during a mean observation period of 25 months (from 8 to 36 months). Two patients irreversibly rejected their grafts during cyclosporine treatment (patients S.G. and Z.F.). In one of these patients, cyclosporine treatment was withdrawn at six weeks after transplantation because of thromboembolic occlusion of the femoral artery.

In this patient acute rejection occurred on day 7 after discontinuation of systemic immunosuppression. One patient experienced one reversible acute rejection episode during cyclosporine treatment, and another irreversible episode 21 months after discontinuation of cyclosporine treatment (patient H.U.). One patient experienced an acute rejection episode during, and another episode after, discontinuation of cyclosporine treatment (patient F.O.). Both these rejection episodes rapidly responded to treatment with i.v. methylprednisolone. Side effects related to systemic immunosuppression with cyclosporine at this low-dose level were minimal (Table 1). In particular, none of the patients experienced renal dysfunction, which otherwise represents the main complication of cyclosporine therapy. The only toxicity observed was development of transient hypertension in three and aggravation of preexisting hypertension in one further patient. These side-effects were equally distributed between patients initially receiving a first i.v. infusion of cyclosporine and those who were started on oral medication.

After cessation of systemic prophylactic immunosuppression, 13 patients had functioning grafts and were followed for 5–33 months (mean follow-up 22.1 months). During this time grafts remained quiescent in eleven patients. Acute rejection episodes occurred in two patients who had already rejected their graft during prophylactic immunosuppression with cyclosporine (patients H.U. and F.O. [Table 1]). No systemic symptoms or precipitating local infections occurred in any of the rejection episodes.

The remaining patient (H.U. [Table 1]) lost the graft 21 months after withdrawal of cyclosporine despite systemic and topical administration of corticosteroids.

The efficacy and toxicity of short-term immunosuppression with cyclosporine was tested in fifteen high-risk cornea allograft recipients. Strong vascularization represented the main risk factor in these cases. Systemic immunosuppression consisted of a three-month course of 5 mg/kg p.o. of cyclosporine per day and was supplemented by topical application of corticosteroids. Of the 15 patients, 12 enjoyed an uncomplicated course and functional restoration during an average period of observation of more than two years. Graft rejection episodes were rare during, and after cessation of systemic immunosuppression four patients experienced a total of six rejection episodes, and only three grafts were lost. Toxic side effects of the three-month treatment with low-dose cyclosporine were minimal and mainly related to transient hypertension in three

and aggravation of preexisting hypertension in one further patient. We thus conclude that low-dose cyclosporine as short-term immunosuppression has an acceptable toxicity and is effective in preventing rejection in high-risk corneal allograft recipients.

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TRANSMISSION OF FATAL HERPES SIMPLEX INFECTION THROUGH RENAL TRANSPLANTATION[1]

Herpes simplex virus (HSV)* infections cause significant morbidity and occasional mortality following organ transplan-

* Abbreviations used: ALAT alanine aminotransferase; ASAT aspartate aminotransferase; CsA cyclosporine; DNA deoxyribonucleic acid; HSV herpes simplex virus; PO postoperative.

tation. The majority of these infections are believed to be reactivation of a latent and remote infection in the recipient (1–3). Rare cases of primary herpes simplex infection following renal transplantation have been reported but the source of infection in these is unknown (4, 5).

Donor kidneys have been shown to be a source of cytomegalovirus infections (2) but evidence has been minimal regarding their role in HSV infections (6,7). We recently documented

one instance of transmission of primary HSV-2 infection by renal transplantation but thought this was a rare occurrence (7), but 21 months after that we had a tragic experience in which two renal recipients died of disseminated herpes infection with fulminant hepatitis after receiving organs from the same donor. Liver and heart from that donor were also transplanted into different recipients. It is our intent in this report to present evidence that donor kidneys in this case also acted as a source of the herpes simplex infection.

Renal recipient No. 1. A 21-year-old man received a cadaveric kidney transplant on June 19, 1986 from the same donor as recipient No. 2. Immunosuppression was with CsA and steroids. On the 7th postoperative (PO) day monoclonal antibody OKT3 (Ortho Pharmaceutical Company, Raritan, NJ) therapy was added for suspected rejection. The patient became febrile from the 10th PO day, with no identified source. On the 14th PO day his hepatic transaminases were elevated (ASAT 1075 U/L, ALAT 1181 U/L). Two days after this he developed severe coagulopathy and gastrointestinal bleeding. OKT3 therapy was stopped and i.v. acyclovir was started because of isolation of HSV from the other renal recipient's blood. No mucosal or skin lesions suggestive of herpes infection were present. Over the next several hours, however, his coagulopathy worsened and he died of hyperkalemic cardiac arrest in spite of hemodialysis. An autopsy was denied.

