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Infusion of Sodium Nitroprusside Induces Platelet Dysfunction In Vitro

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The effect of sodium nitroprusside (SNP) infusion on platelet function was prospectively evaluated in 29 patients undergoing cardiac surgical procedures requiring cardiopulmonary bypass. Any patient receiving preoperative medication known to interfere with platelet function was excluded from this study. Platelet function was evaluated by measurement of platelet aggregation with both adenosine diphosphate (ADP) and epinephrine-induced aggregation tests. Ten patients served as a control population receiving fentanyl anesthesia and no SNP. Nineteen patients received SNP, as clinically indicated, following the induction of anesthesia (and prior to cardiopulmonary bypass) to maintain a mean blood pressure of 80 mmHg. The infusion rate and total dose of SNP delivered was recorded for each patient. Infusion rates of SNP $\geq 3\ \mu g \cdot kg \cdot^{-1} min^{-1}$ resulted in a dose-related decrease in platelet aggregation ($P < 0.05$). This reduction in platelet function was accompanied by a concomitant increase in bleeding time from 5.8 ± 0.6 to 9.3 ± 0.4 min in the patients receiving SNP ($P < 0.05$). In addition, with the administration of a total dose of SNP ≥ 16 mg, a significant reduction in platelet aggregation ($P < 0.05$) was observed. Platelet aggregation studies and bleeding time performed in the control group (following the administration of fentanyl (30 μg/kg) did not reveal any deviation from baseline values. The results from this *in vivo* study support previous *in vitro* data demonstrating a detrimental effect upon platelet function following SNP infusion. (Key words: Anesthesia: cardiac. Coagulation: bleeding time; platelet aggregation. Pharmacology: sodium nitroprusside.)

NORMAL HEMOSTASIS is essential in patients undergoing cardiac surgery. Inhibition of platelet aggregation has been reported following sodium nitroprusside (SNP) infusion.[1] *In vitro* studies using specific methods for measurement of platelet aggregation (adenosine diphosphate [ADP] and epinephrine-induced platelet aggregation) reveal an adverse effect (*i.e.*, disaggregation) on platelet function following SNP administration.[2,3] In addition, *in vivo* experimental data suggest that the infusion of SNP (within the therapeutic range) inhibits platelet aggregation.[4] However, there are conflicting reports regarding the clinical implications of these observations. Graybar *et al.* documented an increased perioperative blood loss when SNP was infused for blood pressure control following coronary artery bypass surgery.[5] In contrast, a retrospective review performed by Snow *et al.* failed to document any increase in the transfusion requirements for patients receiving SNP postoperatively.[6] To date, no prospective controlled study has evaluated the effects of SNP (in clinically relevant doses) on platelet function *in vivo*. Consequently, we designed a controlled prospective study to evaluate the effects of SNP on platelet function in patients undergoing elective cardiac operations.

Methods

Following patient consent, the study was undertaken with a protocol approved by the Human Investigation Committee. Twenty-nine adult patients with a mean age of 63 ± 6.7 yr, scheduled to undergo elective coronary artery bypass surgery were included. Patients were interviewed the evening prior to surgery, and any patient having ingested medications known to interfere with platelet function was excluded from the study area. In addition, prothrombin time, partial thromboplastin time, and standardized (*i.e.*, template) bleeding times were determined upon admission to the hospital.[7] All patients included in this protocol had normal hemostatic function preoperatively.

Patients were premedicated with intramuscular morphine (0.15 mg/kg) and scopolamine (0.4 mg) 90 min prior to arrival in the operating room. No peripheral iv catheters were inserted in the left upper extremity; consequently, fluid administration would not interfere with the results of the template bleeding test, which was always performed on the patient's left forearm.[8]

Induction of anesthesia was performed using fentanyl (30 μg/kg), and pancuronium (0.1 mg/kg) was utilized for muscle relaxation. Following tracheal intubation, mechanical ventilation was maintained using 100% O_2. Anesthesia was maintained using enflurane combined with supplemental fentanyl.[9] To ensure that any abnormalities observed in platelet aggregation were solely the result of SNP, platelet aggregation studies were also performed in a control group of patients (n = 10), anesthetized with fentanyl, but receiving no SNP. The demographic characteristics (age, sex, procedure) were similar within the two groups studied. In addition, the cardiovascular status (as assessed by preoperative ejection fraction) was comparable in each of the study populations.

