

Reduced Platelet Thromboxane Formation in Uremia. EVIDENCE FOR A FUNCTIONAL CYCLOOXYGENASE DEFECT

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Research Article

A qualitative platelet abnormality and a bleeding tendency are frequently associated with renal failure and uremia. We demonstrated previously that uremic patients display an abnormal platelet aggregation to arachidonic acid and reduced malondialdehyde production in response to thrombin and arachidonic acid. The objectives of this investigation were: (a) to compare platelet prostaglandin (PG) and thromboxane (TX) production in whole blood and in platelet-rich plasma (PRP) of 21 uremic patients and 22 healthy subjects; (b) to evaluate the concentration and activity of platelet PG- and TX-forming enzymes; (c) to assess the functional responsiveness of the platelet TXA_2/PGH_2 receptor; (d) to explore the hemostatic consequences of partially reduced TXA_2 production.

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Reduced Platelet Thromboxane Formation in Uremia

EVIDENCE FOR A FUNCTIONAL CYCLOOXYGENASE DEFECT

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ABSTRACT A qualitative platelet abnormality and a bleeding tendency are frequently associated with renal failure and uremia. We demonstrated previously that uremic patients display an abnormal platelet aggregation to arachidonic acid and reduced malondialdehyde production in response to thrombin and arachidonic acid. The objectives of this investigation were: (a) to compare platelet prostaglandin (PG) and thromboxane (TX) production in whole blood and in platelet-rich plasma (PRP) of 21 uremic patients and 22 healthy subjects; (b) to evaluate the concentration and activity of platelet PG- and TX-forming enzymes; (c) to assess the functional responsiveness of the platelet TXA₂/PGH₂ receptor; (d) to explore the hemostatic consequences of partially reduced TXA₂ production.

Platelet immunoreactive TXB₂ production during whole blood clotting was significantly reduced, by ~60%, in uremic patients as compared to age- and sex-matched controls. Exogenous thrombin (5–30 IU/ml) failed to restore normal TXB₂ production in uremic platelets. Uremic PRP produced comparable or slightly higher amounts of TXB₂ than normal PRP at arachidonate concentrations 0.25–1 mM. However, when exposed to substrate concentrations >2 mM, uremic PRP produced significantly less TXB₂ than normal PRP. To discriminate between reduced arachidonic acid oxygenation and altered endoperoxide

metabolism, the time course of immunoreactive TXB₂ and PGE₂ production was measured during whole blood clotting. The synthesis and release of both cyclooxygenase-derived products was slower and significantly reduced, at all time intervals considered. Furthermore, PGI₂ production in whole blood, as reflected by serum immunoreactive 6-keto-PGF_{1α} concentrations, was significantly reduced in uremic patients as compared with healthy subjects. PGH synthase levels, as determined by an immunoradiometric assay, were not significantly different in platelets from uremic patients as compared to control platelets. A single 40-mg dose of aspirin given to five healthy volunteers reduced their serum TXB₂ to levels found in uremic patients. This was associated with a significant increase of threshold aggregating concentrations of ADP and arachidonic acid and prolongation of bleeding time. Substantially similar threshold concentrations of U46619, a TXA₂ agonist, induced aggregation of normal and uremic platelets. Prostacyclin induced a significant elevation of uremic platelet cyclic AMP, which was suppressed by U46619, further suggesting normal responsiveness of the TXA₂/PGH₂ receptor.

We conclude that: (a) an abnormality of platelet arachidonic acid metabolism exists in uremia, leading to a reduced TXA₂ production; (b) the characteristics of this abnormality are consistent with a functional cyclooxygenase defect; (c) reduced TXA₂ production may partially explain the previously described abnormality of platelet function in uremia.

INTRODUCTION

A bleeding tendency is frequently associated with renal failure and uremia (1). The existence of an ac-

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quired platelet abnormality in these patients is widely accepted (2-5). However, the mechanism(s) underlying this abnormality has not been elucidated (see ref. 6 for a review). Previous studies from our laboratories have established an abnormal platelet aggregation to exogenous arachidonic acid and reduced malondialdehyde (MDA)¹ production in response to thrombin and arachidonic acid (7, 8). Both findings suggested that an abnormal platelet prostaglandin (PG) and thromboxane (TX) production might contribute to the observed defect in platelet function. The aims of the present study were: (a) to compare platelet PG and TX production in whole blood and in platelet-rich plasma (PRP), in response to endogenous and exogenous stimuli, in uremic patients and in healthy subjects; (b) to evaluate the concentration and activity of platelet PG- and TX-forming enzymes in an attempt to identify the site(s) of platelet dysfunction; (c) to investigate the functional responsiveness of platelets to TXA₂ and prostacyclin (PGI₂); (d) to explore the hemostatic consequences of partially reduced platelet TXA₂ production.

