PLATELET HYPERSENSITIVITY IN THE NEPHROTIC SYNDROME

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Summary

The possible relationship between low levels of circulating plasma albumin and hypersensitivity to the induction of platelet aggregation by arachidonic acid, was studied in 11 patients with nephrotic syndrome. Similar studies were done in 10 healthy volunteers. All the patients had abnormally low or low normal levels of serum albumin. This protein is a regulator of platelet aggregation and prostaglandin synthesis. The mean threshold concentration of arachidonic acid necessary to induce aggregation in patients’ platelet-rich plasma (PRP) was 0.15mM while this value for normals was 0.53mM. The normal range has been reported elsewhere as between 0.2 and 1.0mM. This difference between patients and normals was highly significant. The 7 patients who had the lowest serum albumin levels (1.1 to 2.7g/dl) had platelets which were highly sensitive to induction of aggregation by arachidonic acid (0.02–0.09mM). In 3 cases, addition of albumin to the patients’ platelet-rich plasma to raise the plasma albumin level to the normal range normalised the threshold aggregating concentration to arachidonic acid. In 2 cases, infusions of albumin in the patients resulted in elevation of the concentration of circulating plasma albumin and normalisation of the threshold aggregating concentration to arachidonic acid.

The data indicate that the low plasma albumin concentration in patients with nephrotic syndrome is an important factor in their hypersensitivity to platelet aggregation by AA in vitro and probably contributes to their thrombotic tendency.

Introduction

The nephrotic syndrome (NS) is frequently complicated by a variety of thrombotic disorders and the major thrombotic episode reported in these patients is renal vein thrombosis[1–5].

Recently, enhanced platelet aggregation in response to collagen and ADP has been described in patients affected by glomerular renal disease and proteinuria [6]. The degree of platelet hyperfunction was correlated with the degree of
proteinuria and it was suggested that abnormal platelet aggregation might be a consequence of urinary loss of plasma proteins normally responsible for inhibition of platelet aggregation. It is known that albumin can inhibit platelet aggregation induced by arachidonic acid (AA) and collagen [7] as well as ADP [7,8]. It was therefore proposed that albumin may be a controlling factor in haemostasis [7]. In this study we investigated the possible relationship between low levels of circulating plasma albumin and hyper-aggregable platelets in patients with NS.

Materials and Methods

Plasma albumin levels were determined as described elsewhere [9]. The following materials were purchased: arachidonic acid sodium salt 90% (Sigma, St. Louis, Missouri); arachidonic acid 99% (Nuchek, Elysian, Minnesota). Solutions of sodium arachidonate (50mM) were prepared by adding AA to a solution of sodium carbonate (100mM) as described elsewhere [10]. All work with arachidonic acid was done in a nitrogen atmosphere. Serum albumin solution (25% albumin) for infusion into humans was Siero Albumina Umama Normale (Sclavo, Siena, Italy). Platelet counts were performed by phase contrast microscopy using the Unopette diluting system (Becton Dickinson, Novate Milan, Italy). Counts for patients and controls were between 3 and 4 x 10^8/ml platelet rich plasma (PRP).

Eleven patients (9 males and 2 females), 6–73 years old with NS, and 10 normal volunteers (6 males, 4 females) 25–58 years old were studied. At the time of admission of patients, clinical evaluations were performed including past history (especially of macroscopic haematuria and oedema). In addition measurements of serum concentration of protein, cholesterol, creatinine, blood urea nitrogen, quantitative urinary excretion of protein and creatinine were done by standard methods in the hospital laboratory [11]; glomerular filtration rate was estimated by the measurement of 24 hr creatinine clearance [11]; initial examination of urinary sediment and percutaneous renal biopsy were done. Histological sections (2 to 3 μ) were stained with haematoxylin and eosin, periodic acid Schiff’s and Masson’s trichrome. Immunofluorescence microscopy using fluorescein conjugated antiserum against IgG, IgA, IgM, IgE, C3 (β1C) and fibrinogen were done. The diagnosis of nephrotic syndrome was made according to the criteria of Schreiner [12]. The types of glomerular disease included membranoproliferative nephritis, minimal change nephritis, segmental and focal hyalnosis and membranous nephritis.

