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Mast cells infiltration and decreased E-cadherin expression in ketamine-induced cystitis

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ABSTRACT

Background: Ketamine is a controlled substance and often illegally used as a recreational drug primarily by young adults. Increasing ketamine abusers associated with lower urinary tract symptoms have been reported at hospitals in recent years. Here we used a murine model to explore the changes of bladder in order to elucidate its pathogenesis.
Methods: ICR mice were randomly distributed into control and ketamine groups and received daily intraperitoneal injection of saline and ketamine (30 mg/kg), respectively. The bladders were excised and processed for histology at 4, 8 and 12 weeks. Tryptase and E-cadherin were investigated by immunohistochemistry in bladder tissues from ketamine-treated and control mice to assess the mast cell activation and junction protein expression.
Results: After ketamine treatment, the bladder changed to be hyperemic, inflamed, and with more fissures in mucosa. Compared with control group, the number of tryptase-positive mast cells significantly increased, which was 6.98 ± 2.89 and 23.00 ± 6.48 cells per field ($100\times$) at 8 and 12 weeks, respectively ($P = 0.016$ and $P = 0.003$, respectively). Additionally, the expression of E-cadherin in ketamine-treated mice bladder tissue was significantly lower than that in the control tissues, $P < 0.001$.
Conclusions: Increased mast cells in bladder wall and downregulated expression of E-cadherin junction protein in epithelial cells were probably associated with interstitial inflammation and fissures in mucosa. It implied that ketamine induced an interstitial cystitis.
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1. Introduction

Ketamine is currently used as an injectable anesthetic in hospitals. In contrast to medical use, it is also abused by young adults in clubs. Illicit use of ketamine has been reported to produce adverse effects on the urinary tract [1], particularly the bladder [2–5]. Symptoms of long term use include dysuria, urgency, nocturia, urothelial ulceration, etc., which are very similar to interstitial cystitis. To our knowledge, how ketamine produces these lower urinary tract symptoms is still not well known. Previous reports have showed the mechanisms of it on the nervous system and have linked it to the glutamatergic N-methyl-aspartate

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(NMDA) receptor [1,6–8]. Although, ketamine, as an NMDA non-competitive antagonist, can induce neurons to commit apoptosis, the effect of ketamine on other organs of the body is still far from clear.

Here, in a study of ketamine addiction induced mice cystitis, we have observed histopathological changes in the urinary bladders wall and urothelium, such as immune cell infiltration, junction protein expression in ketamine-treated bladder tissue. It maybe advances our understanding of the bladder pathophysiology of ketamine addiction.

2. Materials and methods

2.1. Animal breeding, ketamine treatment and tissue sampling

A total of 27 ICR mice weighed 20 g were bought from the Animal Center in Fujian Medical University and housed under a 12-h light/dark cycle with free access to food and water. All experimental protocols were approved by the institutional animal care and use committee. These mice ($n = 6$ for every time point) received daily intraperitoneal injection of ketamine (30 mg/kg) to model the effects of repeated ketamine abuse, or saline for normal subjects ($n = 3$ for every time point). Each week the weight of the living mice are weighed and recorded. After 4, 8 or 12 weeks, mice were sacrificed. Their bladders were harvested and fixed in 4% formaldehyde solution at room temperature for immunohistochemistry assay. If for electron microscopy, the samples should be fixed immediately by immersion in 3% glutaradehyde plus 1.5% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2 at 4 °C for later use.

2.2. Immunohistochemistry

Fixed tissue samples were processed for dehydration in increasing concentrations of ethanol, two changes of xylene, and embedded in paraffin wax. Afterwards, the paraffin blocks were sectioned serially at 4 μm using leica 2235. When analyzed for mast cell tryptase activity and E-cadherin expression, bladder tissue sections were deparaffinized and rehydrated. Subsequently, immunoreactivity was detected using 1:100 dilution of purified rabbit monoclonal anti-tryptase antibody (ab134932, Abcam Inc.) and anti-E-cadherin monoclonal antibody (24E10, Cell Signaling Technology Inc.) or normal rabbit IgG (as a control) at 4 °C overnight, followed by incubation with peroxidase-conjugated anti rabbit IgG for 1 h at room temperature and reacted with DAB according to SABC kit guideline. All stained sections were examined under light microscope. Images were acquired using Olympus DP72. The integrated optical density (IOD) was then measured using Image-Pro plus 5.0 software. In addition, all serial sections were stained with routine hematoxylin and eosin, washed by tap water, dehydrated, and mounted with neutral gum.

