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EFFECT OF HYDERGINE ON CYCLOSPORINE-INDUCED NEPHROTOXICITY IN A RAT KIDNEY TRANSPLANTATION MODEL

Cyclosporine (CsA)* has now been established as a most effective immunosuppressive agent in man and various animal models (*1*, *2*). Apart from one exception in living-related donor renal transplantation in diabetics (*3*), it has never been shown to be inferior, but is mostly superior, to conventional immunosuppression, when considering patient and graft survival. However several side effects have also been reported during CsA therapy—such as hepatotoxicity, nephrotoxicity, effects on the central nervous system (CNS), resulting in convulsion and tremor, anorexia, hirsutism, gum hypertrophy, and increased renin-angiotensin-aldosterone system (RAAS) activity (*4*, *5*).

CsA associated nephrotoxicity, being reported ever since the first study of the use of CsA in clinical renal transplantation (*6*), is one of the most frequently occurring adverse effects of CsA therapy. This nephrotoxicity appears to be reversible and dose-dependent. On the basis of renal scans, CsA nephrotoxicity has been attributed to acute tubular necrosis (*7*). Other studies in rats have proposed that the nephrotoxicity is caused by a glomerular rather than a tubular mechanism (*8*). However, absence of any histological change in patients manifesting nephrotoxicity has also been reported (*9*). The correlation of morphological changes with nephrotoxicity is still not clear.

Various attempts have been made to modify CsA-associated nephrotoxicity. Calne (*10*) suggested induction of forced diuresis with fluid and mannitol and delay of CsA administration until establishment of posttransplant diuresis. However, others showed that this early posttransplantation complication was the result of acute rejection rather than CsA nephrotoxicity and that it reacted well to steroid therapy (*11*). Unsuccessful attempts in transplant patients have been made to correct nephrotoxicity by means of dopamine infusions (*12*). Attempts to accelerate CsA metabolism using hepatic Cyt-P450 enzyme-complex inducer, have been made in rats, resulting in reduced nephrotoxicity (*13*). Generally the strategy has been to reduce the CsA dose or convert to conventional therapy—however, these measures may be followed by rejection of the graft (*14*).

CsA has been reported to increase sympathetic nerve activity in spontaneous hypertensive rats (*5*). This was indicated as leading to stimulation of the RAAS-system, as well as release of angiotensin II, and inhibition of renal prostaglandin synthesis. Prevention of CsA nephrotoxicity in rats with prostaglandins has been described by Makowka et al. (*15*).

* Abbreviations used: BUN, blood urea nitrogen; CNS, central nervous system; CsA, cyclosporine; GFR, glomerular filtration rate; RAAS, renin-angiotensin-aldosterone system; RGFR, relative GFR.

Hydergine is a partial α_1 and α_2 adrenoceptor agonist that also has a stimulating effect on dopamine and an antagonistic activity on the 5HT receptor. Considering these properties of Hydergine, it has been suggested that it might interfere with the proposed mechanism of CsA-induced nephrotoxicity (*5*). The present study, using a rat kidney allograft model, was undertaken to investigate whether Hydergine could prevent or offer partial protection from CsA-induced nephrotoxicity.

Male BN ($RT1^n$) and WAG ($RT1^u$) rats, weighing about 250 g were used as kidney graft donors and recipients, respectively. Kidney transplantations in the BN-to-WAG combination were performed using microsurgical techniques, as described earlier (*16*). Bilateral nephrectomy was done at the time of operation. CsA, diluted in olive oil, was administered i.m. in a volume of 0.2 ml. Hydergine (Sandoz) was administered i.p. in doses of 0.5 mg/kg/48 hr. Glomerular filtration Rates (GFR) were measured as clearances of ^{51}Cr-ethylenediaminotetraacetate using a method described in detail earlier (*17*). The relative glomerular filtration rate (RGFR; expressed as a percentage of normal GFR) was calculated by considering the GFR of WAG rats with both kidneys intact to possess an RGFR of 100% (*17*).

Serum creatinine (normal value 43±4 μmol/L), blood urea nitrogen (BUN, normal value 8.6±1.1 mmol/L), and serum Na and K values, were determined at regular intervals using standard analytical procedures.

The experimental design was as follows: Kidney transplantations in the BN-to-WAG combination were performed and the rats divided into two groups. One group was treated with CsA 15 mg/kg/48 hr from the day of transplantation for a period of 35 days (group 1, n=8). At the end of this period GFRs were determined. From day 42 onward these rats were treated with CsA 100 mg/kg/48 hr for a period of 21–28 days (group 1a). At the end of this period GFRs were determined again. A second group of kidney-allografted rats underwent the same treatment with CsA as the group above, except that they additionally received Hydergine, from the day of transplantation, 0.5 mg/kg/48 hr (groups 2 and 2a, n=8). All through this treatment period serum creatinine, BUN, Na, K, and body weight were determined regularly. The results were analyzed statistically by using the Student's *t* test.

