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NATURE OF THE TOXICITY OF CYCLOSPORIN A IN THE RAT

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Abstract—The potent immunosuppressive agent Cyclosporin A (CyA) causes a spectrum of toxicological effects in rats, of which the most striking is weight loss. Pair-feeding experiments have shown that this is caused, in part, by a short period of anorexia. However, even when the food intake has become normal the rats receiving CyA fail to gain weight. That CyA at the doses used causes increased protein catabolism is also indicated by a fall in serum albumin and a marked rise in blood urea unaccompanied by a corresponding rise in creatinine. CyA is mildly and reversibly hepatotoxic and there is slight nephrotoxicity in the rat on the basis of histology and small elevations in creatinine.

Cyclosporin A (CyA), a new fungal cyclic polypeptide [1] is a powerful immunosuppressive agent, which prevents rejection of transplanted organs [2–7] and graft-versus-host disease following grafting of bone-marrow [1, 8, 9]. Reversible biochemical abnormalities of liver function have been observed in animals [3, 10] and man [2, 3, 8]. These are usually restricted to elevations of serum alkaline phosphatase and bilirubin, but a rise in alanine transaminase has also been attributed to CyA [8]. Focal necrosis has been reported in the liver of dogs receiving CyA [3] but whether this can be attributed to drug toxicity is uncertain. In this study the systemic toxicity of CyA and its reversibility has been investigated by determining biochemical as well as histological changes in the liver and kidney. These have been related to the reduction in food intake found after CyA which was administered at two dose levels of CyA given for either 10, 21 or 50 days.

MATERIALS AND METHODS

Adult male Lister Hooded rats (200–250g) were fed a standard laboratory feed and exposed to a 8–16 hr illumination–darkness cycle. Animals were weighed daily and received either CyA in olive oil by gavage or the same volume of olive oil (0.5ml) for the control animals. Before rats were killed by decapitation, a light ether anaesthetic was given during which they were bled by percutaneous cardiac puncture. Alanine transaminase (ALT optimised UV system, Boehringer Corporation, London, U.K.), alkaline phosphatase, bilirubin aspartate transaminase and serum albumin (Vickers M 300 analyser), urea and creatinine (Technicon SMA III analyser) were measured in the serum. Autopsy was performed and the liver was weighed. Liver and kidney tissue were taken for routine light microscope histological examination and for electron microscopy. Statistical analyses were by the Student's *t*-test.

The first experiment was a short-term study in which CyA was fed for either 10 or 21 days so as to determine whether CyA hepatotoxicity was dose-dependent and reversible. Four groups of nine rats were fed CyA 25 or 50mg/kg/day dissolved in 0.5ml olive oil for either 10 days or 21 days and two control groups received 0.5ml olive oil similarly for 10 or 21 days. During the 21-day study three animals from each group were killed on days 7, 14 and 21.

In the second longer term experiment two groups of 10 male rats were either given CyA 50mg/kg/day for 50 days or the equivalent volume of olive oil. As in the first experiment severe weight loss occurred in the CyA treated animals. Control animals and CyA treated animals in the second experiment were pair-fed to control for the effects of malnutrition.

RESULTS

Short-term experiment

Figure 1 shows the mean change in body weight for experimental and control groups. Control animals had gained 7.0 and 9.2 per cent of initial body weight at the end of 21 days, whereas the experimental groups, receiving CyA, lost 4.3–6.1 per cent body weight during the first six to eight days. Thereafter all rats even those continuing on CyA at low and high dose progressively gained weight so that by 21 days they had almost achieved their starting weights. Rats in which CyA was stopped after 10 days, tended to regain the lost weight more rapidly and after 21 days rats that had received CyA for 10 days had a mean gain in body weight equivalent to control animals.

