Molecular pathways underlying tissue injuries in the bladder with ketamine cystitis

Xiang Xie | Jiayu Liang | Run Huang | Chuang Luo | Jiali Yang | Hongming Xing | Le Zhou | Han Qiao | Erti Ergu | Huan Chen

Public Center of Experimental Technology and The School of Basic Medical Sciences, Southwest Medical University, Luzhou, China

Correspondence
Huan Chen, Public Center of Experimental Technology and The School of Basic Medical Sciences, Southwest Medical University, Luzhou 646000, Sichuan Province, China. Email: huanchen@swmu.edu.cn

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Abstract
Ketamine cystitis (KC) is a chronic bladder inflammation leading to urinary urgency, frequency, and pain. The pathogenesis of KC is complicated and involves multiple tissue injuries in the bladder. Recent studies indicated that urothelium disruption, lamina propria fibrosis and inflammation, microvascular injury, neuropathological alterations, and bladder smooth muscle (BSM) abnormalities all contribute to the pathogenesis of KC. Ketamine has been shown to induce these tissue injuries by regulating different signaling pathways. Ketamine can stimulate antiproliferative factor, adenosine triphosphate, and oxidative stress to disrupt urothelium. Lamina propria fibrosis and inflammation are associated with the activation of cyclooxygenase-2, nitric oxide synthase, immunoglobulin E, and transforming growth factor β1. Ketamine contributes to microvascular injury via the N-methyl-D aspartic receptor (NMDAR), and multiple inflammatory and angiogenic factors such as tumor necrosis factor α and vascular endothelial growth factor. For BSM abnormalities, ketamine can depress the protein kinase B, extracellular signal-regulated kinase, Cav1.2, and muscarinic receptor signaling. Elevated purinergic signaling also plays a role in BSM abnormalities. In addition, ketamine affects neuropathological alterations in

Abbreviations: Akt, protein kinase B; ALK, activin-like kinase; Ang, angiotensin; AP, activator protein; APF, antiproliferative factor; Areg, amphiregulin; ATP, adenosine triphosphate; Bax, B-cell lymphoma 2-associated X protein; Bcl, B-cell lymphoma; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; BSM, bladder smooth muscle; C/M, collagen-to-smooth muscle; CAT, catalase; CCL, CC chemokine ligand; CD, cluster of differentiation; c-fos, cellular fos; CGRP, calcitonin gene-related protein; CHOP, CCAAT/enhancer-binding protein homologous, calcitonin gene-related peptide; c-Jun, cellular jun; CKAP, cytoskeleton-associated protein; COX, cyclooxygenase; CREB, cAMP response element-binding protein homologous, calcitonin gene-related peptide; CYP, cytochrome P450; EP2, prostaglandin E receptor subtype 2; EFS, electrical field stimulation; EMT, epithelial-mesenchymal transition; endoMT, endothelial-mesenchymal transition; eNOS, endothelial nitric oxide synthase; EP2, prostaglandin E receptor subtype 2; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FOXO, forkhead box protein O; G, growth; GAG, glycosaminoglycan; GP, glycoprotein; GSH/GSSG, glutathione/glutathione; IC/BPS, interstitial cystitis/bladder pain syndrome; IEGs, early genes; IgE, Immunoglobulin E; IL-6, interleukin 6; iNOS, inducible nitric oxide synthase; IC/BPS, interstitial cystitis/bladder pain syndrome; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; KC, ketamine cystitis; LPS, lipopolysaccharide; LTCC, L-type calcium channel; LUTs, lower urinary tract symptoms; M, mitosis; MAPK, mitogen-activated kinase; MMPs, matrix metalloproteinases; MOMP, mitochondrial outer membrane permeabilization; NAPDH, nicotinamide adenine dinucleotide phosphate; NAPDH, nicotinamide adenine dinucleotide phosphate oxidase; NGF, nerve growth factor; NGFR, nerve growth factor receptor; NKA, neurokinin A; NMDAR, N-methyl-D aspartic receptor; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NSC, neural stem cell; PDGF, platelet-derived growth factor; PGs, prostaglandins; PTK, phosphotyrosine 3-kinase; RNS, reactive nitrogen species; ROS, reactive oxygen species; Smad, small mother against decapentaplegic; SMCs, smooth muscle cells; SOD, superoxide dismutase; SP, substance P; TGF, transforming growth factor; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; Trk, tropomyosin receptor kinase B; TRP, transient receptor potential; TRPV, transient receptor potential vanilloid; TTX, tetrodotoxin; VEGF, vascular endothelial growth factor; WB, western blot; ZO, zonula occludens.

