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Early treatment with xenon protects against the cold ischemia associated with chronic allograft nephropathy in rats

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Chronic allograft nephropathy (CAN) is a common finding in kidney grafts with functional impairment. Prolonged hypothermic storage–induced ischemia-reperfusion injury is associated with the early onset of CAN. As the noble gas xenon is clinically used as an anesthetic and has renoprotective properties in a rodent model of ischemia-reperfusion injury, we studied whether early treatment with xenon could attenuate CAN associated with prolonged hypothermic storage. Exposure to xenon enhanced the expression of insulin growth factor-1 (IGF-1) and its receptor in human proximal tubular (HK-2) cells, which, in turn, increased cell proliferation. Xenon treatment before or after hypothermia-hypoxia decreased cell apoptosis and cell inflammation after reoxygenation. The xenon-induced HK-2 cell proliferation was abolished by blocking the IGF-1 receptor, mTOR, and HIF-1α individually. In the Fischer-to-Lewis rat allogeneic renal transplantation model, xenon exposure of donors before graft retrieval or recipients after engraftment enhanced tubular cell proliferation and decreased tubular cell death and cell inflammation associated with ischemia-reperfusion injury. Compared with control allografts, xenon treatment significantly suppressed T-cell infiltration and fibrosis, prevented the development of CAN, and improved renal function. Thus, xenon treatment promoted recovery from ischemia-reperfusion injury and reduced susceptibility to the subsequent development of CAN in allografts.

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Transplantation remains the preferred treatment for patients suffering from end-stage renal failure. Despite improved postoperative immunosuppression regimens, the majority of late graft failures are attributable to chronic allograft nephropathy (CAN).[1] Histologically, renal graft with CAN is characterized by intimal thickening of arteries, glomerulosclerosis, tubular interstitial fibrosis, and renal atrophy. The clinical course of CAN is manifested as a progressive deterioration in renal function, in combination with proteinuria and aggravation of *de novo* hypertension.[2] The etiology of CAN remains to be elucidated, but it is widely believed to be the end result of cumulative damage to the renal grafts associated with immune and nonimmune factors.[3] A wide range of clinical evidence has demonstrated that ischemia-reperfusion injury (IRI) is one of the vital events ultimately leading to the development of CAN.[4,5] Investigations into an effective renoprotective strategy are therefore needed in order to enhance early graft functional performance. Consequently, the development of CAN could be prevented.

The noble gas xenon is clinically used as an anesthetics[6] and has recently been shown to possess therapeutic value as an organo-protectant against IRI.[7–11] The aim of the present study was to evaluate the efficacy of xenon in preventing IRI and CAN after allogeneic kidney transplantation in rats.

RESULTS

Prolonged cold ischemia led to an earlier onset of CAN

The relationship between the duration of cold ischemia and development of CAN was first investigated in the Fisher-to-Lewis renal transplantation model. Renal grafts were stored in cold preserving solution for up to 16 h and then transplanted into the recipient. As demonstrated in Figure 1a, more severe tubular injury was found in ischemic allografts than in non-ischemic allografts on day 1 after surgery. In allografts with 16 h cold ischemia, severe tubular damage, interstitial fibrosis, and cell infiltration were observed. Two months after transplantation, tubular, vascular, and glomerular lesions were more evident in

