ENHANCED PROSTACYCLIN AVAILABILITY OF BLOOD VESSELS IN URAEMIC HUMANS AND RATS

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Summary

Recently prostacyclin (PG I$_2$), an unstable prostaglandin with a strong inhibitory effect on platelet aggregation, has been demonstrated in the wall of blood vessels. We estimated the PG I$_2$ availability of arteries and veins in 10 uraemic patients and 12 nephrectomised rats. The production of PG I$_2$ after long-term incubation of the vessels was markedly enhanced and prolonged. This alteration is probably one key mechanism for the deterioration of haemostasis in uraemics. Preliminary results of further studies indicate that substances in uraemic plasma — presumably middle molecules — may enhance the PG I$_2$ availability of normal vessels in vitro.

Introduction

Disturbances of haemostasis, particularly defects in platelet function are quite common in uraemia. Several dialysable compounds such as phenol, phenolic acids, cyclic AMP and guanidinosuccinic acid have been reported to cause these alterations [1–3]. In 1975 Rabiner and Drake suggested that substances which were responsible for platelet abnormalities were handled in a similar manner to ‘middle molecules’ during haemodialysis [4].

As Moncada et al demonstrated in 1976, the vascular endothelium is able to generate prostacyclin, an unstable prostaglandin, from its precursors, the cyclic endoperoxides (PGG$_2$, PGG$_2$) and arachidonic acid [5]. Prostacyclin, also named PG I$_2$, acts as a very potent inhibitor of platelet aggregation. On the other hand thromboxane A$_2$ and B$_2$, which are also synthesised from the cyclic endoperoxides and released by platelets, markedly induce platelet aggregation. Therefore the balance between prostacyclin and the thromboxanes is thought to be an important mechanism for thromboregulation. Hence it was a logical step to elucidate the role of prostacyclin in the uraemic haemorrhagic diathesis.
Material and Methods

For this purpose we estimated the prostacyclin availability of arteries and veins of uraemic humans and rats. To get results suitable for statistical analysis we chose a defined experimental model of acute uraemia in rats. Twelve male Wistar rats with a mean body weight of 250 g and an age of about 6 months were anaesthetised with Pentothal-Na i.p. and bilaterally nephrectomised by laparotomy. After 30 hours the aorta and the inferior vena cava were removed. Blood was taken to determine BUN and creatinine, as well as to test for platelet aggregation. Twelve normal rats served as controls. Vascular rings (14 ± 2 mg of uraemic and 15 ± 3 mg of control rats; wet weight) were kept in tris-HCl buffer (0.05 mol/L, pH 7.5) at 2°C. For the platelet aggregation tests fresh platelet-rich plasma (PRP) was obtained from volunteers who had not taken aspirin-like drugs for at least 14 days, and from the control rats.

\[\text{PROSTACYCLIN} \]

Figure 1. ADP-induced platelet aggregation curves. A: irreversible aggregation by ADP alone; B: addition of prostacyclin markedly inhibits platelet aggregation, even when aggregation seems to be irreversible; C: supernatant of incubated vessel containing prostacyclin is added at the beginning of aggregation and causes a striking inhibition.
Aggregation was induced by the addition of ADP resulting in a final concentration of 2 µmol/L in human PRP and 1 µmol/L in rat PRP. In a Born-type aggregometer the light transmission through the sample was recorded as a measure of aggregation. The aggregation curves were evaluated by measuring the angle between the tangent of the steepest portion of the initial increase of the aggregation curve and the base line, tge, the maximal extent of aggregation (Δ T\text{max}) and the extent 4 minutes after ADP addition (Δ T4 min). The production of prostacyclin was estimated according to the bioassay of Moncada by its inhibitory effect on platelet aggregation (see Figure 1) [5]. For this purpose each of the vascular rings was incubated in 300 µl tris-HCl buffer at 22°C. After 3 and 15 min, samples were taken from the supernatant and tested for their ability to inhibit ADP-induced platelet aggregation. The incubation medium was changed when the vascular samples had been incubated for 1 and 2 hr respectively. The supernatant was then tested again after 15 min of further incubation. The control vessels were examined at the same time with PRP from the same donor. Control aggregations were performed by adding buffer instead of supernatant. Prostacyclin was characterised by its rather short half life, inactivation by short-term boiling, stability after freezing in liquid nitrogen, pH-dependent instability and inhibition by cyclo-oxygenase inhibitors. For statistical analysis we used Student’s t-test.

In experiments with human material, arteries and veins of 10 uraemic patients (4 females and 6 males, aged 15–58 years) were treated in the same way as described above. The vessels were taken from the forearms during shunt operations. At this time the plasma creatinine values were in the range of 7–12 mg/100 ml. Vascular samples for control experiments were taken from 10 heartbeating donors for kidney transplantation.