All patients and controls denied the ingestion of any drugs for at least two weeks before the experiments and were fasted overnight. Nine volumes of blood, drawn from the antecubital vein, were mixed with 1 volume of trisodium citrate (3.8%). PRP was prepared by centrifuging the citrated blood at 220g for 15min at room temperature. All platelet aggregation tests were performed within 2 hr of blood collection by the method of Born and Cross [13] using an Elvi 840 aggregometer (Elvi Logos, Milan, Italy). Sodium arachidonate solutions, in micro-litre amounts, were added to 0.25ml PRP to determine the threshold aggregating concentration (TAC). The TAC of AA was defined as the smallest concentration
which gave irreversible platelet aggregation starting within 3 min of addition of the aggregating agent to PRP.

**Addition of Albumin to Patients’ PRP**

One tenth ml of the 25% albumin solution was added to 1ml PRP to raise the plasma albumin concentration of patients’ PRP to normal levels.

**Infusion of Albumin**

A solution of 25% albumin was infused intravenously into 2 patients at a rate of 1.5ml/min. One patient (C.L.) received 50g albumin, the other patient (I.R.) received 25g albumin.

**Results**

**Hypersensitivity of Platelets to Induction of Aggregation by Arachidonic Acid**

Figure 1 shows that the platelets in PRP of 7 out of 11 patients aggregated in response to 0.09mM AA or less whereas all 10 normal controls aggregated only after 0.27mM AA or higher concentrations. Mean threshold aggregating concentration for patients’ PRP with AA was 0.15mM and for controls 0.53mM. The difference was highly significant (p <0.001).

**Correlation Between Sensitivity to Induction of Aggregation by Arachidonic Acid and Serum Albumin Levels**

In Figure 2 it can be seen that 7 patients whose PRP aggregated to 0.09mM AA or less, had abnormally low plasma albumin levels. Two patients, whose TACs to AA were also below normal (0.18mM), had plasma albumin concentrations around the lower limit of normal (3.7g/dl). The last 2 patients had albumin levels lower than normal with TACs within the normal range.

**Correction of Hypersensitivity to Arachidonic Acid**

*Increasing the concentration of albumin in PRP in vitro* When albumin was added to the PRP of 3 patients with hypersensitivity to AA, to bring the plasma concentration into the normal range, the TAC for AA in each case was elevated to the normal range (Figure 3).

*Intravenous infusion of albumin solution in NS patients to normalise the TAC to AA* Blood samples were obtained from 2 patients prior to infusion of albumin solutions, and 15 min after the completion of each infusion, for determination of serum albumin levels and TAC to AA. For patient I.R. the TAC increased from 0.05mM AA to 0.2mM. The corresponding albumin levels were 1.6g/dl and 2.2g/dl. For patient C.L. the TAC to AA increased from 0.06mM AA to 0.3mM.
Figure 1. Threshold aggregating concentrations obtained on PRP of 11 patients and 10 controls in response to AA. Differences between patients and controls are significant (p < 0.001, Student t test and p < 0.01, Mann-Whitney test, see [17]).

Figure 2. Threshold aggregating concentration (●) on PRP and serum albumin concentration (■) starting with the same sample of blood from 11 different NS patients. Statistical analysis [17] of the data to try to define the correlation between TAC and serum albumin concentration indicated a parabolic regression:

\[ y = A_0 + A_1 \times A_2^2 \]

\[ y = 0.466 + 21.294 \times -34.812^2 \]

(p < 0.025)
The corresponding albumin levels were 1.35 and 2.44 g/dl.

