The therapeutic use of ganciclovir for invasive cytomegalovirus infection in cadaveric renal allograft recipients

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ABSTRACT

Between November 1987 and September 1989, 419 cadaveric renal transplants were performed at our university. Of the patients 36 (8.6%) had invasive cytomegalovirus infection documented by gastric or duodenal mucosal biopsy in 23 (64%), bronchoalveolar lavage in 12 (32%), allograft biopsy or nephrectomy specimen in 5 (14%) and/or liver biopsy in 1 (3%). Cytomegalovirus severity was defined as mild in 27 patients, moderate in 6 and severe in 3. Ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)-guanine] was begun once the diagnosis was confirmed by histology or culture at a median of 56 days from transplantation (range 28 to 133 days). Duration of ganciclovir therapy was a minimum of 7 days or until fever was absent for 5 consecutive days (mean 12.2 ± 3.5 days, range 4 to 21). Ganciclovir was well tolerated and side effects were limited to de novo neutropenia (7 patients), thrombocytopenia (2) and rash (1). Initial clinical improvement was observed in all patients. Two patients had recurrent cytomegalovirus infections that responded to a second course of ganciclovir. The 1-year actuarial patient survival was 100%. At a mean followup of 12.7 ± 6.2 months 19 patients retained allograft function with a mean serum creatinine of 2.5 mg./dl (range 1.2 to 4.6). Ganciclovir appears to be a safe and effective drug for the treatment of tissue invasive cytomegalovirus infection in cadaver renal transplant recipients. Prompt institution of this drug at diagnosis of invasive cytomegalovirus may lower the mortality rate formerly associated with this disease.

Key Words: cytomegaloviruses, kidney transplantation, cadaver, ganciclovir

Renal transplant recipients with tissue invasive cytomegalovirus infection have had as much as a 25% risk of dying, even when immunosuppression is decreased or stopped. Until recently, there was no effective therapy for this group of patients. Ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)-guanine] is metabolized by viral kinases within infected host cells to yield 2' -nor-2' deoxyguanine, a potent inhibitor of herpesvirus deoxyribonucleic acid polymerase. Although previous studies have reported its usefulness for life or sight-threatening cytomegalovirus infections in immunocompromised hosts, the optimum use of ganciclovir in cadaver renal transplant recipients with tissue invasive cytomegalovirus has not yet been established. We report our experience with the use of ganciclovir in treating tissue invasive cytomegalovirus infection following cadaver renal transplantation. The results suggest that this drug is well tolerated and is effective in arresting the progress of invasive cytomegalovirus to life-threatening disease with little or no drug-related morbidity.

Materials and Methods

Patients. Of 419 cadaveric renal allograft recipients who underwent transplantation at our university between November 1987 and September 1989, 36 (8.6%) had tissue invasive cytomegalovirus infection, including 25 men and 11 women (mean age 42.1 ± 11.5 years, range 18 to 71). Cytomegalovirus serology was determined by solid phase fluorescence immunoassay of IgG antibodies to cytomegalovirus. Immunofluorescence titers of less than 20 were considered negative, 20 to 30 equivocal and more than 30 seropositive. In 16 of the 36 patients (44%) an allograft from a cytomegalovirus seropositive donor was transplanted into a seronegative recipient. Nine cases (25%) were seropositive to seropositive, 5 (14%) were seronegative to seropositive and 4 (11%) were seronegative to seronegative. Cytomegalovirus serology was not available from 2 donors. Prophylactic acyclovir was given to all patients at either low dose (200 mg. twice daily in 28) or high dose (up to 3,200 mg./24 hours based on renal function in 8). Immunosuppression. A standard immunosuppressive regimen including cyclosporine (15 to 17.5 mg./kg. orally before transplantation, subsequently tapered to maintain whole blood high performance liquid chromatography levels of 100 to 200 ng./ml), prednisone (tapered to 0.1 mg./kg. by 3 months) and azathioprine (3 to 5 mg./kg. by 3 months) or OKT3. Rejection episodes were defined clinically by an increase in serum creatinine accompanied by fever, decreased urinary output, graft tenderness and/or weight increase and were confirmed by core needle biopsy. Rejection was treated with increased steroid therapy and/or OKT3 5 mg. daily for 10 to 14 days). Definition of cytomegalovirus infection. Cytomegalovirus infection was clinically suspected in patients with unexplained fever 38.3°c or greater for more than 2 days, leukopenia (white blood count less than 4,000/mm.3), thrombocytopenia (platelet count less than 100,000/mm.3) and/or diffuse pulmonary infiltrates. Cultures of blood, urine and pharyngeal washings were performed in all patients. When clinically indicated, the presence of tissue invasive cytomegalovirus was sought by bronchoalveolar lavage, upper gastrointestinal endoscopy with biopsy, liver biopsy, lumbar puncture, bone marrow examination and/or renal allograft biopsy. The diagnosis of tissue invasive...
cytomegalovirus required histological documentation of cyto-
meaglic inclusion bodies and/or positive tissue cultures for
cytomegalovirus. An increase in serum antibody titer and/or
seroconversion was considered insufficient for the diagnosis of
tissue invasive cytomegalovirus. Only those patients with in-
vasive cytomegalovirus infection as defined by these criteria
were considered as candidates for therapy with ganciclovir.

