Sevoflurane Inhibits Human Platelet Aggregation and Thromboxane A₂ Formation, Possibly by Suppression of Cyclooxygenase Activity


Background: Halothane increases bleeding time and suppresses platelet aggregation in vivo and in vitro. A previous study by the authors suggests that halothane inhibits platelet aggregation by reducing thromboxane (TX) A₂ receptor-binding affinity. However, no studies of the effects of sevoflurane on platelet aggregation have been published.

Methods: The effects of sevoflurane, halothane, and isoflurane were examined at doses of 0.13–1.4 μM. Human platelet aggregation was induced by adenosine diphosphate, epinephrine, arachidonic acid, prostaglandin G₂, and a TXA₂ agonist ([1H]epithila-11,12-methano-TXA₂, STA₂) and measured by aggregometry. Platelet TXB₂ levels were measured by radioimmunoassay, and the ligand-binding characteristics of the TXA₂ receptors were examined by Scatchard analysis using a [1H]-labeled TXA₂ receptor antagonist (5Z,7Z-exo-5,4-H)phenyl sulphonilamino-[2,2',1'] bicyclohept-2-exo-y) heptenoic acid, [1H]S145).

Results: Sevoflurane (0.28–0.84 μM) did not significantly affect platelet aggregation induced by adenosine diphosphate and epinephrine. Sevoflurane (0.13–0.91 μM) and halothane (0.49–1.25 μM) inhibited secondary platelet aggregation induced by adenosine diphosphate (1–10 μM) and epinephrine (1–10 μM) without altering primary aggregation. Sevoflurane (0.13 μM) also inhibited arachidonic acid-induced aggregation, but not that induced by prostaglandin G₂ or STA₂, although halothane (0.49 μM) inhibited the latter. Sevoflurane (3 μM) did not affect the binding of [1H]S145 to platelets, whereas halothane (3.5 μM) suppressed it strongly. Sevoflurane (0.26 μM) and halothane (0.98 μM) strongly suppressed TXB₂ formation by arachidonic acid-stimulated platelets.

Conclusions: The findings that sevoflurane suppressed the effects of arachidonic acid, but not those of prostaglandin G₂ and STA₂, suggest strongly that sevoflurane inhibited TXA₂ formation by suppressing cyclooxygenase activity. Halothane appeared to suppress both TXA₂ formation and binding to its receptors. Sevoflurane has strong antiaggregatory effects at subanesthetic concentrations (greater than 0.13 μM; i.e., approximately 0.5 vol%), whereas halothane has similar effects at somewhat greater anesthetic concentrations (0.49 μM; i.e., approximately 0.54 vol%). Isobutane at clinical concentration (0.84 μM; i.e., approximately 1.82 vol%) does not affect platelet aggregation significantly. (Key words: Anesthesia, volatiles; sevoflurane; halothane; isoflurane; Eicosanoids; thromboxane A₂; prostaglandin G₂; arachidonic acid. Blood: platelets, aggregation.)

ANESTHESIA with halothane increases bleeding time and inhibits platelet aggregation in vivo and in vitro. Studies in vivo found that halothane, which shows stronger antiaggregatory effects than do enflurane and isoflurane, inhibits adenosine diphosphate (ADP)- and epinephrine-induced secondary aggregation without altering primary aggregation significantly. Because secondary aggregation is caused primarily by thromboxane A₂ (TXA₂) secreted by stimulated platelets, we examined the effect of halothane on platelet TXA₂ receptors.
and found that it had a strong suppressive effect on the binding affinity of TXA₂ for its receptors. The present study, which compared the effects of volatile anesthetics on platelet aggregation, showed that sevoflurane has strong antiaggregatory effects, even at subanesthetic concentrations, and halothane exerts such effects only at anesthetic concentrations, whereas isoflurane does not exert antiaggregatory effect in the range of clinical concentrations. To identify the mechanism(s) responsible, the effects of sevoflurane on platelet aggregation induced by a TXA₂ analog and the TXA₂ precursors arachidonic acid (AA) and prostaglandin G₂ (PGG₂) were examined, and a radioligand-binding assay of platelet TXA₂ receptors and a radioimmunoassay of the TXB₂ levels of stimulated platelets were performed.

