PART XIV

TRANSPLANTATION COMPLICATIONS

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RADIOLABELLED PLATELETS AND PROSTACYCLIN IN DIAGNOSIS AND TREATMENT OF TRANSPLANT REJECTION

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Summary

The trapping of $^{111}$Indium-oxine labelled autologous platelets by the transplant was recorded by means of a computerised gamma camera and expressed as platelet uptake index (PUI). Eighty-five patients were studied. The PUI increased in acute rejection from $1.13 \pm 0.11$ to $1.74 \pm 0.17$, and decreased again when rejection was reversed. In chronic rejection PUI was significantly lower than in acute rejection, but still higher than in long term stable grafts. Prostacyclin infusions were given to six cases of acute rejection and 12 of chronic rejection. In the majority of patients an improvement in transplant function and a decrease in platelet trapping could be demonstrated.

Introduction

In 1979 Smith and co-workers [1] reported on a deposition of $^{111}$Indium labelled platelets in kidney transplants during acute rejection. This phenomenon was demonstrated by gamma camera imaging and served as a diagnostic sign. These findings accorded with those of Kincaid-Smith [2] who had observed platelet deposition in acute rejection using electron microscopy. In 1980 our group reported the deposition of $^{111}$Indium labelled platelets in chronically rejected grafts, but, of lower intensity than in acute rejection [3]. Arterial obliteration in the graft caused by endothelial cell proliferation is an integral feature of chronic rejection and we suggest that the mitogenic factor released by platelets [4] might enhance this harmful process.

Mundy and co-workers [5] have reported results of experiments with a platelet inhibitor, prostacyclin. They temporarily prevented hyperacute rejection in the dog by infusing synthetic prostacyclin. This success indicated that it was possible to modify graft rejection by prevention of a secondary phenomenon, such as platelet trapping, whilst the immunological aggression itself proceeded unchanged. Stimulated by these promising findings we investigated the effect of prostacyclin in patients with chronic rejection. We chose
this rejection type because the relatively stable clinical condition and medication allowed better evaluation of the effects of additional drugs such as prostacyclin. Encouraged by positive results [6] we have studied the effects of prostacyclin in acute rejection.

Materials and methods
Eighty-five patients, 50 men and 35 women, aged 8–60 years (X ± SD; 35.6 ± 12.9) were collected in three groups; patients with long term good and stable grafts (N=29), those suffering from histologically proved chronic rejection (N=28), and the rest (N=28) observed during the first weeks after transplantation. Informed consent was obtained from all patients according to the declaration of Helsinki. For the labelling of platelets 17ml of blood were drawn by venepuncture into syringes containing 3ml of acid citrate dextrose and 250ng prostacyclin for anticoagulation and prevention of premature platelet activation respectively. After separation of platelet-rich plasma the platelets were isolated, labelled with 250μCi 111 Indium-oxine, resuspended in platelet-poor plasma and reinjected into the patients [7]. This procedure was performed at least once in patients with long term stable grafts (plasma creatinine 1.0–2.0mg/dl) and patients suffering from chronic rejection, both received prednisolone and azathioprine for immunosuppression. In the group examined during the post-operative period, platelet labelling was usually undertaken weekly for at least four weeks, if necessary more frequently. These patients received ATG, prednisolone and azathioprine with the exception of one subject treated with cyclosporin A and prednisolone. The patients were examined 2–3 times daily by gamma camera (Nuclear Chicago) using a computerised programme. For the enumeration of platelet deposition in the graft constant regions of interest were inserted over the transplant. Counts measured in the transplant region during five minutes divided by the counts of the contralateral region of the same size resulted in the so-called platelet uptake index (PUI). In addition the platelet t½ was determined. Acute rejections were diagnosed clinically, some confirmed by percutaneous biopsy. The patients were regularly examined by ultrasonic scanning.

In another series of investigations six patients with acute rejection (histologically proven in three cases) and 10 patients suffering from chronic rejection (confirmed by biopsy) were treated with prostacyclin (kindly provided by Dr O'Grady, Wellcome Research Laboratories, Beckenham, Kent, United Kingdom). For this purpose 5ng prostacyclin/kg body weight/minute were infused by a central vein catheter. In the cases with acute rejection this medication was given in addition to the usual therapy with infusions of 0.5g methylprednisolone, because of ethical reservations. On the other hand in chronic rejection the immunosuppressive therapy remained unchanged. The estimation of PUI was performed as stated above.

Results and discussion
Platelet scan and platelet uptake index
Platelet deposition in long term stable transplants functioning well was usually absent or minimal, as shown by a PUI of 1.08 ± 0.14 (X ± SD) and a platelet
t½ of 102.3 ± 21.3 hr. These values differed significantly (p<0.01) from those with chronic rejection, where a mild to moderate platelet trapping (PUI 1.32 ± 0.16) and shorter platelet t½ (61.6 ± 24.7 hr) indicate platelet consumption in the graft. In this way the obliterator proliferation of intrarenal arteries could be enhanced by the release of platelet mitogen. In addition, we suggest that patients showing relatively high PUI values in spite of normal graft function might be subjected to harmful platelet deposition in the transplant, resulting in chronic rejection.

In the group of 28 patients examined during the post-operative period we observed 13 acute rejections (six histologically proven) in 12 patients. Two of these subjects remained oligoanuric during the whole observation period. During acute rejection the PUI increased significantly (p<0.01) from 1.13 ± 0.11 to 1.74 ± 0.17, while platelet t½ decreased from 87.9 ± 18.6 hr to 42.4 ± 21.0 hr. In patients still producing urine the rise of PUI was observed 0–48 hr (15.5 ± 12.0) before rejection caused an increase in plasma creatinine (Figure 1). The platelet trapping was clearly visible in the gamma camera scans (Figure 2). However, the PUI increase was only small, from 1.3 to 1.5 in one case of acute interstitial rejection, while greater increases were observed in acute vascular rejection. The further course of acute rejection was also associated with PUI.