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1 | INTRODUCTION

Ketamine is a non-competitive N-methyl-D aspartic receptor (NMDAR) antagonist first synthesized in the 1960s. It had been widely used as an anesthetic drug since the 1970s. It has also been increasingly abused as a recreational drug since the 1980s because of its hallucinatory effects. Ketamine abuse causes toxicity to many organs, including cardiovascular, hepatobiliary, respiratory, gastrointestinal, and urinary systems. At least one-third of ketamine abusers develop lower urinary tract symptoms (LUTS), such as severe dysuria, painful hematuria, urinary frequency, urgency, and urge urinary incontinence, which is termed ketamine cystitis (KC).

Since Shahani and colleagues reported the first case of KC in 2007, the number of cases reported by clinicians worldwide has increased.

Previous studies reported that a contracted bladder with urothelium denudation and lamina propria fibrosis and inflammation were the common pathological features. The urothelium is in direct contact with ketamine and its metabolites in the urine, and has shown to suffer a loss of junction proteins such as zonula occludens (ZO)-1 and E-cadherin and to become more apoptotic. Based on these findings and the urothelium pathological features, urothelial barrier dysfunction has been indicated as the major pathogenesis of KC. However, recent studies have made great advances in the pathogenesis of KC and demonstrated that the nerves, microvasculature, and bladder smooth muscle (BSM) in the bladders of patients with KC have pathological changes and display injuries. These ketamine-induced tissue injuries involve various signaling pathways and contribute to the pathogenesis of KC. This review summarizes the molecular signaling pathways underlying these tissue injuries in the bladder of KC, to enhance the understanding of the pathophysiology of this disease and to facilitate the identification of novel pharmacologic targets for KC.

2 | UROTHELIUM DISRUPTION

Urothelial barrier dysfunction is proposed as a major mechanism in the pathogenesis of KC. The barrier function comprises multiple defensive molecules including glycosaminoglycans (GAGs), uroplakin plaques, and tight and adherent junctions. Ketamine causes the urothelium to become leaky by disruption/deficiency of GAGs, uroplakins, and tight and adherent junctions. Abnormal urothelial cell apoptosis can enhance urothelium disruption and lead to barrier dysfunction.

2.1 | Deficiency of GAGs and uroplakins

The apical surface of the urothelial cells is covered with a layer of GAGs, which consists of a thick mucus layer of glycoproteins and proteoglycans. Disruption/deficiency of the GAG layer damages its protective barrier function, giving rise to increased permeability into the bladder wall, thus causing inflammation and pain. Disruption/deficiency of GAGs is commonly observed in the histopathological examination of biopsies from patients with KC. Similar findings were recently reported in rat models wherein ketamine as well as its analog methoxetamine both reduced the GAG intensities on the apical surface of the urothelial cells. Previous studies indicated that high and low doses of ketamine both significantly decreased the levels of glycoprotein 51 (GP51) in the urine. GP51 is a component of GAGs produced and secreted by the transitional epithelium of humans and other mammals. The decreased secretion of GP51 in KC suggests a defect of bladder surface GAGs and impaired bladder barrier function. Intravesical GAG replenishment has been demonstrated to be an effective therapy in patients with KC as a result of the restoration of urothelial barrier function. In healthy individuals, uroplakins are the central glycoproteins of GAGs. Loss of uroplakins in knockout mice has been reported to increase...
the urothelial permeability about twofold. Abnormalities in the amount and location of uroplakins have been confirmed in the bladders from patients with KC and animal models.

