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

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論 文 審 査 の 結 果 の 要 旨

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, 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 prevents acute kidney injury-induced kidney fibrosis in mice, the effects of roxadustat on the 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.

Established an experimental rat model of tacrolimus-induced kidney injury with interstitial fibrosis in 2 weeks. 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 ischemia-reperfusion (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). Rats subjected to I/R injury and treated with tacrolimus for 2 weeks showed higher serum creatinine (Scr), blood urea nitrogen (BUN), serum magnesium (Mg) and serum potassium (K) levels, indicating decreased renal function. In addi-

tion, treatment with tacrolimus combined with I/R injury increased histological injury (tubular vacuolation, glomerulosclerosis, and interstitial fibrosis), as well as expression of α-smooth muscle actin (α-SMA), transforming growth factor-β (TGF-β), and kidney injury molecule-1 (KIM-1) in the renal cortex. The results demonstrate a new method for developing tacrolimus-induced kidney injury with renal interstitial fibrosis in a short time. This model would reduce the time required for renal fibrosis development, and showed decreased kidney function, tubular vacuolization, glomerulosclerosis, as well as increased expression of α-SMA, TGF-β, and KIM-1. In addition, the results showed both tacrolimus and I/R injury 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. Thus, this model may help elucidate the underlying mechanisms of tacrolimus-induced kidney injury in future studies.

Next, 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). The co-administration of roxadustat remarkably prevented tacrolimus-induced kidney injury, as evidenced by reduced Scr levels and expression of KIM-1. In tacrolimus-induced kidney injury rat treated with roxadustat, glomerular area, mesangial matrix area, and mesangial matrix expansion were significantly reduced, indicating that tacrolimus-induced glomerulosclerosis is attenuated by co-administration of roxadustat. Roxadustat also significantly reduced tacrolimus-induced tubule-interstitium fibrosis. In addition, the present results showed roxadustat suppresses tacrolimus-induced inflammation in the kidney cortex. These findings are the first report on the protective effect of roxadustat on the tacrolimus-induced kidney injury in rats. Therefore, this study provides useful information for the prevention of tacrolimus-induced kidney injury.

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