Increased production of prostacyclin after injury to the microvasculature in uraemic patients

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Summary

A recently described method to evaluate the primary haemostatic mechanism under *in vivo* conditions was utilised to investigate thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) production by platelets and vascular endothelial cells, respectively, in patients with severe chronic renal failure. Unlike some previous studies, a decrease in TXA_2 production by uraemic platelets could not be demonstrated. PGI_2 — produced by microvascular endothelial cells after a standardised injury — was, however, 59% higher in patients than controls ($P < 0,05$). An increased local level of this potent platelet inhibitory eicosanoid could play an important role in the bleeding tendency exhibited in chronic renal failure.

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It is well known that uraemia is associated with a bleeding tendency. The incidence of dangerous bleeding episodes has decreased since the introduction of dialysis, but is still a problem for patients needing major surgical procedures or diagnostic biopsies of, for instance, the kidney or liver.[1]

A prolonged bleeding time is still the best, although it is not infallible, indicator of a clinical bleeding tendency.[2] Various aetiological factors have been proposed to explain this defect, e.g. a low packed-cell volume (PCV);[3,4] depressed *in vitro* platelet aggregation;[5] decreased thromboxane A_2 (TXA_2) production by platelets;[6] increased prostacyclin (PGI_2) production by endothelial cells;[7,8] abnormalities of von Willebrand factor[9,10] and parathyroid hormone;[11] and abnormal platelet surface carbohydrates.[12] Conflicting results are regularly published[5,6,13-15] and the true pathogenesis of this defect of primary haemostasis is still unresolved.

A new experimental approach to the evaluation of primary haemostasis has recently been published.[16-19] Blood collected from cuts made for measurement of bleeding time, a standardised injury of the microvasculature, is used for the measurement of different mediators operative in haemostasis, e.g. thromboxane B_2 (TXB_2) — as an end-product of platelet cyclo-oxygenase activity — and 6-keto-prostaglandin $F_{1\alpha}$ (6KPGF) — as an indication of vascular endothelial synthesis of PGI_2.

In the present study *in vivo* production of TXA_2 by platelets and PGI_2 by vascular endothelium was examined in patients with severe chronic renal failure and compared with normal controls.

Patients and methods

The protocol was approved by the Ethical Committee of the Medical Faculty, University of Stellenbosch, and informed consent was obtained from all patients.

Ten patients, 8 men and 2 women, with severe chronic renal failure (serum creatinine value over 600 μmol/l or receiving regular haemodialysis) were examined. Patients on haemodialysis were examined immediately before a dialysis session. No diabetics, patients with nephrotic syndrome or patients receiving drugs that could influence prostaglandin metabolism were included in the study. No patient received recombinant human erythropoietin. Age-comparable healthy volunteers, 7 men and 3 women, on no drugs that could influence prostaglandin metabolism, were used as controls for bleeding time, TXB_2 and 6KPGF determinations.

The bleeding times were determined according to the standardised method of Mielke *et al.*,[20] using a disposable apparatus (Simplate II, General Diagnostics, Morris Plains, NJ, USA). Duplicate horizontal incisions, 5 mm in length and 1 mm deep, were made on the lateral volar aspect of the forearm. All bleeding times were measured by the same investigator.

Blood from bleeding-time cuts was collected, as recently described,[17,18] with some modifications. The blood was sucked up in heparinised capillary tubes containing 60 μM indomethacin (Sigma Chemical Co., Poole, UK), immediately blown out into plastic Eppendorf tubes, centrifuged at 4 000 *g* for 10 minutes in a modified Beckman Microfuge, drawn off and frozen at –80°C until analysis.

Blood was also drawn from an antecubital vein or the dialysis fistula in the absence of heparin. For coagulation tests blood was drawn into 3,8% sodium citrate (9:1 v/v), centrifuged to obtain platelet-poor plasma and tested immediately.

TXB_2 and 6KPGF were measured by commercially available radio-immunoassay kits (Amersham International, Amersham, Bucks, UK). Some 6KPGF measurements were done with half the volumes of specimen, specific antibody, marker and second antibody as recommended by the manufacturers because of the small volume. The validity of the modification was confirmed by finding no significant difference between results using the recommended and modified methodology (41,3 ± 10,6 pg/ml and 43,5 ± 18,2 pg/ml respectively; $N = 13$).

Platelet count, packed cell volume, serum creatinine level, prothrombin time ratio (PTR), activated partial thromboplastin time (APTT) and clottable fibrinogen were determined by routine methods.

