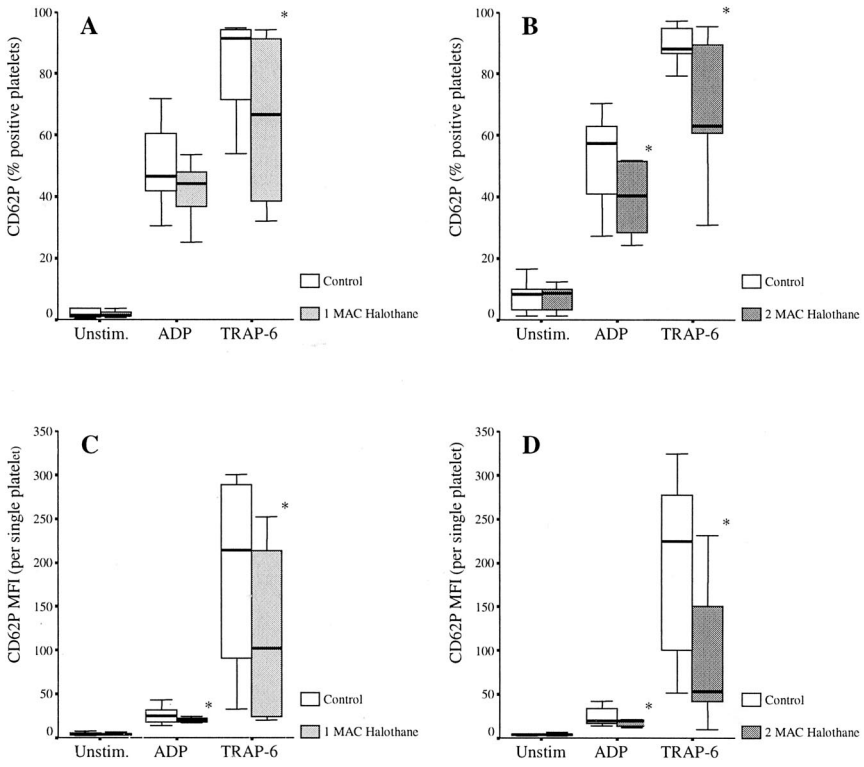

Fig. 2. Dose-dependent effect of isoflurane on unstimulated and agonist-induced (2 μM ADP; 6 μM TRAP-6) expression of CD62P on the platelet surface membrane. Data are presented as percentage of platelets positive for CD62P (*A* and *B*) and the mean fluorescence intensity (MFI) of expressed CD62P in arbitrary units (*C* and *D*). CD62P MFI represents the amount of CD62P epitopes expressed on the surface membrane per single platelet. Box plots show 25th and 75th percentiles, median, and range of 10 independent experiments for each concentration of isoflurane. **P* < 0.05, #*P* < 0.01 compared with control in the absence of isoflurane.

monocytes was unaffected. At 2 MAC halothane, platelet–monocyte adhesion was reduced after stimulation with both agonists. Furthermore, our results indicated that the inhibiting effect of halothane on platelet–monocyte adhesion seems to be mediated by a decreased expression of CD62P on activated platelets. The observed lack of effect on ADP-induced platelet–monocyte adhesion after 1 MAC halothane can be explained by the fact that the percentage of platelets expressing CD62P on its surface was not altered. In this group, only the mean fluorescence intensity of CD62P was moderately reduced by 1 MAC halothane, reflecting a lower amount of CD62P epitopes expressed on the surface membrane of platelets. We suggest that the lower amount of expressed CD62P epitopes on the platelet surface membrane had no influence on platelet–monocyte adhesion because the overall number of activated platelets positive for CD62P remained unchanged.

Interestingly, Fröhlich *et al.*[18] reported an upward regulation of CD62P on the surface of unstimulated platelets in the presence of a halothane concentration of 1 MAC or greater, but halothane did not interfere with the platelet response to ADP stimulation. However, the difference between this particular study and the current study could be because Fröhlich *et al.*[18] used platelet-rich plasma, whereas we used human whole blood, to investigate the effect of halothane on platelets. Furthermore, ADP stimulation was performed with a supramaximal concentration (final concentration 25 μM) that might have prevented the detection of an inhibitory halothane effect on platelet activation.