Categories of cytomegalovirus severity. Disease severity
was based on 6 clinical features defined previously by Peterson et
al: 1) prolonged fever (temperature 38.3°C or greater for more
than 7 days), 2) diffuse pulmonary infiltrates, 3) gastrinomes-
tinal bleeding, 4) pancreatitis, 5) transplant nephrectomy and
6) development of another systemic infection. Patients with
severe cytomegalovirus infection had at least 3 of these 6
features, patients with moderate disease had 2 of these features
and mild cytomegalovirus disease was defined by the presence
of no more than 1 of these features.

Ganciclovir therapy. Ganciclovir was made available with
informed consent to patients with proved tissue invasive cyto-
megalovirus on a compassionate release basis. The protocol
was established, ganciclovir was administered intravenously at
2.5 mg/kg every 12 hours or 1.25 mg/kg every 48 hours. Treat-
ment lasted for a minimum of 7 days and was continued until the
patient was afebrile for 5 consecutive days. Duration of ganci-
clovir therapy was extended if persistent symptoms or evidence
of widely disseminated infection were present. Baseline and
repeat complete blood counts, serum electrolyte determinations
and renal function tests were performed daily in all patients.
Liver function tests were done twice weekly or daily for patients
with hepatitis. End points used to evaluate the outcome of
cytomegalovirus infection included patient survival, resolution
of clinical symptoms, reversal of chest radiographic, hematolo-
gic and liver function abnormalities, and elimination of the
virus from the affected tissue or body fluid as determined
histopathologically or by culture.

Statistical analysis. Actuarial patient and graft survival rates
were determined by life-table analysis using BMDP statistical
software. Potential risk factors were studied for their effects on
cytomegalovirus severity and treatment outcome, and were
compared by chi-square analysis.

RESULTS

Diagnosis of tissue invasive cytomegalovirus infection. A total
of 36 patients with clinically suspected cytomegalovirus infec-
tion had tissue invasive cytomegalovirus a mean of 60.6 days
after transplantation. Cytomegalovirus infection was clinically suspected because of unex-
plained fever in 32 patients (89%), leukopenia in 21 (58%),
thrombocytopenia in 17 (47%) and/or diffuse pulmonary infil-
trates in 9 (25%). Of the 27 patients (75%) who had mild, 6 (17%)
were moderate and 3 (8%) had severe infection (table 1). There
were no lethal infections. The pattern of cytomegalovirus tissue
involvement was stomach/duodenal mucosa in 26 patients
(72%), lung in 14 (39%), allograft in 5 (14%), liver in 1 (3%) and
multiple sites in 11 (31%). Biopsies were considered positive
cytomegalovirus based on viral culture or histology/immu-
nuntissuecytopathology. Tissue diagnosis was accompanied by positive
viral blood cultures in 17 patients (46%), urine cultures in 19
(53%) and pharyngeal washings in 2 (5%). A total of 8 patients
underwent treatment for concurrent infections, includ-
ing oesophageal or gastric candidiasis (4), Pneumocystis carinii
pneumonia (1), pseudomonal pneumonia (1), pseudomonal al-
lograft pyelonephritis (1) and Escherichia coli urosepsis (1).

Management of immunosuppression during cytomegalovirus
infection. Azathioprine was discontinued or temporarily with-
held in 31 patients, decreased in 4 and continued without
change in dosage in 1 following the diagnosis of cytomegalovirus
infection. Cyclosporine was stopped in 6 patients with non-
functioning allografts at cytomegalovirus diagnosis. In the re-
mainder cyclosporine was either temporarily withheld (6, mean
7 days) or continued with (4) or without (20) dosage reduction.
Prednisone dosage was lowered in 23 patients and continued
without change in the remaining 13.

