Establishment of an experimental rat model of tacrolimus-induced kidney injury and protective effects of hypoxia-inducible factor prolyl hydroxylase (HIF-PHD) inhibitors

付, 饒

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Establishment of an experimental rat model of tacrolimus-induced kidney injury and protective effects of hypoxia-inducible factor prolyl hydroxylase (HIF-PHD) inhibitors

（分野名）臨床薬物治療学分野　（学籍番号）3PS18105M　（氏名）付　饒

【目的】

The calcineurin inhibitor tacrolimus is the cornerstone of most immunosuppressive regimens in kidney transplantation. With tacrolimus use, the survival rate of transplants 1 year after kidney transplantation is about 90%, with the incidence of acute rejection being less than 20%. However, long-term use of tacrolimus causes chronic, structural, progressive, and irreversible nephrotoxicity, which is thought to be the primary cause of chronic allograft dysfunction. Tacrolimus causes kidney injury, including arteriolar hyalinosis, tubular atrophy, interstitial fibrosis, and glomerulosclerosis, which can lead to irreversible kidney dysfunction. Renal interstitial fibrosis is a typical feature of tacrolimus-induced nephrotoxicity, and renal fibrosis represents the common pathway of nearly all chronic and progressive nephropathies, limiting tissue regeneration potential, thereby further reducing kidney function. At present, attempts to elucidate the mechanism underlying tacrolimus-induced renal injury have been hindered by the need for a suitable animal model.

Tubulo-interstitial hypoxia is considered to be a common pathway for progressive kidney disease, while hypoxia-inducible factor (HIF) is a well-known master mediator of hypoxia-adaptive responses in a variety of pathophysiological processes in the kidney diseases. Roxadustat is an oral HIF-prolyl hydroxylase inhibitor (HIF-PHD), that can stabilize HIF and increase iron utilization, thereby stimulating erythropoietin production, and thus, is used as a novel treatment for anemia of chronic kidney disease. Although it has been demonstrated that roxadustat ameliorates acute kidney injury-induced kidney fibrosis, the effects of roxadustat on tacrolimus-induced kidney injury have not been explored.

Therefore, the aims of this study are to develop a new rat model of tacrolimus-induced kidney injury with renal interstitial fibrosis and investigate the effects of roxadustat on the tacrolimus-induced kidney injury in rats.

【方法】

In Chapter 1, we investigated whether administration of tacrolimus for two weeks post kidney ischemia-reperfusion (I/R) could produce an experimental rat model of renal injury with interstitial fibrosis. Before drug administration, renal I/R injury was performed according to a previously published protocol. Rats were anesthetized by means of isoflurane inhalation, with a 4% dose for induction and a 1.5% dose for maintenance. The abdominal cavity was then exposed using midline laparotomy. Subsequently, both renal pedicles were cross-clamped for 45 min. During ischemia, the rats were hydrated with saline and maintained at 37°C using heating plates. Renal clamps were removed after 45 min, and all incisions were closed. After surgery, the animals were placed on the heating plates until recovery from anesthesia, and then moved to cages with free

access to water and standard rat chow. Wistar rats (n=30) were randomly divided into four groups: (1) sham group, sham operation on day 1 and treatment with saline for 14 d (n=7); (2) I/R group, renal I/R injury on day 1 and treatment with saline for 14 d (n=7); (3) TAC group, sham operation on day 1 and treatment with tacrolimus (5mg/kg per day) for 14 d (n=9); (4) TAC+I/R group, renal I/R injury on day 1 and treatment with tacrolimus (5mg/kg per day) for 14 d (n=7). The kidney function was evaluated according to serum creatinine (Scr), blood urea nitrogen (BUN), serum magnesium (Mg), serum potassium (K) levels, and kidney injury molecule-1 (KIM-1) expression. Using hematoxylin and eosin (HE), and periodic acid-Schiff (PAS) to evaluated renal morphology. In addition, the kidney fibrosis was evaluated according to Masson's trichrome staining (MTS), α-smooth muscle actin (α-SMA), transforming growth factor-β (TGF-β). All experimental protocols were approved by the Animal Research Committee of the Graduate School of Medicine, Kyushu University (approval number: A30-029-0).