These studies demonstrate that an abnormality of platelet arachidonic acid metabolism exists in uremia, the characteristics of which are consistent with a functional cyclooxygenase defect.

METHODS

Subjects. 21 patients (12 male, M; 9 female, F; ages 22-74 yr; 48±15 yr, mean±SD) with chronic renal failure (due to chronic glomerulonephritis in 9, chronic pyelonephritis in 6 and polycystic kidney disease in 6) on maintenance hemodialysis and 22 normal volunteers (11 M, 11 F; ages 23-50 yr; 36±9 yr, mean±SD) were studied. The 21 patients had serum creatinine concentrations ranging from 9.4 to 17.4 mg/dl (mean±SD: 13.3±2.4), platelet counts ranging from 120,000 to 280,000/ μ l (mean±SD: 185,000±44,000) and serum albumin concentrations ranging from 3.4 to 4.8 g/dl (mean±SD: 4.2±0.3). Uremic patients were receiving 12 m² h of weekly hemodialysis in three sessions. The dialyzers used were: Coil kidney (Vita 2 Belco, Mirandola, Italy) with cuprophan membrane in 11 patients and parallel flow (Gambrö, 11.5 μ M, Lund, Sweden) in 10 patients. All patients were studied immediately before a routine dialysis, i.e., 68 h after the end of the previous session.

A careful interview was entertained with each subject in order to exclude the intake of aspirin-containing medications during the 2 wk preceding the study. Peripheral blood samples were drawn by venipuncture, in the fasting state.

Materials. Sodium arachidonate was prepared by dissolving arachidonic acid (Sigma Chemical Co., St. Louis, MO) in 0.1 M sodium carbonate, pH 10.0. U46619 [(15S)-

hydroxy-11 α ,9 α -(epoxymethano)-prosta-5Z,13E dienoic acid] (kindly supplied by Upjohn Co., Kalamazoo, MI) was dissolved in ethanol. PGI₂ Na salt (kindly supplied by Carlo Erba-Farmitalia, Milano, Italy) was dissolved in 0.05 M Tris buffer, pH 9.4.

Whole blood clotting. Platelet TXA₂, PGE₂, and leukocyte PGI₂ production in response to endogenous thrombin was studied by letting multiple 1-ml aliquots of whole blood to clot at 37°C for 5-60 min, as previously described (9). Additional experiments were performed by letting 1-ml aliquots of whole blood to clot at 37°C for 60 min, in the presence of increasing concentrations of added thrombin (Topostasin, Roche, Milano, Italy) i.e., 5-30 IU/ml. The separated sera were frozen and kept at -20°C until assayed.

Treatments of platelet-rich plasma (PRP). 9 vol of venous blood were mixed with 1 vol of 3.8% sodium citrate. PRP was prepared by centrifugation at 190 g for 15 min at room temperature. 1-ml aliquots of PRP were incubated at 37°C with increasing concentrations of added sodium arachidonate, i.e., 0.25-5 mM. Two min after addition of the stimulus, a 100- μ l sample was removed, immediately added to 400 μ l of buffer containing 2 μ g/ml indomethacin (Merck & Co., Inc., Rahway, NJ), vortexed, and frozen in liquid nitrogen at -80°C. Samples were subsequently thawed, spun at 12,500 g for 3 min and the supernates stored at -20°C until assayed. In 11 uremic patients and 13 normal volunteers the threshold aggregating concentration of the endoperoxide-analog U46619, a relatively stable TXA₂ agonist, was determined. Platelet aggregation was performed as previously described (10). Threshold was defined as the lowest concentration that caused irreversible aggregation within 3 min of addition to PRP. Moreover, in nine uremic patients and four healthy subjects, the functional responsiveness of platelets to TXA₂ was further characterized by examining the ability of U46619 to inhibit PGI₂-stimulated cyclic (c)AMP accumulation, as recently described by Wu et al. (11). PRP was divided into four 4-ml aliquots. In aliquot 1, PRP was incubated with 3 μ M U46619 for 90 s at room temperature. 3 nM freshly prepared PGI₂ was added and incubated for an additional 90 s. In aliquot 2, PRP was incubated with ethanol for 90 s followed by PGI₂ for an additional 90 s. In aliquot 3, PRP was incubated with U46619 for 90 s followed by Tris buffer for an additional 90 s. In aliquot 4, PRP was incubated with ethanol for 90 s followed by Tris buffer for an additional 90 s. The mixtures were quickly frozen in liquid nitrogen and stored at -70°C until assayed.