**Figure 1.** Increase of platelet uptake index (PUI) before increment of plasma creatinine due to acute rejection.
changes. In reversible rejection the PUI declined to $1.34 \pm 0.10$, whilst irreversible rejection resulted in small additional increases to $1.76 \pm 0.18$. The platelet $t^1\frac{1}{2}$ showed inverse changes, namely an increase to $83.0 \pm 26.8$ hr after reversible acute rejection, and to $47.6 \pm 29.9$ hr in irreversible rejection. In spite of the diagnostic help provided by PUI estimation one must be aware of the fact that transplants which are avascular and/or necrotic due to preceding graft rejection will not continue to trap radiolabelled platelets. Therefore a negative platelet scan will not always allow the clinician to refrain from other diagnostic methods, such as transplant perfusion scan or percutaneous biopsy.

Ten patients in the post-operative group demonstrated no rejection during the observation period. They revealed a low PUI of $1.19 \pm 0.14$ and a platelet $t^1\frac{1}{2}$ of $93.1 \pm 21.0$ hr. In two cases an acute rejection was considered because of fever, swelling and tenderness in the graft region, and a decrease of transplant function. The constantly low PUI of about 1.0 did not support this sus-
picion. Some days later it became evident that bacterial transplant infections were responsible for graft dysfunction. Two false positive increases of PUI were observed; one was due to a transplant haematoma, the other developed a recurrence of haemolytic-uraemic syndrome, his basic renal disease, probably in connection with cyclosporin A treatment [8]. As one typical feature of this syndrome, there was marked platelet trapping in the graft, which revealed, however, no signs of rejection in a biopsy specimen.

During this study we found some problems with the method. In one case insufficient platelet labelling probably due to thrombocytopenia did not allow the monitoring of the patient by platelet scan. Since liver and spleen take up the tracer liberated by platelets, hepato- or splenomegaly may cause an overlap between these organs and transplants in the gamma camera imaging. This made the evaluation of a platelet scan impossible in one case. However, in spite of these pitfalls the described method served as a valuable diagnostic tool in the management of transplant patients.

**Prostacyclin therapy in graft rejection**

In the six patients selected for prostacyclin treatment, the acute rejection episode had resulted in an increase in plasma creatinine from 1.5–5.2 to 3.5–8.6 mg/dl in four cases, whereas two patients were oliguric and had to be dialysed. Three rejection episodes were confirmed by biopsy. The PUI increased from 0.97–1.23 to 1.5–2.2. During prostacyclin treatment the PUI decreased in all cases to a value of 1.15–1.36. The oliguric cases remained in this state and had further rejection episodes leading to graft destruction after termination of prostacyclin infusion. On the other hand the improvement in four cases producing urine lasted for at least two months. Verification of the prostacyclin

![Figure 3. Improvement of transplant function, decrease of PUI and prolongation of platelet t½ in chronic rejection treated with prostacyclin](image-url)
effect on acute rejection can only be achieved by a controlled or double blind study. However, in our preliminary study the drug was well tolerated and produced no major side effects with the exception of blood pressure lowering which was not unwelcome.

In the 12 cases suffering from chronic rejection the prostacyclin treatment resulted in a decrease of PUI and a prolongation of platelet t½ indicating a suppression of platelet consumption by the graft. The transplant function improved (Figure 3) with this therapy which could, in part, have been caused by the influence of prostacyclin on renal haemodynamics [9]. We tried to prolong this beneficial effect after the termination of prostacyclin infusion by the oral administration of 200mg sulfinpyrazone and 3 x 75mg dipyridamole per day. However, this therapy failed, and graft function deteriorated in eight of 12 cases six months later.

In our opinion prostacyclin represents a drug which could increase the therapeutic possibilities in graft rejection. The present limitations of its application and effects could perhaps be reduced by the administration of stable prostacyclin analogues.

Acknowledgments

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References

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Open Discussion

RITZ (Heidelberg) Your findings with prostacyclin infusion on transplant function are certainly impressive. However, I have difficulty in sorting out whether this is a haemodynamic phenomenon or some immuno-modulatory event. Could you give us information on the time course of the effect on GFR? An immediate effect would make me inclined to consider the involvement of a purely vasodilatory phenomenon.
LEITHNER It is absolutely right that prostacyclin increases the renal plasma flow. Therefore the improvement of graft function might be in part due to haemodynamic effects. On the other hand, the decrease of the platelet uptake and the prolongation of the platelet half-life suggest that platelet trapping in the transplant was suppressed too. It might be therefore that the vessels of the transplant could in some way be cleared of platelet deposits, I don’t know whether prostacyclin might influence the immunological process.

BURCK (Kiel) How long does the 111Indium stay in the liver and how many times can you repeat the test from the irradiation risk?

LEITHNER Indium has a short half-life of 2.8 days. Within about one week, the indium will be cleared from the liver. Therefore, you can repeat it weekly. If you have an acute rejection you might have to do it more frequently. The radiation risk is about the same as one thorax X-ray. Therefore, in our opinion we can monitor these patients without major radiation risk.

COHEN (London) I wonder if you could tell us whether you found any relationship between peripheral platelet counts and your radio-labelled platelet uptake studies?

LEITHNER Some cases, where the platelet uptake was very high showed rather low platelet counts, but there was no strong correlation between these two parameters.