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CYCLOSPORIN A NEPHROTOXICITY: COMPARISON OF BIOPSY HISTOLOGICAL FINDINGS IN RENAL TRANSPLANT PATIENTS TREATED WITH CYCLOSPORIN A OR AZATHIOPRINE

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Summary

Kidney transplant biopsy specimens from patients experiencing episodes of impaired renal function while under immunosuppression with Cyclosporin A (CyA) or azathioprine were assessed histologically. Results were correlated with a retrospective clinical diagnosis based on the response to anti-rejection therapy or reduction of CyA dose. In acute rejection interstitial haemorrhages and inflammatory changes of arteries were common, while in CyA nephrotoxicity these were absent and oedema occurred less frequently. Hyaline arteriosclerosis had a higher incidence in nephrotoxicity but was found also with chronic renal damage. Interstitial inflammation was often focal in CyA toxicity, whereas in acute rejection it was nearly always diffuse. We confirm that histology is valuable for diagnosis in these circumstances.

Introduction

The fungal metabolite Cyclosporin A (CyA) was found to cause impairment of renal function from the outset of its clinical use as an immunosuppressive agent [1,2], and nephrotoxicity remains its most troublesome side effect. Reliable differentiation of toxicity from rejection is not possible either from the clinical symptoms or from blood levels of the drug. Furthermore, while there is agreement on histological criteria for rejection in people being treated with azathioprine, such is not yet the case for rejection during CyA treatment, or for CyA toxicity.

Cyclosporin nephrotoxicity has been associated with tubular effects [3,4], thrombotic lesions [5–7], or combinations of vascular and tubular damage [8]. We were prompted to re-examine the transplant biopsy histology in end-stage renal failure patients receiving kidney allografts at Dulwich Hospital, to ascertain whether such changes could be demonstrated also in our material, and to seek diagnostic criteria. Several previous investigators have compared the morphology of rejection and nephrotoxicity in patients receiving CyA: in this

retrospective study we compared the renal histology of CyA patients with those on azathioprine, using routine biopsy specimens taken for the same clinical indications, and correlated the findings with the clinical diagnoses.

Treatment protocols

Formerly, immunosuppression for transplantation in our unit was based upon azathioprine and prednisolone, used in conventional doses on patients who had received prior blood transfusions. In mid-1983, Cyclosporin A was substituted and given in an oral loading dose of 5mg/kg, followed post-operatively by 5mg/kg twice daily for three days, and the dose subsequently adjusted to maintain CyA whole blood trough levels at 200–400ng/ml by Sandoz radioimmunoassay; 20mg prednisolone per day was also given, reducing to 10mg/day at one month. For second or subsequent allografts, or sensitized patients, a 10-day course of antithymocyte or antilymphocyte globulin was added.

If the patient suffered unexplained impairment of renal function, percutaneous biopsy of the graft was performed using a Trucut needle, before any treatment was given. The indications for biopsy did not change over the period of the study.

Material

Biopsy sections were taken from the files of 1982 to 1985. Obstructive lesions, infections and ischaemic acute tubular necrosis were excluded, and also any specimens that contained fewer than five glomeruli or were technically unsuitable, but otherwise the cases were unselected. The subjects were as follows:

Azathioprine group Twenty-three biopsies from 23 patients (14 males and 9 females) aged 26 to 68 years, mean 44 years.

Cyclosporine A group Thirty-four biopsies from 31 patients (23 males and 8 females) aged 19 to 67 years, mean 41 years.

Biopsy had been performed less than one month after transplantation in 15 instances, between one and three months in 22, between three and 12 months in eight, between 12 and 18 months in five (azathioprine 1, CyA 4), and more than 18 months in seven (azathioprine 7, CyA nil). The composition of the two groups was broadly comparable as regards the original cause of renal failure, where known, except that among CyA patients there was an excess of polycystic disease (5 against 1), and diabetic nephropathy (4 against 1). Six azathioprine patients and 11 CyA patients had received a second or third allograft.

Donors were live related in three azathioprine and two CyA cases and the remainder cadaveric, and mean donor ages were 39 years and 36 years respectively.

Methods

Laboratory procedures Biopsy specimens were fixed in McDowell and Trump's formalin-glutaraldehyde mixture or buffered neutral formalin and embedded in

paraffin wax; 2μm sections were stained with haematoxylin and eosin, supplemented in a few cases by the periodic acid-Schiff stain or by 1μm epoxy resin sections stained with toluidine blue, and examined by light microscopy.

Histological assessment Findings were analysed by GHN and FED without knowledge of the clinical diagnosis. The features listed in Table I were looked

TABLE I. Histological findings in biopsy material

	Azathioprine (23)		Cyclosporin A (34)		
	Acute rejection (8)	Chronic dysfunction (15)	Nephro-toxicity (11)	Acute rejection (14)	Chronic dysfunction (9)
GLOMERULI					
Capillary thickening (excluding ischaemia)	0/8	8/15	1/11	2/14	0/9
Cell increase ('glomerulitis')	0/8	3/15	0/11	0/14	2/9
Fibrin thrombi	1/8	1/15	0/11	2/14	0/9
LARGE ARTERIES (not present in all specimens)					
Inflammation	1/4	4/8	0/4	4/8	4/6
Fibrinoid change	1/4	2/8	0/4	2/8	1/6
Mucoid change	0/4	1/8	1/4	1/8	0/6
SMALL ARTERIES/ARTERIOLES					
Hyaline change	1/8	5/15	6/11**	1/14	2/9
Contraction	5/8	10/15	7/11	6/14	6/9
Mucoid change	0/8	2/15	1/11	1/14	0/9
TUBULES					
Atrophy	5/8	15/15	11/11	13/14	7/9
Necrosis	5/8	2/15	3/11	5/14	2/9
RBC in lumen	1/8	3/15	4/11	3/14	0/9
Vacuolation	0/8	3/15	0/11	0/14	2/9
Tubulitis	3/8	7/15	2/11	7/14	6/9
INTERSTITIAL TISSUES					
Oedema	7/8	13/15	6/11*	13/14	9/9
Fibrosis	0/8	7/15	5/11	2/14	1/9
Inflammation focal	0/8	3/15	5/11	2/14	1/9
diffuse	4/8	9/15	3/11	11/14	8/9
Haemorrhage	3/8	6/15	0/11**	6/14	2/9