Aspirin study in healthy subjects. To explore the possible hemostatic consequences of a reduced platelet TXA₂ production, five healthy subjects (three F, two M; age 31.4±7.2 yr; range 20-41) received a single 40-mg dose (0.68±0.09 mg/kg) of aspirin (Bayer AG, Leverkusen, West Germany) i.e., a dose of the drug reducing serum TXB₂ concentrations by ~65%, 24 h after oral administration in healthy subjects (12). Bleeding time, platelet aggregation in response to sodium arachidonate and ADP, and serum TXB₂ concentrations were measured prior to and 24 h after oral aspirin administration.

Bleeding time was measured in duplicate by an automatic template device (Simplate II, General Diagnostic, Warner Lambert Co., Morris Plains, NJ).

Immunoradiometric assay for quantitating platelet PGH synthase. Platelet samples from healthy subjects and uremic patients were analyzed for PGH synthase protein concentrations. PGH synthase was solubilized by sonication and homogenization of the platelet sample in 1 ml of 0.1 M Tris-chloride, pH 8.0 containing 1% Tween 20 (Sigma Chemical

¹ Abbreviations used in this paper: MDA, malondialdehyde; PF₃, platelet factor 3; PG, prostaglandin (used variously according to the identification of a given prostaglandin, i.e., PGE₂ or PGI₂); PRP, platelet-rich plasma; RIA, radioimmunoassay; TLC, thin-layer chromatography; TX, thromboxane.

Co.). Three different aliquots of each sample (10-100 μ l) were used in triplicate in the standard assay (13). The characteristics of monoclonal antibodies against PGH synthase, preparation of 125 I-IgG₁ (cyo-3) and IgG_{2b}-(cyo-5)-*Staphylococcus aureus* complexes are described in detail elsewhere (13). Purified sheep vesicular gland PGH synthase (14) was used as the standard. Protein content was determined by the Lowry method (15).

cAMP determinations. 1 ml of 5% TCA was added to 1 ml of PRP and immediately frozen in liquid nitrogen. After thawing at room temperature, platelets were shaken at 4°C for 30 min. The protein precipitate was removed by centrifugation at 2,500 *g* for 30 min. The supernates were extracted with water saturated ether, and the residual ether of the aqueous extract was evaporated under nitrogen at 50°C. Recovery of cAMP was 90±11% (mean±SD) as determined by [3 H]cAMP (New England Nuclear, Boston, MA: 33 Ci/mM), and concentrations were corrected accordingly. cAMP was determined by radioimmunoassay (RIA), according to Steiner et al. (16), using a commercially available kit (Becton Dickinson, Novate Milanese, Milano, Italy).

RIA for TXB₂, PGE₂, and 6-keto-PGF_{1 α} . Serum and plasma TXB₂ (the stable breakdown product of TXA₂), PGE₂, and 6-keto-PGF_{1 α} (the stable breakdown product of PGI₂) concentrations were determined by previously described RIA techniques (17-19). Unextracted samples were diluted in the standard diluent of the assay (0.02 M PO₄ buffer pH 7.4) and assayed in a volume of 1.5 ml, at a final dilution of 1:500-1:15,000 for TXB₂, 1:50-1:1,000 for PGE₂ and 1:150 for 6-keto-PGF_{1 α} . 5,000 dpm of [3 H]TXB₂ (New England Nuclear: 150 Ci/mmol), [3 H]PGE₂ (Amersham International Limited, Buckinghamshire, England: 180 Ci/mmol) or [3 H]6-keto-PGF_{1 α} (Amersham International Limited, 150 Ci/mmol) and appropriately diluted anti-TXB₂ (final dilution 1:1,000,000), anti-PGE₂ (final dilution 1:50,000) or anti-6-keto-PGF_{1 α} serum (final dilution 1:350,000) were mixed and added in a volume of 1.25 ml to each assay tube. The least detectable concentration that could be measured with 95% confidence (i.e., 2 SD at zero) was 2 pg/ml for TXB₂ and PGE₂ assays, and 1 pg/ml for 6-keto-PGF_{1 α} . Consequently, the detection limit was 1 ng of TXB₂, 100 pg of PGE₂, and 150 pg of 6-keto-PGF_{1 α} per milliliter of serum or plasma, respectively. Validation of TXB₂, PGE₂, and 6-keto-PGF_{1 α} measurements was obtained by dilution and recovery studies and by characterization of the chromatographic pattern of distribution of extracted TXB₂, PGE₂, and 6-keto-PGF_{1 α} -like immunoreactivities on thin-layer chromatography (TLC), as described in detail elsewhere (9, 18, 19). The relative cross-reactions of anti-PGE₂ serum GP 356-23/5 and of anti-6-keto-PGF_{1 α} serum 1 with TXB₂, i.e., 0.015 (18), and 0.003% (19), respectively, allow reliable determinations of PGE₂ and 6-keto-PGF_{1 α} even in the presence of 10³ higher TXB₂ concentrations in the same sample.