Renal recipient No. 2. A 30-year-old man received a cadaveric kidney transplant on June 19, 1986. Immunosuppression was with cyclosporine and steroid. He became febrile on the 10th PO day. This was thought to be due to rejection and was treated with bolus steroids. On the 11th PO day his hepatic transaminases were ASAT 824 U/L and ALAT 1135 U/L. At that time he was started on i.v. ampicillin because of a positive blood culture for enterococcus. He developed severe back pain, right-sided abdominal pain, and tenderness from the 14th PO day on. Ultrasound of gallbladder and biliary scintiscan were unremarkable. Over the next few days he continued to be febrile, developed coagulopathy and gastrointestinal bleeding, and required ventilatory support. The true nature of the problem was suspected when a buffy coat culture drawn on the 14th PO day yielded herpes simplex virus, and i.v. acyclovir was begun. No skin or mucosal lesions suggestive of herpes infection were evident. His clinical condition deteriorated with hypoglycemia, hypoxemia, and seizures, and he died on the 19th PO day.

Postmortem examination revealed hepatomegaly (2880 g) with extensive hemorrhagic hepatic necrosis. In addition, massive gastrointestinal hemorrhage with multiple petechial hemorrhages into skin, mucous membranes, heart, and brain, were present with recent right cerebellar and left superior frontal infarcts.

Microscopically, the liver showed extensive coagulative necrosis. The sparse remaining viable liver parenchyma showed well-demarcated areas of necrosis with no particular distribution with respect to the lobular architecture. The necrotic tissue was infiltrated with neutrophils and contained cellular and nuclear debris (Fig. 1). Although viral inclusions were scarce, several foci of multinucleate hepatocytes that contained large intranuclear Cowdry type A inclusions were seen (Fig. 1A). Immunoperoxidase staining of the liver for herpes simplex virus type 2 was strongly positive. Transmission electron microscopy revealed clusters of viral particles with features characteristic of the herpes family (Fig. 1B). Remarkably, the brain and other organs were free of apparent viral infection.

FIGURE 1. Microscopic section of liver, showing extensive hemorrhagic necrosis (H&E; ×200). Intranuclear inclusions typical of herpes simplex were recognized in the sparse remaining viable parenchyma (insert 1A, H&E; 500×) at the edge of necrotic areas. Electron micrographs revealed viral particles characteristic of the herpes simplex group (insert 1B, uranyl acetate and lead citrate, 46,100×).

The cause of death in this patient was determined to be secondary to HSV induced massive hepatic necrosis resulting in coagulopathy, diffuse hemorrhage, and hypotension.

Liver recipient. A 36-year-old man with primary sclerosing cholangitis received an orthotopic liver transplant on June 6, 1986. His postoperative course was complicated by hepatic artery thrombosis and enterobacter septicemia. He was retransplanted on June 18, 1986. Four days after this, he developed tongue and pharyngeal ulcerations from which HSV type 1 was eventually grown. He was started on i.v. acyclovir and these lesions gradually resolved. This patient had 2 liver biopsies on the 8th and 18th PO days after the second liver transplant. Both showed no evidence of herpes hepatitis. His first hepatic allograft, after removal, did not show evidence of herpes simplex infection. Eventually he was discharged from hospital and is currently doing well.

Heart recipient. A 17-year-old boy received an orthotopic heart transplant on June 17, 1986 and required retransplantation on June 18, 1986. He died 3 days following his second transplant of severe cardiovascular instability. No skin or mucosal lesions were evident. Autopsy did not reveal any histological evidence of HSV infection.

Donor. The two kidneys, the second liver, and the second heart in the above transplant procedures were all obtained from the same donor. He was a 26-year-old male victim of an automobile collision with a closed head injury and crush injury to left lower extremity. He was treated in hospital for 3 days with several blood transfusions. Any history of previous herpes infection was unknown. Clinical examination at admission did not reveal evidence of oral, cutaneous, or genital sores.

