Delayed Contralateral Nephrectomy

Subjects: Pathology

Contributor: Peter Hamar

Background: Successful treatment of acute kidney injury (AKI)-induced chronic kidney disease (CKD) is unresolved. We aimed to characterize the time-course of changes after contralateral nephrectomy (Nx) in a model of unilateral ischemic AKI-induced CKD with good translational utility. (2) Methods: Severe (30 min) left renal ischemia-reperfusion injury (IRI) or sham operation (S) was performed in male Naval Medical Research Institute (NMRI) mice followed by Nx or S one week later. Expression of proinflammatory, oxidative stress, injury and fibrotic markers was evaluated by RT-qPCR. (3) Results: Upon Nx, the injured kidney hardly functioned for three days, but it gradually regained function until day 14 to 21, as demonstrated by the plasma urea. Functional recovery led to a drastic reduction in inflammatory infiltration by macrophages and by decreases in macrophage chemoattractant protein-1 (MCP-1) and tumor necrosis factor-alpha (TNF-α) mRNA and most injury markers. However, without Nx, a marked upregulation of proinflammatory (TNF-α, IL-6, MCP-1 and complement-3 (C3)); oxidative stress (nuclear factor erythroid 2-related factor 2, NRF2) and fibrosis (collagen-1a1 (Col1a1) and fibronectin-1 (FN1)) genes perpetuated, and the injured kidney became completely fibrotic. Contralateral Nx delayed the development of renal failure up to 20 weeks. (4) Conclusion: Our results suggest that macrophage activation is involved in postischemic renal fibrosis, and it is drastically suppressed by contralateral nephrectomy ameliorating progression.

Keywords: acute kidney injury ; renal fibrosis progression ; macrophage derived inflammation ; tnf-alpha ; nephrectomy

1. Introduction

Acute kidney injury (AKI) is an acute and serious reduction in kidney function diagnosed by an increase in serum creatinine and/or by decreased urine output (KDIGO) [1]. AKI is a frequent complication among hospitalized patients, but the true incidence of AKI is underestimated [2]. Patients with AKI have a higher risk to subsequently develop chronic kidney disease (CKD) and end-stage renal disease (ESRD) [3][4]. CKD is characterized by constantly declining glomerular filtration rate (GFR), the presence of various markers of kidney damage (e.g., proteinuria) and progressive fibrosis [5].

2. Causes

The leading causes of AKI are sepsis [6][7] and ischemia-reperfusion injury (IRI) [8][9]. Severe impairment of blood supply to the kidney triggers endothelial injury [10] and leads to the release of damage-associated molecular patterns (DAMPs), which increases the expression of monocyte chemoattractant protein-1 (MCP-1), also known as C-C motif chemokine 2 (CCL2). One of the main cytokines produced by macrophages is tumor necrosis factor-alpha (TNF-α). Thus, macrophage infiltration leads to increased TNF-α production [8][11][12][13]. Macrophage infiltration was reduced in mice deficient in C-C chemokine receptor type 2 (CCR2), the receptor of MCP-1. Additionally, these mice were largely protected from IRI-induced tubular necrosis [14]. Macrophages play a central role in the repair process following injury [15], but their sustained activation is a major contributor to the transition from repair to fibrosis. Especially in the setting of unilateral IRI, macrophages persisted beyond the time of repair, and MCP-1/CCR2 signaling played an important role in the cross-talk between injured tubular cells and infiltrating immune cells and myofibroblasts and promoted sustained inflammation and tubular injury with progressive interstitial fibrosis in the late stages of U-IRI [16]. The best-characterized profibrotic cytokine transforming growth factor-beta (TGF-β) activates myofibroblast formation and the production of fibroblast markers [17]. Myofibroblasts—characterized by alpha-smooth muscle actin (α-SMA) expression—are mainly responsible for the extracellular fibrotic matrix (fibronectin (FN) and collagen-1a1 (Col1a1)) deposition [18]. In summary, following severe AKI, the infiltrating macrophages play a central role in the transition of postischemic repair into progressive renal fibrosis characterized by glomerular sclerosis and tubulointerstitial fibrosis [3][19].