There were no significant differences in liver weight (expressed in g/100 *g* body weight, mean ± S.E.M. between control groups and rats which had received 25 or 50mg CyA for 21 days (3.4 ± 0.2 and 3.3 ± 0.1, respectively). The results of biochemical investigations in controls and CyA treated rats are

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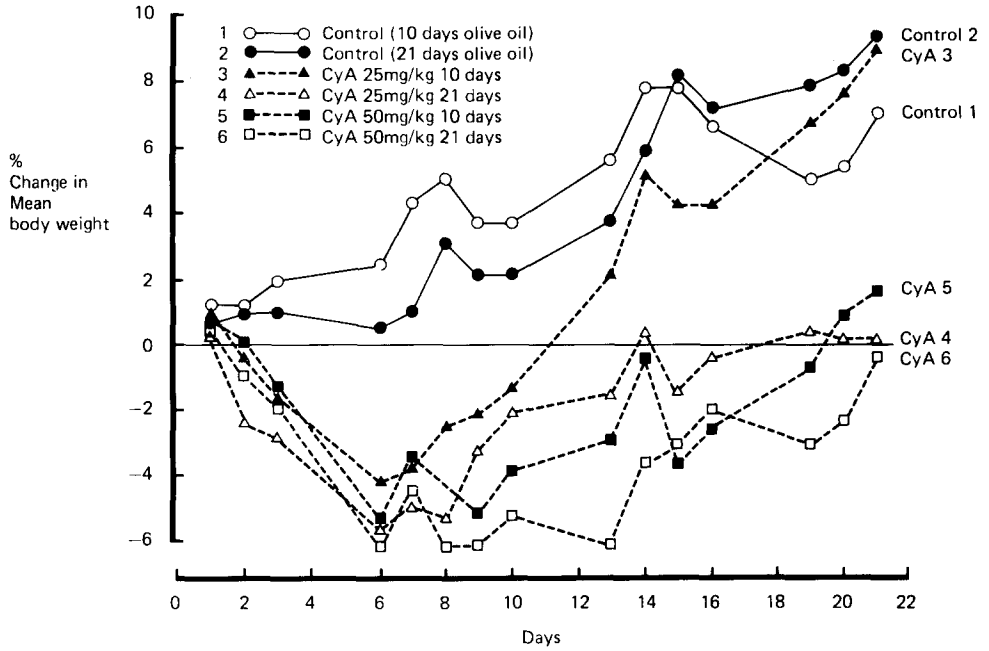

Fig. 1. Changes in weight of rats fed 25 or 50mg/kg/day for either 10 or 21 days. Food intake of controls was *ad libitum*.

shown in Table 1. Alkaline phosphatase in experimental and control animals is shown in Fig. 2. After 21 days of CyA (25 or 50mg/kg) there was significant rise in alkaline phosphatase compared to controls which began at approximately 14 days. In addition, alkaline phosphatase was significantly higher in animals that had received CyA for 21 days than those in whom it had been discontinued after 10 days. Bilirubin levels closely follow those for alkaline phosphatase. Bilirubin was significantly higher in animals who had received CyA (25 or 50mg/kg/day) for 21 days, than controls and animals treated for only 10 days. No significant differences in aspartate transaminase were found between control animals and those receiving CyA for 10 days or 21 days, irrespective of the dose received. There was a significant drop in albumin and the magnitude of this effect increased with dose and time.

CyA at both dose levels caused a significant elevation of plasma urea in treated animals compared to controls (Table 1). Plasma urea was significantly higher in animals that had received CyA 50mg/kg for 10 days than those at the lower dose level but this difference, with respect to dose, was not observed in animals treated for 21 days. Plasma creatinine was significantly higher in animals which received CyA for 21 days than controls and animals treated for 10 days.

Longer term experiment

Body weights and food intake. During the first 10 days, body weight fell in rats receiving CyA

Table 1. Blood biochemistry measured on day 21

Tests	CONTROLS mean ± SEM	CYCLOSPORIN A mean ± SEM			
		25mg/kg		50mg/kg	
		Period of dosing			
		10 days	21 days	10 days	21 days
Alkaline Phosphatase IU/L	291 ± 9	266 ± 24	398 ± 58*†	271 ± 17	456 ± 66*†
Bilirubin μmol/L	1.8 ± 0.3	3.1 ± 0.5	3.5 ± 0.8*	3.1 ± 0.6	4.8 ± 0.4*†
Albumin G/L	39 ± 0.5	38.8 ± 1.3	37.6 ± 1.0*	38.6 ± 0.6	36.1 ± 0.7*†
Aspartate transaminase IU/L	370 ± 40	378 ± 29	400 ± 68	293 ± 29	357 ± 43
Urea mMol/L	5.7 ± 0.3	6.8 ± 0.3	8.7 ± 0.7*	8.1 ± 0.2	8.3 ± 1.3*
Creatinine mMol/L	0.06 ± 0.02	0.07 ± 0.004	0.08 ± 0.01*	0.06 ± 0.003	0.08 ± 0.004*†

* $P < 0.05$ compared to controls.
† $P < 0.05$ CyA 21 says vs 10 days.