Outcome of ganciclovir therapy. A total of 36 patients with
documented tissue invasive cytomegalovirus infection was
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diagnosed was treated for 12.2 ± 3.5 days (range 4 to 21) and followed for an
average of 12.7 months. One patient refused further therapy
after 4 days but showed no evidence of residual or recurrent
disease. Ganciclovir was well tolerated by all 36 patients. Side
effects were limited to transient, de novo leukopenia (white
blood count less than 4,000) in 7 patients, thrombocytopenia
(less than 100,000) in 2 and rash in 1, none of whom required
discontinuation of the drug. Serum creatinine was unaltered by
ganciclovir in patients with functioning grafts (mean 4.1 ± 1.6
mg/dl before and 3.8 ± 1.9 after ganciclovir therapy). Follow-
ing the initial course of treatment with ganciclovir, all 36
patients had complete resolution of symptoms (fever, fatigue,
malaise, cough and abdominal discomfort) associated with cyto-
meagalovirus. A total of 18 patients had positive baseline cul-
tures for cytomegalovirus from at least 1 body fluid with at
least 1 followup culture after ganciclovir treatment. The figure
summarizes the virological data of these patients before and
after ganciclovir therapy. The overall virological responses for
patients with initial viremia and/or viruria are summarized in
table 2. Viral blood cultures were negative 7.5 ± 3.6 days after
starting ganciclovir in 11 of 11 patients (100%) who had positive
blood cultures before therapy. Of the 13 patients with initially
positive urine cultures 11 (85%) became negative at 8.1 ± 4.8
days and 2 remained positive. All 9 patients with initially
positive cultures for blood and urine were cleared after ganci-
clovir. Tissue sampling was repeated in 7 patients after ganci-
clovir therapy. In 1 patient with cytomegalovirus pneumonia
repeat bronchoalveolar lavage was negative for cytomegalovirus
68 days after ganciclovir. Two stomach biopsies were rendered
negative 8.0 ± 9.9 days after treatment and 4 duodenal biopsies
were negative at 29.3 ± 20.9 days.

Factors potentially influencing cytomegalovirus severity and
outcome. There was no correlation between cytomegalovirus
severity and recipient age (more than 45 or 45 or less years),
interval to cytomegalovirus onset after transplantation, prior
rejection, OKT3 use, use of low dose versus high dose prophy-
lactic acyclovir, or donor/recipient cytomegalovirus serology
status (table 3). Graft survival, however, was higher in inverse
proportion to disease severity, with 17 of 27 grafts (63%) still
functioning in patients originally having mild, 5 of 6 (83%) in
those with moderate and 0 of 3 (0%) in those with severe
disease (p < 0.02).

Graft losses. There was a total of 17 graft losses in this series:
4 were lost at the onset of cytomegalovirus infection (primary
graft nonfunction in 2 and chronic rejection in 2). The remain-
ing 13 graft losses occurred at a median of 5 weeks (range 10
days to 16 months) following invasive cytomegalovirus infec-
tion. One patient underwent allograft nephrectomy after im-
unosuppression was withheld for transverse myelitis 2
months after treatment with ganciclovir. Concurrently, he had
benign cerebrospinal fluid and no evidence of ongoing cyto-
meagalovirus infection. One patient lost the graft to rejection
concurrent with acute pancreatitis (with negative cytem-
egalovirus evaluation) 2 months after treatment, and 1 patient
had recurrent oxalosis and graft failure 1 year after ganciclovir.
Two patients had primary graft nonfunction and the remaining 8 grafts were lost to rejection.

Cytomegalovirus and rejection episodes. A total of 29 patients (81%) with invasive cytomegalovirus infections had been previously treated for rejection with either increased steroid therapy alone in 18 (50%), OKT3 (5 mg. daily for 10 days) alone in 2 (6%), or OKT3 and steroids in 9 (25%). Prophylactic OKT3 was used in an additional 8 patients. Overall, 19 of the 36 patients (53%) received an average 13.2-day course (range 6 to 19 days) of OKT3 before contracting cytomegalovirus. Of 7 patients who had cytomegalovirus without prior rejection (86%) still have functioning allografts, compared to only 13 of 29 (45%) treated for rejection before cytomegalovirus infection occurred. Of the 13 graft losses that occurred after cytomegalovirus infection (median time 5 weeks) 10 (83%) were due to either acute (2) or chronic (8) rejection.

