Proc EDTA–ERA (1985) Vol 22

CYCLOSPORIN A AND URINE GLUTATHIONE-S-TRANSFERASE

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Summary

Urines from rats given toxic doses of Cyclosporin A and renal transplant patients were examined for glutathione-S-transferase a proximal tubule protein. Rats given 10mg/kg of Cyclosporin A had minimal histological changes and negative urine; those given 20mg/kg had tubular vacuolization and four of nine had positive urine; those given 50mg/kg had severe tubular changes and interstitial inflammation, with eight of nine urines positive. Two patients given higher doses of Cyclosporin A developed urinary glutathione-S-transferase after three to four days. This enzyme may be a marker for Cyclosporin A nephrotoxicity in animal models and patients.

Introduction

Glutathione-S-transferase (GST) is a cytosolic enzyme of the proximal nephron [1]. It is normally undetectable in urine but appears in the urine following tubular injury with several nephrotoxins [2,3]. A major side effect of Cyclosporin A is renal tubular toxicity [4]. We therefore looked for urinary GST in rats given varying toxic doses of Cyclosporin A and in renal transplant patients given this drug, to evaluate GST as an indicator of Cyclosporin A toxicity.

Methods

Animal studies were performed in male Lewis rats weighing 250–300g, housed in metabolic cages and given standard rat chow and free access to tap water. The rats were given daily IP injections of Cyclosporin A, either 10 (Group I), 20 (Group II), or 50 (Group III) mg/kg in saline for six days. Serial 24-hour urines were collected for GST and osmolality beginning one day prior to the start of the injections, so that each rat served as its own pre-Cyclosporin A control. Serum was obtained for creatinine determination during the control period and at four and six days following the start of injections. The animals

were sacrificed following the sixth day of Cyclosporin A, and the kidneys examined for histological changes.

Random urine specimens were collected and tested for GST on eight renal transplant patients, six of whom received Cyclosporin A.

Urine GST was measured as previously described [3]. Specimens were concentrated 25-fold by dialysis, and one hundredμL were added to a cuvette containing 1mmol glutathione and 1mmol 1-chloro-2,4-dinitrobenzene in potassium phosphate buffer at pH 6.5. Change in optical density per minute was read at 344nm in a Spectronic 2000® double-beam recording spectrophotometer (Bausch & Lomb, Rochester, NY, USA). A blank cuvette containing reagents but no sample was used to subtract out non-catalysed conjugation. A test was considered positive if a consistent increase in optical density was recorded over three minutes.

Urine osmolality was measured on an Advanced Digimatic® Osmometer, Model 3D1 (Advanced Instruments, Needham Heights, MA, USA). Serum creatinine was measured by AutoAnalyzer. The results for serum creatinine and urine osmolality are expressed as the mean±standard error for that group on that day.

Results

Animal studies

As can be seen in Table I, rats in Group 1 (10mg/kg) had no significant change in either urine osmolality or serum creatinine, and their urines were consistently negative for GST. Animals in Group II (20mg/kg) also had no rise in creatinine, but had a progressively lower mean urine osmolality. By day three to four two of them had positive urine GST, and by day five to six, four of nine urines had GST. Group III (50mg/kg) rats fared the worst: one died after day three to four, and three died after day six, prior to sacrifice. There was a steady decline in urine osmolality and a rise in serum creatine. By day three to four, four of nine urines were positive for GST, and by day five to six, seven of the surviving eight were positive.

Table II shows the pathological findings in the rats' kidneys after six days of Cyclosporin A. Glomeruli and arteries were normal in all three groups. Group I kidneys showed only mild margination of leucocytes in interstitial capillaries. Proximal tubules in Group II had mild patchy cytoplasmic vacuolization. Vacuolization of proximal tubules was severe in Group III animals, with secondary narrowing of tubular lumens and distortion of cell membranes and brush borders.

This group also had a mild interstitial infiltrate of mononuclear leucocytes.

Patient studies

Of eight renal transplant patients (Table III), two had never received Cyclosporin A and had no urinary GST. Two of the six given the drug had detectable urinary GST 72–96 hours after starting Cyclosporin A. These patients had been given an IV dose of Cyclosporin A and then 14–15mg/kg per day orally; those with negative urines got only 10–10.6mg/kg daily. There was no clinical evidence of acute rejection or Cyclosporin A toxicity.