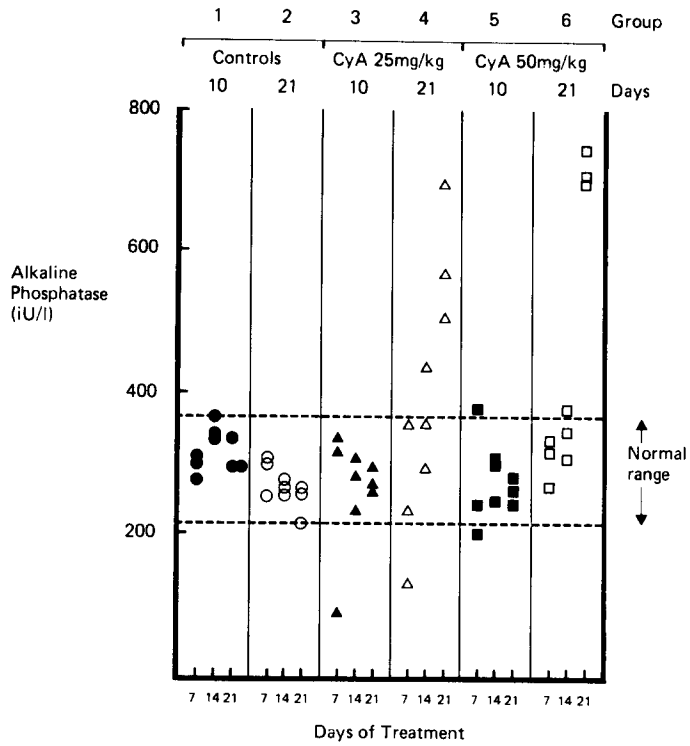

Fig. 2. Alkaline phosphatase activity at different times during treatment with CyA (at 25 and 50 mg/kg/day) for either 10 or 21 days.

(10.9 ± 1.9%) and in pair-fed control rats (7.5 ± 1.9%) but this difference was not significant (Fig. 3). By 18 days the control rats had regained this loss, whereas in the CyA rats this was not achieved until day 26. The difference between body weights of control and CyA treated rats became significant on day 33 (controls 14.3 ± 1.8%, $P < 0.05$). Mean food intake fell abruptly during the first week and was lowest on day 4 (6.0 ± 0.9g/day). Subsequently food intake progressively increased until a plateau was

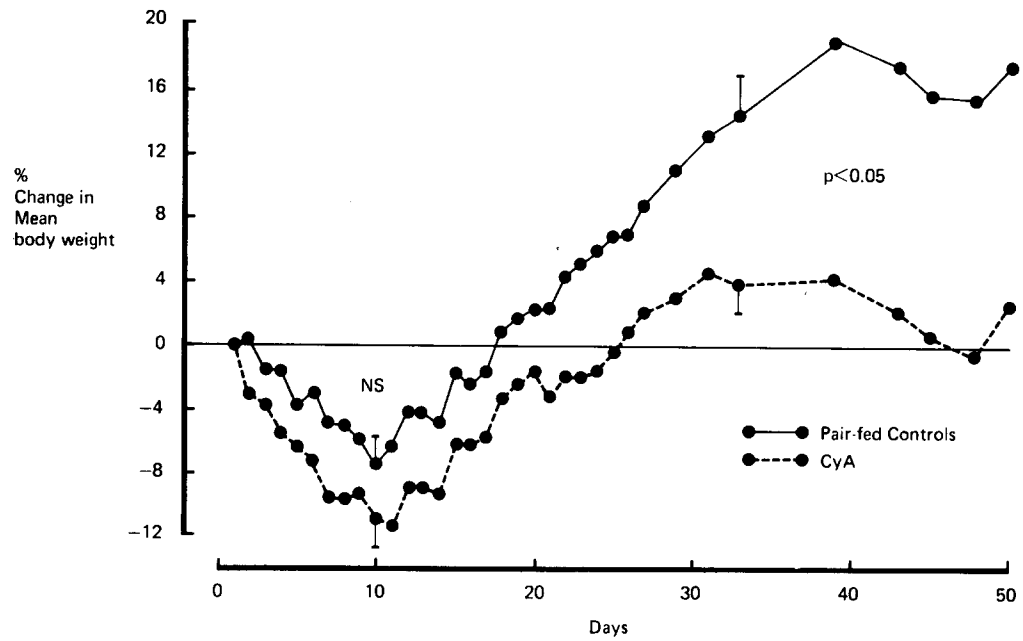

Fig. 3. Changes in weight in rats fed 50mg/kg/day CyA for 50 days compared with 'pair-fed' controls.