2.3. Electron microscopy

After 2 h fixation at 4 °C as described above, the tissue samples were washed 3 times with PBS and trimmed into small pieces, then postfixed with 1% OsO4–1.5% potassium ferrocyanide in phosphate buffer for 1.5 h. After 3 times wash and dehydration in graded ethanol series, the specimens was embedded in epoxy resin. One micrometer thick sections were first examined by light microscopy in order to locate the area of interest (semi thin section). These sections were cut using a Leica UC-6 ultramicrotome with a diamond knife into ultrathin sections and mounted on copper grids and were counterstained with uranyl acetate and lead citrate, then examined by PHILIPS EM208 electron microscope.

2.4. Statistical analysis

The data were presented as means ± standard deviations. All data were analyzed using one-way ANOVA test bilaterally. When significance was found, between-group differences were further analyzed by Student–Newman–Keuls analysis. P values less than 0.05 were considered statistically significant. SPSS 11.5 statistics software (SPSS, Chicago, IL, USA) was used for all statistical analysis.

3. Results

3.1. Body weight changes of mice and general changes of bladder

After treatment of ketamine, the mice showed prominent swaying and ataxia in about 30 min. The increases in body weight for both the ketamine and control groups were similar at 4 and 8 weeks. But weight gain in ketamine-treated mice became slower after 12 weeks. However, the difference was not statistically significant (data not shown). The bladders in ketamine groups appeared hyperemic and inflamed (arrows indicating where the ketamine tissue was inflamed), compared with the normal bladders in control group (Fig. 1A and C). The ketamine-treated animals developed dramatically histological changes in bladder. The bladder epithelia in the experimental mice were impressed with more ulceration and fissure than those in control mice. Epithelium shedding was often noted in the ketamine-treated bladders. Also, with HE staining, fissures of mucosa were calculated under light microscopy. Compared with fissures per field in control group (0.14 ± 0.35), fissures in ketamine group increased at 4 weeks (0.18 ± 0.39), 8 weeks (0.21 ± 0.41), and especially at 12 weeks (1.28 ± 1.15) ($P < 0.001$), respectively (Fig. 1B and C).

3.2. Increased mast cells trypase enzymes immunoreactivity

Tryptase-positive mast cells were found mainly in the bladder wall of ketamine-treated mice and were observed initially after 8 weeks of administration of ketamine. There were sparsely one or no tryptase-positive cells in the same place of control mouse. The numbers of tryptase-positive mast cells in the ketamine group were 3.16 ± 2.71, 6.98 ± 2.89 and 23.00 ± 6.48 cells per field (100×) at 4, 8 and 12 weeks, respectively. While the corresponding