Statistics. A non-parametric method (Mann-Whitney *U*-test) was applied to compare TXB_2 and 6KPGF values between patients and controls and $P < 0,05$ was regarded as statistically significant. All results are expressed as mean ± SD.

Results

Haematological values (Table I)

The PTR (normal range 1,0 - 1,3), APTT (normal range 25 - 40 s) and plasma fibrinogen (normal range 2 - 4 g/l) were within normal limits in all patients. The blood platelet count was normal in 9 patients and decreased to 55×10^9/l in 1.

Bleeding time (Table I)

Six patients had a prolonged skin bleeding time (normal range 2 - 9 min) of over 12 minutes, while it was at the upper limit of the normal range (8 - 9 minutes) in the

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TABLE I.
Formation of TXB_2 and 6KPGF in blood obtained from template bleeding-time incisions and patient bleeding times, haemoglobin levels and platelet counts

	Patients					Controls	
Subject No.	TXB2 (pg/ml)	6KPGF (pg/ml)	Haemoglobin (g/l)	Platelet count ($\times 10^9$/l)	Bleeding time (min)	TXB_2 (pg/ml)	6KPGF (pg/ml)
1	1 010,9	53,8	38,9	283	8	1 232,0	23,1
2	679,3	71,7	71	141	> 12	373,1	16,2
3	407,1	81,9	48	55	> 12	684,4	20,4
4	645,4	86,5	98	394	> 12	1 148,3	21,4
5	607,6	84,6	69	491	> 12	479,6	27,4
6	1 758,1	45,2	77	251	7	636,9	54,9
7	654,1	25,2	78	233	> 12	477,6	35,2
8	919,3	37,1	64	207	> 12	555,7	51,7
9	988,7	42,7	102	268	9	443,1	67,1
10	846,9	64,1	93	258	7	652,9	55,1
Mean	851,7 ± 371,0	59,3* ± 21,7	73,9 ± 20,5	258 ± 121		668,4 ± 292,9	37,3 ± 18,3

*$P < 0,05$.

other 4 patients. All the controls had bleeding times that were within normal limits.

TXB_2 and 6KPGF in blood from bleeding time cuts (Table I)

In the controls 668,4 ± 292,9 pg/ml TXB_2 and 37,3 ± 18,3 pg/ml 6KPGF was measured in blood collected over the whole duration of the bleeding time. The amount of TXB_2 measured in uraemic patients (851,7 ± 371,0 pg/ml) did not differ significantly from that of the controls ($P > 0,05$). The production of 6KPGF was, however, increased by 59% in uraemic patients (59,3 ± 21,7 pg/ml) compared with controls and this difference was statistically significant ($P < 0,05$). The TXB_2/6KPGF ratio did not differ significantly between the patient (17,4) and the control (23,5) group. There was no significant correlation between haemoglobin levels, bleeding times, platelet counts and either TXB_2 or 6KPGF levels. Because of the small number of patients in the study, this cannot be regarded as absolute proof of non-correlation.

Discussion

Prostaglandin metabolites of the cyclo-oxygenase pathway play a very important role in the aggregation of platelets. TXA_2 is strongly stimulatory[21,22] and PGI_2 strongly inhibitory[23] to platelet aggregation. Both have short half-lives *in vivo* and the stable breakdown products TXB_2 and 6KPGF are measured *in vitro* as an indication of *in vivo* production.

It has recently been shown that the measurement of different mediators of haemostasis in blood collected from bleeding-time cuts is an excellent method for *in vivo* evaluation of the primary haemostatic mechanism.[17,18] These measurements are indicative of events at the level of the primary haemostatic mechanism and, unlike measurements of TXB_2 and 6KPGF in venous blood or urine, are not an indication of production in the whole vascular system and other sources, e.g. the kidneys.

Results of *in vitro* experiments, e.g. the determination of PGI_2 production by isolated vascular tissue of uraemic patients, should be verified, if possible, by *in vivo* methods, e.g. as used in the present study. The *in vivo* method is also sensitive to abnormal cyclo-oxygenase activity, as has been shown by measurement of TXB_2 after treatment with low dose aspirin.[18]

The results of the present study are an indication that *in vivo* production of TXA_2 by uraemic platelets is not reduced in comparison with normal controls. In patients on maintenance haemodialysis specimens were obtained immediately before the next dialysis and platelet activation during dialysis could thus not account for the failure to demonstrate decreased production of TXB_2. The presence of a functional cyclo-oxygenase defect, one of the proposed mechanisms of the uraemic bleeding tendency, could thus not be confirmed by this *in vivo* evaluation.

