253

Lin C[1], Lin A[1], Yang A[1]
1. *Taipei Veterans General Hospital*

KETAMINE ACTIVATE OF THE MTOR DEPENDENT SIGNALING PATHWAY IN THE ENDOTHELIAL INJURY OF KETAMINE CYSTITIS

Hypothesis / aims of study

ketamine-induced cystitis (KC) are chronic inflammation with eroded urothelium, eosinophil infiltration, and neovascularization in the urinary bladder with resultant fibrotic change. Nevertheless, the exact pathogenic signaling pathway remains obscure and controversial. Recently, the microvascular injury in urinary bladder was reported in the patients with ketamine abuse.[1] This study is to investigate the pathogenic role of the mammalian target of the rapamycin (mTOR) activation in the ketamine induced microvascular injury.

Study design, materials and methods

In human study, there were 23 patients with ketamine-induced cystitis (KC) and 16 control volunteers were recruited between September 2010 and August 2011. Bladder tissues were obtained from both groups by cystoscopic biopsies. Phospho-S6 ribosomal protein (p-S6RP), an end product of the mTOR pathway, was stained in the urinary bladder from both groups. Endothelial cells of the urinary bladder (HBdMECs) were examined by western blotting analysis of phospho-mTOR, phosphor-Akt, phospho-S6 ribosomal protein, and phospho-p70 S6 kinase to investigate the in vitro activation of the mTOR pathway. With reference to the clinical application and abuse dose of patients, concentrations of ketamine of 1, 10, 100, and 1000 μM (equivalent to 0.01, 0.1, 1, and 10 times the clinical plasma concentration) were selected as the administered dosages for endothelial cells in this study.[2] Double immunofluorescence stain were used for the co-expression of the endothelial marker [cluster of differentiation 31 (CD31)] and the mesenchymal marker [fibroblast-specific protein 1 (FSP-1)]. Rapamycin (100ng/ml), mTOR inhibitor, was applied with ketamine treatment for blocking the activation of mTOR pathway. Differences in immunohistochemistry staining between the KC and control groups were analyzed by means of the Mann–Whitney U test, with comparisons of HBdMECs between groups analyzed using a one-way analysis of variance (ANOVA) followed by post hoc comparisons using the Bonferroni method.

Results

Expression of p-S6RP increased significantly after ketamine exposure more in the vesical microvessels of KC patients (2.26 ± 0.14) than in control volunteer (0.50 ± 0.18). (Table 1) In HBdMECs treated with 100 μM Ketamine, time-dependent activation of the mTOR pathway occurred, with significantly increased levels of the phosphorylated forms of mTOR at 30 min and of S6RP and p70S6 kinase (p70S6K) at 6 h. The increased level of p-S6RP returned to baseline within 2 days after ketamine exposure.(Figure 1) The co-expression of CD31 and FSP-1 implied that EndMT was present in HBdMECs at 7 days after ketamine treatment. Furthermore, when the mTOR inhibitor rapamycin was administered with ketamine to the HBdMECs, the expression of FSP-1 decreased significantly.

Interpretation of results

The expression of phosphorylated S6RP was presented more significantly in KC patients. Cultured HBdMECs embedded with ketamine showed activation of mTOR pathway. The co-localization of CD31 and FSP1 in the endothelial cell of urinary bladder showed positive finding of EndMT.

Concluding message

Ketamine induces activation of the mTOR pathway and subsequent mesenchymal phenotypic expression (FSP1) in HBdMECs

Table 1. Immunohistochemical analysis of phosphorylated S6RP

	KC	Control	*p*
Number	n = 23	n = 16	
Gender (M:F)	8:15	4:12	NS*
Mean age (years)	27.61 (±4.78)	43.69 (±10.28)	0.016
Immunohistochemical analysis of phosphorylated S6 ribosomal protein			
Mucosa			
Mean Grading of staining	1.83 (± 0.20)	1.56 (± 0.20)	NS*
Endothelial cells			
Mean Grading of staining	2.26 (± 0.14)	0.50 (± 0.18)	<0.001

Figure 1.