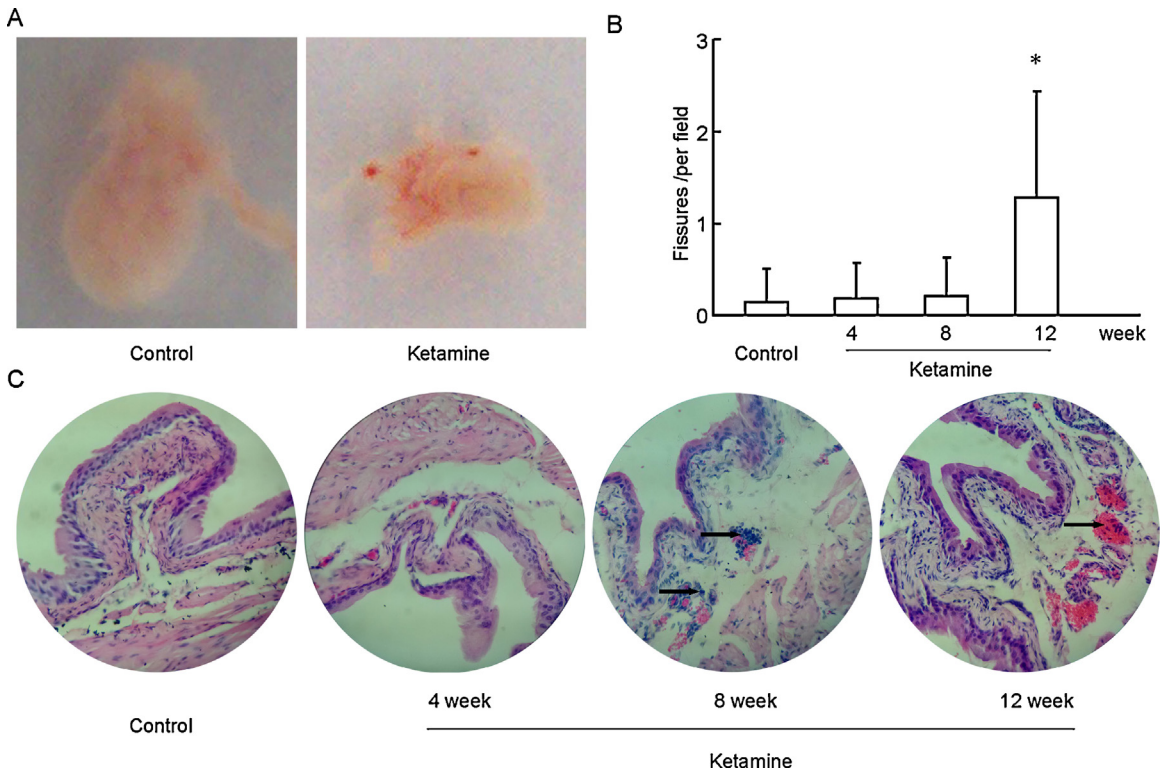

Fig. 1. Effect of ketamine treatment on bladder wall. (A) The normal gross bladder and blood congested ketamine-induced bladder. (B and C) Paraffin sections of bladders in ICR mice, stained with hematoxylin–eosin, showing morphology of bladder tissue. Compared with the control group, ketamine abuse resulted in remarkable increased fissures in bladder mucosa, especially at 12 weeks. *A significant difference between "12 weeks" and the others. Original magnification 200×.

numbers in control groups were 1.29 ± 0.68, 1.17 ± 0.77 and 2.67 ± 0.85 cells per field. As shown in Fig. 2A and B, there were significant increases of tryptase-positive mast cells at 8 and 12 weeks in ketamine group than those in control mice ($P = 0.016$ and $P = 0.003$, respectively). This phenomenon was also confirmed by electron microscopy, as presented in Fig. 2C.

3.3. E-cadherin junction protein expression

The E-cadherin moleculars were widely distributed at cell–cell junction in the superficial layers of the urothelium in normal state. The expression of E-cadherin in ketamine-treated mice bladder tissue was significantly downregulated, compared with that in the control mice ($P < 0.001$). As shown in Fig. 3, the expression of E-cadherin in the control group was standardized to be 100 ± 8.81, At 4, 8 and 12 weeks, those in ketamine groups were decreased to be 96.43 ± 5.77 ($P = 0.41$), 64.30 ± 8.36 ($P < 0.001$) and 77.25 ± 6.01 ($P < 0.001$), respectively.