Results

In our rat experiments the uraemic as well as the normal vessels obviously produced the same amount of prostacyclin during the first 15 min of incubation. This resulted in a marked inhibition of the ADP-induced platelet aggregation particularly at 15 min (see Figure 2). When the incubation medium was replaced after 1 hr and the vessels were incubated for 15 min again, a difference between both groups appeared. This difference increased and became highly significant (P < 0.001) when the change of incubation medium and reincubation were repeated 2 hr after the start of the experiment. At this time the prostacyclin availability of normal vessels was nearly exhausted. On the other hand uraemic vessels were still able to inhibit platelet aggregation very effectively by the production of prostacyclin. In the human material this behaviour — enhanced prostacyclin synthesis by uraemic blood vessels — was similar, but the differences between the individual cases were more pronounced than in the experimental model. The possibility that other prostaglandins were responsible for the observed phenomena was excluded by using rat PRP for platelet aggregation too, and by the short half time of our compounds.
Discussion

Our results demonstrate an increased release of prostacyclin by uraemic blood vessels after long-term incubation. Particularly in the experimental model of acute uraemia [6] we confirmed the findings of Remuzzi et al, who examined veins of two patients with acute and of one with chronic uraemia [7]. The enhanced prostacyclin production by uraemic vessels is probably one key mechanism for the deterioration of haemostasis in renal insufficiency. However it must be emphasised that our tests are in vitro tests. In the uraemic organism other, up to this time unidentified, mechanisms may be involved too. Further experiments in the search for explanations for the altered prostacyclin metabolism in uraemia are in progress. For this purpose we are examining the effect of diluted uraemic platelet-poor plasma (PPP) on the generation of prostacyclin by normal blood vessels. The preliminary results indicate that
uraemic PPP is far more active in the stimulation of prostacyclin generation than PPP of healthy persons. After tests using plasma fractions which were separated by crude gel chromatography, we suggest that 'middle molecules' are responsible for this effect of uraemic plasma.

Acknowledgments

This study was supported by a grant from 'Kammer der Gewerblichen Wirtschaft'.

References

1 Rabiner, SF and Molinas, F (1970) *Amer. J. Med.*, 49, 346
4 Rabiner, SF and Drake, RF (1975) *Kidney Int.*, 7, S-144
5 Moncada, S, Gryglewski, RJ, Bunting, S and Vane, JR (1976) *Nature*, 263, 663

Open Discussion

REMIZUZZI (Bergamo) Do you believe that the role of platelets in prostacyclin generation by endothelium is important or not? Because we have had similar results, using platelet-poor plasma from uraemic patients. These results both support the work of McIntyre et al who found that cell-free plasma stimulated prostacyclin synthesis.

LEITHNER I think the experiments with uraemic platelet-poor plasma is surely related only to one of several factors which might be responsible for the altered prostacyclin metabolism in uraemia. One of several, surely not the most important.

REMIZUZZI Which buffer pH did you use in your studies?

LEITHNER It was a pH of 7.4.

REMIZUZZI With a high pH, 8.5 or 9, prostacyclin is more stable. This makes the experiments much easier.

NENOV (Varna) Have you tried to measure if there is some difference between prostacyclin from renal vessels and the prostacyclin activity from vessels of other origin? Which kind of vessels do you use?

LEITHNER In our human material we use arteries and veins from the forearm. We have only a little experience with other arteries. I cannot answer for the renal arteries. Renal biopsy material should be used I think for histological examination of pathological alterations and I think this is too important for
the patient to use portions of tissue for prostacyclin tests.

WOODS (London) As you know, we have been using synthetic prostacyclin in extracorporeal circulation. John Vane and Salgo Macadam have suggested that the prostacyclin which is generated by vascular endothelium is an endogenous anti-thrombotic agent. But you and the group from Italy have suggested that the levels in uraemic patients are higher than in normal. Yet we know that patients with uraemia have an accelerated rate of atherosclerosis compared to a normal population. Can you possibly comment on this, and fit it into some sort of hypothesis or theory as to why this occurs? Is it in fact a response of uraemia to some other stimulus to atherosclerosis and to the normal physiological response or do you possibly agree with Moncada et al that the generation of prostaglandin endoperoxide by platelets contributes to this higher prostacyclin production by uraemic patients?

LEITHNER It's a very interesting theory that the enhanced prostacyclin synthesis by uraemic vessels is a response against development of atherosclerosis.

WOODS I agree that these are speculations but there are other lines of evidence that suggest that patients with uraemia, whether on long-term dialysis treatment or not, do have a coagulopathy that is not in fact purely a haemostatic defect but is an increased tendency to clot and the increased prostacyclin production might be postulated as some form of a protective mechanism in response to that.

LEITHNER We could discuss this for one hour or more. I think rather that the altered pathway of metabolism is in part a result of the fatty acid metabolism in uraemia, and in part perhaps a result of the 'middle molecules'.

WOODS Well, that was the answer I'd hoped you'd come around to, talking about fatty acids and fat, because I think that this abnormality you have demonstrated, or this change in prostaglandin production must remind us that we must pay a lot more attention to the use of fatty acid manipulation if we are going to get on top of the problems of atherosclerosis in uraemia.

RITZ (Heidelberg) In response to Dr Wood, perhaps it is pertinent to mention the results of Bagdade's (Seattle) studies which demonstrate in the plasma of uraemic patients the presence of some stimulatory activity on arterial smooth muscle cells. Do you have any information as to whether delivery of arachidonic acid is the rate limiting step for the synthesis of PGI2? I am asking this because when we studied the composition of subcellular membranes (sarcoplasmic reticulum), we found a marked increase in polyunsaturated fatty acids in subcellular membranes. These increased pools might favour increases in synthesis of PGI2.