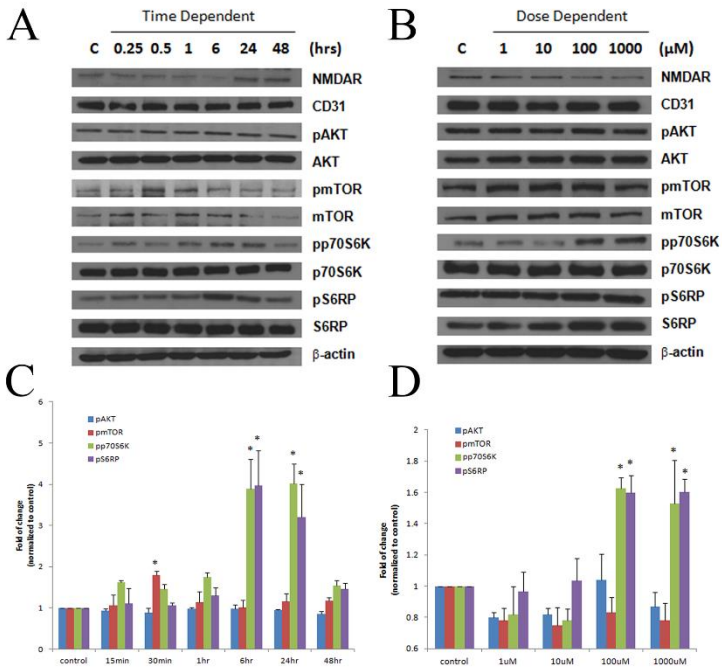
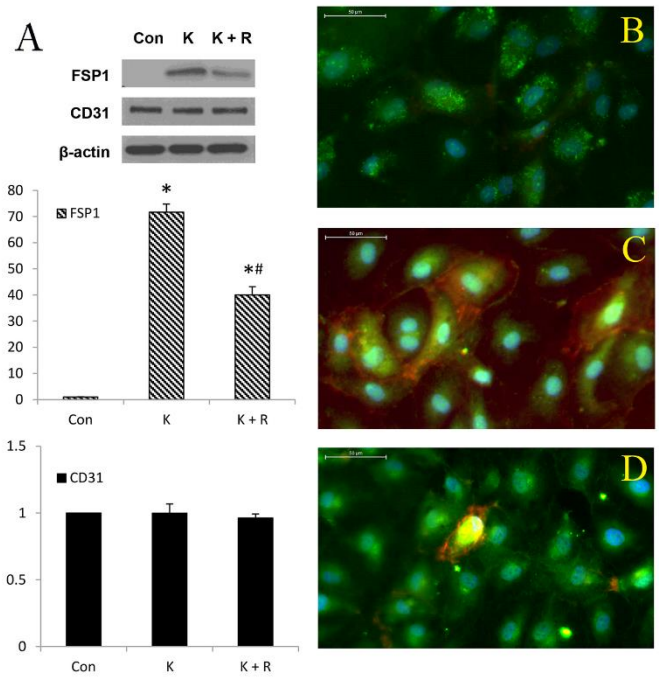


Figure 2.



References

1. Lin CC, Lin ATL, Yang AH, Chen KK: Microvascular Injury in Ketamine-Induced Bladder Dysfunction. PLoS ONE 2016; 11: e0160578
2. Chen RM, Chen TL, Lin YL, Chen TG, Tai YT: Ketamine reduces nitric oxide biosynthesis in human umbilical vein endothelial cells by down-regulating endothelial nitric oxide synthase expression and intracellular calcium levels. Critical Care Medicine 2005; 33: 1044-1049

Disclosures

Funding: This study was supported, in part, by a grant (V104C-189) from Taipei Veterans General Hospital to Chih-Chieh Lin. **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** Institutional Review Board and Ethics Committee of the Taipei Veterans General Hospital **Helsinki:** Yes **Informed Consent:** Yes