

ARE CYCLOSPORIN A DEPOSITS IN RENAL ALLOGRAFTS OF CLINICAL SIGNIFICANCE?

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Summary

To examine the clinical significance of Cyclosporin A (CyA) deposits in renal parenchymal cells, we analysed 94 fine needle aspiration biopsies in 32 graft recipients.

CyA deposits were found in 44 (47%) fine needle aspiration biopsies. No significant correlation was found between CyA deposits and cytological findings of drug toxicity.

Clinical nephrotoxicity was seen in only five cases in which CyA deposits were associated with blood trough levels ≥ 500 ng/ml. This study shows that CyA deposits alone cannot be considered as an indicator of drug toxicity.

Introduction

The nephrotoxic effect of Cyclosporin A (CyA) is well known and seems to be related to CyA blood trough levels and to be reversible after dose reduction [1].

CyA deposits have been described in renal parenchymal cells obtained by fine needle aspiration biopsy and directly correlated with morphological changes and with clinical drug nephrotoxicity [2,3].

Our aim was to verify whether the presence of CyA deposits in renal fine needle aspiration biopsies could be of clinical value.

Patients and methods

Study group Thirty-two renal cadaveric graft recipients, receiving CyA (5mg/kg/day intravenously for 5 days, followed by 14mg/kg/day orally, then adjusted to maintain blood trough levels between 200 and 500ng/ml) and low dose steroids.

Control group Eight patients receiving conventional therapy (azathioprine 1-3mg/kg/day and methylprednisolone 20mg/day).

Ninety-four fine needle aspiration biopsies (61 in the first 3 months, 33 between the fourth and the tenth month) from 32 patients and 24 fine needle aspiration biopsies from controls were obtained as previously described [4]. Renal and capillary blood samples were cytocentrifuged. Three blood and six renal specimens from each case were stained with May Grünwald Giemsa stain. At least three slides from renal specimens were used for immunohistochemistry. After acetone fixation and methanol + H₂O₂ incubation, an indirect immunoperoxidase technique was applied. A sheep anti-CyA antiserum (Sandoz, dilution 1:50 in phosphate buffered saline) and a peroxidase conjugated rabbit anti-sheep gammaglobulin (DAKO, dilution 1:80) were used. Immunoreaction procedures were carried out at room temperature and developing was obtained with diaminobenzidine, counterstaining with Mayer's haematoxylin. Control tests included replacement of the anti-CyA antiserum with phosphate buffered solution or various antibodies, e.g. IgG, factor VIII, fibrinogen, alpha-2-macroglobulin, EMA (DAKO).

Results

Morphological changes and CyA deposits

Isometric vacuolization, swelling and cytoplasmic inclusions, suggestive of CyA-toxicity, were observed in 29 of 94 (31%) fine needle aspiration biopsies.

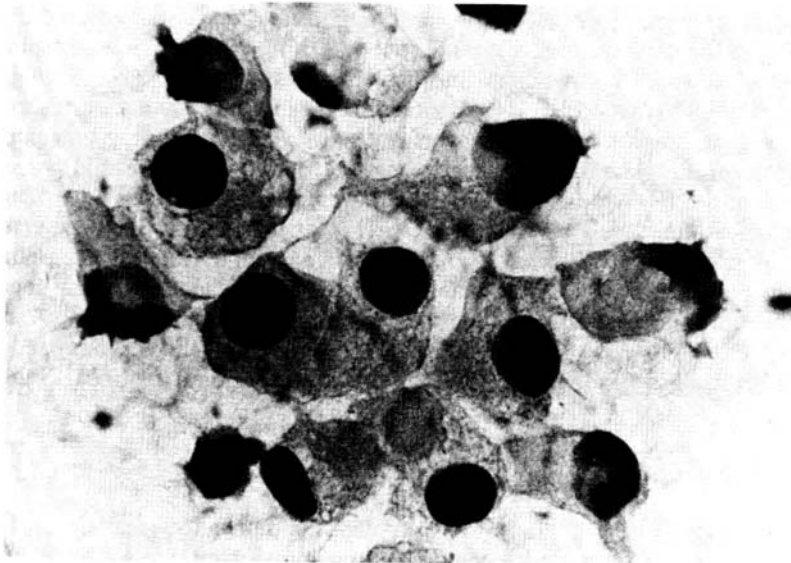


Figure 1. Immunoperoxidase staining showing CyA deposits in parenchymal cells (x 1000 – reduced for publication)

Immunocytochemistry on material obtained by fine needle aspiration biopsy, revealed anti-CyA antiserum positivity in epithelial and more rarely endothelial cells of the CyA treated patients (Figure 1). No positivity was observed in parenchymal cells of the control group. Positivity appeared as diffuse or granular in the cytoplasm and as thin staining on the cell membrane. Positive epithelial cells were observed both as isolated elements or in nests. A positivity cytoplasmic and membrane staining for IgG, EMA and fibrinogen was observed in epithelial cells from both CyA-treated patients and controls. Staining for alpha-2-macroglobulin and factor VIII (DAKO) gave negative results.

Deposits of CyA were found in 44 of 94 (47%) fine needle aspiration biopsies (Group A), while 50 of 94 (53%) did not reveal CyA deposits (Group B).

Cytological signs of CyA toxicity were more frequent in group A (41%; 18 fine needle aspiration biopsies) than in group B (22%; 11 fine needle aspiration biopsies) ($p=NS$; χ^2 test).

Correlation of CyA deposits to the CyA dose and to the CyA blood levels

At the time when fine needle aspiration biopsy was performed, CyA blood levels in group A were 411 ± 35 ng/ml (mean \pm SEM) compared to 347 ± 47 (mean \pm SEM) in group B ($p=NS$; Student's 't' test). CyA daily dose was 10.4 ± 4 mg/kg (mean \pm SD in group A and 11.2 ± 4 mg/kg in group B; $p=NS$).

