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Platelet aggregation is impaired during anaesthesia with sevoflurane but not with isoflurane

Purpose: Halothane suppresses platelet aggregation *in vitro* and *ex vivo*, and prolongs bleeding time. In a previous *in vitro* study we demonstrated that sevoflurane had a more suppressive effect on platelet aggregation than did halothane. The present study investigated whether the clinical use of sevoflurane affected platelet aggregation *ex vivo*.
Methods: Thirty-eight patients undergoing minor elective surgery were divided randomly into sevoflurane and isoflurane groups. Anaesthesia was induced with thiopentone *iv*, and was maintained with sevoflurane or isoflurane with nitrous oxide. Blood was collected to measure platelet aggregation induced by adenosine diphosphate (ADP) and epinephrine. The first (control) blood collection was performed in the operating room before induction of anaesthesia, and the second 5–10 min after tracheal intubation but before the start of surgery, when the end-expiratory sevoflurane or isoflurane concentrations had stabilised at 1–1.5 times the minimum alveolar concentration (MAC) and mean arterial pressures were between 80–120% of preanaesthetic values.
Results: In all samples obtained during sevoflurane anaesthesia (n= 15), ADP and epinephrine could not induce secondary aggregation, although they did induce primary aggregation. In contrast, in the isoflurane group, both primary and secondary aggregation were observed in 14 out of 15 patients, and secondary aggregation was abolished in only one of the samples obtained during anaesthesia.
Conclusion: Sevoflurane, but not isoflurane, alters platelet aggregation in the clinical situation, possibly by suppression of thromboxane A_2 formation.

Objectif : L'halothane inhibe l'agrégation plaquettaire *in vitro* et *in vivo* et prolonge le temps de saignement. Nous avons antérieurement démontré que le sévoflurane avait un effet inhibiteur *in vitro* plus important sur l'agrégation plaquettaire que l'halothane. La présente étude a pour but de vérifier si l'usage clinique du sévoflurane affecte l'agrégation plaquettaire *in vivo*.
Méthodes : Trente-huit patients soumis à une chirurgie élective mineure répartis au hasard en groupe sévoflurane et groupe isoflurane participaient à l'étude. L'anesthésie était induite au thiopental *iv*, et entretenue au sévoflurane ou à l'isoflurane avec du protoxyde d'azote. Du sang était recueilli pour la mesure de l'agrégation plaquettaire induite par le diphosphate d'adénosine (ADP) et l'épinéphrine. Le premier échantillon sanguin (contrôle) était recueilli en salle d'opération avant l'induction de l'anesthésie et le second, 5–10 min après l'intubation trachéale et avant le début de l'intervention après stabilisation des concentrations télé-expiratoires de sévoflurane et d'isoflurane à 1–1,5 fois la concentration alvéolaire minimale (MAC) de même que de la pression artérielle moyenne à 80–120% des valeurs préanesthésiques.
Résultats : Malgré une agrégation primaire, l'ADP et l'épinéphrine n'ont induit l'agrégation secondaire dans aucun des échantillons recueillis sous anesthésie au sévoflurane (n= 15). Par contre, on a observé dans le groupe isoflurane une aggrégation tant primaire que secondaire chez 14 des 15 patients, l'agrégation secondaire n'ayant été abolie que chez un seul des patients.
Conclusion : En clinique, le sévoflurane contrairement à l'isoflurane altère l'agrégation plaquettaire possiblement par suppression de la formation de thromboxane A_2.

SOME anaesthetics possess strong antiaggregatory action and their use may increase surgical bleeding or haemorrhagic complications. However, they may exert a beneficial action by decreasing the risk of thrombogenetic complications during the perioperative period. Previous investigators have demonstrated the anti-aggregatory effects of halothane in vitro[1-4] and in vivo.[5-8] Dalsgaard-Nielson et al.[6] showed that platelet aggregation is affected in blood obtained during halothane anaesthesia, which resulted in prolongation of bleeding time. Gibbs[7] demonstrated that isoflurane, in contrast to halothane, did not affect platelet function ex vivo. However, information regarding the effect of newer anaesthetics, including sevoflurane, on platelet function is limited.