In Chapter 2, Wistar rats (n = 25) were randomly divided into four groups: (1) sham group, sham operation on day 1 and treatment with saline and vehicle (n=8); (2) ROX group, sham operation on day 1 and treatment with saline and roxadustat (3 mg/kg per 2d, n=4); (3) TAC group, renal I/R injury on day 1 and treatment with tacrolimus (5 mg/kg per day) and vehicle (n=6); (4) TAC+ROX group, renal I/R injury on day 1 and treatment with tacrolimus (5 mg/kg per day) and roxadustat (3 mg/kg per 2d, n=7). Roxadustat was gavage administered two days before renal I/R injury. All experimental protocols were approved by the Animal Research Committee of the Graduate School of Medicine, Kyushu University (approval number: A22-049-0). The kidney function was evaluated according to Scr, BUN, serum Mg and K levels, and KIM-1 expression. PAS was evaluated to assess glomerulosclerosis. In addition, the kidney fibrosis was evaluated according to MTS, α-SMA, and F4/80 for kidney inflammation.

【結果】

Chapter 1 Establishment of an experimental rat model of tacrolimus-induced kidney injury accompanied by interstitial fibrosis

Rats subjected to I/R injury and treated with tacrolimus for 2 weeks showed decreased kidney function, demonstrated by significantly increased Scr and BUN levels, which indicate damaged glomerular function, and increased serum Mg and K as well as uric acid levels, which indicate damaged tubular dysfunction. The increased KIM-1 expression in the TAC+I/R group also indicate kidney injury. According to the Banff 2018 classification, HE staining revealed higher tubular vacuolization score, while PAS staining revealed noticeable glomerulosclerosis in the TAC+I/R group. In addition, our results showed a significant increase in α-SMA and TGF-β expression, as well as increased MTS-positive cells in the TAC+I/R group, as compared to those in the sham, I/R, and TAC groups, indicating that tacrolimus induced interstitial fibrosis (**Figure 1**).

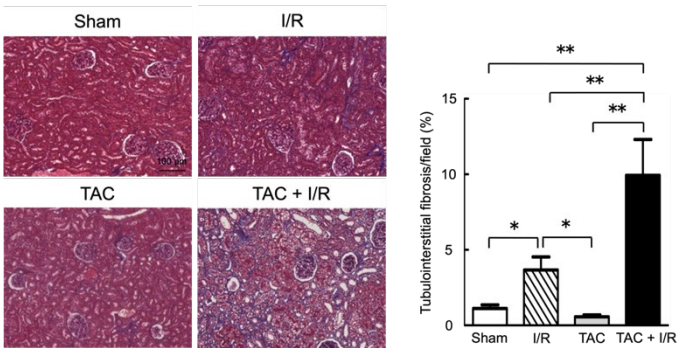

Figure 1. Tacrolimus-induced kidney fibrosis

Chapter 2 The hypoxia-inducible factors prolyl hydroxylase inhibitor roxadustat attenuates tacrolimus-induced renal interstitial fibrosis in rats

The co-administration of roxadustat administration attenuated tacrolimus-induced kidney injury, as evidenced by reduced Scr levels and expression of KIM-1. According to PAS staining, in tacrolimus-induced nephrotoxicity rat treated with roxadustat, glomerular area, mesangial matrix area, and mesangial matrix expansion were significantly inhibited, which indicate that tacrolimus-induced glomerulosclerosis is attenuated by roxadustat administration. MTS staining revealed roxadustat administration significantly reduced MTS-positive cells in tacrolimus-induced nephrotoxicity rat, indicate tacrolimus-induced renal fibrosis is attenuated by roxadustat administration (**Figure 2**). In addition, our results showed roxadustat suppresses tacrolimus-induced inflammation in the kidney cortex proved by reduced F4/80 expression (**Figure 3**).

