Role of Poly-(ADP-Ribose) Polymerase in Transplant Acute Tubular Necrosis and Its Relationship With Delayed Renal Function


ABSTRACT
The enzyme poly(ADP-ribose) polymerase (PARP-1) participates in the repair of DNA damaged by genotoxic agents such as oxygen-derived free radicals. If the allograft suffers pretransplant cold ischemia and subsequent ischemia–reperfusion injury (IR), overactivation of PARP-1 can be induced, which may lead to an increase in acute tubular necrosis (ATN) and a delay in total recovery of renal function (RRF) of the transplanted organ. We studied the nuclear expression of PARP-1 in tubular cells by immunohistochemistry with the monoclonal antibody PAR01 in 104 kidney transplant biopsies from allografts with ATN. In 50% of biopsies with ATN, >50% of tubular nuclei were PARP-1 positive; only 9.6% of biopsies were negative. The increase in the immunohistochemical expression of PARP-1 showed a statistically significant relationship with the duration of cold ischemia, with serum creatinine levels, and with the time required to achieve effective diuresis (P < .0001, Spearman test). Cold ischemia of >24 hours and serum creatinine levels >1.7 mg/dL showed a statistically significant relationship with the highest PARP-1 expression levels (2.83 ± 0.4 vs 1.36 ± 0.8, P < .0001, Mann–Whitney U test). We conclude that PARP-1 plays an important role in ATN and RRF and is related to the extent and severity of ATN and to the renal allograft function.

PARP-1 is a nuclear zinc-finger DNA-binding protein with a molecular weight of 113 kd that specifically detects DNA-strand breaks or nicks produced by different genotoxic agents in mammalian cells. PARP-1 catalyzes the ADP ribosylation of proteins using NAD(+) as the substrate. The activation of PARP is a consequence of ischemic injury and results in a depletion of intracellular NAD(+) which can only be replenished via a reaction that consumes ATP. Ischemia–reperfusion (I/R) injury that results in substantial DNA degradation requires cells to consume large amounts of ATP to support poly ADP-ribosylation. For this reason, whereas moderate activity of PARP protects cellular genome integrity, its excessive activation can lead to cell death secondary to ATP depletion.

Acute failure of the transplanted kidney is a major problem in the early posttransplant phase and is acknowledged as a cause of allograft loss. Early renal transplant dysfunction is mainly due to ischemic damage (acute tubular necrosis [ATN]), rejection, infection, and cyclosporine toxicity. Renal ischemia is a major cause of acute renal failure, whether or not caused during transplantation, initiating a complex and interrelated sequence of events resulting in injury and eventual death of renal cells. Salahudeen et al recently studied 6465 kidney transplant patients using UNOS data and concluded that prolonged cold ischemia is a significant predictor of long-term graft loss. The prognosis is complicated by the fact that reperfusion, although essential for the survival of ischemic renal tissue, causes additional damage (reperfusion injury) that contributes to the renal dysfunction and injury associated with I/R of the kidney. Within the kidney, the proximal tubule appears to be particularly susceptible to...
I/R injury, leading to ATN, which plays a pivotal role in the pathogenesis of early transplanted kidney dysfunction.\textsuperscript{2,9,12} Ischemia–reperfusion injury is a common process that triggers a pathophysiologic cascade, including an inflammatory response with the release of cytokines and oxygen-derived free radicals. PARP-1 was recently shown to be involved in the pathogenesis of various forms of I/R injury in animal models.\textsuperscript{13} In addition, the pharmacologic inhibition of PARP-1 reduces reperfusion injury in the kidney and other organs of the rat.\textsuperscript{14} The present study examined whether increased tubular expression of PARP-1 in human allograft kidneys with ATN contributes to a subsequent delayed renal function.

MATERIALS AND METHODS

One hundred four kidney biopsies from 104 transplant patients at Hermanos Almeijeras Hospital, La Habana, were fixed in 10% buffered formalin and embedded in paraffin to determine glomerular, vascular, and tubulointerstitial lesions, according to the Banff scheme.\textsuperscript{15} Biopsies were taken between day 5 and day 11 of the transplant (mean 7.3 days) for oliguria in 101 patients (97.1%) and/or increased renal volume in 20 patients (19.2%). The nuclear expression of PARP-1 was characterized by incubating sections for 16 hours at 4°C with PARP-1 monoclonal antibody (Clone PAR01) (LabVision, Fremont, Calif). Subsequently, the immunohistochemistry study was done on an automated immunostainer (LabVision), using the streptavidin–biotin–peroxidase method followed by development with dianminobenzidine (Master Diagnóstica, Granada, Spain). The positivity and intensity of the immunostaining was calculated semiquantitatively using a 0 to 3 scale (0, absence of positive tubular nuclei; 1, 1% to 9% tubular nuclei positive; 2, 10% to 49% positive; and 3, >50% positive). Data were gathered on renal function parameters (serum creatinine levels at time of biopsy and end of follow-up), age, cold ischemia, effective diuresis, and immunosuppression regimens. The Kolmogorov–Smirnov test was used to assess the normal distribution of the variables. After the descriptive analysis, Spearman correlation and Mann–Whitney \(U\) test analyses were performed to determine statistical significance. The confidence interval was 95% (\(P < .05\)). The statistical analysis was performed using SPSS v11.0 (SPSS, Inc, Chicago, Ill).