Viral studies. Urine specimens, throat swabs, and buffy coats were inoculated onto monolayer cultures of human foreskin fibroblasts and also onto rabbit kidney cells. Presumptive identification of HSV was made by characteristic cytopathology appearing in both human and rabbit cells. The isolates were confirmed as HSV and typed with monoclonal antibodies (Syva, Genetic Systems Corp., Seattle, WA) using direct immunofluorescence (Table 1). Sera from the donor, both renal recipients and the liver recipient, were available and were assayed for the

TABLE 1. Results of virological cultures in liver and kidney recipients

	Throat swab	Buffy coat blood	Urine	Liver
Renal recipient No. 1	—	HSV II (16th day)	HSV II (16th day)	Not done
Renal recipient No. 2	HSV II (16th day)	HSV II (14th day)	—	HSV II (autopsy)
Liver recipient	HSV I (7th day)	—	—	—

presence of neutralizing antibodies to HSV-1 and HSV-2 (8); the results are as shown in Table 2.

Viral DNA isolation. Five viral isolates were available for analysis. These consisted of isolates from urine and buffy coat specimens from renal recipient No. 1, liver and throat swab from renal recipient No. 2, and a tongue isolate from the liver recipient. All isolates from the renal recipients were characterized as HSV-2 by monoclonal antibody fluorescence staining. The tongue specimen (liver recipient) was identified as HSV-1. The 4 HSV-2 isolates were examined for type and intratype nucleotide sequence variation by restriction endonuclease analysis. Standard HSV-1 (strain F) and HSV-2 (strain G) were included for comparison. In the electropherograms shown in Figure 2, a and b the strains are denoted by patient (1 or 2), source of isolate (urine [UR], buffy coat [BC], throat swab [TS] and liver [LV]) and clinical isolate number. Arrows indicate the presence or absence of specific fragments that distinguish strains. Arrows 1 and 3 of Fig. 2a denote differences between patients' strains and HSV-2; arrows 2 and 4, between patients' strains and HSV-1. In Figure 2b, arrows refer to restriction fragments that differentiate patient DNAs from standard HSV-2 (strain G). The apparent differences between the patterns in the region to the left of arrow 1 in Fig. 2b were not consistently seen and are apparently due to incomplete restriction. Restriction endonuclease profiles clearly defined all isolates as HSV-2. Isolates from patients Nos. 1 and 2 revealed similar cleavage patterns. While minor heterogeneities in digestion patterns were observed between the 2 patients, similar differences were also noted between isolates from a single individual. Herpes simplex-2 DNA isolated from unrelated patients was also analyzed by the same methods (data not shown). The restriction patterns showed a variety of distinctly different profiles.

A large number of patients show serological evidence of past HSV infection at the time of renal transplantation (1-3), and the great majority of HSV infections occurring after transplantation are believed to be reactivation of these past infections (2). Both of our renal recipients had no prior history of herpetic lesions and their pretransplant sera did not have neutralizing antibodies to HSV-1 or -2. This is strong evidence that these infections were primary; however, the liver recipient had a high titer of neutralizing antibodies to both subtypes before his first transplant and thus was at risk for reactivation.

Both renal recipients had identical incubation periods and similar clinical courses, and both died of fulminant hepatitis. In one of them autopsy showed massive necrosis of the liver with characteristic inclusion bodies, and herpesvirus particles were seen on electron microscopy. HSV-2 was isolated from buffy coat cultures taken before death. The DNA restriction endonuclease patterns of the viral isolates from both are very similar except for minor variations. These provide evidence that the virus strains in these patients had a common source. Surgical teams for both patients were different, and patients had minimal contact with each other on the ward. Neither of the renal recipients received blood at operation. Thus, evidence

TABLE 2. Serum neutralizing antibody titers to HSV I and HSV II

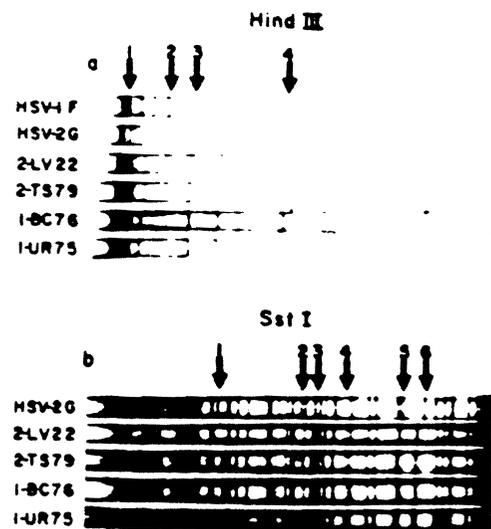| Date	Renal recipient No. 1		Renal recipient No. 2		Liver recipient		Donor	
	HSV I	HSV II	HSV I	HSV II	HSV I	HSV II	HSV I	HSV II
6/6/86					>256	>256		
6/18/86	<4	<4	<4	<4			>256	4
6/26/86	<4	<4	<4	<4				
7/3/86	<4	<4	≤4	<4				