In previous in vitro studies[9,10] we found that the anti-aggregatory potencies of volatile anaesthetics were in the order sevoflurane > halothane > isoflurane and enflurane, on a minimum alveolar concentration (MAC) basis. Sevoflurane suppressed ADP- and epinephrine-induced secondary platelet aggregation strongly, possibly by suppression of cyclooxygenase activity. However, no study has evaluated the effect of sevoflurane on platelet aggregation during clinical use. The present study was conducted to investigate the effects of sevoflurane on ex vivo platelet aggregation and its mechanism in the clinical setting. Isoflurane was used in the control group because it is known not to affect platelet function.[7,9,10]

Methods

After approval of the institutional ethics committee, and obtaining informed consent, 38 patients undergoing minor elective surgery were included in the study. The patients were randomly divided into sevoflurane and isoflurane groups. None of the patients had a history of haematological disorder and none had taken drugs known to affect platelet aggregation for at least two weeks. Preoperative measurements including complete blood count, platelet count, prothrombin time, partial thromboplastin time, electrolyte concentration, and arterial blood gas tensions were within normal limits. The patients were premedicated with 50 mg ranitidine im.

Anaesthesia was induced with 3–5 mg·kg^{-1} thiopentone and 0.1–0.15 mg·kg^{-1} vecuronium iv, and was maintained with the volatile anaesthetic to be tested with 66% nitrous oxide in oxygen. After tracheal intubation, inspiratory and end-expiratory vapour gas concentrations were monitored (Datex Capnomac Ultima, DATEX Instrumentarium Corp, Helsinki, Finland). Inspiratory concentrations of the volatile anaesthetics were adjusted to maintain the end-expiratory concentrations at between 1 and 1.5 MAC and mean arterial pressures between 80–120% of their preanaesthetic values. Lactated Ringer's solution was infused and no other drug was administered during this period. We used a warmer blanket to prevent hypothermia and body temperature was maintained between 35 and 37°C in all cases. Patients in whom preanaesthetic control values indicated impaired platelet aggregation and in those for whom mean arterial pressure could not be maintained within 80–120% of the preanaesthetic value during the test period were excluded from further studies.

Venous blood was obtained from large veins in the forearm and placed into tubes containing a 10% volume of trisodium citrate 3.8% w/v. The test tubes were sealed tightly with parafilm to minimize evaporation of the anaesthetics. In our previous study,[10] we confirmed by gas chromatography that the concentration of halothane and isoflurane in the sealing tubes were maintained >90% for 30 min at 37°C. The blood was centrifuged at 160 g for 10 min to prepare platelet-rich plasma (PRP), or at 1600 g for 30 min to prepare platelet-poor plasma (PPP). Both the PRP and PPP were then stored at room temperature for 30 min. An aliquot (200 μl) of PRP was placed into a siliconized glass tube, warmed to 37°C for a few minutes before analysis, and stirred continuously both before and during the experiments. Platelet aggregation induced by ADP (1–10 μM) and epinephrine (1–10 μM) was measured at 37°C by recording the increase in light transmission using an 8-channel aggregometer (MCM Hematracer VI MC Medical Inc., Tokyo, Japan). The light transmission of untreated PPP was taken as 100%. The drugs used were sevoflurane (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan), isoflurane (Dainabot, Osaka, Japan), epinephrine hydrochloride (Sigma, St. Louis, MO, USA) and ADP (Sigma). In a preliminary study, the concentrations of volatile anaesthetics in the platelet suspension contained in parafilm-sealed tubes were not significantly altered by the 30 min incubation at 37°C.

Data are expressed as means ± SD and were analyzed by Student's t test for unpaired data. Male/female and ASA physical status were analyzed by Fisher's exact probability test. Differences at $P < 0.05$ were considered to be significant.