4. Discussion

Ketamine abuse has become more and more popular in clubs for youngsters. Lower urinary tract damage and symptoms of cystitis were frequently reported in literature in recent years. To investigate the underlying mechanism, ketamine at 30 mg/kg was administered interperitoneally (i.p.) to mice. Higher dosage (60–150 mg/kg) could cause severe incordination of hind limbs of mice and anesthetic effects according to documents [9]. At 4, 8 and 12 weeks, bladders were collected and effects of this amount of ketamine on urinary bladder morphology and histology were investigated firstly. As expected, all specimens in experimental group were characterized by apparent hyperemic mucosa lesions with naked eyes. It was further confirmed by histology with microscope. The mucosa had been ulcerated to fissure and was infiltrated of inflammatory cells surrounding the capillaries. This resembles interstitial cystitis [10] in clinical practice, which also has reddish dotted blood congestion and bladder mucosal ulcerative damage [11,12] under cystoscope. It indicated ketamine-treated mice bladder was associated with interstitial inflammation and mucosa damage, which demonstrated the successful mice model.

Which kind of immune cells involved in this process was important. Previous reports about interstitial cystitis showed mast cells could directly cause vasodilation and bladder mucosa damage [12–14]. Immune staining using antibodies against tryptase which presented in the granules of mast cells has been considered to be the most preferential method for identification of mast cells. In our study we used this method to characterize the mast cell infiltration in bladder tissue. Expectedly, mast cell counts were elevated in the bladders of ketamine-treated mice than in control mice. Electron microscopy also approved