**Materials and Methods**

In accordance with the human research standards of our institutional ethics committees, venous blood was obtained from six healthy volunteers (who, for at least 2 weeks, took no drugs known to affect platelet aggregation) and placed in tubes containing a 10% volume of 3.8% wt/vol trisodium citrate. The blood was centrifuged at 160g for 10 min to prepare platelet-rich plasma (PRP) or at 1,600g for 30 min to prepare platelet-poor plasma (PPP). The platelet count of the PRP was adjusted to 300,000/mm³ by adding PPP, and then PRP and PPP were stored at room temperature. To prepare washed platelets, a 10% volume of 100 mM EDTA (pH 7.4) was added to PRP and the mixture was centrifuged at 900g for 15 min. The platelet pellet was suspended in buffer A (composed of 8 mM Na₂HPO₄, 2 mM NaH₂PO₄, 10 mM EDTA, 5 mM KCl, and 135 mM NaCl [pH 7.2]) and recentrifuged at 900g for 15 min. The platelet pellet was finally suspended in buffer B (composed of 25 mM Tris-HCl, 1 mM EGTA, 5 mM MgCl₂, and 138 mM NaCl [pH 7.5]), and the platelet count was adjusted to 1,000,000/mm³ by adding buffer B for the binding assay. Washed platelets were stored in Ca²⁺-free buffer at 0°C for 1-3 h until a few minutes before being used. Platelet-rich plasma was used for the aggregation study and radioimmunoassay, and washed platelets were used for the binding assay.

**Aggregation Study**

A 200-μl aliquot of PRP was placed into a siliconized glass tube, warmed to 37°C for a few minutes before analysis, and stirred continuously before and during the experiments. An aliquot of halothane (0.16, 0.49, 0.98, and 1.25 mM), sevoflurane (0.13, 0.26, 0.65, 0.91, and 1.3 mM) and isoflurane (0.28, 0.56, 0.84, 1.12, and 1.4 mM) were added directly to the PRP-containing tubes, the tops of which were immediately sealed tightly with parafilm to minimize evaporation of the anesthetics, and incubated at 37°C for 10 min. To test the reversibility of the effect of sevoflurane, three tubes of platelet suspension with sevoflurane (0.26 mM) were sealed and incubated for 10 min, then unsealed and incubated for another 45 min before inducing platelet aggregation. Platelet aggregation induced by adding 1 μM (+)-9,11-epithia-11,12-methano-TXA₂ (STA₂), 1 mM AA, 15 μM PGG₂, 1-10 μM ADP, and 1-20 μM epinephrine was measured at 37°C by recording the increase in light transmission using an eight-channel aggregometer (MCM Hematracer VI; MC Medical, Tokyo, Japan) (n = 3 each). The light transmission of untreated PPP was taken as 100%.

**Binding Assay**

Each anesthetic was added directly to test tubes containing 100 μl washed platelets and 700 μl buffer B, and each tube was sealed immediately. After incubation for 10 min at 37°C, [³H]-labeled TXA₂, receptor antagonist (5Z-7-(3-endo-(1-ring-¹⁴H) phenyl) sulphonylaminomethyl-2,2,1 bicyclohept-2-exo-y) heptenoic acid, [³H]-S145 (5 nM) was added and incubation at 37°C was continued for 30 min. The reaction was terminated by adding 5 ml of 5 mM Tris-HCl buffer (pH 7.4) which had been precooled to 0°C. Each mixture was then filtered in vacuo through a Whatman GF/C filter, which was washed three times with 5 ml precooled Tris-HCl buffer, and the radioactivity on the filter (n = 3 for each agent) was determined by a liquid scintillation analyzer (Tri-Carb 1900 CA; Packard Instruments, Meriden, CT). For Scatchard analysis, the samples were incubated with various concentrations (1.25-200 nM) of [³H]-S145 in the absence and presence of halothane (3.3 mM) or sevoflurane (3 mM).