Experimental protocol

Blood collection was carried out in the operating room immediately before induction of anaesthesia (preanaesthetic control, T0) and at 5–10 min after tracheal intubation when the end-expiratory sevoflurane or isoflurane concentration was between 1.0–1.5 MAC (2.1–3.1% and 1.2–1.8%, respectively) (T1).[11,12] The threshold concentrations of ADP and epinephrine used to induce secondary aggregation were determined in the preanaesthetic control PRP, and a PRP obtained during anaesthe-

sia was exposed to these agonists at concentrations higher than their preoperative thresholds.

Results

There were no differences between the two groups in age, body weight, sex, American Society of Anesthesiologists (ASA) physical status (Table I), or in blood pressure or heart rate (Table II). In three patients, in the isoflurane group, and one in the sevoflurane group, the mean arterial pressure at T1 was outside the 80–120% of preanaesthetic value range. These patients were excluded from further studies. Doses of anaesthetics administered at T1, and blood pressure and heart rate at T0 and T1, are shown in Table II.

Four patients had impaired platelet aggregation at T0, and were excluded from further study. Seven minute measurement of ADP (1–10 µM) and epinephrine (1–10 µM) induced platelet aggregation, using preanaesthetic

control PRP obtained from the remaining 30 patients at T0, showed that primary aggregation was followed by secondary aggregation. The threshold concentrations of ADP and epinephrine capable of inducing secondary aggregation averaged 3.75 ± 1.68 µM and 3.93 ± 2.18 µM, respectively, in preanaesthetic control (T0) PRP. Seven minute measurement of platelet aggregation using samples obtained during sevoflurane anaesthesia at T1, revealed that ADP and epinephrine at 1.2 times the preanaesthetic threshold concentration could not induce secondary aggregation, although they did induce primary aggregation in the all cases (n=15) (Figure 1). In contrast, in the isoflurane group, both primary and secondary aggregation was observed in 14 out of 15 patients, and secondary aggregation was abolished in only one of the samples obtained during anaesthesia (Figure 2). Volatile anaesthetics inhibited platelet secondary aggregation completely or did not affect it at all at least in our experimental condition.

TABLE I Demographic data of patients studied

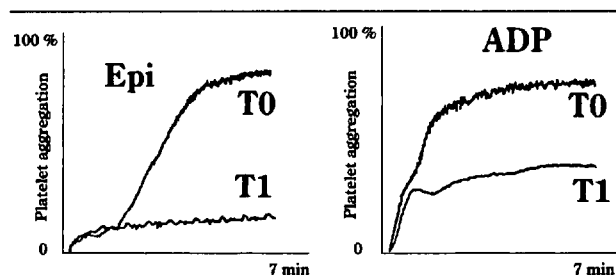|	Age	M/F	Body weight	ASA I/II	Platelet count (× 10,000)
Sevoflurane	45.5 ± 12.6	1/14	52.5 ± 8.3	10/5	25.7 ± 6.4
Isoflurane	48.2 ± 17.4	3/12	57.2 ± 11.5	12/3	24.7 ± 6.0

P = NS between groups.

FIGURE 1 Typical recordings of epinephrine (3 µM)- and ADP (3 µM)-induced platelet aggregation of PRP obtained right before induction of anaesthesia (T0) and during anaesthesia with sevoflurane (T1).
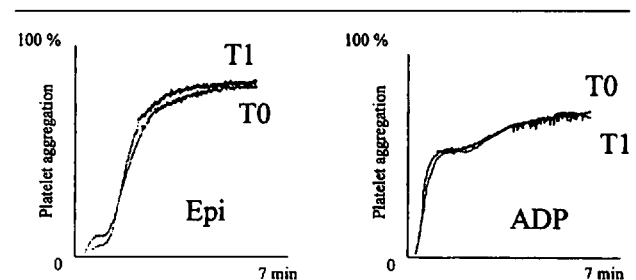

FIGURE 2 Typical recordings of epinephrine (3 µM)- and ADP (3 µM)-induced platelet aggregation of PRP obtained right before induction of anaesthesia (T0) and during anaesthesia with isoflurane (T1).

