DIFFERENTIAL DIAGNOSIS OF CYCLOSPORIN A NEPHROTOXICITY VERSUS REJECTION BY FINE NEEDLE ASPIRATION BIOPSY

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Summary

Twelve transplants with signs of graft failure and five with good function on CyA, were aspiration biopsied and analysed for (a) the presence of inflammation, (b) morphological changes in the graft parenchymal cells and (c) by indirect immunofluorescence for the presence of CyA in the renal tubular cells. Four reaction patterns were recorded: (i) good transplants with a normal serum creatinine, no evidence of inflammation, no parenchymal cell changes and no deposits of CyA in the graft; (ii) patients with elevated serum creatinine, no inflammation but distinct parenchymal cell changes and massive deposits of CyA (i.e., nephrotoxicity); (iii) patients with elevated serum creatinine, distinct inflammation in situ, modest tubular cell changes and no CyA in the graft (i.e., rejection); and (iv) patients with elevated serum creatinine, distinct inflammation in situ, distinct tubular cell changes and concomitant deposits of CyA (i.e., nephrotoxicity and rejection). CyA deposits in the graft had only a marginal relationship to drug dose and concentration of the drug in serum. When the dose was reduced, the deposits rapidly disappeared and tubular cell changes resolved. We recommend the FNAB for differentiating between nephrotoxicity and rejection in renal transplants.

Introduction

Among the practical difficulties in the clinical use of Cyclosporin A (CyA) are the nephrotoxic and hepatotoxic effects of this drug. These difficulties are even more pronounced when a differential diagnosis of rejection must be made, especially if the transplant is complicated by prolonged acute tubular necrosis (ATN). Although some investigators have claimed that specific histological changes are recorded in renal biopsies [1,2] others have failed to confirm this [3,4].

Fine needle aspiration biopsy (FNAB) is an atraumatic method of assessing intragraft events in parenchymal organ transplantation in man [5,6]. In this
study we have analysed 27 consecutive aspiration biopsies performed on human renal allografts in cases of suspected Cyclosporin toxicity, rejection and quiescence. In addition to analysing the cytological changes in graft parenchymal cells, we have analysed the deposition of CyA in the grafts by indirect immunofluorescence and correlated these changes to the serum creatinine, dose of CyA and serum concentration of the drug.

Materials and methods

Thirteen of the 27 patients received initially only CyA (10mg/kg/d) i.v. adjusted to produce a plasma concentration of approximately 200–300ng/ml after i.v. administration for three days; the remaining also received initial methylprednisolone (MP), 3.6mg/kg/d tapered to 0mg in nine days. All rejection episodes were treated by elevating the oral dose of MP to 3.6mg/kg/d until inflammatory cells disappeared from the aspirates. Repeated episodes necessitated routine administration of approximately 0.1–0.5mg/kg/d of MP for basic immunosuppression to 24 of these patients from the third week onwards.

Plasma concentrations of CyA were determined by radioimmunoassay, using the standard Sandoz RIA-kit nr. 031180 (Sandoz Pharmaceuticals, Basle) [7].

Fine needle aspiration biopsies were performed as previously described [6], and several cytocentrifuged cell preparations were made from these specimens. One of the preparations was stained with May-Grünwald-Giemsa [6] to quantify the morphological changes in the graft parenchymal component and to assess, if any, the inflammatory events in the graft.

One or more duplicate FNAB specimens were fixed with cold acetone for 15 min, treated with 1:1000 diluted rabbit anti-Cyclosporin serum (courtesy of Sandoz Co), and subsequently with 1: 20 diluted FITC-conjugated swine anti-rabbit IgG (Dakopats, Copenheagen). The antiserum has been reported to be specific for CyA and to a number of (still unidentified) split products of this drug [7]. Occasionally indirect PAP-peroxidase technique was used instead of immunofluorescence. The frequency and amount of CyA in the graft parenchymal cells was assessed from coded FITC-stained specimens mixed with normal rabbit serum treated control specimens by scoring the intensity of reaction from 0 to 3+.

On three occasions, a concomitant needle and/or open biopsy was performed. Part of the biopsy was snap-frozen in liquid nitrogen, cut and immunoperoxidase or immunofluorescence evaluation was performed from the frozen section specimens as above.

Results

In good grafts, with entirely normal serum creatinine, there was no inflammation in the graft, no or only very modest tubular cell changes and no or only faint deposits of CyA demonstrable by immunofluorescence.

Four different patterns of alterations were delineated in cases of elevated serum creatinine (Table I).

The first pattern consisted of no inflammation, distinct though not extreme
TABLE I. Differential diagnosis of renal transplant failure by FNAB

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*The possibility that CyA deposits occasionally induce some inflammation, is not entirely excluded. (From von Willebrand and Hayry, Transplant Proc 1983; In press)

Tubular cell changes and no CyA deposits in the graft. All these cases fell within the postoperative period, and because they coincided with the clinical signs of postoperative acute tubular necrosis, they were interpreted as being due to ATN.

In the second pattern distinct changes, including swelling, vacuolisation and inclusions, were recorded in the tubular cells. This pattern was associated with distinct deposits of CyA in the graft but no inflammation in the specimen. These cases were interpreted as being due only to CyA toxicity.

In the third pattern, the biopsy was dominated by pronounced inflammation with modest tubular cell changes and no CyA deposits in the graft. These cytological alterations entirely coincided with those described previously for acute renal allograft rejection.