No evidence indicates that ketamine can directly disrupt GAGs and uroplakins on the apical surface of the urothelial cells. However, a number of in vivo and in vitro studies have demonstrated that ketamine inhibits the urothelium proliferation and causes urothelial cell injury and death, leading to a decrease in urothelial cell count, and subsequently affecting the urothelium’s ability to produce and secrete GAGs and uroplakins. Uroplakins are also the end products and differentiation markers of the urothelial cells, which decrease in the bladder of KC, indicating that ketamine has detrimental effects on the urothelial cells, consequently affecting the production and secretion of GAGs and uroplakins. Several recent articles confirmed the detrimental effects of ketamine on urothelial cells mediated by the antiproliferative factor (APF), adenosine triphosphate (ATP), and oxidative stress.

2.2 Defect of tight and adherent junctions

Tight junctions seal the space between adjacent urothelial cells to maintain the permeability barrier. ZO-1 and claudin, as essential components of tight junctions, have important roles in maintaining epithelial barrier function and the impermeability of the bladder urothelium. E-cadherin is the core protein of adherent junctions, which are more basal than tight junctions and play important roles in establishing cell-cell adhesion in the urothelium. The integrity of the tight and adherent junctions is vital to maintaining basic epithelial function; a defect of these represents a pivotal step in epithelial denudation and barrier dysfunction. Clinic and animal studies indicate a defect of tight and adherent junctions and separated cell-cell contacts with reduced expression of ZO-1, claudin, and E-cadherin in the bladder with KC.

2.2.1 APF suppress the expression of junction proteins

Increasing evidence indicates that the expression of junction-associated proteins is regulated by APF. Urinary APF levels increased in ketamine-treated rats within 30 h after administration. In vitro studies showed that APF decreased the expression of ZO-1 and claudin, blocked the growth (G2-mitosis (M) phase cell cycle, and inhibited the proliferation of bladder epithelial cells. APF can quickly activate p38/mitogen-activated kinase (MAPK) activity. It plays an important role in the modulation of tight and adherent junction function by regulating the expression of junction proteins including ZO-1, claudin, and E-cadherin. Treatment with p38/MAPK inhibitors has been shown to abrogate the suppressive effect on cell proliferation with APF and rescue the loss of ketamine-induced junction proteins, suggesting that APF suppresses urothelial cell proliferation and decreases junction protein expression by stimulating the p38/MAPK signaling pathway in the bladder of KC. Moreover, ketamine has been demonstrated to directly activate the p38/MAPK signaling pathway in an APF-independent manner.

In addition, previous studies found that APF bound to a high-affinity cell surface receptor cytoskeleton-associated protein (CKAP)-4/p63 and inhibited the protein kinase B (Akt) signaling pathway, resulting in the inhibition of urothelial cell proliferation and alteration of the expression of E-cadherin and ZO-1. This finding suggested that an interaction between APF and this cognate protein might play roles in the urothelium disruption in the bladder of KC. Epithelial cells treated with antagonists of APF or with inhibition of its downstream signaling pathway could recover the altered ZO-1 and claudin expression and rescue epithelium permeability and epithelial cell proliferation, indicating that APF and its downstream signaling pathway could be promising treatment targets for patients with KC.

2.2.2 Oxidative stress disrupt junctions

Oxidative stress is another key factor responsible for the alternations in expression, localization, and function of junction proteins. It is associated with the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which were commonly observed in the bladder of KC.

Under normal conditions, the cells control ROS levels by balancing the generation of ROS with their elimination using antioxidant enzymes, which consist of superoxide dismutase (SOD) and catalase (CAT). SOD destroys the free radical superoxide by converting it into peroxide, which, in turn, is broken down by CAT to water and oxygen. However, an animal study demonstrated that ketamine treatment significantly reduced the expression of Cu/Zn-SOD, Mn-SOD, and CAT in the bladder. In addition, ketamine stimulates rapid ATP release from the urothelium, and thus more ATP molecules are needed to maintain the urothelium function. A previous study found that ATP levels increased in ketamine-treated mice as a result of increased activity of the respiratory chain for ATP synthesis. Liu et al demonstrated the overexpression of the respiratory enzymes in mitochondria for ATP synthesis in ketamine-treated mice, leading to increased leakage of electrons for ROS generation. Therefore, ketamine enhances the production and accumulation of ROS in the urothelium by the interruption of oxygen homeostasis via increasing the activity of oxidative respiratory chain and decreasing the expression of antioxidant enzymes.