RESULTS

Of the 104 biopsies studied, 99 (95.1%) corresponded to cadaveric donors and 5 (4.8%) to living donors. The mean age of the patients was 46.5 ± 11.4 years, all of whom had altered serum creatinine levels (at time of biopsy: 1.73 mg/dL, range 1.45 to 1.90 mg/dL; at end of follow-up: 2.01 mg/dL, range 1.55 to 2.90 mg/dL). The mean duration of cold ischemia was 22 hours (range 1 to 31 hours). No immunosuppressive regimen exerted a statistically significant influence on renal function parameters studied. Ten patients developed an acute rejection episode, 3 with concomitant ATN and 7 without ATN, and 12 patients (all with ATN) showed borderline changes. The mean time to effective diuresis was 13.7 days (range 0 to 24 days).

According to the Banff scheme, 12 biopsies were diagnosed with borderline changes (11.5%) and 10 with acute rejection (9.6%; 5 grade I and 5 grade II). ATN was present in 94 (90.3%) of the 104 cases, and changes suggestive of cyclosporine nephrotoxicity were present in 2 (1.9%). Only the kidneys with ATN (n = 94) were selected for the statistical study.

The frequency distribution of the renal biopsies according to their immunohistochemical expression of PARP-1 was as follows: zero, 9.6%; one, 11.5%; two, 28.8%; and three, 50%. PARP-1 expression showed a statistically significant relationship with the duration of cold ischemia (rho coefficient = 0.806, \(P = .0001\), Spearman test), time to effective diuresis (rho coefficient = 0.774, \(P = .0001\), Spearman test), and serum creatinine levels (rho coefficient = 0.649, \(P = .0001\), Spearman test). Kidneys from ATN patients who failed to reduce serum creatinine levels to <1.7 mg/dL after transplantation showed a different intensity of PARP-1 expression (creatinine <1.7, PARP 1.71 ± 0.58 vs creatinine >1.7, PARP 2.40 ± 1.05, \(P < .0001\), Mann–Whitney \(U\) test).

DISCUSSION

A prolonged cold ischemia time is a strong risk factor for delayed graft function (DGF) and graft loss.\textsuperscript{10,16} This present study revealed a severe intensity of immunohistochemical expression of PARP-1 in the tubular cells of human transplant kidneys with ATN and demonstrated a relationship between the highest tubular expression of PARP-1 and delayed renal function. In addition, transplanted kidneys in patients with serum creatinine levels that did not fall to <1.7 mg/dL presented almost double the intensity of PARP-1 expression. To our knowledge, this is the first time that the degree of PARP-1 activation has been related to the extent of human renal tubular injury and to renal function.

Activation of the PARP-1 pathway, a recently discovered cell injury mechanism,\textsuperscript{17–19} is currently regarded as the final common effector in the pathogenesis of various types of tissue injury, including systemic inflammation, circulatory shock, and I/R. A major contributor to the development and progression of I/R-induced renal failure is the loss of functioning tubular epithelial cells through cell deletion or cell death processes (necrosis or apoptosis). Donor kidneys inevitably undergo a period of ischemia. In our series, the periods of cold ischemia ranged from 1 to 31 hours. The variable resistance to ischemia of the heterogeneous renal cell population is well known. The proximal straight tubule and, to some extent, the thick ascending limb of the loop of Henle are more sensitive to ischemia. It can be hypothesized that these cells tend to suffer more necrosis in comparison with less sensitive cells,\textsuperscript{20} and that PARP-1 activation may be one of the pathogenic mechanisms. Thus, in our 94 patients with ATN, the kidneys that tolerated a long period of cold ischemia had the highest levels of PARP-1 (\(<24\) hours: 1.71 ± 0.62; \(\geq 24\) hours: PARP-1: 2.86 ± 0.350). In fact, the lowest PARP-1 expression (1% to 9% of tubular nuclei positive) was only observed in kidneys with
<20 hours of cold ischemia (mean 16.36 hours, range 12 to 20).

Delayed renal function after kidney transplantation may be due to various factors, such as the condition of the transplanted kidney and the compliance of the vascular system in the renal graft or recipient. The functional capacity of renal tubular cells contributes significantly to adequate renal function. Hence, measures taken to ameliorate the condition of these cells may also improve the outcome of kidney transplantation.21 We found a statistically significant relationship between the highest expression of PARP-1 and delayed renal function, with a moderate or severe PARP-1 expression (mean 2.64 ± 0.68) in all kidneys suffering >10 hours of cold ischemia and a mild expression (1.26 ± 0.86) in those with ≤10 hours of cold ischemia.

Although long-term inhibition of PARP activity is likely to be harmful to the cell, it has been proposed that its transient inhibition after I/R injury may prevent cell death.5 Recovery from renal function depends not only on the replacement or regeneration of deleted cells, the theme of many recent studies, but also on the protection of cells from death. In vitro, necrotic cell death was induced by the exposure of LLC-PK-1 tubular cells to H₂O₂, whereas 3-aminobenzamide, a PARP inhibitor, completely prevented this H₂O₂-induced necrosis.22 Moreover, in a heterotopic rat heart transplant model of early I/R injury, the administration of PARP-1 inhibitors reduced the release of cardiospecific damage markers.23,24 These results suggest a role for PARP-1, with subsequent NAD(+) and ATP depletion, in acute tubular necrosis in renal transplantation. The administration of PARP-1 inhibitors may represent a therapeutic option to reduce damage from I/R in donor kidneys with intense PARP-1 expression by preventing or minimizing ATN.

REFERENCES