FIGURE 2. (a and b) Restriction endonuclease profiles of DNA from standard HSV strains and isolates from 2 kidney transplant recipients. The laboratory strains are HSV-1 (strain F) and HSV-2 (strain G); the clinical isolates are denoted by patient (1 or 2), site of isolation (urine [UR], buffy coat [BC], throat swab [TS], and liver [LV]) and specimen number. The restriction endonucleases used are listed above each gel. The arrows refer to restriction fragments that differentiate patients' DNA from standard strains.

is strong implicating the donor kidneys as the common source of infection for both our renal recipients.

Donor organs have not been considered as a potential route of transmission of HSV. There is a recent report presenting evidence that donor kidneys could act as a vehicle for transmission of HSV (7). HSV normally enters the body through abraded skin or mucosal surfaces. The virus is known to establish latency in ganglionic neurons after primary infection (9, 10). Following genital HSV infection, it is possible that the virus could reach the ureter and kidney along rich periureteric and perinephric autonomic nerve plexuses and remain latent there. Other possible routes to the kidney would be viremia during a primary HSV infection (11), or via an ascending route from the external genitalia in a catheterized patient. Although one report discusses the isolation of HSV from 5 of 10 normal kidneys (6), the author did not actually isolate the virus in

tissue culture, and other investigators have not been able to confirm their results. Thus it remains unclear whether HSV can establish latency in the kidney. It is interesting that we did not isolate HSV or find histological evidence of HSV in the one donor kidney examined at autopsy. However, it is well known that the kidneys may transmit cytomegalovirus infection without overt evidence of infection in the kidney (12). It is interesting that the donor had a low titer of neutralizing antibodies to HSV-2. This might indicate that the infection was recently acquired. Although no mucocutaneous herpetic lesions were evident, they might have been missed in a polytrauma victim. The fact that the liver recipient did not develop HSV-2 infection may result from any one of the following: acyclovir therapy started fortuitously for his tongue lesions, which eventually grew HSV-1; his high titer of neutralizing antibodies to HSV-2; and inability of herpes simplex to establish latency in liver tissue, and hence not be transmissible through transplantation.

This is the second documented instance of transmission of HSV infection by renal transplantation at our institution in 2 years. Unlike the first case, these cases had a fulminant course and a fatal outcome. Identification of these cases was more difficult because of the absence of skin and mucosal lesions. Whether transmission of HSV can happen through a liver or heart transplant is not known at present, but with efficient utilization of organs from a single donor becoming more common, this type of transmission could have a devastating effect on several recipients. HSV should be considered as a possible pathogen whenever fulminant hepatitis occurs in a transplant recipient. Knowledge of donor and recipient serological status for HSV may be helpful in identifying transplant recipients at risk for such infections and prophylactic acyclovir therapy should strongly be considered whenever a kidney is transplanted from a seropositive donor to a seronegative recipient.

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THE EFFECT OF CYCLOSPORINE ON THE PHARMACOKINETICS OF PREDNISOLONE IN RENAL TRANSPLANT PATIENTS

Immunosuppressive drugs have an important role in the prevention of graft rejection following renal transplantation. Traditionally, immunosuppressive therapy consisted of the administration of azathioprine and corticosteroids (1). The introduction of cyclosporine has greatly improved graft survival for cadaver transplants (2–4). Cyclosporine is now widely used as immunosuppressive therapy in renal transplantation in combination with corticosteroids.

Some investigators have suggested that cyclosporine inhibits the elimination of prednisolone when both drugs are administered concurrently (5, 6). Possible mechanism(s) for this interaction include: (1) cyclosporine-induced reduction in the amount of hepatic cytochrome P_{450}, (2) cyclosporine-induced

hepatotoxicity, and (3) competition between cyclosporine and prednisolone for hepatic drug metabolizing enzymes (5).

Previously published studies that evaluated the cyclosporine-prednisolone interaction did not evaluate changes in the pharmacokinetics of free prednisolone. This latter variable may be particularly important since prednisolone plasma binding is nonlinear and may be affected by renal disease.

The purpose of the present study was to evaluate the effect of cyclosporine on the pharmacokinetics of free and total prednisolone following its intravenous administration to renal transplant patients.

The effect of cyclosporine on prednisolone pharmacokinetics was evaluated in six patients (5 female), ranging in age from