Statistical analysis. Results were analyzed using parametric analysis of variance and Student's *t* test.

RESULTS

Platelet TXB₂ production during whole blood clotting was significantly ($P < 0.0005$) reduced in uremic patients as compared to age- and sex-matched controls. Serum TXB₂ concentrations averaged 120±67 ng/ml (mean±SD, $n = 17$; range 29-255) in uremic patients vs. 317±76 ng/ml (mean±SD, $n = 17$; range 180-450) in healthy subjects. When calculated as TXB₂ pro-

duced/10⁶ platelets, mean (±SD) values were 0.65±0.36 and 1.44±0.35 ng, respectively. 13 of the 17 uremics had serum TXB₂ concentrations <2 SD of the normal mean. TXB₂-like immunoreactivity of uremic and control sera met classical criteria of specificity for RIA measurements: i.e., it was recovered quantitatively when added to serum, and its concentrations decreased in a linear fashion upon dilution. Furthermore, when subjected to extraction and TLC, the vast majority (>90%) of the recovered immunoreactivity (50-60%) cochromatographed with authentic TXB₂.

Because thrombin is a major stimulus to platelet aggregation and release during whole blood clotting (20), the possibility of a defective thrombin formation as the cause of reduced TXB₂ production was examined by letting multiple 1-ml aliquots of whole blood to clot in the presence of increasing concentrations of exogenous thrombin. As shown in Fig. 1, uremic platelets produced significantly ($P < 0.0005$) lower TXB₂ than normal platelets, throughout the whole range of thrombin concentrations tested. This finding suggested a more likely abnormality of the enzymes of the arachidonic acid cascade. Defective phospholipase activity would result in reduced arachidonic acid release in response to endogenous as well as exogenous stimuli. Therefore, we studied the effects of a wide range of added substrate concentrations on TXB₂ production in PRP. As shown in Fig. 2, uremic PRP produced comparable or slightly higher amounts of TXB₂ than normal PRP at arachidonate concentrations 0.25-1 mM. However, when exposed to substrate concentrations >2 mM, uremic PRP produced significantly ($P < 0.01$) less TXB₂ than normal PRP. These results were suggestive of either PGH synthase or TX synthase deficiency. In order to discriminate between reduced arachidonic acid oxygenation and altered endoperox-

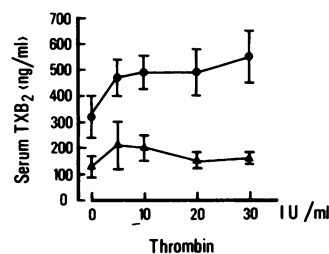


FIGURE 1 TXB₂ production during whole blood clotting in the presence of varying concentrations of thrombin. Nine normal subjects (●) and six uremic patients (▲) were studied and each bar represents mean±SD. Because no statistically significant differences were found in platelet counts, TXB₂ production is expressed as actually measured serum TXB₂ concentrations. TXB₂ levels were significantly ($P < 0.0005$) lower in uremic than in normal sera throughout the whole range of thrombin concentrations.