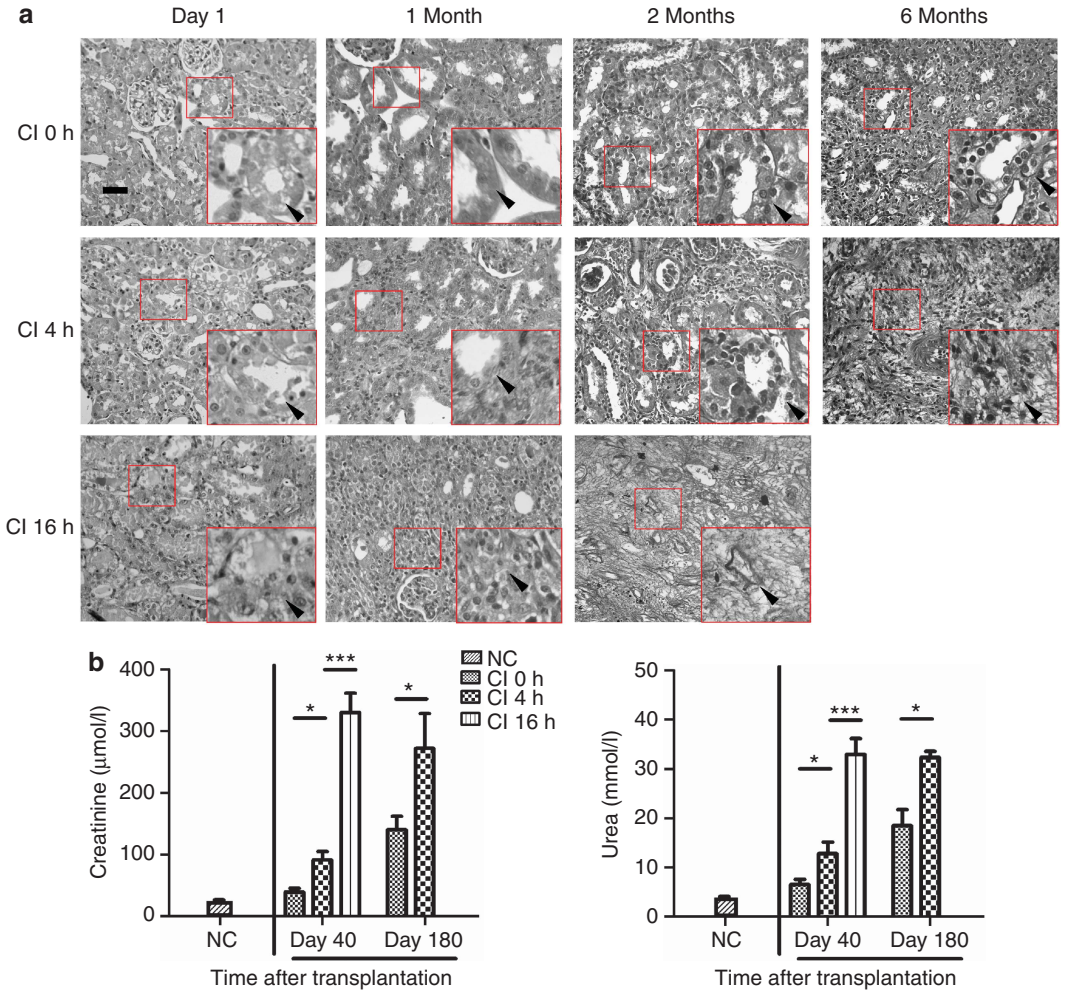

Figure 1 | Prolonged cold ischemia (CI) induced early onset of chronic allograft nephropathy. The Fischer renal graft was stored in 4 °C Soltran preserving solution for 0 h (quick flush, immediate engraftment), 4 h, or 16 h (CI) and then transplanted into Lewis recipient. (**a**) Histology (H and E staining) of the allograft with CI 0–16 h on day 1, 1 month, 2 months, or 6 months after surgery. (**b**) Serum creatinine and urea concentration on days 40 and 180 after surgery. Data are expressed as mean ± s.d. ($n = 4$, $^{*}P < 0.05$ and $^{***}P < 0.001$). Bar = 50 μm. Arrow indicates injury in renal tubules. NC, naive control.

ischemic allografts than in non-ischemic allografts. No grafts with 16 h cold ischemia were able to survive beyond 3 months. Six months after surgery, tubular atrophy and vascular obliteration were more widely observed in ischemic grafts than in non-ischemic grafts. On day 40 after transplantation, significantly higher serum creatinine or urea levels were recorded in recipients with ischemic grafts than in non-ischemic grafts. Renal function deteriorated more rapidly in ischemic grafts than in non-ischemic grafts 6 months after transplantation (Figure 1b).

Xenon exposure caused upregulation of insulin growth factor-1 (IGF-1) and IGF-1R

Increased production of IGF-1 was observed 24 h after xenon exposure on human proximal tubular cell line HK-2 ($P < 0.05$; Figure 2a). Flow cytometry analysis demonstrated that the expression of IGF-1R increased 24 h after gas exposure ($P < 0.05$; Figure 2b). Activation of the IGF-1 signaling pathway is involved in cell cycle progression,[12,13] and the potential role of xenon treatment in relation to IGF-1 in this respect was then explored. The cell cycle transition regulatory proteins cyclin D and cyclin E were found to be increased in HK-2 cells after xenon exposure (Figure 2c and d); this suggested that xenon treatment promotes progression of the cell cycle through enhanced IGF-1/IGF-1R signaling, and that cyclin D and and cyclin E are possibly involved in the signaling network of IGF-1.

Xenon exposure reduced cell death and cell inflammation after hypoxia-reoxygenation

Xenon treatment induced continuous increase in the number of Ki-67^{+} cells (a marker of proliferation) up to 24 h after exposure (Figure 3a and b). Hypoxia-inducible factor-1α (HIF-1α) is the established downstream mediator of trophic stimuli of IGF-1 and promotes cell survival and cell proliferation.[14] To confirm the increased proliferation of HK-2 cells as a direct consequence of HIF-1α activation, the localization of HIF-1α and Ki-67 on HK-2 cells was assessed