3. Models

There are two distinct models of ischemic AKI in animals, the bilateral and the unilateral IR, induced by occluding the renal pedicles [11][20]. The contralateral kidney can remain intact [21] or can be removed either during the surgery [22][23] or later [24][25]. Surprisingly, delayed contralateral nephrectomy (Nx) induced partial functional recovery of the postischemic kidney, even following a severe (30-min) IRI, as described first by Finn WF [26] and, recently, by Skrypnyk et al. [24]. However, the mechanisms of functional recovery of the postischemic kidney are unknown. Furthermore, the roles of increased MCP-1 expression, consequent macrophage infiltration and enhanced TNF-α production after Nx have not yet been studied.

Our aim was to evaluate the magnitude and time-course of molecular mechanisms that contribute to the functional recovery of a postischemic and nonfunctioning kidney after Nx. For this reason, we investigated molecular events at various times up to three weeks after Nx. In a long-term experiment, we also followed the development of ESRD in the Nx group. ESRD of the postischemic kidney was delayed to about 140 days. Thus, delayed contralateral nephrectomy is an excellent model to study the mechanisms of renal fibrosis progression/reversal. Markers of renal fibrosis, oxidative stress and, especially, macrophage infiltration were drastically reduced by nephrectomy, suggesting a central role for macrophage activation in the development of ESRD in the postischemic kidney.

The main finding of the study is that the kidney becomes atrophic after unilateral ischemic injury in the presence of a healthy kidney. The postischemic kidney did not recover from the ischemic insult, as the plasma urea substantially increased for several days upon removal of the healthy kidney. Such a functional failure was related to increases in inflammatory, fibrotic and oxidative processes in the postischemic kidney. However, contralateral Nx induced a slow recovery of the postischemic kidney, leading to a drop in the plasma urea. In parallel, the expression of several pathogenic molecules, especially those of macrophage-driven inflammation drastically decreased. Similar studies have demonstrated previously that contralateral Nx at the time of renal ischemia [27] or two weeks later [28] can delay the progression of postischemic injury to end-stage renal fibrosis. However, these authors selected different time points for removal of the functional right kidney, and they did not investigate the time-course of progression and its amelioration by contralateral Nx or the long-term outcome with a late follow-up to demonstrate the development of fibrosis in the absence of a noninjured kidney.

Regarding the pathomechanisms behind the observed rapid fibrosis in IR-S mice and the halted progression following Nx in IR-Nx mice, we investigated inflammatory, hypoxia-driven and fibrotic processes.

We observed ongoing inflammatory processes in the postischemic kidney (IR-S group) at day 8 marked by TNF-α, MCP-1, IL-6 and C3 mRNA production, reaching their peak on day 10. The strongest upregulation was observed in the case of C3 (280-fold on day 10) and MCP-1 (125-fold on days 8–14). Although the C3 peak was the highest, it was also the shortest, as C3 upregulation diminished already by day 14. IL-6 and C3 elevations were much lower on day 28 than before. On the contrary, TNF-α remained as elevated as before even on day 28, and MCP-1 also remained significantly elevated, accompanied by strong inflammatory and F4/80+ infiltration throughout the observation period.

Thus, in postischemic kidneys after a contralateral sham operation, there was a strong complement (C3) upregulation. C3 as an anaphylatoxin is a potent proinflammatory mediator [29]. Although, C3 is produced mainly by hepatocytes [30][31], immune cells (including monocytes and tissue resident macrophages) can also secrete C3 [32]. Local C3 synthesis in the kidney has been linked to the progression of renal diseases [33][34]. C3 peak was the shortest, but—together with a sustained MCP-1 upregulation—macrophages were attracted to the inflamed kidney, as demonstrated by the F4/80 staining. Macrophages may be the main source of TNF-α in this setting, as C3, MCP-1, TNF-α and F4/80 infiltration reached their peak simultaneously on day 10, and both F4/80-positive cells and TNF-α and MCP-1 mRNA remained elevated on day 28, when the other inflammatory markers (C3 and IL-6) were fading away.