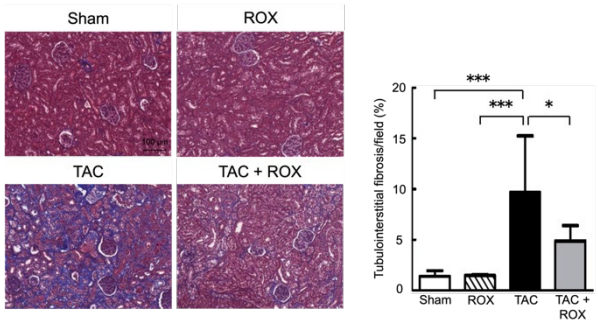

Figure 2. Tacrolimus-induced kidney fibrosis is attenuated by roxadustat administration

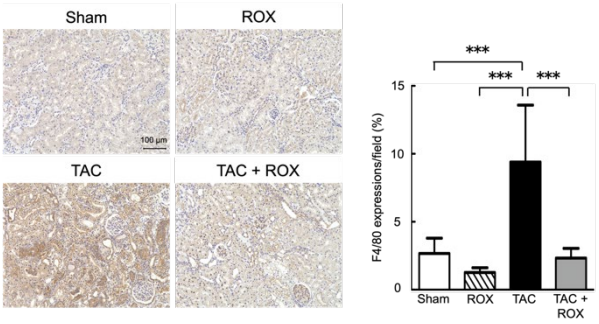

Figure 3. Tacrolimus-induced inflammation is attenuated by roxadustat administration

【考察】

In Chapter 1, we developed a new rat model of tacrolimus-induced kidney injury with renal interstitial fibrosis by 2-week tacrolimus administration following renal I/R. This model would reduce the time required for renal fibrosis development, and showed decreased kidney function, tubular vacuolization, glomerulosclerosis, as well as increased α-SMA, TGF-β, and KIM-1 expression. In addition, our results showed both tacrolimus and I/R injury are induce nephrotoxicity, but tacrolimus or I/R injury alone cannot induce nephrotoxicity, indicating that I/R injury contributes significantly to the development of tacrolimus-induced nephrotoxicity. The injury caused by I/R is inevitable in renal transplant recipients and several studies have already shown that I/R-induced acute kidney injury can lead to renal fibrosis and is an important risk factor for chronic kidney injury. Thus, a possible mechanism is that after I/R injury, the defects in the progression of the cell cycle may switch tubular cells to a pro-fibrotic phenotype, and along with the subsequent damage from tacrolimus. These tubular cells may continue produce pro-fibrogenic growth factors that stimulate fibroblast proliferation and collagen production, thereby accelerating tacrolimus-induced fibrosis in rats.

In Chapter 2, we demonstrated that roxadustat remarkably prevented the tacrolimus-induced kidney injury

via reducing tubule-interstitium fibrosis, glomerulosclerosis, and inflammation. Considering our results in combination with previous reports, we speculate that the mechanisms of the protective effect of roxadustat on tacrolimus-induced kidney injury are as follows: (1) inhibiting the NF-kB pathway thereby reduce inflammation; (2) activating the Nrf2/HO-1 pathway, heme oxygenase-1 (HO-1) which is a free radical scavenger reduces oxidative stress, which is a well-known cause for tacrolimus-induce kidney injury; (3) the activation of HIF enhancing angiogenesis via up-regulation vascular endothelial growth factor thereby reduces tubule-interstitium fibrosis, glomerulosclerosis, and inflammation caused by capillary rarefaction and protects kidney function. To the best of our knowledge, our findings are the first report on the protective effect of roxadustat on the tacrolimus-induced kidney injury in rats. Therefore, this study offered a clinical potential approach for the prevention of tacrolimus-induced kidney injury.

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