In Table 1 the RGFRs of the groups treated with CsA and CsA + Hydergine are given. At the end of the first 35 days of treatment with low doses of CsA, the mean RGFRs of the CsA (group 1) and CsA + Hydergine groups (group 2) were 34.4± 8.2 and 28.2±16.7, respectively. This difference is not statistically significant. After completion of the treatment with a dose

TABLE 1. Relative glomerular filtration rates (RGFR) of kidney allografts after treatment with CsA + hydergine[a]

Group	Treatment	RGFR[b] (Mean ± SD)
1.	15 mg/kg CsA	34.4±8.2
2.	15 mg/kg CsA + Hydergine	28.1±6.9
1a.	100 mg/kg CsA	23.9±5.8[b]
2a.	100 mg/kg CsA + Hydergine	16.7±4.3

[a] The animals in group 1 were treated with 15 mg/kg CsA on alternate days for 35 days. The rats in group 2 received the same CsA treatment and were additionally given Hydergine in a dose of 0.5 mg/kg on alternate days. The animals in group 1a are the same animals as in group 1; they were treated with 100 mg/kg CsA on alternate days for 28 days after completion of the first CsA treatment period. The rats in group 2a are the same animals as in group 2; they were given 100 mg/kg CsA + Hydergine for 28 days.

[b] RGFRs are glomerular filtration rates expressed in percentages of normal values, they were determined after completion of the low and high CsA treatment schedules. Normal GFR of WAG rats, weighing 250 g is 1.04±0.22 ml/min.

[c] Of 8 animals, 5 died—on days 13, 14, 17, 21, and 21.

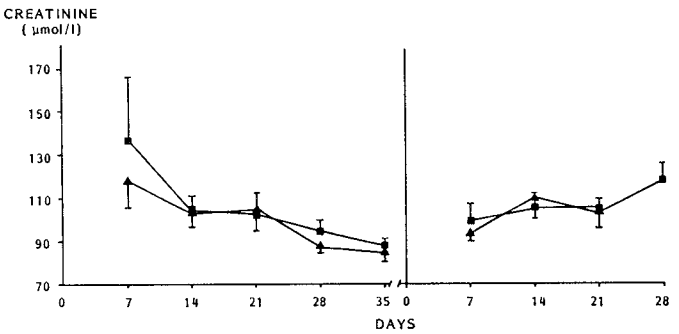

FIGURE 1. Creatinine levels (µmol/L) of kidney-allografted rats treated with 15 mg/kg CsA and 100 mg/kg CsA (▲) on alternate days for 35 and 28 days, respectively, or with the same dosages of CsA combined with Hydergine 0.5 mg/kg on alternate days (■).

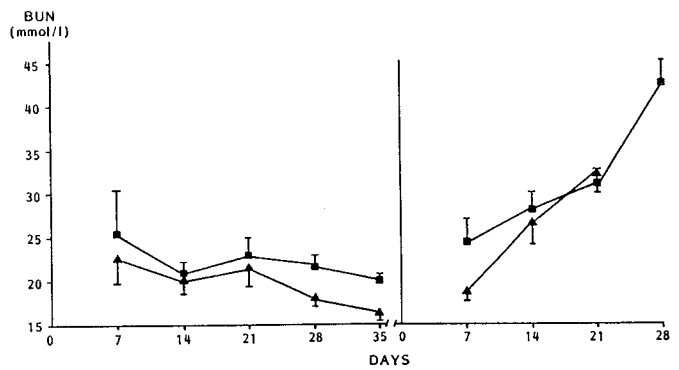

FIGURE 2. BUN levels (mmol/L) of kidney allografted rats treated with 15 mg/kg CsA and 100 mg/kg CsA (▲) on alternate days for 35 and 28 days, respectively, or with the same dosages of CsA combined with Hydergine 0.5 mg/kg on alternate days (■).

of 100 mg/kg CsA the RGFRs had deteriorated markedly, indicating the dose relationship of CsA associated nephrotoxicity. Although there were only 3 animals left for GFR determination in the CsA group (group 1a) there was no indication of a better RGFR in the CsA + Hydergine group (group 2a).

In Figures 1 and 2, the serum creatinine and BUN values are given, respectively. They confirm the dose-relationship of CsA-associated nephrotoxicity. The commencement of treatment with 100 mg/kg CsA evoked a steady rise in BUN and serum creatinine levels in both groups. There was no significant difference in BUN and serum creatinine values between the CsA and CsA + Hydergine group.

In the CsA + Hydergine group, all 8 rats survived the entire observation period. In the group treated with CsA alone, only 3 of 8 animals outlived the experiment. For 5 rats, the treatment with 100 mg/kg of CsA proved to be fatal, they died after 13, 14, 17, 21, and 21 days.

Serum K levels showed an upward trend with the progress of the experiment, though there was no significant difference between the CsA and CsA + Hydergine groups. There also was no appreciable difference in Na values among the various groups. In the course of the experiment there was marked reduction in body weight, with a mean weight loss of 45±6 g at the end of the study, but there was no significant difference among the groups.