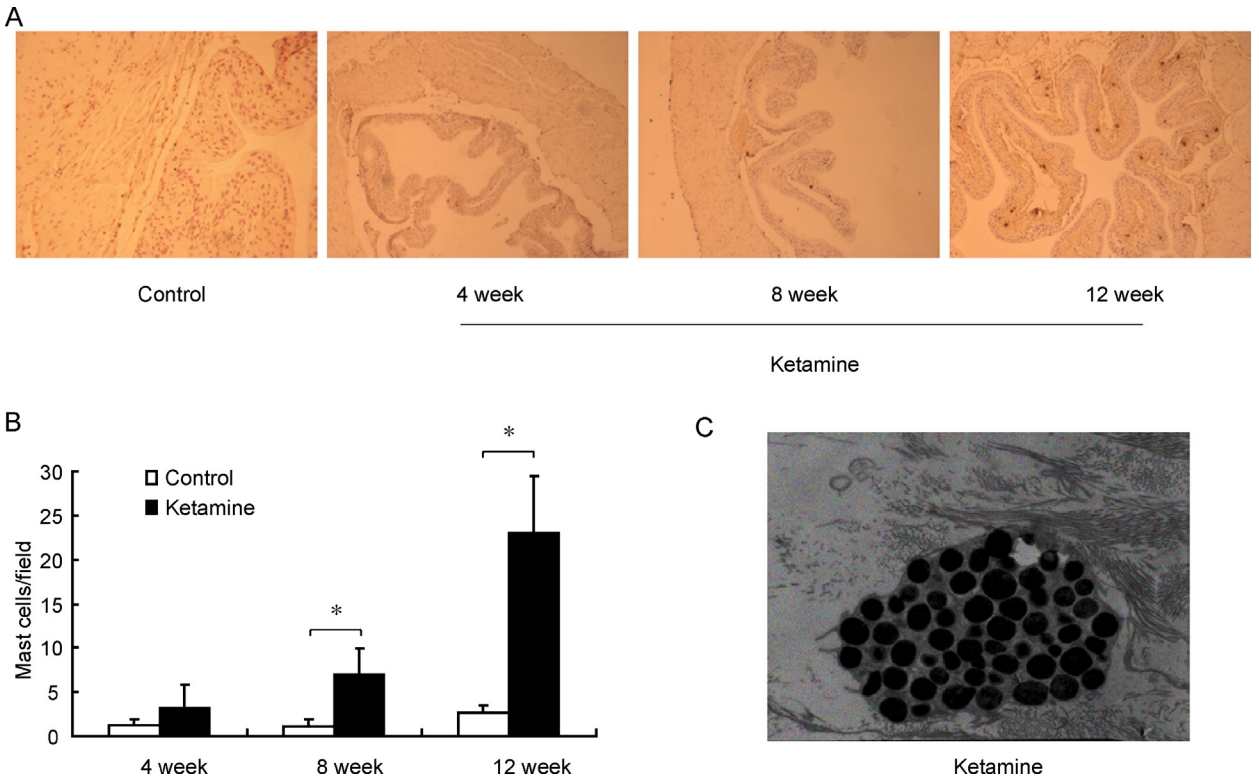

Fig. 2. Increased mast cells were observed in ketamine-treated mice bladder. (A and B) More tryptase-positive mast cells were identified as brown particles on ketamine-treated bladder wall sections compared with the control group. Mast cells increased mainly at 8 and 12 weeks. All figures were 100×. *$P < 0.05$. (C) Electron photomicrograph of mast cell on ketamine-treated mice bladder at 12 weeks. Note intact mast cell with cytoplasm full of homogeneous electron-dense granules with speckled patterns. Original magnification 6300×.

the existence of mast cells. These results suggest that mast cells may participate in bladder inflammation and consequently play an important role in the pathogenesis of bladder cystitis.

The urothelia serve as an effective blood–urine permeability barrier to the penetration of urine into the bladder stromal. The barrier function is maintained by tight junctions that seal the space between adjacent superficial cells. Detection of E-cadherin is a valuable tool to investigate cellular adhesion status of urothelium [11,15]. So recently Lee reported that decreased expression of E-cadherin was shown in ketamine-related cystitis of 16 patients [16]. It was coincidently consistent with our results in mice model and approved its importance. After 4 weeks of daily ketamine injection, the mice started to show obviously decreased E-cadherin expression. Especially after 8 weeks and 12 weeks, the lost of E-cadherin was gradually aggravated. This suggests that ketamine showed time-dependent effect to mice cystitis. In the long term, decreased E-cadherin expression may damage the layer of the bladder mucosa and make it vulnerable for urine to penetrate into the mucosa and cause inflammation.

On the whole, the study of the model of ketamine-treated mice demonstrated that ketamine induced cystitis in a time-dependent manner. The more exposure time to ketamine, the more changes to bladder wall and E-cadherin

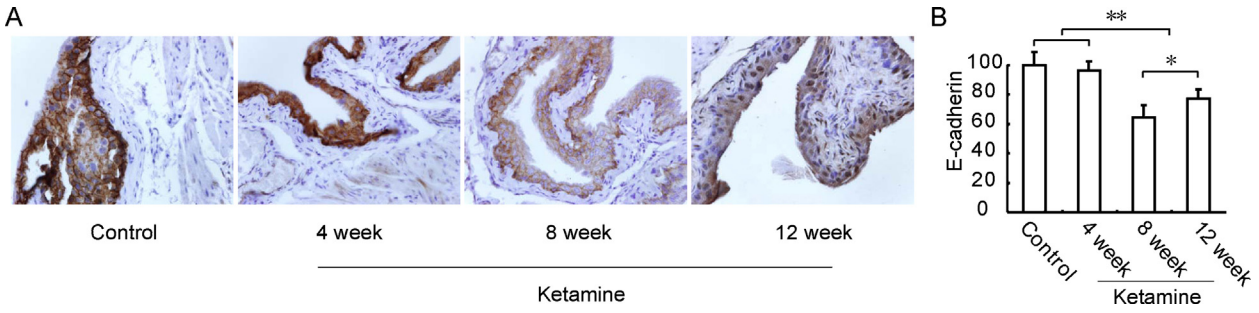

Fig. 3. The expression of E-cadherin decreased in ketamine-treated bladder. (A) Representative E-cadherin immunostaining in bladder wall of the control group and ketamine group. (B) In ketamine-treated mice, especially at 8 and 12 weeks, there was a significant decrease of E-cadherin immunostaining on bladder wall. The *P* value of * and ** were all no more than 0.001. Original magnification 400×.

expression in mucosa occurred. Ketamine induced apparent interstitial cystitis. Here we have developed a mice model that predicts what is observed in humans and that it could then be used to further investigate mechanisms or therapies/interventions for patients in clinic.

Declaration of interest

The project is in part supported by innovation foundation for college undergraduate students (2012). No potential conflicts of interest were disclosed.

Transparency document

The Transparency document associated with this article can be found in the online version.

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