The production of PGI_2 by injured vascular endothelial cells of uraemic patients is, however, significantly increased. The above findings confirm the results of Kyrle *et al.*[8] who used a similar method of investigation. The lower TXB_2/6KPGF ratio in the patients, in spite of higher levels of TXB_2 than the controls, also suggests that the increased PGI_2 levels are more important than the increase in production of TXA_2.

The cause of the increased production of prostacyclin has not been determined. It has been shown that normal platelet-poor plasma contains a factor that causes release of prostacyclin from vascular endothelial cells in culture. Platelet-poor plasma from uraemic patients causes an increased rate of synthesis and release of prostacyclin in the same system.[24] The precise nature of this factor, which is present in normal and uraemic plasma, has not been determined. Prostacyclin is a potent vasodilator and inhibitor of platelet aggregation and the findings of this study could thus explain the defective platelet-endothelial interaction and consequent prolonged bleeding time of uraemic patients. A decreasing effect of treatment modalities known to improve the uraemic bleeding diathesis, e.g. haemodialysis, on prostacyclin production by vascular endothelial cells *in vivo* could help to substantiate this.

The clinical application of these results is still uncertain. Selective pharmacological inhibition of vascular endothelial cell cyclo-oxygenase or prostacyclin synthetase would be the theoretical solution to the problem, but such an agent is not yet available. It has been shown in *in vitro* experiments that vitamin K_1 can inhibit prostacyclin production by bovine vascular endothelial cells.[25] As far as could be ascertained, this substance has not been clinically used for the treatment of the uraemic bleeding tendency. This widely available and inexpensive form of treatment, that could correct one of the basic pathophysiological defects of haemostasis in the uraemic patient, should be evaluated in a clinical trial.

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Anaesthesia without tears

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Summary

Rectal induction of anaesthesia is a useful method but is not widely used in South Africa. We studied the onset of action and side-effects of 1% methohexitone administered rectally in a dose of 20 mg/kg to 110 preschool children. Ninety-one per cent were adequately sedated for inhalation induction by mask within 10 minutes, and all by 15 minutes, of drug administration. There was no evidence of significant cardiovascular or respiratory depression and only minor complications such as faecal soiling (11,8%) and hiccough (3,6%) were noted.

The technique has been favourably received by parents, surgeons and nursing staff and has now become routine practice.

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The smooth induction of anaesthesia in preschool children presents a specific challenge to the general anaesthetist in a busy peripheral or day hospital. Oral premedication, favoured by many because it provides anxiolysis and facilitates the induction of anaesthesia, may have serious unwanted side-effects. In addition, appropriate timing is essential for it to be effective.

Rectal induction is an alternative not commonly used or taught in South Africa. It has been described as probably the least disturbing method of inducing anaesthesia, since it involves neither a painful injection nor swallowing of suspicious-looking potions.[1]

We therefore investigated the use of 1% methohexitone for rectal induction of anaesthesia for both day surgery and inpatient surgery in a peripheral hospital setting.

Methods

Preschool children, ASA physical status I and II, presenting for elective surgery during a 6-month period were studied prospectively. Both outpatients and inpatients were involved. Children under the age of 6 months or those in whom a 'difficult airway' was suspected were excluded from the study.

During the routine pre-operative visit the procedure was explained to both parent and child and the age, sex and weight were noted. No additional pharmacological premedicant was prescribed.

At the time of surgery the child was accompanied to the operating room by a parent or caring adult. In the operating room the child was placed in the lateral position while the parent talked to and comforted the child as required.

A freshly prepared 1% methohexitone solution in a dose of 20 mg/kg to a maximum of 500 mg was injected rectally via a soft red rubber catheter (Jacques) using a 10 ml or 20 ml plastic syringe. The catheter was lubricated with KY jelly and was inserted to a depth of 2 cm. The time of injection was noted. Following the injection, the buttocks were taped together in an attempt to prevent any loss of medication.

At 10 and 15 minutes after the injection the level of consciousness of the child was assessed. 'Asleep' was defined as no response to the application of a face-mask, 'sedated' as response to the mask or the introduction of halothane, and 'awake' as resistance to the face-mask.

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