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# LONG-TERM KETAMINE ABUSE INDUCE INTERSTITIAL CYSTITIS IN RAT BY IMPAIRING BLADDER EPITHELIUM BARRIER

Hypothesis / aims of study

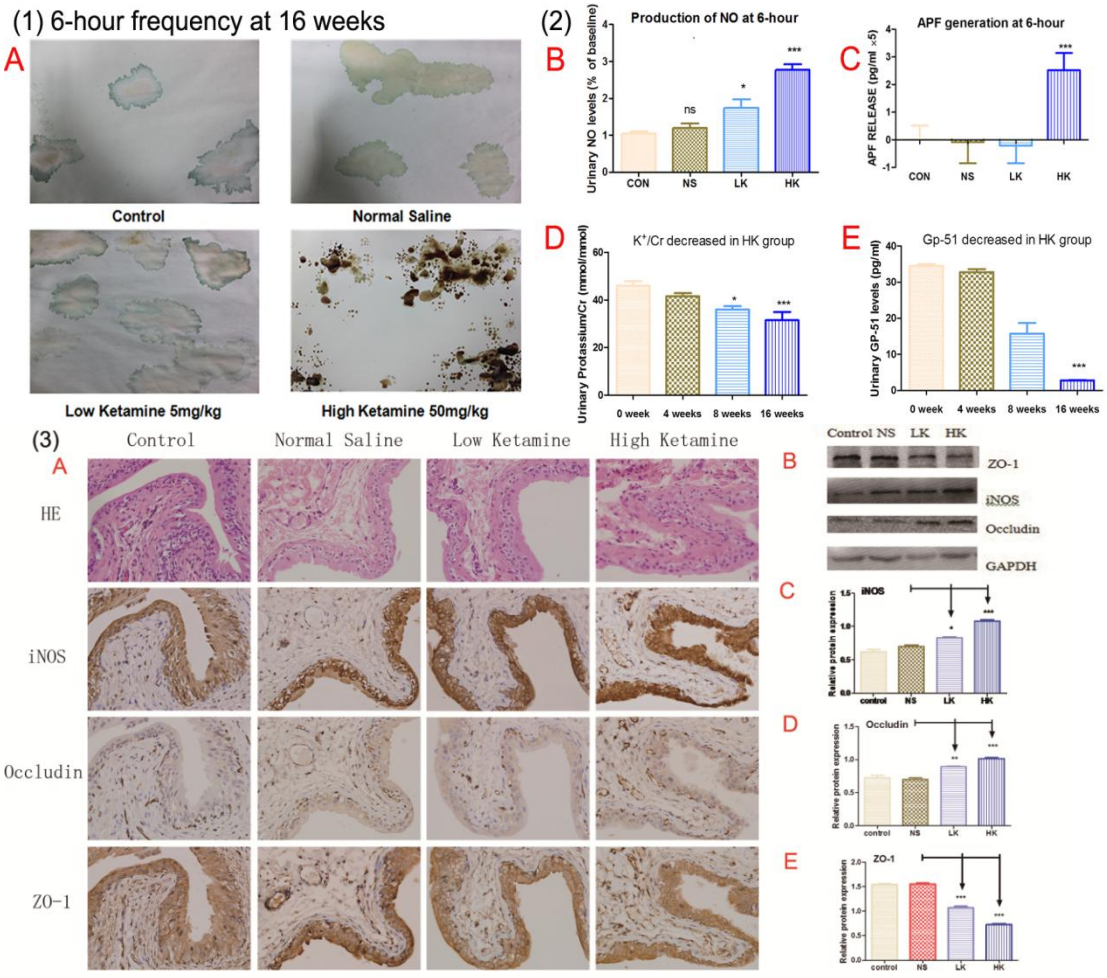
Ketamine has increasingly been abused as a recreational drug. Long-term ketamine abuse can affect urinary system, causing lower urinary tract syndrome, such as frequency, urgency, suprapubic discomfort and times hematuria. However, the pathophysiology and causative mechanism of this ketamine associated cystitis is not clear. Since the clinical and laboratory features as well as the cystoscopy and biopsy findings of the ketamine abusers are all similar to those with interstitial cystitis (IC) in clinic [1], we hypothesized that ketamine and its metabolites in the urine might cause direct toxic effect on the bladder epithelial barrier, gradually generating IC-like symptom. In this present study, we aim to substantiate the existence of ketamine associated cystitis and characterize this disease as “IC-like”, both behaviorally and histologically.

