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Ketamine induced renal fibrosis in a ketamine addition rat model

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ABSTRACT

Objective: The toxicity of ketamine to the genitourinary system not only involves the lower urinary tract but also the upper urinary tracts. Reports showed that ketamine abuse may cause hydronephrosis and renal function deterioration. However, the exact pathophysiological mechanism of renal injury has not been investigated. The aim of the present study is to investigate the renal injury after ketamine addition in an animal model.

Materials and methods: Thirty Sprague–Dawley rats were divided into three groups: control group, K-14D group, and K-28D group. The K-14D and K-28D groups received ketamine (25 mg/kg/d, intraperitoneally) through daily injections for a period of 14 days and 28 days, respectively. Masson's trichrome stains were carried out to show the histological changes. Western blotting and reverse transcription polymerase chain reaction were carried out to examine the expressions of profibrosis markers and fibrosis-associated proteins.

Results: Masson's trichrome stain showed that no significant renal hydronephrosis occurred after ketamine treatment. However, ketamine treatment increased the fibrosis of the tubule-interstitium and increased collagen deposition and fibronectin expression. These alterations were accompanied by increases in the expression of inflammatory and fibrosis markers, fibronectin, and type I collagen after ketamine treatment. The profibrosis marker, transforming growth factor-β (TGF-β), increased significantly after 14 days and 28 days of ketamine treatment both in terms of mRNA and protein levels.

Conclusion: Ketamine treatment not only induced cystitis-like syndrome, but also renal fibrosis. These renal interstitial fibrosis changes may be induced by the TGF-β pathway. These preliminary results can provide valuable information from a clinical perspective.

1. Introduction

Ketamine is an anesthetic drug used in human and veterinary procedures.[1] Ketamine is also used for pediatric anesthesia and conscious sedation in asthmatic patients. Recently, people have started using ketamine as a recreational and dissociative drug, especially in nightclubs and dance parties.[1,2] At present, increasing numbers of ketamine abusers are found with severe lower urinary tract symptoms and ulcerative cystitis.[1–3] In addition to injury to the lower urinary tract, ketamine and its metabolite also devastate the upper urinary tract, leading to varying degrees of hydronephrosis and renal function impairment.[4] Clinical observations showed that long-term ketamine addition may induce ureteral stricture and hydronephrosis, but internal double-J stenting could reverse the severity of hydronephrosis and reverse renal function deterioration.[5] However, currently there is no evidence showing

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that ketamine administration diminishes the glomerular filtration rate and promotes tubulointerstitial fibrosis on intrarenal renin–angiotensin system. On the contrary, some studies have shown that ketamine could suppress hypoxia-induced inflammatory responses in the late-gestation fetal kidney cortex.[6] The pros and cons of ketamine effects on the kidney are still unclear.

A noncompetitive antagonist, ketamine can inhibit the reuptake of serotonin, dopamine, and norepinephrine,[7,8] and *N*-methyl-D-aspartate (NMDA) receptor blockage might cause neurotoxicity, especially in the developmental period of synaptogenesis. In addition, NMDA antagonists could also induce neurons to commit apoptosis.[9–11] In human embryonic kidney cells, ketamine could inhibit monoamine transporters expressed in a dose-dependent manner. These results suggested that the ketamine-induced inhibition of monoamine transporters might contribute to its psychotomimetic and sympathomimetic effects by potentiating monoaminergic neurotransmission.[12]

Transforming growth factor-β (TGF-β) signaling controls a diverse set of cellular processes, including cell proliferation, recognition, differentiation, apoptosis, and specification of developmental fate, during embryogenesis as well as in mature tissues. TGF-β acts as a fibrogenic master cytokine and plays a pivotal role in the progression of a variety of chronic fibrotic diseases by promoting myofibroblastic differentiation, stimulating the synthesis of extracellular matrices (ECMs), and downregulating ECM degradation. Nevertheless, the role of TGF-β in regulating collagen deposition in the kidney, especially after ketamine stimulation, is still unknown.

Whether ketamine can cause renal inflammation and fibrosis remains unclear. To date, there are only a few studies investigating the molecular pathway of ketamine-associated renal injury. The major aim of the present study was to evaluate the renal inflammation and fibrosis after ketamine treatment using a ketamine-induced cystitis rat model. The results obtained could provide valuable information from a clinical perspective.