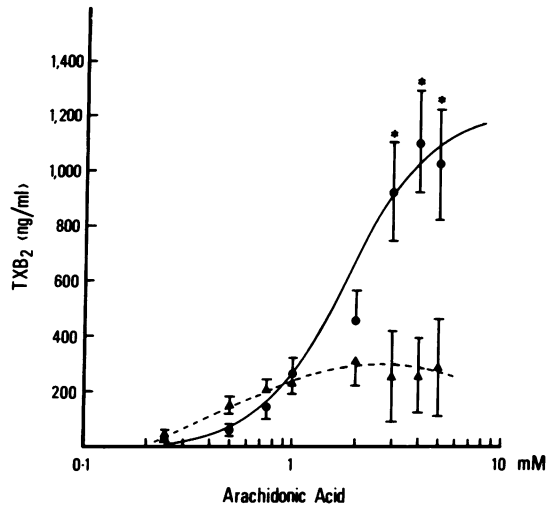


FIGURE 2 TXB₂ production in PRP in response to arachidonic acid. 15 normal subjects (the solid line, ●) and 15 uremic patients (the broken line, ▲) were studied and each bar represents mean±SD. * *P* < 0.01: uremics vs. normals.

ide metabolism, we measured the time course of TXB₂ and PGE₂ production during whole blood clotting in 6 uremic patients and 15 healthy subjects. As shown in Fig. 3, the synthesis and release of both cyclooxygenase-derived products was slower and significantly

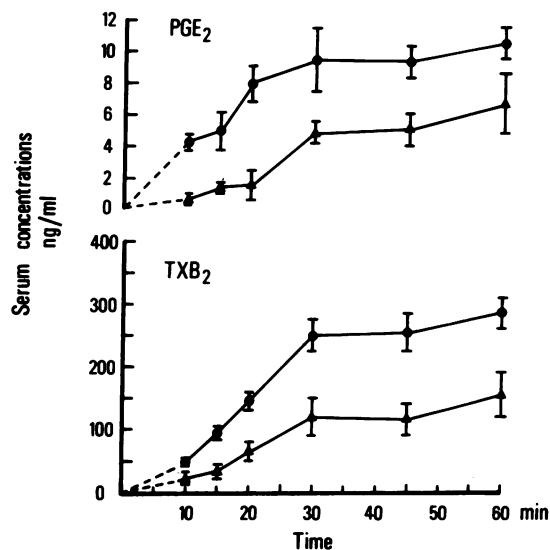


FIGURE 3 Time course of PGE₂ (upper panel) and TXB₂ (lower panel) production during whole blood clotting. 15 normal subjects (●) and six uremic patients (▲) were studied and each bar represents mean±SD. PGE₂ and TXB₂ levels were significantly (*P* < 0.005) lower in uremic than in normal sera at all time intervals considered.

(*P* < 0.005) reduced at all time intervals considered, thus suggesting a more likely PGH synthase defect.

In order to discriminate between reduced enzyme activity and decreased enzyme protein levels, we used an immunoradiometric assay for quantitating PGH synthase protein concentrations in uremic and control platelet samples. PGH synthase levels averaged 1.82 ± 1.63 U/mg protein in platelets from uremic patients as compared to 2.28 ± 1.47 (mean±SD, *n* = 10, *P* = NS) in control platelets. Only 3 of 10 uremic platelet samples analyzed had PGH synthase levels <1 SD of the normal mean. In 10 paired samples, serum TXB₂ determinations were simultaneously obtained. The results of these analyses are detailed in Table I. Uremic platelets produced significantly (*P* < 0.001) lower amounts of TXB₂ than control platelets in whole blood, though having comparable enzyme protein levels in all but one sample. TXB₂ production did not correlate with PGH synthase concentrations to any statistically significant extent. PGI₂ production in whole blood, as reflected by serum 6-keto-PGF_{1α} concentrations, was significantly (*P* < 0.01) reduced in uremic patients (0.59 ± 0.48 ng/ml, mean±SD, *n* = 5) as compared with healthy subjects (1.18 ± 0.13 ng/ml).

A single 40-mg dose of aspirin given to five healthy volunteers significantly (*P* < 0.005) reduced their serum TXB₂ to levels found in uremic patients, i.e., from 385 ± 86 to 122 ± 36 ng/ml (mean±SD). Such a partially reduced platelet TXA₂ production was associated with a statistically significant increase of threshold aggregating concentrations of ADP ($1.3 \pm 0.7 \rightarrow 4.4 \pm 2.2$ μM) and arachidonic acid ($0.3 \pm 0.1 \rightarrow 0.7 \pm 0.5$) and prolongation of bleeding time ($246 \pm 83 \rightarrow 336 \pm 116$ s).