Table 2. Blood biochemistry on day 50 following administration of 50mg/kg/day of CyA

TEST	PAIR-FED CONTROLS mean ± SEM	CYCLOSPORIN A mean ± SEM
Alkaline phosphatase IU/L	264 ± 23	495 ± 111*
Bilirubin μmol/L	1.8 ± 0.8	9.0 ± 6
Albumin G/L	40 ± 1.4	35 ± 1*
Aspartate Transaminase IU/L	255 ± 24	279 ± 17
Alanine Transaminase IU/L	13 ± 2.3	14 ± 2.0
Urea mMol/L	8.0 ± 0.6	15.6 ± 1.4*
Creatinine mMol/L	0.06 ± 0.01	0.08 ± 0.02*

Controls were pair-fed.
* $P < 0.05$

reached at day 24 (18.6 ± 1.3g/day) which was equivalent to the normal food intake of *ad lib.* fed controls. Although the initial weight loss during CyA treatment closely followed the fall in food intake, mean weight in the treated rats was always lower than controls and was significantly lower by day 33, after which no further increase in mean body weight was observed.

Four rats receiving CyA died during the study, 2 on day 12 and 2 others on day 15 and day 18. Weight loss in these animals was dramatic. The rats that died on day 12 had lost 19 per cent and 21 per cent of their initial body weight and those dying on days 15 and 18 had lost 22 and 30 per cent of initial body weight, respectively. These rats failed to gain weight after the tenth day of treatment unlike the CyA treated survivors. Severe malnutrition was clearly a major factor leading to death in these animals, although two also had severe pulmonary infection.

As in the 21-day study no significant differences in liver weight were observed between control rats (3.4 ± 0.1g/100g body weight) and CyA treated rats (3.5 ± 0.2g/100g body weight). The results of biochemical investigations are shown in Table 2. Alkaline phosphatase was significantly higher in CyA treated rats than in controls. This confirmed the findings of the 21-day study and suggests that alkaline phosphatase remains elevated during prolonged administration of CyA, although levels after 50 days were not significantly greater than after 21 days treatment. Only one CyA treated rat had overtly raised bilirubin (38 moles/l). Although bilirubin again tended to be higher in CyA treated rats than in controls, this difference was not significant.

In the 21-day study there was a wide range of values for aspartate transaminase and it was felt that this might be attributed to cardiac puncture. It was decided, therefore, to measure alanine transaminase in the 50-day study, an enzyme which is a more specific marker of liver damage. However, neither the aspartate nor alanine transminases were significantly higher in CyA treated rats than controls.

As in the 21-day study, serum albumin was significantly lower in animals treated with CyA than in controls despite pair-feeding dietary restriction. This suggests that the reduced serum albumin of CyA treated animals cannot be attributed to a protein-deficient diet.

Urea and creatinine were significantly higher in CyA treated animals than in controls (Table 2). Urea was also significantly higher after 50 days' treatment with CyA than after 21 days whereas no further increase in creatinine was observed.

Histopathological findings

Liver. Centrilobular fatty change and the presence of acidophil bodies representing necrotic hepatocytes were the only histological abnormalities in the liver seen by light microscopy and neither lesion was judged to be severe. In the short-term study fatty change was found in four of the nine animals (44%) that received CyA 50mg/kg/day for 21 days and in five of the nine animals (56%) who received the same dose for only ten days. It occurred in only one animal which received CyA 25mg/kg/day for 21 days and in one control. In the rats in the longer term experiment, centrilobular fatty change was observed in three of the ten animals treated with CyA, all of whom died during the study (two on day 13 and one on day 15). One animal in the control group had mild fatty change but this could not be related directly to the protein–calorie restriction of pair-feeding since the mean food intake of this animal was always above the group mean. Acidophil bodies, indicative of hepatocyte necrosis, were only seen in the liver of one rat receiving 50mg/kg and which died on day 12. There was no evidence of cholestasis. The livers from two rats receiving 50mg/kg, both of which appeared normal on light microscopy, did not show significant ultrastructural abnormalities when examined by electron microscopy.

