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THE ENHANCEMENT OF CYCLOSPORIN A-INDUCED NEPHROTOXICITY BY GENTAMICIN

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Abstract—Normal rats receiving treatment with either cyclosporin A or gentamicin show dose-dependent nephrotoxicity, the changes with cyclosporin A centring on the straight segment of the proximal tubule, while gentamicin affects the convoluted segment. When the two drugs are given together, nephrotoxicity is enhanced with significant reductions in renal function even at therapeutic dosage. The study emphasises the caution needed when potentially nephrotoxic agents are administered in combination.

Both because of their primary disease and following therapeutic immunosuppression, patients requiring a variety of organ and tissue transplants show an enhanced risk of serious infection and thus commonly receive antibiotic chemotherapy. The aminoglycoside gentamicin is active against a broad spectrum of both Gram-positive and negative organisms and is often employed in such situations. Gentamicin is, however, potentially nephrotoxic [1, 2] and care must be taken to gauge the dose of this drug against indications of renal function.

Although the new immunosuppressant cyclosporin A (Cy A) has a number of advantages over conventional immunosuppressants such as cytotoxic agents and corticosteroids [3, 4], it does have certain unwanted side effects, the most important of which is nephrotoxicity [3, 5].

The present study, performed in rats over a 7-day period, set out to examine the effects of combined administration of gentamicin and Cy A on renal function and structure. It follows the recent observation that significant renal failure can occur in human bone marrow transplant recipients when these two drugs are exhibited together [6].

MATERIALS AND METHODS

Adult male Sprague–Dawley rats (mean weight 250 g) were housed in a temperature-controlled environment and received Oxoid pasteurised rat and mouse breeding diet with tap water *ad lib.* except as detailed below.

Cy A (Sandoz Ltd., Basel, Switzerland) was dissolved in ethanol and then olive oil and administered to the conscious rat by gastric intubation. Gentamicin sulphate (Nicholas Laboratories Ltd., Slough, U.K.) was administered by intraperitoneal injection. Control animals received the ethanol/olive oil vehicle by gastric intubation.

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Groups of six animals received Cy A alone at 25, 50 or 100 mg/kg or gentamicin alone at 25 or 50 mg/kg or various combinations of the two drugs. (The minimum effective immunosuppressant dose of Cy A in the rat is 25 mg/kg per day.) The drugs were administered every 24 hr for 7 days. Analyses of serum and urine were conducted immediately before starting treatment and at 3 and 7 days: on day 7, the animals were killed by ether anaesthesia, the kidneys being removed for light and electron microscopic examination.

Whole blood samples (1 ml) were collected into plain tubes from the cleaned tail tips anaesthetised with diethyl ether. Serum expressed from the clotted blood was stored at −20° until assayed. Estimations of serum urea and creatinine, of glomerular filtration rates of urea and creatinine and of urinary *N*-acetyl-β-D-glucosaminidase (NAG) were conducted as described previously, as was the preparation of the tissues for microscopy [7].

The significance between means was established using the Student's *t*-test for unpaired samples, with P values <0.05 considered significant.

RESULTS

Biochemical findings

The effect on renal function of Cy A alone, gentamicin alone and the two drugs in combination are given in Tables 1 and 2.

Cy A alone caused a dose-related elevation in serum urea levels, significant with 50 or 100 mg/kg at 3 days and with all three doses at 7 days. Serum creatinine levels tended to parallel the changes in urea, although to a lesser extent with no significant changes being observed. The clearance rates of both urea and creatinine were progressively reduced by increasing doses of Cy A. A similar dose-dependent relationship occurred in the rise in urinary NAG levels seen when the immunosuppressant was administered: NAG enzymuria was maximal at day 3, but still significantly elevated by day 7.