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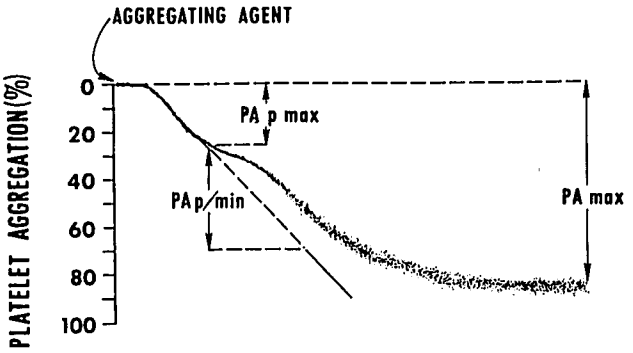

FIG. 1. The typical platelet aggregation curve following the addition of an aggregation agent (ADP). A method for the measurement of the rate of aggregation of the primary wave (PA p/min) the maximal extent of aggregation for the primary waveform (PA pmax) and the maximal effect of platelet aggregation (PA_{max}) are demonstrated.

Following induction of anesthesia, a baseline bleeding time and platelet aggregation studies were performed. Bleeding times were measured using a Simplate-II® system (General Diagnostics). A freshly prepared solution of SNP was made from 50 g of anhydrous powder diluted with 5% dextrose in water. All SNP infusion solutions were protected from direct light and utilized within 4 h of preparation. SNP was infused when clinically indicated following induction to maintain a mean arterial pressure = 80 mmHg (infusion rate of 1–8 $\mu g \cdot kg^{-1} \cdot min^{-1}$). In the control group of patients, blood pressure control was achieved with inhalational anesthesia (enflurane). No patient received any halothane or isoflurane (because these agents have been shown to prolong bleeding time) during the study period.[9–11] In addition to baseline determinations, blood samples for determination of platelet aggregation and bleeding times were obtained 30 min after starting the SNP infusion and 90 min following the institution of SNP therapy. The infusion rate of SNP was held constant for 5 min before samples for platelet function were drawn. The infusion rate and total amount of SNP delivered was recorded at each of these study intervals.

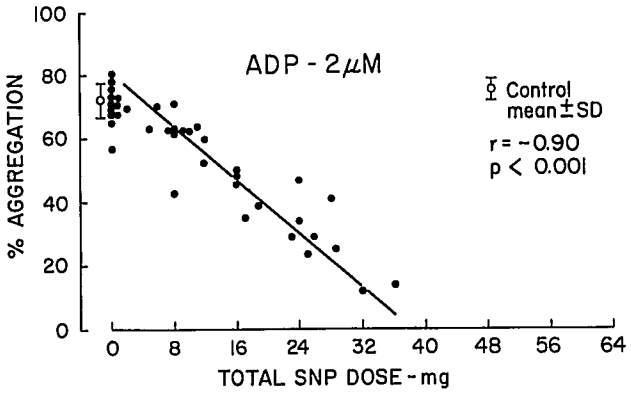

FIG. 2. Percent of platelet aggregation with ADP (2 μM) as measured as a function of total dose of SNP (mg). A significant negative correlation is observed between SNP delivered and platelet aggregation.

To obtain platelet rich plasma, a 15-ml sample of blood was anticoagulated with sodium citrate and centrifuged for 20 min at room temperature. All equipment used to handle the blood or platelet-rich plasma was plastic except for the aggregometer tubes, which were untreated glass. All aggregation studies were performed on aliquots of platelet-rich plasma in which the platelet concentration was $\geq 300{,}000/mm^3$. Aggregation studies were performed by exposure of the platelet-rich plasma to epinephrine (5 μM) or ADP (2 μM). Measurement of aggregation was performed using a Chrono-log® aggregometer, model #540. The ADP was kept frozen as a stock solution of 20 μM and was diluted at the time of aggregation studies. Maximal aggregation (PA_{max}) was read as the percentage increase of light transmission observed at 5 min from the strip chart recorder after the aggregation agent had been added. The rate of aggregation (PA p/min, %) and the extent of aggregation (PA_{pmax}, %) of the primary wave (height of maximum platelet aggregation) were calculated for each determination (fig. 1).[12] All samples obtained for measurement for platelet function were drawn prior to administration of heparinization and the institution of cardiopulmonary bypass. No parenteral infusions known to alter platelet function were administered at any time during the study period.

Data are expressed as the mean ± SD. Statistical analysis was performed using one-way analysis of variance and correlation coefficient with $P < 0.05$ considered significant.