2. Materials and methods

2.1. Animals and ketamine administration

Experiments were performed on adult female Sprague–Dawley (SD) rats (animal center of BioLASCO, Taipei, Taiwan) weighing between 200 g and 250 g. These rats were housed under a 12-hour light/dark cycle at a controlled temperature of 21°C with free access to food and water. Thirty SD rats were divided into three groups: control group, K-14D group, and K-28D group. The K-14D and K-28D groups received ketamine (25 mg/kg/d) intraperitoneally (IP) via daily injections for a period of 14 days and 28 days, respectively. Control rats were given IP injections of normal saline. Rats were weighed once in the beginning of every week for adjustment of the amount of ketamine administered. This study was approved by the Animal Care and Treatment Committee of Kaohsiung Medical University. All experiments were conducted according to the guidelines for laboratory animal care. All efforts were made to minimize animal stress/distress and suffering, and to use the minimum number of animals.

2.2. Ketamine metabolites assay in urine and serum

At the termination of the experiment, 1 ml of blood was obtained from rat tail for ketamine and norketamine analyses. Blood was separated by centrifugation at 4°C. The concentration of ketamine and its metabolites (norketamine and hydroxynorketamine) in urine and serum were determined by using a slightly modified version of the high-performance liquid chromatography (HPLC) method.[2,13,14] After extraction and purification by liquid–liquid extraction using ethyl ether, the samples were chromatographed on a reversed-phase column, and the two molecules (ketamine and norketamine) were detected at 200 nm by UV spectrophotometry. This study was carried out according to ISO 9001:2000 requirements.

2.3. Biochemical studies

Daily urine samples were collected via individualized metabolic cages. At the end of the study, rats were sacrificed, and blood samples were drawn from the inferior vena cava for biochemical analysis. Serum creatinine and blood urea nitrogen content were determined using a commercial enzyme-linked immunosorbent assay kit (Millipore Launches Test kits; Millipore, Billerica, MA, USA). Urinary sodium and creatinine were measured using the Synchron CX Delta system (Beckman, Fullerton, CA, USA) according to the manufacturer's protocol.[15,16] The creatinine clearance rate (CCr) was calculated as follows: urine creatinine × urine volume/serum creatinine. The calculation was used to evaluate renal function in different groups.

The fractional excretion of sodium (FE_{Na}) is the percentage of the sodium filtered by the kidney that is excreted in urine. In clinical use, FE_{Na} can be calculated as part of the evaluation of acute renal failure in order to determine if hypovolemia or decreased effective circulating plasma volume is a contributor to renal failure. The flow rates cancel out in the above equation, simplifying to the standard equation[17]:

$$FE_{Na} = 100 \times \frac{sodium_{urinary} \times creatinine_{plasma}}{sodium_{plasma} \times creatinine_{urinary}}$$

2.4. Histological study with Masson's trichrome stain

Experimental rats were perfused with saline solution through the left ventricle, and the kidneys were removed and further fixed overnight. The tissue samples were embedded in paraffin blocks within the same area in different groups, and serial 5-μm-thick sections were obtained. Deparaffinized sections were stained with Masson's trichrome stain (DAKO, Glostrup, Denmark; Masson's Trichrome Stain Kit). The standard Masson's trichrome staining procedure was followed to stain connective tissue in blue and renal tissue in red color. The transverse section of each specimen was captured with a digital camera in five random nonoverlapping frames at 400× magnification, and the entire bladder wall thickness was included in each analyzed region. The color setting and image-associated quantification were determined using an image analysis software (Image-Pro Plus; Media Cybernetics, Rockville, MD, USA).

2.5. RNA isolation and complementary DNA synthesis

Renal cortex was dissected, and total RNA was isolated from each section using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription for complementary DNA synthesis was performed using the reverse transcription system (Transcriptor First Strand cDNA Synthesis kit; Roche Diagnostics, Mannheim, Germany).

2.6. Statistical analysis

Analysis of variance followed by Bonferonni's test for individual comparisons was used in the experiments. Calculations of mean, standard deviation, and p values were performed on triplicate experiments. Student t test was used to calculate p values for comparison. The significant statistics was set at $p < 0.05$.