In the final pattern, distinct tubular cell changes were associated with CyA deposits in the graft and, in addition to this, a marked inflammation in situ containing T and B blast cells, lymphocytes and mononuclear phagocytes. On these occasions, an inflammatory response of rejection was apparently going on in the graft in spite of maximal cyclosporin therapy.

CyA deposits in the graft had no correlation to the drug dose and only a marginal correlation (p > 0.05) to the concentration of CyA in the serum.

In the concomitantly obtained immunofluorescence and immunoperoxidase specimens, deposits of CyA were also seen particularly prominent in the proximal tubular cells.

Discussion

Our results demonstrate that deposits of CyA are invariably seen in a renal allograft during episodes of clinical Cyclosporin nephrotoxicity. Similar deposits have been seen in the liver and kidney of liver transplant recipients and in the kidney of bone marrow transplant recipients during CyA therapy. In general, the kidney rather than the liver seems to be the more sensitive organ and a
limiting factor for the administration of CyA.

Cyclosporin deposits in the kidney are associated with marked changes in the
graft tubular cells and more modest changes in the vascular endothelial cells. These changes are, as expected, non-specific in nature and similar though more
modest changes, are seen, e.g., during acute tubular necrosis and in advanced
rejection. Thus direct demonstration of CyA in a renal transplant, is the only
clear-cut method of assessing the deposition of this drug in the transplant.
Deposition of CyA is not followed, per se, by any inflammation. This is
clearly seen in cases where serum creatinine is strongly elevated, extreme deposits
of CyA are seen in the kidney while no inflammation is present in the graft. This
observation makes it possible to differentiate between the three most commonly
encountered clinical disorders: acute tubular necrosis, rejection and CyA toxicity.

Acknowledgments

Supported by grant 1RO1 AM26882-03 from the National Institutes of Health,
Bethesda, Maryland and grants from the Sigrid Juselius Foundation, and the
Association of Finnish Life Insurance Companies, Helsinki, Finland.
The authors thank Ms Leena Saraste for secretarial help.

References

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Open Discussion

WILLIAMS (Chairman) Did you do a control in which you put a rabbit serum,
or rabbit immunoglobulin on the cell to prevent it having antibody specificity?

HÄYRY Absolutely, yes.

TAUBE (London) I wonder if you have looked at the mononuclear cell infiltrates
in your graphs in which you showed rejection, and Cyclosporin A toxicity, with
monoclonal antibodies directed against helper and cytotoxic suppressor cells?

HÄYRY Yes, we have been analysing that, you mean the composition of
infiltrates during rejection under Cyclosporin?

TAUBE Yes, if you’ve analysed individual episodes of rejection.
HÄYRY As most people have found, in most rejection episodes the suppressor killer T-cells infiltrate the graft predominantly. To my knowledge there is one single exception to this rule. This is a paper by Tusveson in Uppsala. They claim that the T-helper cells predominate. We found that in eight out of 10 cases there is a clear suppressor cytotoxic T-cell predominance in the graft regardless of whether we are treating the patient with Cyclosporin or with azathioprine. However, in the remaining two of 10 cases we have found cases where the helper T-cells predominate the infiltrate. Most of these cases were on Cyclosporin, and most of those ended up in irreversible rejection.

Secondly, you asked about the mononuclear cell infiltrates under Cyclosporin. It is a very strange thing, but we see mononuclear phagocyte and lymphocyte dominated inflammatory episodes regularly but very late under Cyclosporin therapy. These may or may not be related to Cyclosporin deposits in the kidney. We don't know if these episodes finally represent a reaction to Cyclosporin, or if they are a strange uncommon type of reaction.

DE VECCHI (Milan) In our experience in several cases of Cyclosporin A toxicity white blood cells in the renal infiltrate have a foamy aspect in their cytoplasm just like that observed in tubular cells. Do you confirm my still small and preliminary experience? What is the time interval between Cyclosporin A dose reduction and improvement in renal tubular cell damage?

HÄYRY To answer the first question, our findings are exactly similar. We can also demonstrate similar changes in really severe cases by classic histology or by frozen section histology. We have seen the foamy tubular cells you have seen and in addition mononuclear phagocytes which also incorporate Cyclosporin.

With regard to the second comment, if you reduce the Cyclosporin dose these deposits disappear in a couple of days, in other words extremely quickly. However, about half of the kidneys where you decrease the administration of Cyclosporin end up in rejection. The rejections you can see by the appearance of inflammatory blast cell and lymphocytes as well as mononuclear phagocytes into the graft. That may complicate at least sometimes, the post-reduction course. If you have a clean case with no episode of rejection after reduction of Cyclosporin dose, the serum creatinine level falls rapidly.

ZAZGORNIK (Vienna) Can you give us a comment about the differential diagnosis of Cyclosporin A nephrotoxicity versus rejection in patients with graft tubular damage caused by prolonged warm or cold ischaemia and primary transplant insufficiency?

HÄYRY It is in these situations where we have found aspiration cytology most informative and important. You can easily recognise the changes related to acute tubular necrosis in the graft tubular cells and these changes disappear when the graft resumes its normal function. If you, during this period of time, see inflammatory cells appearing in the graft, this is a rejection. In these cases, in the lack of clinical signs, we treat the inflammation. If you find Cyclosporin deposits
in the kidney you know that it is a sign of nephrotoxicity and you must reduce the dose of Cyclosporin. These different patterns make the differential diagnosis very easy. Another thing related to your comment is that there is no difference between initially functioning and non-functioning kidneys to Cyclosporin A. Both do exactly as well or as badly under Cyclosporin therapy.