Studies demonstrated that inducible NOS (iNOS) and endothelial NOS (eNOS) were overexpressed in the bladders of
KC. Increased urinary NO production by NOS was reported in ketamine-treated rats. Excessive NO secondary to NOS induction in the bladder can interact with ROS, such as O$_2$ and O$_2^-$, to form peroxynitrite (ONOO$^-$); it is the source of RNS in the bladder of KC.

ROS and RNS have been shown to disrupt junction proteins and cause urothelium barrier dysfunction via modulating reduced glutathione/oxidized glutathione (GSH/GSSG) homeostasis. When the cells are subjected to ROS and RNS, the GSH/GSSG ratio tends to decrease by oxidizing GSH into GSSG. The oxidation of the thiol group of glutathione results in the inhibition of protein tyrosine phosphatases, further activating tyrosine kinases. The activation of tyrosine kinase leads to the phosphorylation of junction proteins, such as ZO-1, occludin, and E-cadherin, leading to the epithelial barrier disruption. However, it can be prevented by treatment with tyrosine kinase inhibitors. In addition, the redistribution of F-actin at the cell periphery was observed after ketamine treatment. The actin cytoskeleton is essential for the assembly and maintenance of tight and adherent junctions. RNS and ROS are inducers of actin cytoskeleton reorganization in endothelial cells, leading to the opening of junctions and gap formation between endothelial cells. Thus, ketamine can disrupt junctions by rearranging the actin cytoskeleton in an ROS- and RNS-dependent manner. RNS and ROS can also initiate secondary oxidation products, such as 4-hydroxynonenal, to influence tight junction stability by modulating the glutathione levels and the p38/MAPK signaling pathway. Furthermore, studies performed by Han showed that decreased epithelial barrier function with reduced expression of tight junction proteins, such as ZO-1, ZO-3, and occludin, could be modulated by the NO and/or ROS scavenger, suggesting that scavenging NO and ROS could be helpful in KC.

### 2.3 Urothelial cell apoptosis

In vitro studies using cultured human urothelial cells demonstrated time- and dose-dependent responses to ketamine on urothelium death and found decreased expression of B-cell lymphoma (Bcl)-2 and increased expression of Bel-2-associated protein (Bax) and caspase-3 activity. Another in vitro study also found a clear sign of apoptosis and thinning of the urothelium after 72 hours of exposure to ketamine. In concert with the in vitro study, in vivo studies showed thin urothelium and increased terminal deoxynucleotidyl transferase dUTP nick end labeling signaling in the bladders of patients with KC and in animal models. These studies implied that ketamine could induce the apoptosis of urothelial cells to affect the ability of urothelium to make new cells. Ketamine enhance urothelium apoptosis via multiple signaling pathways. Both in vivo and in vitro studies showed that urothelium exhibited enhanced apoptosis with activated caspase-3, caspase-8, caspase-9, and caspase-12 after ketamine treatment. Caspases play an essential role in apoptosis. However, different caspases involve different pathways. The caspase-12/caspase-3 is classified as inflammatory caspase and involves apoptosis through the endoplasmic reticulum (ER) stress. The caspase-8/caspase-3 is essential for death receptor signaling-induced apoptosis. The caspase-9/caspase-3 is the central caspase of the mitochondria signaling pathway. Therefore, ER-, death receptor-, and mitochondria-dependent pathways all might contribute to urothelium apoptosis in KC.