TABLE I. Urine GST in rats given Cyclosporin A

	Baseline	Day 1–2	Day 3–4	Day 5–6
Group I (10mg/kg) (n=7)				
Serum creatinine (mg/dl)	0.2±0.08	–	0.2±0.07	0.1±0.07
Urine osmolality (mOsm/kg)	1354±91	1169±113	1143±126	1204±116
Urine positive for GST	0/7	0/7	0/7	0/7
Group II (20mg/kg) (n=9)				
Serum creatinine (mg/dl)	0.3±0.02	–	0.03±0.04	0.3±0.05
Urine osmolality (mOsm/kg)	1626±106	1408±181	1258±129	841±174
Urine positive for GST	0/9	0/9	2/9	4/9
Group III (50mg/kg) (n=9)				
Serum creatinine (mg/dl)	0.2±0.08	–	0.6±0.2	0.9±0.3*
Urine osmolality (mOsm/kg)	1460±42	1178±134	1068±102	760±118*
Urine positive for GST	0/9	0/9	4/9	7/8

GST = glutathione-S-transferase; *$p<0.05$ compared to baseline

Discussion

Urine GST has been well described as a marker for nephrotoxic proximal tubular injury [2,3,5]. In this study high-dose Cyclosporin A was also found to induce GST in the urine. Four of nine rats given an intermediate dose of the drug and eight of nine given a high dose had enzymuria, which preceded the nadir of urine osmolality in many cases. Renal toxicity was confirmed by falling urine osmolality in both higher-dose groups and a rising serum creatinine in the highest-dose rats.

The histologic changes in renal tubules of patients with Cyclosporin A nephrotoxicity include vacuolization and necrosis of epithelium [6] and interstitial inflammation [7]. Animals, on the other hand, tend to be resistant to Cyclosporin A toxicity. A recent study in the rat found vacuolated tubular cells after

TABLE II. Pathological findings in rats given Cyclosporin A

	Glomeruli	Tubules	Vessels and Interstitium	% GST-Positive Urines
Group I (10mg/kg)	Normal Appearance	Normal Appearance	Mild margination of mononuclear leucocytes. Normal Arteries	0
Group II (20mg/kg)	Normal Appearance	Mild patchy cytoplasmic vacuolization in proximal tubules	Mild peritubular inflammation and mild-moderate margination of mononuclear leucocytes. Normal Arteries	44
Group III (50mg/kg)	Normal Appearance	Severe patchy vacuolization of proximal tubules. Secondary narrowing of tubular lumens. Distortion of cell membrane and brush border	Mild interstitial inflammation. Mild-moderate margination of of mononuclear leucocytes. Normal Arteries	89

TABLE III. Urine GST in transplant patients

Patient	IV Cyclosporin (mg/kg)	Oral Cyclosporin (mg/kg 1 day)	Post-transplant course	Urine GST
1	0	0	Polyuria Stable at 5 days	Negative
2	0	0	Polyuria Stable at 5 days	Negative
3	5	14	Polyuria Stable at 10 days	Positive
4	5	15	Polyuria Stable at 10 days	Positive
5	0	10	Oliguria Stable at 18 days	Negative
6	5	10	Early Oliguria Stable at 31 days	Negative
7	5	10	Oligo-anuria Stable at 21 days	Negative
8	0	10.6	Delayed function	Negative

21 days of Cyclosporin A, 100mg/kg [8]. This was also found by Iaina et al after 60mg/kg/day of Cyclosporin A plus renal ischaemia [9].

The rats in our study had similar findings after lower doses of Cyclosporin A for only seven days. This was dose-related: findings were minimal at 10mg/kg, striking at 50mg/kg, and intermediate at 20mg/kg (Table III). We also found interstitial inflammation, which has been reported in patients with Cyclosporin A toxicity [6] but only in one previous animal study with this durg, using Fisher rats [10]. This strain and the Lewis rats in our study six appear to be more sensitive to Cyclosporin than in other strains. They may represent a better model for Cyclosporin A nephropathy.

Two of six renal transplant patients who had received Cyclosporin A had GST in their urine three to four days after initiation of the drug. These two patients had been given an IV dose (which we no longer do) and were on a highe r per-kg regimen of Cyclosporin A than the others. Acute tubular necrosis prior to transplantation could account for this enzymuria; however, these kidneys functioned promptly and had no acute renal failure or rejection during this time. The results of our animal study indicate that Cyclosporin A-induced proximal tubular toxicity can lead to detectable GST in the urine. It is possible that the appearance of this urinary GST represents subclinical tubular injury, similar to that reported with administration of iodinated radiocontrast media, which can be dose-related [2,3].

We are investigating further the usefulness of urinary GST determinations in Cyclosporin A-treated patients. It would appear, however, that GST in the urine correlates with the degree of proximal tubular damage in the Lewis rat model of Cyclosporin A nephrotoxicity. This could prove useful in future studies.

Acknowledgments

This study was supported in part by a grant from the Ancient Egyptian Arabic Order Nobles of the Mystic Shrine of North and South America and Its Jurisdictions, Inc. The authors thank Ms M Prasek, Ms E Meyer, and Ms P Gomez for technical assistance and Ms H Mortimer for assistance in completing the manuscript.

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