TABLE I
Whole Blood TXB₂ Production and Platelet PGH Synthase Levels in Paired Samples from Five Healthy Subjects (HS) and Five Uremic Patients (UP)

Subject	Serum TXB ₂	PGH synthase
	ng/10 ⁸ platelets	U/10 ⁸ platelets
HS 1	1.22	4.50
HS 2	1.22	—
HS 3	1.13	9.99
HS 4	1.83	7.04
HS 5	0.94	11.25
Mean±SD	1.27±0.33	8.2±3.0
UP 1	0.28	12.21
UP 2	0.72	0.70
UP 3	0.39	9.30
UP 4	0.08	6.21
UP 5	0.21	7.32
Mean±SD	0.34±0.24*	7.1±4.3

* *P* < 0.001, UP vs. HS.

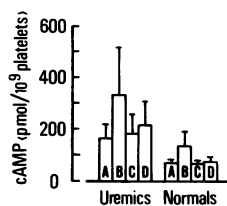


FIGURE 4 Changes in platelet cAMP content in response to PGI₂ and U46619. Nine uremic patients and four healthy subjects were studied and each bar represents mean±SD. A, control; B, PGI₂; C, U46619; D, PGI₂ + U46619. B vs. A, $P < 0.01$ for both uremics and normals.

In addition, we have investigated the possibility that TXA₂/PGH₂ receptor abnormalities might contribute to the observed defect of uremic platelet function. Substantially similar threshold concentrations of the TXA₂ agonist U46619 induced aggregation of normal and uremic platelets. Threshold aggregating concentration of U46619 averaged $0.25 \pm 0.11 \mu\text{M}$ (mean±SD, $n = 11$) for uremic platelets as compared to $0.17 \pm 0.09 \mu\text{M}$ (mean±SD, $n = 13$, $P = \text{NS}$) for normal platelets. To further substantiate the normal responsiveness of the platelet TXA₂ receptor, the effects of PGI₂ and U46619 on platelet cAMP were investigated in nine uremic patients and four healthy subjects. Basal cAMP levels in uremic platelets ($165 \pm 54 \text{ pM}/10^9$ platelets; mean±SD) were significantly ($P < 0.005$) higher than in normal platelets ($68 \pm 14 \text{ pM}/10^9$ platelets). PGI₂ induced a statistically significant ($P < 0.01$) twofold increase of uremic platelet cAMP, which was suppressed by U46619 (Fig. 4), as seen in normal platelets.

DISCUSSION

Uremic bleeding is associated with abnormalities of platelet function tests. These include prolonged bleeding time, decreased platelet adhesiveness to foreign surfaces, reduced platelet aggregation in response to various agents and abnormal PF₃ availability (4, 5). Because arachidonic acid metabolism via the cyclooxygenase pathway plays an important role in modulating platelet function (21), we investigated this metabolic pathway in uremia. This study demonstrates that platelet PG and TX production is reduced in uremic patients, in response to exogenous as well as endogenous stimuli. Recently, Smith and Dunn (22) have reported a substantial reduction of ADP-induced platelet aggregation and TXB₂ production in seven undialyzed or inadequately dialyzed uremic patients when compared to three adequately dialyzed patients or three healthy subjects.

Since a different pattern of response can be obtained depending upon the concentration of the exogenous

stimulus (8), we have investigated platelet PG and TX production in response to endogenous thrombin (9). Whole blood clotting is a model that perhaps more closely mimics the in vivo situation. Under these circumstances thrombin is a major stimulus to platelet aggregation and release (20). Both the absolute amount of TXB₂ produced after whole blood clotting and the time course of its formation were significantly altered in uremic patients.

Since TXA₂ reacts with plasma proteins to form a large proportion of covalently bound derivatives, obviously unavailable to further hydrolysis to TXB₂, this compound is more likely to reflect the biologically active fraction of released TXA₂ (9). The high degree of reproducibility of serum TXB₂ levels within a group of 45 healthy subjects (9) indicates that a relatively constant fraction of platelet TXA₂ normally escapes such covalent linkage to human serum albumin. Although changes in serum TXB₂ concentrations might reflect alterations of these binding phenomena, this seems unlikely to account for reduced serum TXB₂ levels in uremia in view of substantially unchanged albumin levels and comparably reduced serum PGE₂ and 6-keto-PGF_{1 α} concentrations.