#### 2.3.1 ER stress signaling pathways in urothelial apoptosis

ER stress is a major factor affecting the initiation of apoptosis. Besides caspase-12, other ER stress markers, including ER-chaperone 78-kDa glucose-regulated protein and ER-associated apoptosis protein CCAAT/enhancer-binding protein homologous protein (CHOP), are also upregulated in the urothelium of the bladder with KC, indicating activated ER stress. Ketamine stimulates ATP release from urothelial cells, which then binds to the purinergic receptor on the cell surface, allowing inositol trisphosphate to trigger calcium release from ER. Prolonged elevation of calcium in the cytoplasm promotes ER stress. In addition, the internal environment of cells is normally highly reducing, and an increase in ketamine-induced oxidative stress can also act as an important trigger to induce ER stress. Therefore, ketamine induces ER stress in urothelial cells through the stimulation of urothelial ATP release and oxidative stress. ER stress can activate caspase-12 through the ER-specific apoptosis pathway. The activated caspase-12 can activate caspase-9 that in turn cleaves and activates caspase-3, consequently triggering apoptosis in a cytochrome c-independent manner. In addition, ER stress can also trigger the mitochondria-dependent pathway to initiate urothelium apoptosis. ER stress leads to a massive and/or prolonged influx of calcium into mitochondria, which can activate the mitochondrial permeability transition pore complex, resulting in the release of cytochrome c into the cytosol. This results in the activation of caspase-9, which then triggers the apoptosis by the activation of caspase-3. The involvement of mitochondria in caspase-9/3 activation via ER stress in response to ketamine in the human urothelium has been previously reported.

#### 2.3.2 Mitochondria-dependent pathway in urothelial apoptosis

In addition, the oxidative stress-triggered mitochondria-dependent pathway leading to urothelium apoptosis has also been reported in the bladder of KC. The balance of
proapoptotic proteins (Bax) that translocate to the mitochondria and anti-apoptotic proteins (Bcl-2) that reside in the mitochondrial membrane regulate mitochondrial outer membrane permeabilization (MOMP), leading to the release of proapoptotic proteins and subsequent caspase activation and apoptosis. However, ketamine can cause decreased expression of Bcl-2 and increased expression of Bax in the urothelium by the induction of oxidative stress. This is followed by mitochondrial membrane depolarization and an increase in MOMP, which allows the release of proapoptotic proteins including cytochrome c into the cytosol. Cytochrome c then forms the apoptosome complex in the cytosol with procaspase-9, leading to caspase-9 activation. Caspase-9-catalyzed activation of caspase-3 executes the final steps of apoptosis.

2.3.3 | Death receptor pathways in urothelium apoptosis

The caspase-8/caspase-3 is essential for the death receptor signaling-induced apoptosis. In an in vitro study showed that the expression of tumor necrosis factor (TNF)-α was elevated in ketamine-treated human uroepithelial cells. Fan et al also reported that patients with KC had a higher level of TNF-α compared with healthy volunteers. TNF-α interacts with the tumor necrosis factor receptor (TNFR)1 and stimulates the formation of a cytoplasmic of tumor necrosis factor receptor type 1-associated death domain protein complex, containing Fas-associated protein with death domain and pro-caspase-8, leading to the activation of caspase-8 and the initiation of an apoptotic signaling cascade. In addition, oxidative stress can also activate death receptors, such as Fas, and TNF-related apoptosis-inducing ligand receptor, to induce apoptosis.

2.4 | Urothelial disruption is more than leading barrier dysfunction

The urothelium has classically been thought of as a passive barrier to ions and solutes. A leaky epithelium leads to the migration of water, urea, and toxic substances. In particular, potassium passes into the underlying tissue, depolarizes nerves and muscles, and generates symptoms of frequency, urgency, and bladder pain during bladder filling and voiding. Other large molecules and toxic substances leaking into lamina propria and muscular layer lead to inflammation, fibrosis, and other pathological changes. Recently, a number of novel properties have been attributed to urothelial cells. Studies have revealed that the urothelium acts as a sensor and transducer that can respond to chemical and mechanical stimuli and release chemical mediators.