Kidney. In view of the elevated urea and creatinine observed in both experiments, the kidneys were examined histologically. The only histological abnormality seen in the kidney consisted of zones of cytoplasmic vacuolation and swelling of tubular cells associated with the presence of colloid casts. These abnormalities were confined almost entirely to the

proximal tubules. None of the controls or the low-dose group treated for 10 days developed these lesions. However, of the animals that received CyA 50mg/kg/day, five of nine (56%) treated for 21 days and four of nine (44%) treated for 10 days had these abnormalities. Four of nine (44%) of animals receiving CyA 25mg/kg/day for 21 days also had these tubular abnormalities. Four of the ten (40%) animals scheduled to receive 50mg/kg/day for 50 days had lesion in the kidney, three of whom were among the four fatalities for this group. Again, the kidneys of the controls were histologically normal.

Other organs. Sections of spleen, oesophagus, stomach, large and small intestine, pancreas, adrenal and testis were taken from the animals in which the renal lesions were severe but no abnormality was found.

DISCUSSION

CyA is a very potent immunosuppressive agent [1–9] and its use in bone-marrow and organ transplantation is likely to increase but is limited by toxicological effects. This study in the rat has shown a variety of toxic actions of this drug; particularly striking are the effect of food intake, body weight and blood urea. There were changes in hepatic and renal structure and function but these were neither severe nor progressive. The striking rise in blood urea does not appear to be wholly explicable by renal damage which on the basis of raised creatinine and histology was not severe.

The striking observation was the profound weight loss in treated animals which appeared to be related to the dose and duration of treatment. One cause of this weight loss was clearly shown in the pair-feeding experiment when it became apparent that treated animals ate less food. In addition, despite pair-feeding, treated animals failed to achieve the control weight. These findings suggest, firstly that CyA diminishes appetite, particularly early in treatment, and secondly, that it may promote catabolism as an additional separate effect and this may contribute to the low albumin and raised urea levels. Weight loss in dogs receiving CyA 50mg/kg/day had been reported [3] and weight was regained when the dose reduced, although the mechanism was not discussed. Four animals in the pair-feeding experiment died during the study, all of whom had profound weight loss, between 19 and 30 per cent of their initial body weights. This was presumably a major causative factor in their deaths. Failure to gain weight after 10 days treatment with CyA in the rat may be a useful index of CyA toxicity. Whether this is applicable to man remains to be established.

CyA clearly causes biochemical and mild structural abnormalities in the liver, which appear to be related to the dose and the duration of treatment. Elevation of alkaline phosphatase was the most obvious biochemical abnormality often associated with mild but significant elevations of bilirubin, although only one rat became overtly jaundiced. These abnormalities have been previously reported in animals [3] and man [2, 3, 8, 9] but direct involvement of CyA as the cause of these abnormalities could not be established with certainty as other factors, such as drugs or graft-versus-host disease (GVHD) were almost invariably present. The mechanism by which CyA causes cholestasis in previously published studies is unknown but histological examination of the liver did not show any evidence of damage to small bile ducts as occurs in GVHD after bone-marrow transplantation [11, 12]. CyA or one of its metabolites may inhibit hepatic NaK- and Mg-ATPase, a mechanism which has been postulated to account for the cholestasis due to chlorpromazine [13]. Alternatively, cholestatic drugs have been shown to alter the physical characteristics of liver plasma membrane [14], another proposed mechanism of cholestasis without clear histological damage.

Although centrilobular fatty change occurs in starvation [15], its absence in pair-fed controls suggests that this was a direct effect of CyA.

Similarly, the significant fall in serum albumin in treated rats cannot be attributed solely to malnutrition as it was not observed in the pair-fed controls. This study suggests that in addition to causing intrahepatic cholestasis, CyA has an effect on albumin metabolism which may include a reduction in synthesis, increased breakdown or both. A similar effect on albumin metabolism has been described with the drug dapsone [16]. The only other histological abnormality seen was hepatocyte necrosis in one treated rat, although aspartate and alanine transaminase levels were not higher than controls. It would appear that hepatocyte necrosis is an infrequent and minor occurrence during CyA treatment in the rat, although this lesion has been attributed to CyA in the dog [3].

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