**Radioimmunoassay of Thromboxane B₂**

Platelet aggregation was induced by epinephrine, ADP or AA, in the presence or absence of an inhalational anesthetic. Seven minutes after adding an aggregating agent, the reaction was terminated by adding one-tenth volume of 100 mM EDTA precooled to 0°C, and the suspension was immediately placed in an ice bath. The suspension was then prepared at +20°C until further use. The platelets were washed three times with 0.15 M NaCl in a modified Earle's solution, and the platelet radioimmunoassay kit (Biorad, Hemel Hempstead, Herts, UK) was used for the analysis.

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Fig. 1. (A: top left and right) Typical recordings of epinephrine (3 μM)- and adenosine diphosphate (ADP; 1 μM)-induced platelet aggregation in the absence (control) and presence of sevoflurane (0.13 min). (B) Typical recordings of STA2 (1 μM)-induced platelet aggregation in the absence (control) and presence of sevoflurane (0.26 min) and halothane (0.49 min).

The drugs used were halothane (Takeda Pharmaceutical, Osaka, Japan), sevoflurane (Maruishi Pharmaceutical, Osaka, Japan), isoflurane (Dainabot, Osaka, Japan), epinephrine hydrochloride (Sigma Chemical, St. Louis, MO), ADP (Sigma Chemical), arachidonic acid (Nacalai Tesque, Kyoto, Japan), and prostaglandin G2 (Cayman Chemical, Ann Arbor, MI). [3H]S145 was a gift from Shionogi Research Laboratories (Osaka, Japan), and STA2 was a gift from Ono Pharmaceuticals (Osaka, Japan). GF/C filters were obtained from Whatman International (Maidstone, UK), and the radioimmunoassay kit used was a...
thromboxane B\textsubscript{2} [\textsuperscript{3}H] assay system, code TRK 780 (Amersham International, Buckinghamshire, UK).

The inhalational anesthetics were diluted with ethanol when necessary and the aliquot was added directly to test tubes. The final concentrations of ethanol in the reaction mixtures were less than 0.5% vol/vol, which does not affect platelet aggregation.\textsuperscript{10,11} The concentrations of the volatile anesthetics in the liquid phase in each test tube were confirmed by gas chromatography (model 5890A; Hewlett-Packard, Palo Alto, CA). In a preliminary study, the concentrations of volatile anesthetics in platelet suspension in paraffilm-sealed tubes were not significantly altered by 30 min of incubation at 37°C.

The data are expressed as means ± SD and were analyzed by one-way analysis of variance and the Scheffé’s test. Differences at $P < 0.05$ were considered significant.

### Results

Epinephrine (1 - 20 \textmu M) and ADP (1 - 10 \textmu M) induced primary aggregation, which was followed by secondary aggregation (fig. 1A). Pretreatment with sevoflurane (0.13 - 0.91 mM) and halothane (0.16 - 1.25 mM) did not significantly alter the primary aggregation induced by these agonists. However, the secondary aggregation induced by epinephrine (1 - 10 \textmu M) and ADP (1 - 10 \textmu M) were suppressed by sevoflurane (0.13 - 1.3 mM) and halothane (0.49 - 1.25 mM), but not by halothane (0.16 mM) and isoﬂurane (0.28 - 0.84 mM) (fig. 1A and table 1). The secondary aggregation induced by a high concentration (20 \textmu M) of epinephrine was suppressed only by higher concentrations of sevoflurane (0.26 - 0.91 mM) and halothane (0.98 - 1.25 mM). Sevoflurane and isoﬂurane at higher concentrations (> 1 mM) suppressed platelet aggregation nonspecifically. Aggregation was not inhibited in the tubes to which sevoflurane (0.26 mM) was added and 10 min later were unscaled and incubated for 45 min more ($n = 3$), indicating that the effects of sevoflurane were reversible.

Sevoflurane (0.13 mM) completely inhibited platelet aggregation induced by AA (1 mM), whereas concentrations as much as 0.26 mM did not affect that induced by PGG\textsubscript{2} (15 \textmu M) or STA\textsubscript{2} (1 \textmu M) (fig. 1B, tables 1 and 2), and halothane (0.49 mM) inhibited both AA- and STA\textsubscript{2}-induced aggregation (figs. 1B and 2A).