Following gentamicin, there was no increase in serum creatinine levels: only at a dose of 50 mg/kg

Table 1. The effects of cyclosporin A or gentamicin on renal function

	Duration of treatment (days)	Serum urea (mmole/l.)	Serum creatinine (μmole/l.)	Urea clearance (ml/hr)	Creatinine clearance (ml/hr)	Urine NAG activity (u/mg)
Pretreatment (80)	—	7.1 ± 1.1	54 ± 8	42 ± 7	86 ± 14	644 ± 127
Vehicle alone (8)	3	6.5 ± 0.9	51 ± 5	37 ± 6	79 ± 12	569 ± 139
	7	6.4 ± 0.9	54 ± 7	43 ± 12	92 ± 26	548 ± 150
Cy A (25 mg/kg) (8)	3	6.9 ± 1.1	44 ± 6	33 ± 3	72 ± 11	1006 ± 242**
	7	11.3 ± 2.3**	49 ± 7	24 ± 7	64 ± 12	1005 ± 220**
Cy A (50 mg/kg) (8)	3	8.5 ± 1.8†	48 ± 3	27 ± 7**	72 ± 13	1673 ± 663**
	7	12.1 ± 3.8**	52 ± 8	22 ± 4**	63 ± 13†	1265 ± 537**
Cy A (100 mg/kg) (8)	3	8.8 ± 0.9†	54 ± 9	32 ± 8*	62 ± 14†	2769 ± 1144**
	7	16.4 ± 3.5**	61 ± 7	20 ± 9**	57 ± 13*	1639 ± 493**
Gentamicin (25 mg/kg) (8)	3	5.9 ± 1.1	43 ± 3†	38 ± 11	88 ± 16	1470 ± 347**
	7	6.1 ± 0.7	42 ± 4†	38 ± 4	91 ± 7	953 ± 259**
Gentamicin (50 mg/kg) (8)	3	7.5 ± 0.8	51 ± 5	28 ± 9*	67 ± 14†	516 ± 94
	7	8.5 ± 0.6†	49 ± 3	29 ± 6**	85 ± 16	1068 ± 190**

Results expressed as mean ± 1 S.D. The number of animals in each group is given in parentheses. Values compared to pretreatment levels by Student's *t*-test for independent samples; †, $P < 0.05$; *, $P < 0.01$; **, $P < 0.001$.

Table 2. The effects of cyclosporin A and gentamicin on renal function

	Duration of treatment (days)	Serum urea (mmole/l.)	Serum creatinine (μmole/l.)	Urea clearance (ml/hr)	Creatinine clearance (ml/hr)	Urine NAG activity (u/mg)
Pretreatment (80)	—	7.1 ± 1.1	54 ± 8	42 ± 7	86 ± 14	644 ± 127
Vehicle alone (8)	3	6.5 ± 0.9	51 ± 5	37 ± 6	79 ± 12	569 ± 139
	7	6.4 ± 0.9	54 ± 7	43 ± 12	92 ± 26	548 ± 150
Cy A (25 mg/kg) +	3	8.5 ± 1.3†	47 ± 3	22 ± 7**	67 ± 19	1502 ± 401**
gentamicin (25 mg/kg) (8)	7	17.7 ± 3.2**	56 ± 12	15 ± 9**	48 ± 21**	2266 ± 997**
Cy A (25 mg/kg) +	3	9.8 ± 1.9*	57 ± 9	24 ± 3**	64 ± 5**	1371 ± 337**
gentamicin (50 mg/kg) (8)	7	31.2 ± 15.8**	186 ± 120**	7 ± 3**	17 ± 14**	6709 ± 4022**
Cy A (50 mg/kg) +	3	11.4 ± 1.7**	55 ± 10	21 ± 4**	56 ± 14**	2560 ± 1178**
gentamicin (50 mg/kg) (8)	7	44.5 ± 20.8**	293 ± 53**	7 ± 5**	14 ± 10**	1265 ± 537**
Cy A (100 mg/kg) +	3	17.3 ± 4.1**	82 ± 17**	12 ± 6**	40 ± 20**	3409 ± 1022**
gentamicin (50 mg/kg) (8)	7	96.6 ± 25.2**	666 ± 196**	1 ± 1**	2 ± 2**	7806 ± 102**

Results expressed as mean ± 1 S.D. The number of animals in each group is given in parentheses. Values compared to pretreatment levels by Student's *t*-test for independent samples; †, $P < 0.05$; *, $P < 0.01$; **, $P < 0.001$.

was a significant increase in serum urea seen with a concomitant reduction in urea clearance. Gentamicin produced significant NAG enzymuria at 25 mg/kg on both days 3 and 7, but only on day 7 at the higher dose.

