HAEMOLYTIC URAEMIC SYNDROME: REPORT OF A CASE AND NEW PATHOGENETIC CONCEPTS

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Summary

Haemolytic uraemic syndrome (HUS) is a severe clinical condition characterised by thrombocytopenia, microangiopathic haemolytic anaemia and renal impairment [1].

At histological examination hyaline microthrombi occluding terminal arterioles and capillaries are generally found.

Other syndromes share major clinical and histological diagnostic criteria with HUS. In particular, there is no method at present which clearly differentiates HUS from thrombotic thrombocytopenic purpura (TTP) [2]. We propose therefore the term thrombotic microangiopathy (TMA) to discuss both syndromes.

The pathogenesis of TMA is unclear and many forms of therapy have been attempted without definitive proof of efficacy. The recently-reported [3] successful results with exchange transfusion and plasma infusion represented a consistent advance in the management of these diseases. The beneficial effect of these procedures was attributed to replacement of an unknown 'missing' factor in plasma.

This finding generated recent observations possibly relevant in understanding the pathogenesis of these diseases.

Introduction

Lian et al [4] reported a platelet aggregating factor in plasma from patients with TTP, which is inhibited in vitro by normal plasma. Our group presented evidence suggesting that patients with thrombotic microangiopathy may lack a physiological plasma component which stimulates vascular prostacyclin (PGI₂), a potent endogenous inhibitor of platelet aggregation [5].

We describe here a patient with HUS and two of this patient's four offspring, all with a similar plasma defect. Our observations support a previous suggestion that some people have a congenital or hereditary predisposition to thrombotic microangiopathy [6] and further support the 'missing factor' hypothesis of the pathogenesis of TMA [7].
Case Report

The proposita is a 54-year old woman admitted to the Nephrology Division of our hospital for acute renal failure. HUS was diagnosed on the basis of thrombocytopenia, microangiopathic haemolytic anaemia and neurological signs. Renal biopsy showed a picture of cortical necrosis. After plasma exchange and infusion, marked improvement was seen in both clinical condition and laboratory test results. One month later the patient was discharged and remained asymptomatic with a compensated haemolytic state during the following ten months. This patient has been described in detail elsewhere [8]. Plasma activity stimulating vascular prostacyclin was studied while the patient was in hospital and at subsequent out-patient follow-up. The patient’s family was also investigated as regards the property of plasma to stimulate prostacyclin production.

Methods

Blood samples were collected by clean venipuncture into plastic tubes. Platelet-free plasma was obtained by differential centrifugation of citrated venous blood [9]. One volume of 0.126 M trisodium citrate was added to nine volumes of blood; this proportion was modified according to the haematocrit, in order to ensure comparable anticoagulant concentrations in all plasma samples tested. All plasma samples from the patient and her offspring were fresh-frozen and tested simultaneously.

The capacity of plasma to stimulate prostacyclin production was tested on cultured pig endothelial cells [10]. Prostacyclin production was measured by radio-immunoassay of its stable derivative 6-Keto-PGF$_{1\alpha}$ [11]. Each plasma sample to be tested was mixed with 5u/ml heparin and diluted to 5 in HEPES buffered cell culture medium [12]. Portions of 0.3ml diluted plasma (or control medium) were added to the wells of a multi-well culture dish containing about $10^5$ endothelial cells/well. Following incubation for 1 hour at 37°C (under constant rotation) 10–200µl plasma or medium were removed and 6-Keto-PGF$_{1\alpha}$ was assayed as specified above. Each sample was tested on 3–4 wells. Results were expressed as pg 6-Keto-PGF$_{1\alpha}$/10$^5$ cells, after subtraction of the values found in the corresponding control medium.

Results

Figure 1 shows that both before and three hours after treatment by plasma infusion the patient’s plasma could not stimulate prostacyclin production by endothelial cells. In the days following treatment, the patient’s plasma stimulating activity markedly increased, although remaining below the range of activity of six control normal plasmas. Plasma collected at subsequent out-patient follow-up again showed a fluctuating deficiency of prostacyclin-stimulating capacity.

The capacity to stimulate prostacyclin activity was normal in two of the patient’s offspring (BA, female, 25 years old, and BD, male, 30 years old), but very low in the other two (BE, female, 32 years old, and BG, male, 16 years old). The patient’s husband was unavailable for testing, but plasma from a daughter
Figure 1. Production of 6-Keto-PGF$_1\alpha$ by endothelial cells incubated for one hour with culture medium containing 20% v/v plasma. Dotted lines show range of values obtained with plasma samples from 6 control subjects; histograms show mean values of 3–4 replicate determinations made on samples from the proposita, four offspring and a grandaughter. B — sample taken before treatment; other times shown refer to the time since treatment

(AF) of BE behaved normally (Figure 1). None of the subjects examined had any history or clinical signs of microangiopathic disorders [13].