Patient and graft survival. The overall 1-year actuarial patient and graft survival rates were 100% and 56%, respectively, with a mean follow-up of 12.7 ± 6.2 months. As indicated previously, survival was significantly better for allografts not treated for rejection before the onset of invasive cytomegalovirus infection (86% versus 45%, p < 0.05). Those grafts still functioning have a mean serum creatinine of 2.5 mg./dl. (range 1.2 to 4.6). The 1-year actuarial patient and graft survival rates for all cadaveric renal transplants (419) performed at our institution during the same period were 95% and 80%, respectively.

DISCUSSION

Cytomegalovirus infection is a serious and potentially lethal complication in immunocompromised patients. Renal transplant recipients with cytomegalovirus infection have had mortality rates as high as 25% with even higher death rates (up to 100%) reported for patients with cytomegalovirus pneumonia.3,12 Until recently, the only therapeutic approach for this group of patients was to withhold immunosuppression and provide supportive care. Ten years ago the reported incidence of overt cytomegalovirus in renal transplant recipients was 40 to 50%.3,14,15 In this earlier era aggressive use of certain agents, such as prophylactic antilymphocyte preparations, was associated with the development of severe cytomegalovirus infection.10 Despite ongoing refinements in immunosuppressive protocols, the incidence of cytomegalovirus infection in renal transplant recipients (cadaveric and living related) has been recently reported to be as high as 29%.11 A successful approach to reducing the incidence of overt cytomegalovirus in renal transplant patients has been the use of prophylactic acyclovir.11 However, a finite number of patients will still have cytomegalovirus infection despite acyclovir prophylaxis. The severity of subsequent cytomegalovirus is not lessened by prophylactic acyclovir in these patients.11 Attempts to treat established cytomegalovirus disease with intravenous acyclovir as well as other antiviral agents have been unsuccessful.

Ganciclovir selectively inhibits the replication of herpes group viruses and is much more potent than acyclovir in vitro. This drug has been used to treat severe cytomegalovirus infection in patients with the acquired immunodeficiency syndrome (AIDS), with initial clinical improvement observed in 73 to 77%.1,3,19 However, these patients experience significant relapse rates (up to 80%) with up to 100% mortality often due to disseminated cytomegalovirus. Early experience with the use of ganciclovir for life or sight-threatening cytomegalovirus infection in bone marrow14 and solid organ allograft recipients16,18 has been encouraging, with response rates ranging from 36 to 70%. These patients have responded better to ganciclovir than those with AIDS with less frequent relapse. The use of
TABLE 2. Effect of ganciclovir on cytomegalovirus viremia and viruria

<table>
<thead>
<tr>
<th>Culture Site</th>
<th>Before Ganciclovir</th>
<th>After Ganciclovir</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Ob.</td>
<td>No. Cultures Pos. (%)</td>
</tr>
<tr>
<td>Blood</td>
<td>33</td>
<td>17 (52)</td>
</tr>
<tr>
<td>Urine</td>
<td>31</td>
<td>19 (61)</td>
</tr>
</tbody>
</table>

TABLE 3. Influence of potential risk factors on severity of cytomegalovirus infection

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cytomegalovirus Severity</th>
<th>P Value (chi-square)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No.</td>
<td>Mild (No. %)</td>
<td>Moderate (No. %)</td>
</tr>
<tr>
<td>Pl. age yrs.:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 45</td>
<td>23 (64)</td>
<td>18 (47)</td>
</tr>
<tr>
<td>45 or more</td>
<td>13 (36)</td>
<td>9 (26)</td>
</tr>
<tr>
<td>Prophylactic acyclovir:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low dose</td>
<td>28 (78)</td>
<td>21 (75)</td>
</tr>
<tr>
<td>High dose</td>
<td>8 (12)</td>
<td>6 (75)</td>
</tr>
<tr>
<td>Days to cytomegalovirus onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transplantation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 40</td>
<td>6 (17)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>40-100</td>
<td>25 (69)</td>
<td>19 (76)</td>
</tr>
<tr>
<td>100 or more</td>
<td>5 (14)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Prior rejection:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>29 (81)</td>
<td>21 (72)</td>
</tr>
<tr>
<td>Yes</td>
<td>7 (19)</td>
<td>6 (86)</td>
</tr>
<tr>
<td>Prior OKT3:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>19 (53)</td>
<td>14 (74)</td>
</tr>
<tr>
<td>Yes</td>
<td>17 (47)</td>
<td>11 (64)</td>
</tr>
<tr>
<td>Graft loss:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>19 (53)</td>
<td>16 (84)</td>
</tr>
<tr>
<td>Yes</td>
<td>17 (47)</td>
<td>11 (64)</td>
</tr>
<tr>
<td>Serologic status of donor/recipient:*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neg./neg.</td>
<td>4 (11)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Neg./pos.</td>
<td>5 (14)</td>
<td>4 (80)</td>
</tr>
<tr>
<td>Pos./neg.</td>
<td>16 (44)</td>
<td>11 (69)</td>
</tr>
<tr>
<td>Pos./pos.</td>
<td>9 (25)</td>
<td>8 (89)</td>
</tr>
</tbody>
</table>