Neutrophil respiratory burst and recruitment of neutrophils to sites of inflammation are modulated upon mutual contact with activated platelets.[9,10] Although binding between platelets and neutrophils is primarily mediated *via* the CD62P/PSGL-1 adhesion proteins, a reduction in ADP and TRAP-6 induced platelet–neutrophil adhesion was observed in our study only after exposure to 2 MAC halothane. A possible explanation could be that platelet–neutrophil adhesion is partially mediated by a non-CD62P mechanism. Kirchhofer *et al.*[22] demonstrated complete inhibition of platelet–neutrophil adhesion by using a CD62P-blocking antibody in the presence of a GPIIbIIIa antagonist but only partial inhibition in the absence of a GPIIbIIIa antagonist. Because platelets can bind fibrinogen *via* the activated GPIIbIIIa receptor and neutrophils can bind fibrinogen *via* CD11b/CD18,[23,24] it is possible that platelet–neutrophil adhesion also involves a fibrinogen bridging mechanism. However, it remains to be determined whether halothane interacts with fibrinogen binding between platelets and neutrophils.

Isoflurane is known to have no effect on platelet aggregation,[17] but an increase in the expression of CD62P on the surface membrane of resting platelets was observed at concentrations of 2 MAC and greater.[18] The current study confirms and extends these findings by showing that isoflurane also enhances agonist-induced expression of CD62P. Furthermore, the enhanced ADP- and TRAP-6–induced expression of CD62P after exposure to 2 MAC isoflurane contribute to the observed increase of platelet–lymphocyte and platelet–neutrophil

conjugation formation. However, platelet–monocyte adhesion was not altered. Therefore, it remains possible that the enhancing effect of isoflurane on the formation of platelet–lymphocyte and platelet–neutrophil conjugation may partly be mediated by a CD62P/PSGL-1 independent pathway.

Evidence suggest that binding of activated platelets to either monocytes or neutrophils has an important role in the regulation of inflammatory responses. Recently, it was demonstrated that activated platelets induced monocyte cytokine synthesis of interleukin (IL)-1β, IL-8, and monocyte chemotactic protein (MCP-1) after adhesion *via* CD62P.[11,12] The proinflammatory cytokines IL-1β and IL-8 are important in the pathophysiology of the local and systemic inflammatory response of the host defense. IL-1β triggers a broad range of inflammatory responses, including induction of further cytokines, upregulation of adhesion molecules, activation of T lymphocytes, and respiratory burst and lysosomal enzyme release by neutrophils.[25,26] IL-8 promotes chemotaxis,[27] release of neutrophil lysosomal enzymes,[28] neutrophil rolling,[29] and adherence to endothelial cells,[30] as well as transendothelial migration.[29,31] MCP-1 enhances monocyte chemotaxis.[32] Neutrophils are the first line of defense against bacterial infections by engulfing and digesting bacteria. Interaction between platelets and neutrophils also leads to the induction of neutrophil respiratory burst[9] and recruitment of neutrophils[10] to sites of vascular or inflammatory injury. Reduced or missing respiratory burst activity, as seen in chronic granulomatous disease, leads to repeated and life-threatening infections, such as pneumonia or multiple abscesses in the lungs and liver. Therefore, the ability of halothane to inhibit binding of activated platelets to monocytes and neutrophils, as well as the enhancement of platelet–neutrophil adhesion by isoflurane, might contribute to a disturbance of the inflammatory response to a microbial injury. However, the physiologic inflammatory response consists of an initially proinflammatory phase followed by an antiinflammatory phase, which is necessary to manage infections. Therefore, it is uncertain whether the modulation of the platelet–leukocyte adhesion by halothane and isoflurane may have deleterious or beneficial effects on the perioperative immune function.

Limitations of the Study

In this study, we investigated agonist-induced platelet–leukocyte adhesion in static flow conditions. Studies in more physiologic conditions of shear stress of endothelium could have produced different results. Furthermore, platelets are known to modulate leukocyte function also by soluble mediators, such as CD40L[33] or TGFβ-1.[34] However, to evaluate the effect of halothane and isoflurane on these platelet-released mediators is beyond the scope of this study.

The model used in this study allows for analysis of the *in vitro* effect of volatile anesthetics on platelet–leukocyte adhesion. The findings indicate that halothane inhibits, whereas isoflurane enhances, adhesion of agonist-activated platelets to leukocytes. The effects seem to be partly mediated by a altered expression of CD62P on the surface of platelets.

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