Prior to the functional recovery of the affected kidney, nephrectomy (Nx) decreased the expression of proinflammatory mRNAs (IR-Nx group). Based on the extreme elevations in C3, MCP-1 and TNF-α expression, we suspected a central role for macrophages. This hypothesis was supported by the extent of macrophage infiltration, as demonstrated by F4/80 staining. Following Nx, macrophage infiltration decreased in the kidney, strongly suggesting a pivotal role of macrophage-driven inflammation in ischemia-induced renal fibrosis. These observations are in-line with several previous results showing that, upon IRI, proinflammatory cytokines were significantly upregulated in the kidney [8][35][36]. Especially in the setting of unilateral IRI, persisting MCP-1 production was held responsible for sustained macrophage and myofibroblast infiltrations in the injured tubulointerstitium promoting fibrosis [16], supporting our conclusion that macrophages play a central role in this setting.

There is a long debate on hypoxia as a driving factor in the progression of CKD [37][38][39][40]. In our study, two isoforms of hypoxia-inducible factor were studied: HIF-1α and HIF-2α. At this late time interval (8–28 days), after the ischemic insult, the expression of these hypoxia-inducible factors did not seem to play a major role anymore, as their upregulation was only 1.7-fold. This observation suggests that hypoxia was mild when the kidneys regained their functional activity. It has been demonstrated, however, that HIFs exert their effect mainly by nuclear translocation [41], and their activity is not primarily regulated at the level of gene expression. Furthermore, Nx prevented HIF-1α and -2α upregulation, suggesting a pathogenic role of the ongoing mild HIF upregulation.

NRF2 elevation was somewhat higher (2.4-fold) than HIF and was similarly and constantly upregulated during the observation period, including day 28, suggesting a mild, ongoing oxidative stress in the kidney undergoing fibrosis (IR-S group). These observations are in-line with numerous previous studies, reporting protective effects of NRF2 in IRI [42]. Our findings also correlate with the results of Skrypnyk et al. [43], as a 28-day antioxidant treatment dose-dependently reduced fibrosis in the same animal model as used in our study.

In postischemic kidneys after a contralateral sham operation, profibrotic factors (TGF-β, α-SMA, Col1A1 and FN1) were already elevated by the beginning at day 8 and remained similarly elevated throughout the observation period, until day 28, suggesting an ongoing fibrogenesis. Nx significantly reduced the expression of all of these profibrotic factors but did not reduce them to control levels. Thus, ESRD developed in the postnephrectomy (IR-Nx) animals as well, but in 140 instead of 28 days.

Nx also reduced α-SMA mRNA from day 14, suggesting that myofibroblasts or their activity was reduced two weeks after Nx. Myofibroblasts are a main source of ECM deposition during fibrogenesis [44]. Accordingly, the progressively increasing effect of Nx on the production of extracellular matrix proteins (Col1A1 and FN1) suggests that Nx reversed the fibrotic matrix deposition. However, the already deposited matrix, as well as the sustained TGF-β and Col1A1 production by myofibroblasts, were enough to finally lead to ESRD. Similar ongoing fibrosis leading to ESRD was demonstrated by Chancharoenthana et al., who found high serum creatinine 20 weeks after 50-min IRI followed by delayed Nx in CD-1 male mice [45].

As macrophage infiltration-driven inflammation, as well as myofibroblast-driven matrix deposition and hypoxia, were significantly inhibited by Nx, the ongoing renal tubular damage was reversed, as demonstrated by Lcn-2, which began to decrease on day 14. Simultaneously, the excretory function of the postischemic kidney recovered, as demonstrated by continuously decreasing blood urea retention. Lcn-2 fluctuated in parallel with plasma urea during the study, and both markers increased sharply on the last week, indicating the development of renal failure.

In conclusion, severe unilateral ischemia-reperfusion injury with delayed contralateral nephrectomy offers a reproducible model to investigate the functional recovery of a nonfunctioning, fibrosing kidney. According to the results of our study, macrophage-driven inflammatory processes and subsequent reductions of the fibrotic matrix production and oxidative stress play important roles in the observed functional recovery and delayed progression to end-stage fibrosis.

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