Study design, materials and methods

Twenty-four 2-month-old male Sprague-Dawley (SD) rats were randomly assigned to blank control, normal saline (NS), low-dose (5mg/kg) and high-dose (50mg/kg) ketamine groups (n=6/group). Rats were injected intraperitoneally with 50mg/kg or 5mg/kg ketamine hydrochloride or the same volume of normal saline every morning at 9.am.

(1).Urinary frequency measurement. Rat was housed individually in the special lab-made metabolic cage for urinary frequency measurement and urine collection. A special made stained paper placed under the cage was used to record the 24-hour frequency. The papers were changed every six hours to elevate accuracy. (2).Measurement of Urine markers. Antiproliferative factor (APF) and Glycoprotein-51 (GP-51), which are the evidenced urine markers of IC [2], were measured in the urine of rat by ELISA method. Urinary nitric oxide (NO) levels were measured by Griess reaction method. Potassium levels were measured by Ion Selective Electrode method. (3).Histopathological and immunohistochemical analysis. 16 weeks after the ketamine treatment, all the rats were executed. Histopathological changes in bladder were observed by haematoxylin and eosin staining. The expressions of tight junction protein ZO-1 and occludin, which are thought to be important for epithelial barrier function, together with the expression of induced nitric oxide synthesis (iNOS), were assessed by immunohistochemistry. Western blot analysis was used to confirm the results.

Results



Interpretation of results

(1) Both low and high dose of ketamine abuse induced significant increases in 24-hour frequency when compared to NS group at 16 weeks. Three of six rats in the HK group appeared haematuria. (mean ± SEM, NS: 16.4 ± 0.8 spots; LK:21.0 ± 0.7 spots; HK28.6 ± 1.4 spots ; LK vs NS: $P < 0.05$; HK vs LK: $P < 0.001$; n=6 fig.1A).

(2) Urinary NO and APF levels were instantly increased after Ketamine treatment (fig2B,C). When cells are under pathological condition, inducible NOS (iNOS) in mitochondria are activated to produce large amounts of NO as a defence mechanism. The increased production of NO proved the treatment of ketamine has an acute toxic effect on urothelium. The generation of APF, which is produced by bladder epithelial cells only and inhibits self-proliferation [2], implied the direct toxic effect of ketamine on bladder epithelium as well. Urinary GP-51 and potassium levels were significantly decreased after 16 weeks of ketamine treatment. It suggested that the integrity of the bladder epithelium barrier was impaired and the leaky barrier allowed increased potassium absorption in the bladder wall (values presented as mean ± SEM, *P<0.05, ***P<0.001, n=6, fig2D,E).

(3) The images from immunohistochemistry and western blot analysis were both quantified by using Image-Pro Plus v6.0 software and showing consistent results (fig3A,B). The expression of iNOS in urothelium was significantly increased, indicating the activation of iNOS after ketamine treatment and suggesting the toxic effect was from the bladder lumen. As showed in the fig3A, the expression of occludin was mainly localized to the endothelial cells of small vessels in the bladder interstitium, while ZO-1 was mainly localized to the urothelial cells layer. The increased expression of occludin suggesting the vascular proliferation in the bladder interstitium after long-term ketamine abuse, and the decreased expression of tight junction protein ZO-1 implying the impaired integrity of the bladder epithelium barrier (values presented as mean ± SEM, *P<0.05, *P<0.01, ***P<0.001, n=6, fig3C,D,E)

Concluding message

In the present study, we substantiated and monitored the process of how ketamine abuse damaged the bladder epithelium and induced cystitis in rat. Ketamine and its metabolites in urine had direct toxic effect on the bladder epithelium cells, generating defending nitric oxide and antiproliferative factor. The accumulative toxicity and sustained release of APF inhibited the proliferation and self-healing of bladder epithelial cells. Thus, the integrity of the bladder epithelium barrier was impaired, presenting as the decrease expression of glycoprotein GP-51 and ZO-1 on the bladder epithelium layer. Then, the permeability of the barrier was increased. Urine constituents, such as urea, potassium, can therefore penetrate into the bladder interstitium and muscle layer, leading to inflammation and vascular proliferation. The diffusion of potassium depolarized nerves and muscles, provoking IC-like symptoms [3]. This Long-term ketamine abuse rat may also provide a good animal model for IC study.

References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Disclosures


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