Discussion

This study shows that platelets in the PRP of patients with nephrotic syndrome are more sensitive to induction of aggregation by AA than platelets in the PRP of normal subjects. The hypersensitivity to AA is striking and raises the question whether other aggregating agents have similar effects. We have found that platelets in PRP from patients with NS aggregate at significantly lower concentrations of ADP than normals and have a tendency toward hypersensitivity to collagen (unpublished observations of the authors). This is in accord with a previous report of Bang et al [6]. An important difference between normal PRP and PRP from NS patients is that plasma from the patients contains abnormally low levels of albumin. This plasma protein has been reported to inhibit aggregation of normal PRP [7], regulate platelet prostaglandin metabolism [14,15] and, in the present study, it has been shown to normalise the hypersensitivity to AA of platelets in PRP from NS patients. While platelets in PRP of normal subjects aggregate only in response to 0.2 to 1 mM AA, washed platelets suspended in aqueous medium without albumin will do so in response to micromolar concentrations of AA [7,14,15]. Arachidonic acid, the substrate for prostaglandin and thromboxane synthesis by platelets, if bound by albumin would not be available for such synthesis. It is known that albumin suppresses the formation of products of the platelet cyclo-oxygenase pathway [15]. Thus, in the presence of high levels of albumin both platelet aggregation and platelet metabolism of AA are diminished, whereas in the presence of low levels of albumin the platelets become hypersensitive to aggregating agents and metabolise AA very actively. These findings provide an explanation for the earlier report of Bang et al [6] who showed that in NS patients there was a statistically significant correlation between enhanced platelet aggregation in response to ADP and collagen and a variety of indices of
plasma protein derangement, including total amount of plasma protein lost in the urine in 24 hr, and serum albumin level. They concluded that the enhanced platelet aggregation in patients with glomerular disease was a consequence of a plasma protein abnormality rather than an intrinsic platelet defect. We found that these patients have abnormally low plasma albumin levels, resulting in platelet hypersensitivity to aggregating agents and this may promote a tendency toward thrombosis. Our results suggest that the determination of the threshold aggregating concentration to AA would be of value in predicting the thrombotic risk for the patients. The present findings might also have therapeutic implications. In view of the possible crucial role of plasma albumin levels in the pathogenetic mechanism sustaining the thrombotic tendency, therapy aimed at increasing the level of circulating plasma albumin or reduction of platelet and plasma arachidonate by appropriate dietary control might result in diminishing the chances of thrombosis in NS patients at high risk [14,16].

Although it has been suggested that plasma albumin levels can play a role in regulating the metabolism of AA by platelets [14], they have so far not been considered as a possible factor controlling the thrombotic tendency in patients with NS. It is now apparent that the hypersensitivity to platelet aggregation seen in NS patients is related to their low levels of circulating plasma albumin and may be an important factor in the thrombotic tendency seen in these patients.

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References

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Open Discussion

NICHOLLS (Aberdeen) To support the concept that a low albumin alone causes platelet hypersensitivity have studies been made of the aggregation of normal platelets suspended in nephrotic plasma? If they were hypersensitive to aggregating agents in nephrotic plasma this would support your hypothesis.

REMUZZI Can I show a slide please? This is an experimental study with adriamycin-induced nephrotic syndrome in rats, which gives some answer to your question. You can see that in platelet-rich plasma of nephrotic animals we have very high malondialdehyde production in comparison with untreated animals. When washed platelets were resuspended in saline solution we have a similar response between adriamycin and control. When we have control platelets resuspended in adriamycin plasma we have a very high production of malondialdehyde. In contrast, with adriamycin platelets suspended in control plasma, we have a normal production of malondialdehyde. This is an additional proof that plasma is an important factor in inducing platelet hypersensitivity to aggregating agents, whereas platelets themselves in the nephrotic syndrome are essentially normal in this respect.

BJORNSON (Oslo) What were the levels of anti-thrombin III in your patients? Six of your patients had reduced renal function and in these patients you might find hypersensitivity of your platelets. Did they differ from the rest? The final question is: would you advocate the use of platelet inhibitors in addition to anticoagulant therapy for prevention of thrombosis in these people?

REMUZZI We have not measured antithrombin III in these particular patients. We have measured antithrombin III in other patients with nephrotic syndrome and therefore we have not correlated platelet hypersensitivity with antithrombin III. I know that M Stewart has done this kind of study and she does not find correlation between platelet hypersensitivity and antithrombin III.

However, low levels of antithrombin III, if present, may be an important additional factor in promoting the tendency of the patient to thrombosis. As far as your second question is concerned we have measured TAC in a small number of patients with renal insufficiency. The minimum creatinine clearance in these patients was 12ml/min and therefore we did not have impairment of platelet function due to uraemia. Your last question was about antiplatelet agents. I think that increasing the concentration of albumin or appropriate diet with low intake of arachidonic acid or maybe antiplatelet agents should be possible therapeutic procedures on the basis of these results. However, because we have not studied the incidence of very low TAC's of AA in patients with clinical evidence of thrombosis, especially renal vein thrombosis, I cannot provide a definitive answer to this question. I think that the possible use of anti-platelet agents is an interesting suggestion for future studies.
VAN DER HEM  Did you notice a difference in patients with different types of nephrotic syndrome for example with selective and non-selective proteinuria?

REMUZZI  We have not done this kind of statistical study because ours is a small group of patients and I think not sufficient for an appropriate statistical evaluation, on different sorts of nephrotic syndrome. I think it would be interesting to study this in the future for us.