When Cy A and gentamicin were given in combination (Table 2) renal function was more severely affected. The effects were again dose-related and in general increased with time. Even at the lowest dosage combinations, there were significant elevations of serum urea and creatinine and urinary NAG by day 3, increasing by day 7. At the highest dosage combinations, there was acute onset renal failure by day 7. At all doses, NAG enzymuria was massive and progressive.

Microscopical findings

There were no light microscopical abnormalities in the kidneys of control animals receiving vehicle alone or in those treated only with gentamicin. Cy A-treated animals showed abnormalities restricted to the straight segment of the proximal tubule and consisting of cytoplasmic vacuolation. With increasing dose, more tubular profiles were involved, more of the cells comprising each profile affected and the vacuoles were larger. Electron microscopy demonstrated that these vacuoles arose by dilatation of the endoplasmic reticulum of affected cells. Electron microscopy also revealed additional ultrastructural abnormalities in both test groups, although not in the controls; most of the cells of both convoluted and straight segments of the proximal tubules contained increased numbers of lysosomes, cytosegresomes and myeloid figures, with occasional large basal lipid droplets. These additional changes were more notable in animals treated with Cy A than in those given only gentamicin.

In the animals receiving both Cy A and gentamicin at the lowest dosage (25 mg/kg), the histological and ultrastructural renal appearances were similar to the group receiving only Cy A at this dose, viz. vacuolation of some cells in a proportion of straight tubular profiles along with a general increase in lysosomes. In the other three combination groups, the renal structural changes were more severe, progressing with increasing dose from gross course vacuolation of tubular cells to full blown proximal tubular necrosis with the formation of tubular casts.

DISCUSSION

The results of this study confirm the known nephrotoxic properties of the two pharmacological agents examined and demonstrate how these effects are magnified by combined drug therapy.

Gentamicin affects specifically the cells of the convoluted segment of the renal proximal tubular in a dose-dependent manner, the ensuing damage ranging from minor ultrastructural abnormalities to total cellular necrosis [1]. The degree of cellular damage can be predicted by measurement of urinary NAG, a lysosomal enzyme released by the affected cells [8].

Cy A also affects the renal proximal tubule, but its toxic manifestations centre on the straight segment. In previous experimental studies, we have demonstrated the dose-dependent nature of the tubular vacuolation, which is caused by dilatation of the endoplasmic reticulum, and of the increased lysosome production associated with this change [9]. In man, the nature of nephrotoxic properties of Cy A is still unclear; despite numerous accounts of its renal effects [3, 5], there are no precise accounts of tubular enzymuria and few of renal biopsy appearances in the clinical situation, although glomerular changes have been described [10].

When the two potentially nephrotoxic drugs are administered together, both the biochemical and the morphological parameters of renal damage are altered more than would be expected by simple summation of their individual effects. There is significant reduction of renal function even at the lowest dose combinations, while high dose therapy causes acute renal failure due to acute tubular necrosis. The augmented toxicity induced by concomitant administration of both drugs is seen not only in the kidneys, but also in other organs, notably the liver and the lymphoid tissues [11]. These extra-renal effects are almost certainly due to Cy A rather than gentamicin, since they also occur when Cy A is given at doses higher or more prolonged than used here: gentamicin toxicity, on the other hand, is largely restricted to the kidney.

Although we have not measured serum levels of either drug, it seems likely that the effects they cause when given together are a reflection of higher than usual serum concentrations of one or both due presumably to reduced renal clearance, although other alterations of drug metabolism cannot yet be excluded. High serum trough levels of Cy A combined with the therapeutic amounts of aminoglycoside have led to serious nephrotoxicity in human bone marrow transplant recipients [6].

The study reported here emphasises the caution required when known nephrotoxic agents are employed at the same time. When there is no other choice but to proceed with such dual therapy, continual monitoring of renal glomerular and tubular function is mandatory, combined when possible with measurement of circulating drug levels. Better would be avoidance of nephrotoxic antibiotics in patients being immunosuppressed with Cy A. We are in the process of evaluating the effects of three different cephalosporins on Cy A nephrotoxicity; all three agents share a similar broad antimicrobial spectrum with gentamicin, but each appears to enhance the unwanted renal affect of Cy A less than does the aminoglycoside.

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