The effect of indomethacin, a known cyclooxygenase inhibitor, was examined for comparison. Indomethacin (1 \textmu M) suppressed AA (1 mM)-induced platelet aggregation and platelet secondary aggregation induced by ADP and epinephrine without altering primary one and did not affect PGG\textsubscript{2} (15 \textmu M)- and STA\textsubscript{2} (1 \textmu M)-induced platelet aggregation. Table 1 summarizes the aggregation study results.

Sevoflurane (5 mM) and isoﬂurane (2.5 mM) had minimal effects on [\textsuperscript{3}H]S145 binding to platelets, whereas halothane (3.3 mM) suppressed it strongly. Scatchard analysis of [\textsuperscript{3}H]S145 binding showed that sevoflurane affected neither the $B_{max}$ nor $K_d$ values, whereas $K_d$...
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Fig. 2. (A) Typical recordings of arachidonic acid (1 mM)-induced platelet aggregation in the absence (control) and presence of sevoflurane (0.13 mM) and halothane (0.49 mM). (B) Typical recordings of prostaglandin G (15 μM)-induced platelet aggregation in the absence (control) and presence of sevoflurane (0.26 mM).

was increased markedly by halothane (3.3 mM) without significantly altering B_max (fig. 3).

The TXB_2 level in the PRP supernatant was increased markedly by stimulation with AA (1 mM) from 102 ± 49 pg/10^7 platelets in basal level to 19,093 ± 5,838 pg/10^7 platelets (n = 5 each), and this increase was suppressed significantly by sevoflurane (0.26 mM) and halothane (0.98 mM), whereas isoflurane (0.56 mM) did not affect the TXB_2 level significantly (table 2). Thromboxane B_2 levels in supernatant of PRP stimulated by AA under the presence of sevoflurane, halothane, and isoflurane were 4,354 ± 2,081 pg/10^7 platelets, 3,459 ± 2,516 pg/10^7 platelets, and 22,697 ± 11,278 pg/10^7 platelets, respectively.

Discussion

The binding of weak agonists, such as ADP and epinephrine, to platelets activates phosholipase A_2 to re-

Fig. 3. Scatchard analysis of [3H]s145 binding to washed platelets. B = bound; F = free.

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inhibited AA-induced platelet aggregation but not that induced by PGG₂ or STA₂ (table 1) suggests strongly that sevoflurane suppressed the conversion of AA to PGG₂ catalyzed by cyclooxygenase. We also confirmed that indomethacin, a known cyclooxygenase inhibitor, showed similar effect on platelet aggregation with sevoflurane. In contrast to sevoflurane, halothane suppressed both platelet TXA₂ receptor-binding affinity and TXA₂ synthesis, suggesting that the antiaggregatory effect of halothane is due to the reduction of platelet TXA₂ receptor-binding affinity,⁹ the reduction of TXA₂ formation, or both.

The minimum alveolar concentration values of halothane, sevoflurane, and isoflurane, which were 0.75, 2, and 1.2 vol/%, respectively, correspond to 0.68, 0.49, and 0.66 MM.¹⁷ Therefore the minimum concentrations of sevoflurane and halothane that suppressed platelet aggregation in the present study correspond to approximately 0.26 and 0.72 MAC, respectively, whereas isoflurane concentrations as great as 1.52 MAC had no significant effect. The present study also demonstrated the complete reversibility of the antiaggregatory effect of sevoflurane. Platelets once exposed to sevoflurane but left in unsealed tubes for 45 min to let sevoflurane evaporate spontaneously showed normal aggregability.

Sevoflurane and halothane, at clinically relevant concentrations, suppressed secondary aggregation of human platelets in vitro. This effect of sevoflurane appeared to be caused by suppression of cyclooxygenase activity, whereas suppression of TXA₂ synthesis and reduced TXA₂ receptor-binding affinity may contribute to the antiaggregatory effect of halothane.

References

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