Results

All measurements of aggregation (PA_{max}) performed during the pre-SNP infusion period (baseline) were found to be in the clinically acceptable range for normal platelet function, with a mean of 73 ± 4.6% ADP aggregation. In addition, platelet counts in the samples (baseline) ranged from 180,000 to 240,000, with a mean of 198,000 ± 16,000. There was no significant difference in platelet counts among the patients at any of the sampling intervals. The aggregation curve generated by the response of platelets exposed to ADP (before and after the infusion of SNP) reveals that greatest percentage of aggregation occurred during the control period. Following SNP infusion, inhibition of both primary and secondary waves

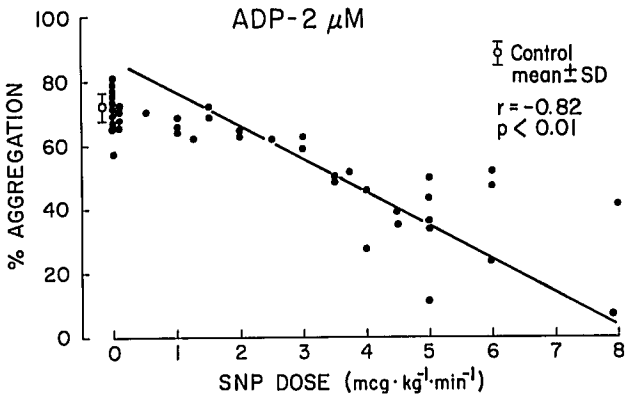

FIG. 3. Percent of platelet aggregation with ADP (2 μM) as measured at varying rate of SNP infusion in $\mu g \cdot kg^{-1} \cdot min^{-1}$. A reduction in platelet function is seen at infusion rates of SNP ≥ 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$.

of platelet aggregation were observed at all sampling intervals (30, 60, and 90 min). An associated reduction in maximal aggregation (PA_{max}) was also observed (as compared to baseline). Platelet function, as assessed by epinephrine-induced aggregation, parallel the data obtained with ADP. A significant negative correlation between the total SNP dose delivered (mg) and ADP platelet aggregation ($r = -0.92$, $P < 0.001$) was observed. Due to the short half-life of SNP, the infusion rate is frequently changed to adapt to varying clinical situations. Therefore, it is necessary to note the cumulative dose of SNP administered at each sampling interval. A 30% decrease from the level of baseline ADP platelet aggregation is noted at a cumulative dose of SNP ≥ 16 mg (fig. 2).

The infusion of SNP at a rate of ≥ 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ resulted in a significant reduction in platelet aggregation ($P < 0.05$). At this infusion rate, ADP aggregation studies revealed a 33% reduction in platelet activity as compared with baseline values (fig. 3).

Alteration in bleeding time measurements were also evident following SNP infusion. Baseline bleeding time values were 5.8 ± 0.6 (SD) min (normal range, 4–8 min). Following the initiation of SNP therapy, a significant prolongation of the bleeding time (as compared with baseline) was seen at an SNP infusion rate ≥ 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (table 1). However, bleeding time outside of the normal range (*i.e.*, 8 min) was observed only at doses of SNP ≥ 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$.

Platelet function, as assessed by the addition of epinephrine to platelet-rich plasma, yields maximum aggregation. However, addition of epinephrine to platelets that have been exposed to SNP resulted in platelet disaggregation with a concomitant decrease in the maximal aggregation (PA_{max}).

TABLE 1. SNP Infusion Rate and Template Bleeding Time (n = 29)

SNP Infusion Rate ($\mu g \cdot kg^{-1} \cdot min^{-1}$)	Bleeding Time (min)
0 (baseline)	5.8 ± 0.6
1 (n = 2)	6.0 ± 0.5
2 (n = 3)	6.3 ± 0.8
3 (n = 3)	7.4 ± 0.3*
4 (n = 8)	7.6 ± 0.6*
5 (n = 4)	8.5 ± 0.3*
6 (n = 6)	8.8 ± 0.2*
7 (n = 2)	9.1 ± 0.6*
8 (n = 1)	9.3 ± 0.4*

* $P < 0.05$ compared with baseline.

Results obtained from the control group of patients (n = 10) anesthetized with fentanyl but receiving no SNP failed to reveal any abnormalities in platelet aggregation before or after fentanyl administration. Aggregation data obtained at baseline and 90 min following administration of fentanyl at 30 μg/kg revealed no statistically significant change in platelet aggregation (table 2). In addition, measurement of bleeding times performed within the control group (following fentanyl infusion) failed to detect any increase from baseline values.

