# 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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氏　　名　：付　饒

論文題名　：Establishment of an experimental rat model of tacrolimus-induced kidney injury and protective effects of hypoxia-inducible factor prolyl hydroxylase (HIF-PHD) inhibitors（タクロリムス誘発性腎障害の実験的ラットモデルの確立および HIF-PHD 阻害剤の保護効果に関する研究）

区　　分　：甲

## 論　文　内　容　の　要　旨

**Background**

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 chonic kidney disease. Although it has been demonstrated that roxadustat ameliorates AKI-induced kidney fibrosis, the effects of roxadustat in tacrolimus-induced kidney fibrosis have not been explored.

Therefore, the aims of this study are to develop report a new method for developing tacrolimus-induced nephropathy with renal interstitial fibrosis in a shorter time; and investigate the effects of roxadustat in the tacrolimus-induced nephropathy rats.

**Method and Results**

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

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 addition, tacrolimus treatment combined with I/R injury increased histological injury (tubular vacuolation, glomerulosclerosis, and interstitial fibrosis), as well as $\alpha$-smooth muscle actin ($\alpha$-SMA), transforming growth factor-β (TGF-β), and kidney injury molecule-1 (KIM-1) expression in the renal cortex.

**Chapter 2 Effect of roxadustat in the tacrolimus-induced nephrotoxicity rat model**

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). Tacrolimus administration increased Scr, BUN, and KIM-1 levels as well as kidney injuries, such as tubular dilation, vacuolization, renal interstitial fibrosis, glomerulosclerosis, and inflammation in the rats. Roxadustat administration attenuated tacrolimus-induced kidney fibrosis and the associated abnormalities.

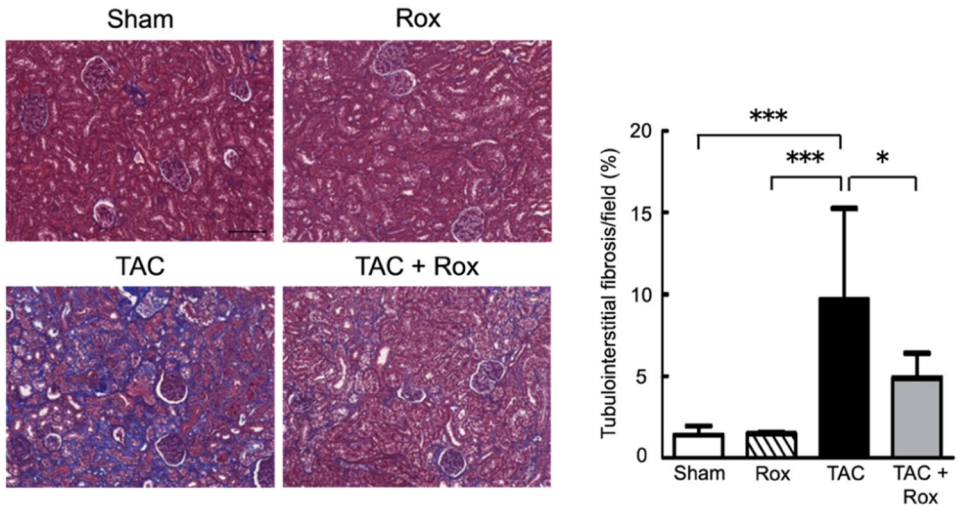


**Figure 9.** Tacrolimus-induced kidney fibrosis.

**Discussion**

In chapter 1, we report a new method for developing tacrolimus-induced nephropathy with renal interstitial fibrosis in a short time. In addition, we found that α-SMA, TGF-β, and KIM-1 were expressed in the kidneys of these tacrolimus-induced nephropathy rats. We hope that this model will help elucidate the mechanism underlying tacrolimus-induced nephropathy in future studies. In chapter 2, we demonstrated that roxadustat remarkably ameliorated tacrolimus-induced kidney injury via reduced tacrolimus, which caused tubule-interstitium fibrosis, glomerulosclerosis, and inflammation, thereby improving kidney function. As per our knowledge, this is the first report on the effect of roxadustat on the tacrolimus-induced nephrotoxicity rats. Therefore, our findings offered a clinical potential approach for the treatment of tacrolimus-induced nephrotoxicity.