Differential resolution of inflammation and recovery after renal ischemia–reperfusion injury in Brown Norway compared with Sprague Dawley rats

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To investigate mechanisms conferring susceptibility or resistance to renal ischemia, we used two rat strains known to exhibit different responses to ischemia–reperfusion. We exposed proximal tubule cells isolated from Sprague Dawley or Brown Norway rats, to a protocol of hypoxia, followed by reoxygenation *in vitro*. The cells isolated from both rat strains exhibited comparable responses in the disruption of intercellular adhesions and cytoskeletal damage. *In vivo*, after 24 h of reperfusion, both strains showed similar degrees of injury. However, after 7 days of reperfusion, renal function and tubular structure almost completely recovered and inflammation resolved, but only in Brown Norway rats. Hypoxia-inducible factor-dependent gene expression, ERK1/2, and Akt activation were different in the two strains. Inflammatory mediators MCP-1, IL-10, INF-γ, IL-1β, and TNF-α were similarly induced at 24 h in both strains but were downregulated earlier in Brown Norway rats, which correlated with shorter NFκB activation in the kidney. Moreover, VLA-4 expression in peripheral blood lymphocytes and VCAM-1 expression in kidney tissues were initially similar at 24 h but reached basal levels earlier in Brown Norway rats. The faster resolution of inflammation in Brown Norway rats suggests that this strain might be a useful experimental model to determine the mechanisms that promote repair of renal ischemia–reperfusion injury.

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Acute tubular necrosis (ATN) due to ischemia–reperfusion (I/R) is observed in important clinical settings, such as patients in intensive care units and those undergoing kidney transplantation.[1] In this situation, ATN contributes to most cases of delayed graft function, is related to higher immunogenicity and is a predictive factor for poor allograft outcome.[2]

ATN is characterized by proximal epithelial cell shedding, cell death inflammation in renal parenchyma and kidney dysfunction.[3] Mechanisms responsible for ATN development have been identified by experimental models, such as the widely used 45-min of bilateral renal ischemia in rats. In this model, analogous to humans, moderate damage occurring mainly in the proximal tubules and recovery after some days of reperfusion were observed.[4] *In vitro* models have also shed light on the mechanisms underlying proximal tubule cell response to ischemia.[5] However, in spite of the advances in experimental models, not much progress has reached clinical practice.

Genetically modified animals could be used to identify new targets to improve ATN outcome. Unfortunately, genetic modification in rats is difficult. Therefore, some rat strains have been selected for their different response or susceptibility to ischemia. Indeed, some reports have identified inbred Brown Norway rats as more resistant to cardiac ischemia than other rat strains,[6,7] including the outbred Sprague Dawley rats. Resistance to the development of tubular damage after renal ischemia was also described in Brown Norway rats.[8,9] These reports indicated higher levels of heat-shock proteins and ablated oxidative stress in Brown Norway rats as putative mechanisms contributing to this resistance.

In this study, we used Brown Norway and Sprague Dawley rats, as well as primary cultures of their proximal epithelial cells to elucidate new mechanisms underlying the resistance/susceptibility to renal ischemia and to identify novel targets for experimental intervention in ATN. We demonstrate that Brown Norway rats recovered earlier after I/R, thus suggesting Brown Norway rats as a useful experimental model to design new therapeutic approaches for ATN resolution.

RESULTS

Renal damage and dysfunction in Brown Norway and Sprague Dawley rats

We performed 45 min of bilateral ischemia and 24 h of reperfusion in male Brown Norway and Sprague Dawley rats obtained from our own breeding facility. Renal injury is maximal at this time point in this model.[10] Sham-operated animals were used as controls. Renal function was estimated by urea and creatinine levels in serum. After periodic acid-Schiff (PAS) staining of paraffin-embedded renal sections, histological damage was scored according to the following criteria: proximal tubule cell morphology alterations, brush border loss, proximal tubular dilation and denudation, as well as the presence of casts and infiltrates.

Sprague Dawley and Brown Norway rats showed similar high urea and creatinine levels at 24 h after ischemia (Figure 1a). Accordingly, histological examination after PAS staining also showed patched ATN in both Sprague Dawley and Brown Norway rats at 24 h of reperfusion (Figure 1b). In addition, phalloidin staining of renal tissue cryosections showed similar brush border loss and actin cytoskeleton disorganization (Figure 1b). Histopathological scoring confirming similar I/R injury is shown in Table 1.