TABLE II Doses of anaesthetics administered and cardiovascular variable at test period

	Doses administered between T0 and T1		Volatile anaesthetics Concentration (MAC)	Blood pressure (mmHg)		Heart rate (l·min⁻¹)	
	Thiopentone (mg·kg⁻¹)	Vecronium (mg·kg⁻¹)		T0	T1	T0	T1
Sevoflurane	3.62 ± 0.69	0.126 ± 0.024	1.41 ± 0.185	86.1 ± 9.2	87.6 ± 13.3	77.4 ± 15.0	79.7 ± 13.4
Isoflurane	3.92 ± 0.67	0.130 ± 0.027	1.44 ± 0.146	89.2 ± 10.8	80.5 ± 12.3	75.8 ± 13.1	81.7 ± 14.1

T0 and T1 represents time when the first (pre-anaesthesia) and second measurements were performed.
Mean ± SD
There were no difference in doses of anaesthetics or cardiovascular variables between groups.

Discussion

In the present study, platelet aggregation was suppressed in all 15 patients after <20 min anaesthesia with sevoflurane at 1 to 1.5 MAC, but was suppressed in only one out of 15 patients anaesthetized with isoflurane. These results indicate that the effect of sevoflurane on platelet function is greater than that of isoflurane even at clinical anaesthetic concentrations.

In PRP obtained during sevoflurane anaesthesia, ADP- and epinephrine-induced secondary aggregation was selectively affected without altering primary aggregation. The binding of weak agonists, such as ADP and epinephrine, to platelets activates phospholipase A_2 to release arachidonic acid. Arachidonic acid is then converted to prostaglandin G_2 (PGG_2) and finally to thromboxane A_2 (TXA_2),[13] which plays a major role in the induction of secondary aggregation. Therefore, inhibition of secondary aggregation without any alteration in primary aggregation is considered to result from suppression of the formation and/or function of TXA_2. The present findings are in agreement with our previous *in vitro* findings.[9,10]

Previously, we showed that sevoflurane suppressed platelet aggregation by inhibiting TXA_2 formation but did not affect TXA_2 function as sevoflurane suppresses arachidonic acid-induced platelet aggregation and TXB_2 formation but does not affect TXA_2 analog-induced aggregation.[10] The present findings that clinical concentrations of sevoflurane suppressed platelet secondary aggregation without affecting primary aggregation are in agreement with the above mechanism, indicating that inhibition of TXA_2 formation occurs during anaesthesia with sevoflurane.

Although aggregation induced by lower concentrations of epinephrine was suppressed by halothane and sevoflurane, the aggregation induced by higher concentrations of epinephrine was reversed. During general anaesthesia, surgical stress induces endocrine responses and sympathetic activation. Therefore, during or after invasive surgical procedures, the anti-aggregatory effect of anaesthetics may be overcome by increased catecholamine concentrations. This, in addition to the large variability of individual conditions encountered in clinical situations, may explain why only a few investigators have confirmed the increased blood loss during general anaesthesia when compared with epidural anaesthesia.[14–17]

We speculate that the suppressive effects of anaesthetics on platelet aggregation might be deleterious in certain clinical situations, for example in patients with known platelet disorders or massive haemorrhage requiring massive transfusion. Conversely, these suppressive effects on platelets could be beneficial in patients with complications such as ischaemic heart disease, as TXA_2 released from platelets would be expected to increase myocardial damage in patients with acute myocardial ischaemia.[18–22]

We conclude that sevoflurane but not isoflurane can suppress platelet aggregation even in the clinical situation. We should take the present findings into consideration when we choose anaesthetics for patients in certain situations. Further studies under more varied conditions, including invasive surgical interventions may be required to confirm this.

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