Discussion

The major hemostatic action of platelets can be divided into three categories: adherence, aggregation, and release. The adherence of platelets is provoked by exposure to damaged endothelium. This process requires glycoprotein I complex, as well as Von Willebrand factor, calcium, and cyclic adenosine monophosphate (cAMP). Platelet aggregation requires fibrinogen binding (to change platelet shape) and glycoprotein. Finally, both alpha and dense granules participate in the platelet release process. The control of platelet activation is complex and is dependent upon the balance of calcium (Ca^{+2}) and cAMP. Acquired disorders of platelet function, such as those seen with SNP administration, are a common problem with variable clinical significance. These acquired defects may only be apparent by abnormal platelet aggregation studies. Most ac-

TABLE 2. Platelet Aggregation in the Control Group Following Fentanyl Administration (30 μg/kg) (n = 10)

Time Following Fentanyl	% Aggregation	
	ADP	EPI
Baseline	76 ± 0.3	81 ± 0.5
30 min post fentanyl	73 ± 0.5	83 ± 0.2
60 min post fentanyl	77 ± 0.2	80 ± 0.6
90 min post fentanyl	78 ± 0.6	79 ± 0.3

quired clinical disorders of platelet function are multifactorial, involving abnormalities of both the adherence and aggregation mechanisms. As a result of the complexity of these disorders, little is known about the precise mechanism of action of most of the medications that are suspected to cause platelet aggregation abnormalities.

In an attempt to elucidate the effect of SNP upon platelet function *in vivo*, we designed a study using the doses of SNP most frequently encountered in the management of cardiac surgical patient (*i.e.*, 1–8 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Our results demonstrate that the use of SNP, at clinically relevant doses, is associated with a statistically significant alteration in platelet aggregation. This decrease in platelet function was seen as a decrease in platelet aggregation as measured using both ADP and epinephrine platelet studies. These assays provide the most sensitive modalities for evaluating *in vitro* platelet function. These alterations in platelet function were accompanied by a significant prolongation of the template bleeding time. This increase in bleeding time may be independent of the alteration in platelet function. The prolongation in bleeding time may occur as the result of diminished vasoconstriction of several vessels secondary to a direct effect of SNP upon vascular tone. Results from our control population support earlier data, which demonstrate no abnormalities in platelet function following the administration of fentanyl.[9]

The importance of vasodilator therapy in the treatment of perioperative hypertension and low cardiac output syndromes is well documented.[13–15] The beneficial effects of SNP in this setting are the result of direct smooth muscle relaxation with a secondary reduction in vascular resistance.[16] However, during cardiac surgery it is crucial that the agents chosen to accomplish these therapeutic goals do not adversely alter normal hemostatic mechanisms. This is especially important in the intraoperative and early postoperative period when excessive bleeding may result in significant patient morbidity.

Previous studies have shown that SNP inhibits ADP and epinephrine-induced aggregation *in vitro*.[17] The mechanism of this inhibition of platelet aggregation is postulated to occur as the result of direct inhibition of thrombosthenin, a smooth muscle-like protein.[18] However, clinical studies regarding the importance of these alterations in platelet function have yielded conflicting results.

Graybar *et al.*[5] compared the perioperative blood loss during cardiac surgical procedures in patients receiving SNP and nitroglycerin for control of perioperative hypertension. Their data suggested that the use of SNP may result in a greater operative blood loss than occurred in patients receiving nitroglycerin. However, the authors used the number of units of blood transfused rather than the amount of actual blood loss as their end point.[5] During cardiac surgery and following cardiopulmonary bypass, postoperative blood replacement is influenced by a variety of factors in addition to platelet function. These include temperature, liver function (*i.e.*, factor II, and V levels), serum calcium concentrations, type of bypass circuits (*i.e.*, membrane or bubble oxygenator), and the duration of cardiopulmonary bypass. In the Graybar *et al.* study,[5] none of these variables were quantitated; therefore, it is difficult to determine their precise impact on overall blood loss. However, regardless of the etiology, a significantly greater perioperative blood loss was seen in patients receiving SNP.

In contrast to the Graybar *et al.* study,[5] Snow *et al.*[6] failed to demonstrate any significant increase in postoperative blood loss in patients receiving an SNP infusion during cardiac surgery. The doses of SNP infused in the study by Snow *et al.*[6] were 0.03–0.06 $\mu g \cdot kg^{-1} \cdot min^{-1}$, well below the usual therapeutic range of SNP administered in the perioperative period. We did not attempt to quantitate blood loss in our patient population because of numerous technical and logistical difficulties imposed during the postoperative cardiac surgical setting. The clinical practice of scavenging and transfusion autologous blood makes the exact measurement of postoperative blood loss and perioperative transfusion volume difficult. Also, variations in surgical technique combined with the lack of a precise definition of an "acceptable" hematocrit further complicate efforts to ascertain specific blood replacement requirements.

In summary, in situations in which SNP therapy is administered, the clinician must be aware of the potential for the inhibition of platelet aggregation. In the management of the cardiac surgical patient in which perioperative blood loss is a major concern, this alteration in platelet function may be of significance.

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