Discussion

Several reports suggest that exchange transfusion in patients with TMA is followed by rapid clinical and laboratory recovery [3,14–17]. The suggestion that the beneficial effects of this procedure was due to removal of a 'toxic' substance [15] was challenged by Byrnes and Khurana [16] who described a patient with thrombotic microangiopathy in whom multiple plasma infusions repeatedly induced remission. We previously reported two patients with TMA, including the proposita, in whom the activity of prostacyclin, a potent inhibitor of platelet aggregation, was absent in venous specimens obtained during relapse but was restored shortly after plasma treatment [5].

Stimulation of prostacyclin activity by normal human plasma was first reported by MacIntyre et al [18] and confirmed by our group [19]. It was suggested that patients with TMA lack the plasma component(s) to stimulate prostacyclin production, resulting in widespread formation of platelet thrombi in the microcirculation [5,20].

We report here that such a defect persisted in one of these patients during a ten month follow-up, while the patient was asymptomatic with a compensated haemolytic state. Moreover, two of the proposita's four offspring showed a similar plasma defect, though they were asymptomatic. These findings suggest
that the defect may be genetically determined. It is recognised that thrombotic microangiopathic episodes may be triggered by agents such as infection [21]. It appears likely that the congenital failure of plasma to stimulate prostacyclin generation by vascular cells would not necessarily result in any clinical signs of haemolysis or thrombocytopenia as long as no aetiological agents were encountered to initiate a pathogenetic sequence leading to thrombotic occlusion of the microcirculation.

Machin et al [22] observed remission in a case of TTP following extensive plasma exchange. At admission the patient had undetectable plasma levels of 6-Keto-PGF$_{1\alpha}$ (the stable breakdown product of PGI$_2$) and no plasmatic activity stimulating PGI$_2$. After plasma exchange the plasma level of 6-Keto-PGF$_{1\alpha}$ became normal and the patient’s plasma stimulated production of normal amounts of PGI$_2$.

These findings further support the hypothesis that TMA is due to the deficiency of a plasma factor usually required for vascular PGI$_2$ synthesis. The nature of this factor remains to be defined. The low prostacyclin stimulating activity could derive from increased lipid peroxidation.

It was reported that during the metabolism of arachidonic acid, free radicals are formed which selectively inactivate PGI$_2$ synthetase [23]. Moreover, free radical scavengers prevented the inhibition of PGI$_2$ synthetase by hydroperoxides [24].

In patients with HUS increased lipid peroxidation and reduced plasma level of vitamin E (a potent free radical scavenger) were both observed [25].

Finally, a recent study indicates that production of PGI$_2$-like substances was significantly reduced in the aorta of rats fed a vitamin E deficient diet. In this model a significant accumulation of lipid peroxides was observed in the vessel walls [26]. Our data and these considerations suggest that the plasma from patients with HUS may have reduced antioxidant power thus leading to a predominance of platelet aggregating thromboxane over vascular prostacyclin generation. The resulting intravascular platelet activation may lead to the release of beta-thromboglobulin which further inhibits PGI$_2$ production by the vessel wall [27].

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References

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Open Discussion

WOODS (Kuwait) This description here of low prostacyclin in TTP makes me wonder, since you were one of the first people to describe high prostacyclin in renal failure in uraemic people, whether you think we might be working on a similar hypothesis and that in uraemia there may be retention of an antioxidant which in fact favours increased prostacyclin generation by the vascular tissues.

REMUZZI The problem is very complicated. One possibility to consider is that phenols, free radical scavengers, are retained in great amount in uraemia. So it may be that in uraemia and in haemolytic uraemic syndrome, differences in the antioxidant potential of plasma result in differences in prostacyclin formation. I am sorry this is terribly speculative; of course, we have no proof so far.

REES (London) Have you got any sequential studies of patients with haemolytic uraemic syndrome looking at 6-oxo-PGI2 level? I ask the question because we have a patient who had low levels when she had post partum renal failure and normal levels on recovery.

REMUZZI This is very interesting and is what we would expect. We have not studied the 6-oxo level in plasma. We studied production of prostacyclin activity from vascular tissues and the capability of plasma to stimulate prostacyclin generation.