The present study indicates that Hydergine is incapable of abolishing the nephrotoxicity of CsA in this renal transplant model. GFR is a very sensitive parameter for kidney function, yet we could not observe any better renal function in rats treated with Hydergine + CsA, as opposed to rats treated with CsA alone. The same conclusion could be reached from the BUN and serum creatinine values of the two groups.

It has been postulated that treatment with CsA leads to increased sympathetic nerve activity, which in turn can induce RAAS stimulation and increased angiotensin II release, which is responsible for hypertension and inhibition of renal prostaglandin synthesis (*5*). This cascade of events may result in altered renal circulation and autoregulation disturbances leading to nephrotoxicity. The properties of Hydergine discussed above might modify this chain of events. The fact that, with the dose of Hydergine used, no effect was apparent in our model could be due to the transplantation itself, the resulting loss of kidney innervation being a major factor. The study of Siegl in rats and dogs (*5*), leading to the abovementioned hypothesis, was performed in animals with intact kidneys. However, results similar to ours have recently been obtained in a rat model with intact kidney innervation in which damage to the kidney was evoked by warm ischemia. Here also no inhibitory effect of Hydergine on CsA nephrotoxicity was observed (*18*).

The finding that all 8 rats of the group treated with the high dose of CsA + Hydergine survived the entire experiment, while only 3 out of 8 rats of the other group could endure the CsA treatment to its conclusion, suggests some beneficial effect of Hydergine. During this study the rats showed a variety of toxic effects of CsA therapy, such as profound weight loss and CNS toxicity (tremors). This may have played a major role in the death of 5 of the 8 rats in the CsA group, while Hydergine in the other group may have affected the rats in a positive manner—e.g., by diminishing the toxic effects of the CNS.

In conclusion we were unable to observe an inhibitory effect of Hydergine on CsA-associated nephrotoxicity in our renal transplantation model. However, Hydergine may have neutralized some general adverse effects of CsA. To solve the problem of CsA-associated nephrotoxicity it is mandatory to elucidate the precise etiology of impaired renal function under CsA therapy.

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HEPATITIS-ASSOCIATED APLASTIC ANEMIA AFTER LIVER TRANSPLANTATION

We recently cared for a child who developed lethal aplastic anemia following liver transplantation for fulminant non-A, non-B hepatitis. Because of the increasing success of liver transplantation for fulminant hepatitis, the association of hepatitis with aplastic anemia needs to be brought to the attention of the transplant community.

A seven year old child was well until April 1985, when he was seen at his local hospital with a three week history of icterus and fatigue. Hepatitis evaluation was negative for cytomegalovirus (CMV),* Epstein-Barr virus (EBV), hepatitis A or B infection, alpha-1 antitrypsin deficiency, Wilson's disease, sickle cell disease, or G-6-PD deficiency. Skin tests were reactive to Candida at 48–72 hr, with negative purified-protein derivative control.

Over the next two weeks, the clinical course was compatible with a diagnosis of subacute hepatic necrosis. He developed progressive hepatic dysfunction, severe metabolic encephalopathy (stage 3), and a profound coagulopathy, and required mechanical ventilation and continuous infusions of fresh frozen plasma and 35% dextrose solutions. During this time, he had a transient drop in his white blood cell count to 3800 coincident with a five-day course of acyclovir given prophylactically for chickenpox exposure, but he recovered, with subsequent counts ranging from 7100–14,000. His platelet count gradually dropped as well, also in association with acyclovir therapy and presumed sepsis.

After transfer to the University of Minnesota Hospitals, a liver trasplant was performed on May 28, 1985. His native liver demonstrated massive hepatocellular necrosis. Immediate graft function led to reversal of his encephalopathy within 48 hr. Although liver function was normal within 72 hr his posttransplant course was complicated by presumed sepsis, (manifest by fevers, tachycardia, decreased peripheral vascular resistance, and elevated white blood count) treated empirically with chloramphenicol, vancomycin, and amikacin for five days, and acyclovir for 12 days. Chloramphenicol levels were always within the therapeutic range. Bacterial culture results were negative after 5 days. Mild hypertension was treated with captopril and propranolol. His white count dropped from 13,000 on the 3rd posttransplant day to 3800 by the 12th posttransplant day. His hemoglobin was stable at 8.0 g/dL, and his platelet count remained at 50,000–100,000/μL. Immunosuppression consisted of cyclosporine, prednisone, and azathioprine. Cyclosporine (CsA) levels (whole-body levels measured by high-pressure liquid chromatography) were 319 ng/ml on the 14th and 462 ng/ml on the 19th posttransplant days, respectively; no dosage adjustments were made. Azathioprine was decreased, and then

* Abbreviations used: CMV, cytomegalovirus; CsA, cyclosporine; EBV, Epstein-Barr virus.