Role of Poly (ADP-Ribose) Polymerase in Kidney Transplant and Its Relationship With Delayed Renal Function: Multivariate Analysis


ABSTRACT

Kidney allografts undergo pretransplant cold ischemia and consequent ischemia-reperfusion injury (IR). Poly (ADP-Ribose) polymerase (PARP-1) overactivation leads to massive NAD+ consumption and ATP depletion with induction of cellular necrosis under ischemic conditions, which may lead to an increase in acute tubular necrosis (ATN) and a delay in total recovery of renal function (RFR) of the transplanted organ.

Materials and Methods. Nuclear PARP-1 immunohistochemical expression (clone: PARP01) was studied in 155 paraffin-embedded renal biopsies from suboptimal donors and 95 kidney allograft biopsies with histopathological diagnosis of ATN.

Results. In 50% of ATN biopsies, more than 50% of tubular nuclei were immunostained for PARP-1. PARP-1 expression was higher in ATN biopsies than in those from suboptimal donors (2.40 ± 0.74 vs 0.92 ± 1.13, P = 0.0001 Mann-Whitney). PARP-1 showed a statistically significant relationship with the time required to achieve effective diuresis (Rho:0.779), with serum creatinine, and with duration of cold ischemia (Rho: 0.803). These relationships were stronger in the biopsies with ATN.

In conclusion, multivariate analysis demonstrated that PARP-1 expression and cold ischemia duration in kidney biopsies with ATN predicted the short-term delay in total recovery of renal function and serum creatinine in the first month.

A CUTE 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.1 Renal ischemia is a major cause of acute renal failure, whether or not caused during transplantation, initiating a complex and interrelated sequence of events that result in the injury and eventual death of renal cells.2,3 Salahudeen et al recently studied 6465 kidney transplant patients using UNOS data and concluded that prolonged cold ischemia is a strong risk factor for delayed graft function and is also a significant predictor of long-term graft loss,4 as previously reported.5,6 The prognosis is complicated because reperfusion, although essential for the survival of ischemic renal tissue, causes additional damage (reperfusion injury).7,8 Which contributes to the renal dysfunction and injury associated with ischemia-reperfusion (I/R).2,4 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.1,3,8

Poly(ADP-ribose)polymerase (PARP-1) is a nuclear zinc-finger DNA-binding protein with a molecular weight of 113 kDa that specifically detects DNA-strand breaks or nicks produced by different genotoxic agents in mammalian cells.9 PARP-1 catalyzes the ADP ribosylation of proteins using NAD(+) as the substrate.10 The activation of PARP is a consequence of ischemic injury and results in a depletion of intracellular NAD(+).11 which can only be replic-
ished via a reaction that consumes ATP. 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 PARP activity protects cellular genome integrity, its excessive activation can lead to cell death secondary to ATP depletion\textsuperscript{12–14} (for general review see Virag and Szabo\textsuperscript{15} and Nguewa et al\textsuperscript{16}). Our group previously demonstrated PARP-1 expression in the tubules of kidneys from aging donors and its significant statistical relationship with parameters of functional reserve (serum creatinine and time required to achieve effective diuresis).\textsuperscript{17}

Mangino et al\textsuperscript{18} recently demonstrated in an experimental canine model that: (1) renal cortical PARP enzyme activity increases with cold-storage time after the tissues are reperfused; (2) cold storage without reperfusion does not increase PARP activity; (3) the cold storage/reperfusion-induced increase in PARP activity is abrogated when the tissues undergo prior warm ischemia; and (4) PARP activity in cold-stored renal tissue may be dependent on the degree of preservation stress because PARP increased after reperfusion after 24 hours of cold storage only in kidneys flushed with Lactate Ringer solution compared with University of Wisconsin-flushed kidneys.

The present study provides statistical evidence in support of the hypothesis that increased tubular expression of PARP-1 in human allograft kidneys that are suboptimal or develop posttransplant ATN contributes to a subsequent delayed renal function.

MATERIALS AND METHODS

One hundred fifty-five human kidney wedge biopsies from suboptimal donors at our hospital were fixed in Glyofix (Pacisa-Giralt, Barcelona, Spain) and paraffin-embedded by a microwave-accelerated technique\textsuperscript{19}; 95
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Paraffin-embedded kidney cylinders were fixed in 10% buffered formalin and paraffin-embedded by standard procedure to determine the degree of preservation stress because PARP increased after reperfusion after 24 hours of cold storage only in kidneys flushed with Lactate Ringer solution compared with University of Wisconsin-flushed kidneys.

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model of short-term renal function according to tubular expression levels of PARP-1, time of cold ischemia, and delay in total recovery of renal function in patients with kidneys from suboptimal donors or kidneys with ATN (R = 0.599, P = 0.06; R = 0.667 P = 0.001 [ANOVA]; respectively).

DISCUSSION

A prolonged cold ischemia time is a strong risk factor for delayed graft function, graft loss\(^4\)\(^-\)\(^10\) and long-term changes after kidney transplantation.\(^1\)\(^8\) The present study revealed a variable intensity of immunohistochemical expression of PARP-1 in tubular cells of kidneys from suboptimal donors and demonstrated that a lesser tubular expression of PARP-1 was related to an earlier recovery of renal function. Likewise, this research revealed a high intensity of PARP-1 immunohistochemical expression in the tubular cells of human transplant kidneys with ATN compared with those from suboptimal donors and demonstrated a relationship between a higher tubular expression of PARP-1 and a delayed renal function. In addition, transplanted kidneys in patients with serum creatinine levels that did not reduce to below 1.7 mg/dL presented almost double the intensity of PARP-1 expression. These findings indicate that the degree of PARP-1 activation may be related to the extent of human renal tubular injury and to the renal function.

Activation of the PARP-1 pathway, a recently discovered cell injury mechanism,\(^20\)\(^-\)\(^2\) 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 via 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 were significantly longer for the kidneys in patients with ATN than in those from suboptimal donors (Table 1). 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,\(^22\)\(^-\)\(^24\) 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 (cold ischemia ≤24 hours, PARP-1 = 1.71 ± 0.62 vs cold ischemia ≥24 hours, PARP-1 = 2.86 ± 0.350). In fact, the lowest PARP-1 expression (1%–9% of tubular nuclei positive) was only observed in kidneys with less than 24 hours of cold ischemia (mean, 16.36 hours range, 12 to 20 hours).\(^25\)

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.\(^26\) We found a statistically significant relationship between a higher expression of PARP-1 and delayed renal function, observing a moderate or intense 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 cold ischemia of ≤10 hours.

In conclusion, multivariate analysis demonstrated that PARP-1 expression and duration of cold ischemia in kidney biopsies with ATN predicted the short-term delay in total recovery of renal function and serum creatinine in the first month.

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REFERENCES