Since these patients are known to have a defective prothrombin consumption, as a consequence of abnormal PF₃ availability (5), we determined whether reduced serum TXB₂ concentrations merely reflected defective thrombin formation. Failure of exogenously added thrombin to restore normal TXB₂ production suggested an abnormality of PG- and TX-forming enzymes.

Thrombin-induced platelet activation is associated with the release of arachidonate from membrane phospholipids, most likely through the sequential activation of a phosphatidylinositol-specific phospholipase C and a diglyceride lipase (23). A defect of these enzyme activities seems unlikely in view of the failure of exogenous arachidonic acid to restore normal TXB₂ production in uremic PRP or whole blood.

Given a normal substrate availability, either a defect of PGH synthase or TX synthase might account for the reduced TXA₂ synthesis. A reduced conversion of arachidonic acid because of a PGH synthase defect would result in reduced formation of the PG-endoperoxide intermediates and therefore similarly reduced synthesis of TXA₂ and PG. On the other hand, a reduced conversion of the PG endoperoxides because of a TX synthase defect would result in decreased TXA₂ and normal or increased PG synthesis. In fact, pharmacologically induced inhibition of platelet TXA₂ synthesis by a selective TX synthase inhibitor in man is associated with markedly enhanced PGE₂ formation in whole blood (24). Thus, the finding of similarly impaired PGE₂ and TXB₂ production during whole blood

clotting can be interpreted as reflecting defective formation of platelet PG endoperoxides in uremia. The results of the immunoradiometric assay indicate substantially unaltered levels of PGH synthase reactivity in the vast majority of uremic platelet samples examined. Thus the integrated information derived from these experiments is consistent with a functional defect of platelet cyclooxygenase activity being the main cause of reduced TXA₂ formation in uremia. The possibility that elevated cAMP levels found in uremic platelets may contribute to the reduced TXA₂ production has been considered, in view of previous studies demonstrating that cAMP may regulate arachidonic acid oxygenation (25, 26). However, it should be noted that inhibition of TXB₂ (27) or MDA (28) formation and oxygen consumption (28) by cAMP was found at low, but not at high, substrate concentrations, a finding not consistent with the results of the present study. The results of the aspirin study further indicate that partial acetylation of platelet cyclooxygenase by low-dose aspirin (29) can reproduce the abnormality of TXA₂ production described in uremics. Since PGH synthase reactivity is not influenced by inactivation of the enzyme with aspirin (13), our results are compatible with the presence of an endogenous inhibitor sharing with aspirin the same reaction site(s) on the platelet enzyme. Furthermore, the finding of significantly reduced concentrations of 6-keto-PGF_{1α} in uremic sera suggests that PGH synthase of PGI₂-producing leukocytes (30) is inhibited to a similar extent as the platelet enzyme. An alternative explanation consistent with this finding is that reduced PGI₂ production in whole blood simply reflects reduced availability of platelet-derived PG endoperoxides feeding PGI₂-synthase. The hypothesis of an endogenous inhibitor of cyclooxygenase activity is consistent with preliminary findings of Smith and Dunn (22) that the defect in platelet aggregation and TXB₂ production can be induced in normal platelets incubated with uremic platelet-poor plasma.

As for the relevance of a partially reduced platelet TXA₂ production to the bleeding tendency frequently associated with renal failure and uremia, the effects of low-dose aspirin in healthy subjects suggest that this abnormality may have clinically relevant hemostatic consequences.

Finally, we have investigated whether an abnormal platelet response to TXA₂ might contribute to the uremic defect in platelet function. Our finding that similar threshold concentrations of U46619 (an endoperoxide analog that mimics TXA₂ activity) aggregate uremic and normal PRP argues against such a disorder. Further evidence for intact TXA₂/PGH₂ receptor responsiveness in uremic platelets was obtained by demonstrating that the same analog was capable

of suppressing cAMP elevation induced by PGI₂, similarly to its effect in normal platelets (11).

We conclude that: (a) an abnormality of platelet arachidonic acid metabolism exists in uremia, leading to a reduced TXA₂ production; (b) the characteristics of this abnormality are consistent with a functional defect at the cyclooxygenase level; (c) defective TXA₂ production may partially explain the previously described abnormality of platelet function in uremic patients.

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