sistent viruria has been reported in up to 70% of the patients treated with ganciclovir, with no adverse impact on clinical outcome. Treatment of cytomegalovirus with ganciclovir was accomplished with only modest reductions in immunosuppression (usually azathioprine). The most common side effect observed was leukopenia in 7 patients (19%), followed by thrombocytopenia in 2 (5%) and skin rash in 1. Leukopenia and thrombocytopenia were transient and did not require cessation of ganciclovir therapy. De novo anemia was not observed. In patients with a functioning graft, renal function was likewise not adversely affected by ganciclovir, with mean serum creatinine of 4.1 mg./dl before and 3.8 mg./dl after therapy.

There were no deaths in this series, with an actuarial 1-year patient survival rate of 100%. All patients had clinical improvement with course 1 of ganciclovir despite concurrent infections in 8 (22%). Relapses occurred in 2 patients within 9 weeks of the initial course of ganciclovir but both were successfully retreated. The overall virological clearance rate of 92% compares favorably with other reported series and usually occurred within 1 week of treatment. Although the natural history of viral shedding without treatment of cytomegalovirus is not well documented, these results suggest that ganciclovir has an antiviral effect in vivo.

The overall graft survival of 56% in this series is similar to that reported previously in cytomegalovirus infected patients. However, this series differs from these earlier reports in several major respects. The definition of cytomegalovirus (and therefore the use of ganciclovir) was restricted to patients with positive proof of tissue invasive disease, not merely seroconversion and/or viral shedding. All of our patients received acyclovir prophylaxis, which has recently been shown to lower the incidence (but not the severity) of clinical cytomegalovirus in renal transplant recipients. Thus, our patients acquired cytomegalovirus despite acyclovir prophylaxis and, therefore, they may be regarded as a high risk group. Whether the patients had received low dose or high dose prophylactic acyclovir had no influence on the severity of cytomegalovirus infection (p = 0.85). Of the patients in our series 81% had been treated for rejection before acquiring cytomegalovirus. More than 50% of the patients had received OKT3 either as prophylaxis for or treatment of rejection. Therefore, this population was already at higher risk of graft loss even had cytomegalovirus infection not occurred.

These results have led us to a more aggressive approach in patients with proved tissue invasive cytomegalovirus infections. We believe that most are able to tolerate ganciclovir with only a modest (25 to 50%) decrease in the dose of azathioprine. In our experience cyclosporine can be continued at therapeutic doses and steroid doses may be tailored individually. We have continued to decrease or withhold immunosuppression in patients with moderate or severe infections and those with nonfunctioning allografts. The efficacy of ganciclovir in the treatment of tissue invasive cytomegalovirus infection in renal transplant recipients would best be studied in a controlled trial. Our initial experience with ganciclovir suggests that early, aggressive treatment of invasive cytomegalovirus infection can be accomplished with little or no reduction in immunosuppression, thus, affording the opportunity for graft salvage and optimum patient survival (100% in our series). We observed no
progression of cytomegalovirus infection to life-threatening disease with this approach. Reservation of ganciclovir treatment only for patients with severe cytomegalovirus has met with poor results. The optimum use of this drug will require further study and enthusiasm for its use must be tempered by recent observations of the emergence of cytomegalovirus strains resistant to ganciclovir. However, the limited toxicity and prompt, uniform eradication of symptomatic disease observed in our series indicate that ganciclovir is a valuable addition to the therapeutic armamentarium in the management of cadaveric renal transplant recipients with tissue invasive cytomegalovirus infection.

REFERENCES