Statistical significance by χ^2 of differences between CyA nephrotoxicity and combined acute rejection groups: *$p<0.05$; **$p<0.02$; other differences not significant

for in the glomeruli, large arteries (arcuate and above), small arteries and arterioles, tubules, and interstitial tissues. Assessment of leucocytes in intertubular capillaries was abandoned when interpretation proved to be uncertain. Abnormalities were recorded semi-quantitatively using an arbitrary scale of 0, + and ++; any slight, borderline or doubtful findings were scored as negative, and for the purposes of this paper, the + and ++ grades were amalgamated.

Clinical assessment The cause of impaired function in each patient was determined from the response to therapy, and placed in one of three diagnostic categories defined as follow:

1. *Acute rejection* was marked by a rise of serum creatinine that was reversed within two weeks by intravenous methylprednisolone given on three consecutive days, or high dose oral prednisolone subsequently tapered.

2. *CyA nephrotoxicity* was characterized by a rise of serum creatinine that was reversed within two weeks by reducing the CyA dose, or that responded to this manoeuvre after anti-rejection treatment had failed.

3. *'Chronic dysfunction'* was distinguished by an elevated serum creatinine that did not fall within two weeks of anti-rejection treatment and of the lowering of CyA dose. This group included cases of chronic rejection and conceivably of irreversible damage mediated by CyA.

These definitions encompassed, in addition, delayed primary function (except for that due to acute tubular necrosis of ischaemic origin).

Adoption of these categories resulted in division of the case material into five groups: acute rejection and chronic dysfunction in azathioprine and CyA patients, and CyA toxicity.

Results

The analysis is given in Table II. Findings for *acute rejection* were similar in the two treatment groups: vascular inflammation or interstitial haemorrhage was often present, while interstitial inflammation was usually diffuse. In *CyA toxicity*,

TABLE II. Histological features useful in differential diagnosis between acute rejection and CyA nephrotoxicity

In favour of acute rejection	In favour of Cyclosporin A nephrotoxicity
	Major features
Interstitial haemorrhages	Combined absense of arterial and interstitial inflammation
Inflammatory cells in arteries	
Diffuse interstitial inflammation	Focal interstitial inflammation
	Hyaline arteriosclerosis (if donor young, and biopsy not late)
	Minor differences
Tubulitis	Absence of oedema
	Interstitial fibrosis (except at late stages)

interstitial haemorrhages and inflammation of arteries were absent, although the number of large vessels available for assessment was insufficient for the difference to be statistically significant; interstitial inflammation was more often focal. In addition, nephrotoxicity was accompanied by greater vascular hyaline but less oedema than in acute rejection; there appeared also to be less 'tubulitis' (inflammatory cell invasion of tubular epithelium) and more interstitial fibrosis, although neither of the last two differences reached statistical significance. With *chronic dysfunction*, glomerular capillary thickening and interstitial fibrosis were common only in azathioprine patients: this is attributable to the long survival of grafts biopsied in this group (7 out of 8 had been transplanted 4 to 13 years before). 'Glomerulitis' was seen only with chronic dysfunction.

Discussion

The case material proves to have included a large number with long-standing grafts among the azathioprine patients, all with chronic dysfunction, but this does not invalidate comparisons between cases of acute rejection and CyA nephrotoxicity. Differences in the sex ratio and incidence of cases of end-stage disease seem unlikely to have affected the issue.

The principal findings in acute rejection were those of vascular inflammation, diffuse interstitial inflammatory infiltration and interstitial haemorrhages, while in CyA toxicity there was a higher incidence of hyaline arteriosclerosis and of focal interstitial inflammation.

The differences observed between CyA nephrotoxicity and the other groups support the previous findings of others [4,8] that histology is of value in the differential diagnosis of these conditions. The greater abnormalities in Cyclosporin A damage seen in the Swiss patients [8] may relate to the higher dosage used in that series. Our results are similar in many respects to those of Sibley et al [4], apart from the question of distribution of inflammatory cells between capillaries and interstitium, which we found difficult to assess. Vacuolation of tubular epithelium did not appear a helpful feature, although greater use of resin sections might have made it more easily recognizable.

Our findings do not provide criteria that distinguish CyA toxicity from acute rejection in all circumstances, but the following guidelines are useful (Table II): interstitial haemorrhages or inflammatory lesions of blood vessels point to rejection, and so to a lesser extent does diffuse interstitial inflammation. CyA toxicity is likely if the major manifestations of rejection are absent, and is also favoured if interstitial inflammatory infiltration is focal, or hyaline arteriosclerosis present; the latter, however, is not uncommonly found with chronic damage, and is also a frequent accompaniment of ageing or hypertension: it is already present in many kidneys from donors in the fifth decade or later, and therefore is of diagnostic value only if these factors can first be ruled out. Other features that suggest nephrotoxicity are interstitial fibrosis (except at late stages) and the absence of oedema or tubulitis.

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