In 61 fine needle aspiration biopsies performed in the first three months, a higher incidence of CyA deposits was associated with CyA blood levels ≥ 300 ng/ml (21; 65.6%) compared to CyA blood levels < 300 ng/ml (11; 34.4%) ($p < 0.05$; χ^2 test).

In 21 patients, fine needle aspiration biopsies were obtained at the sixth month, in the presence of stable renal function. Nine of 21 fine needle aspiration biopsies showed CyA deposits: in these patients CyA dose (11 ± 3 mg/kg/day; mean \pm SD), CyA blood levels (398 ± 66 ng/ml; mean \pm SEM) and serum creatinine levels (1.3 ± 0.2 mg/dl; mean \pm SD) were not statistically different when compared to those of 12 patients in which fine needle aspiration biopsies did not reveal CyA deposits (CyA dose 9.1 ± 2.9 mg/kg/day; blood trough levels 380 ± 42 ng/ml, serum creatinine levels 1.4 ± 0.4 mg/dl). In 12 patients, a first fine needle aspiration biopsy did not discover CyA deposits; a second fine needle aspiration biopsy performed 10–90 days later revealed the appearance of CyA deposits.

The appearance of CyA deposits was correlated with higher CyA blood levels (530 ± 58 ng/ml versus 238 ± 76 ng/ml) ($p < 0.01$; Student's 't' test) and with lower CyA dose (9 ± 4 mg/kg/day versus 13.2 ± 2 mg/kg/day) ($p < 0.01$; Student's 't' test).

Clinical nephrotoxicity

In five cases (11%) of group A, CyA deposits and cytological signs of drug toxicity were associated with renal function impairment due to acute nephrotoxicity. All these cases were associated with higher CyA blood levels (624 ± 47 ng/ml; mean \pm SEM) when compared to the remaining 39 cases of group A (384 ± 37 ng/ml; mean \pm SEM; $p < 0.05$; Student's 't' test).

Discussion

Cyclosporin A deposits occur frequently in the transplanted kidney, being found in 47 per cent of fine needle aspiration biopsies. No correlation was observed between CyA deposits and cytological signs of acute drug toxicity, which were detectable even in the absence of CyA deposits.

In 11 per cent of cases the CyA deposits, associated with signs of cytotoxicity, coincided with a clinical episode of acute nephrotoxicity. All these cases were verified in the presence of significantly higher blood levels of the drug, ≥ 500 ng/ml.

On the whole, the appearance of deposits does not correlate either with average daily dose of the drug or its blood levels.

A major incidence of CyA deposits is found in fine needle aspiration biopsies performed during the first three months, associated with CyA-blood trough levels ≥ 300 ng/ml. It is, however, important to stress that in four cases, the CyA deposits were associated with unmeasurable levels of the drug.

Furthermore, in 12 patients, whose first fine needle aspiration biopsy results were negative, CyA deposits subsequently appeared and coincided with significantly higher blood levels of the drug. Repeating the fine needle aspiration biopsy

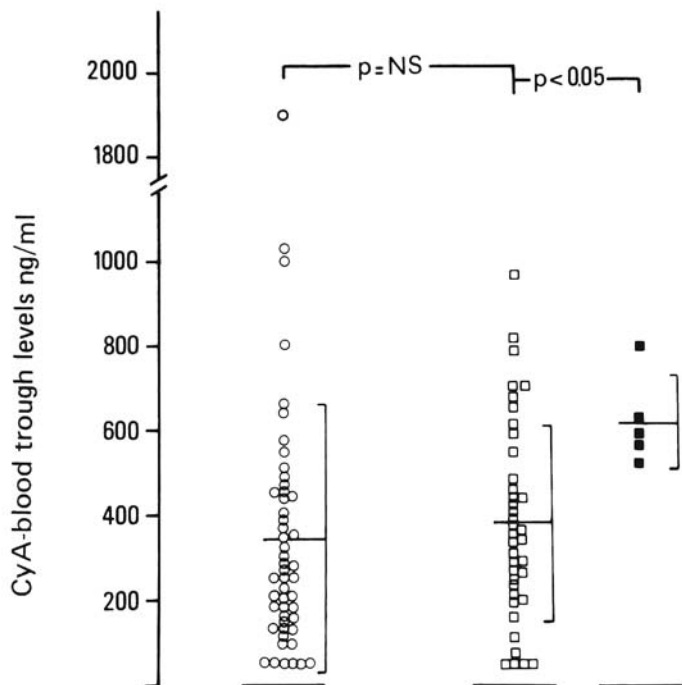


Figure 2. CyA deposits, CyA blood levels and nephrotoxicity in 32 patients. \circ =fine needle aspiration biopsies (50) without CyA deposits; \square =fine needle aspiration biopsies (39) with CyA deposits; \blacksquare =fine needle aspiration biopsies (5) with CyA deposits and associated clinical nephrotoxicity

at the sixth month has shown how CyA deposits are compatible with good renal function.

Our conclusions are therefore:

1. The presence of CyA deposits cannot be considered an indicator of nephrotoxicity and there is no significant correlation with the features of morphological toxicity. Furthermore, CyA deposits are present during clinical stability with good renal function. The CyA deposits coincided with clinical nephrotoxicity only when signs of cytotoxicity and blood levels of the drug $\geq 500\text{ng/ml}$ were associated (Figure 2).
2. No relation has been observed between CyA deposits and drug dose. Even the relationship with the blood levels of the drug is not always evident. It seems likely that the high frequency of CyA deposits is due to the high affinity of the drug for the tissues, as observed experimentally [5].

References

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