To verify that this unexpected susceptibility of Brown Norway rats was not a feature of our own colony, we also studied Brown Norway rats obtained from two widely recognized commercial suppliers (Harlan Laboratories Models, Boxmeer, The Netherlands and Charles River Laboratories International, Margate, UK) (Figure 1c). Renal function at 24 h of reperfusion was similarly affected in Brown Norway rats from our colony and from the other two suppliers: BN/RijHsd (Harlan Laboratories Models) and BN/OrlCrl (Charles River Laboratories International). Accordingly, similar injury was observed in PAS-stained tissue sections of Brown Norway rats obtained from both suppliers (data not shown).

These results demonstrate that both Sprague Dawley and Brown Norway rats develop similar acute renal injury after I/R.

Primary proximal tubule cells from both Sprague Dawley and Brown Norway rats exhibit damage in response to hypoxia/reoxygenation

We further analyzed the response of Sprague Dawley and Brown Norway rats to I/R using primary cultures of their proximal tubule cells after hypoxia/reoxygenation. We previously demonstrated that this protocol provokes, in the rat proximal cell line NRK-52E, cytoskeleton alterations, focal adhesion disassembly, cell–cell adhesion impairment, and cell shedding, all features evidenced in the I/R rat model.[5]

Primary cells under hypoxia/reoxygenation were stained with phalloidin and anti-zonula occludens-1 (ZO-1) antibody to observe actin cytoskeleton organization and determine cell–cell adhesion integrity, respectively. Hypoxia/reoxygenation induced marked actin cytoskeleton disorganization and depolymerization in primary cells from both strains. The lower signal for F-actin detectable at 3 h of reoxygenation in both cases, indicating actin depolymerization (Figure 2a) is noted. Cytokeratin immunostaining was used as an epithelial marker. In addition, ZO-1 internalization from the plasma membrane was evident early during reoxygenation in cells from Sprague Dawley and Brown Norway rats, indicating that in both cases, cell–cell adhesion is similarly affected in response to hypoxia/reoxygenation (Figure 2b).

These results demonstrate that proximal tubular cells from Sprague Dawley and Brown Norway rats exhibit similar injury in response to hypoxia/reoxygenation and support our *in vivo* data.

Sprague Dawley and Brown Norway rats exhibit differential intracellular signaling in response to ischemia

To analyze the renal response of both strains to ischemia, we extended the studies up to 15 days of reperfusion when recovery of renal function is observed in this *in vivo* model.[10] We studied the activity of signaling pathways involved in cell survival and proliferation, oxidative stress response, and adaptation to hypoxia, such as the extracellular signal-regulated kinase (ERK1/2), serine–threonine protein kinase Akt (AKT), manganese superoxide dismutase (MnSOD), and the hypoxia-inducible factor-1 alpha (HIF-1α) target genes: prolyl-hydroxylase-3 (*PHD-3*), vascular endothelial growth factor (*VEGF*), and thrombospondin-1 (*TSP-1*).

Western blot of total renal lysates showed clear differences in cell signaling triggered by I/R in both strains. As shown in Figure 3a, Brown Norway rats had a marked induction of p-AKT at 24 h and 5 days of reperfusion. Notably, p-ERK 1/2 was markedly induced after ischemia in Brown Norway rats. MnSOD levels were slightly higher in Brown Norway than in Sprague Dawley rats, in the whole I/R kinetic.

Using real-time PCR, we assessed the expression of HIF-1α target genes, namely *VEGF*, *TSP-1*, and *PHD-3* (Figure 3b). Both strains showed a similar expression at 24 h, but *VEGF* and *PHD-3* remained higher in Brown Norway, later in reperfusion. In contrast, TSP-1, which has been described as a marker of renal ischemic damage,[11] was higher in Sprague Dawley rats.

Although I/R-induced injury is similar in both Brown Norway and Sprague Dawley rats, these results indicate that both strains exhibit a different response to ischemia and recovery mechanisms might be differently triggered in time. Therefore, a distinct outcome might be expected.

Brown Norway rats show faster recovery from I/R injury

Differences in gene expression and cell signaling could account for a different outcome of ischemic injury as suggested above. Thus, we studied renal function and kidney structure during I/R up to 15 days after ischemia.

As presented in Figure 4, serum urea and creatinine values, as well as creatinine clearance, normalized in both rat strains after 5–7 days of reperfusion, consistent with reported data.[10] However, the improvement in renal function measured by serum creatinine clearance was observed earlier in Brown Norway rats, that is, around day 3.