3 | LAMINA PROPRIA INFLAMMATION AND FIBROSIS

Inflammation and fibrosis in lamina propria are the key pathological changes widely observed in the bladder from KC animal models and patients. In a cohort study, a majority of patients (62.5%) showed moderate inflammation in bladder tissue, with the remainder showing severe (26.1%) or mild (8.7%) inflammation. The bladders of all patients exhibited varying significance of fibrosis. Further histopathological studies showed inflammation and fibrosis in the lamina propria. Consistent with other histopathological assessments of the bladders from KC animal models and patients, the infiltrating inflammatory cells and collagen deposition concentrated in the lamina propria. Multiple signaling pathways have been reported to be involved in these key pathological changes in the bladder of KC.

3.1 | Lamina propria inflammation

3.1.1 | NO-mediated inflammation

NO released from iNOS is a signaling molecule that plays a key role in the pathogenesis of inflammation. Ketamine induces the upregulation of iNOS and produces high levels of NO in the bladder. Further immunohistochemistry studies showed that iNOS was mainly expressed in the mucosa and submucosa layer in the bladders of patients with KC, which positively correlates with the levels of inflammation in the
lamina propria.30 The synthesis of NO by iNOS is reported to be delayed but occurs for a longer period,35 indicating that iNOS is responsible for a chronically inflammatory process in the bladders of patients with KC. In contrast to iNOS, the release of NO from eNOS occurs in small amounts and for a short duration in response to stimulation.58,96 eNOS is expressed in the urothelial and suburothelial layers of the bladder.97-99 eNOS was upregulated in the early phase of inflammation in KC,58 suggesting that eNOS might involve the development of early inflammation in the lamina propria of bladders of patients with KC. Generally, the neuronal NOS (nNOS) is upregulated following neuronal injury and inflammation. A recent study revealed no significant change in the expression level of nNOS in rats after ketamine treatment.58

Large amounts of NO trigger a redox imbalance, leading to the production of nitrated proteins, inhibition of mitochondrial respiration, depletion of cellular energetics, DNA damage, apoptosis, and necrotic cell death, resulting in cellular/tissue injury.100101 With the onset of cellular/tissue injury, damaged cells activate inflammatory cells and trigger inflammatory cascades, most commonly with the nuclear factor kappa-light-chain-enhancer of activated B cells, MAPK, and the cytokine-activated Janus kinase signal transducer and activator of transcription pathways.102 These cascades can increase the expression of genes encoding growth factors, inflammatory cytokines, and chemokines, which attract inflammatory cells including neutrophils, eosinophil, monocytes, lymphocytes, and mast cells into the damaged area. These cells are frequently observed in the lamina propria from human bladder biopsies after long-term ketamine use.10,30,34 In addition, inflammatory cytokines such as TNF-α and interleukin (IL)-6 can further lead to the expression of iNOS, resulting in NO synthesis and secretion.102 Studies showing the importance of NO in inflammation indicated that both inhibitors of NO synthase and NO donors protected against some forms of injury. Indeed, the treatment with NOS inhibitors reduced the degree of inflammation in rats with acute inflammation or adjuvant arthritis, whereas L-arginine enhanced it.103 Furthermore, the inhibition of NOS prevented the progression of cyclophosphamide (CYP)-induced cystitis in rats, but L-arginine had opposite effects.104,105 Therefore, pharmacological intervention modulating NO production and bioactivity could be used in managing KC.

3.1.2 | Cyclooxygenase-mediated inflammation

Cyclooxygenase (COX) enzyme is well known as a key inflammatory mediator.106 Ketamine has been reported to initiate the upregulation of COX-2 at the protein and mRNA levels in the bladder of rats.94 Increased expression of COX-2 in the bladders of KC suggested that the COX pathway might be involved in the development of inflammation. Two types of COX have been identified in the bladder. The constitutive COX-1 plays a cytoprotective role in maintaining a normal physiological process, which is not altered or changed minimally in the bladder under pathophysiological conditions.107 However, the inducible COX-2 is expressed at the site of inflammation and leads to the release of large quantities of prostaglandins (PGs).59 Aoki et al reported that PGs contributed to inflammation by making a positive feedback loop consisting of COX-2-prostaglandin (PG)E2 prostaglandin E receptor subtype 2 (EP2)-NF-κB117 and were involved in inducing chemokines and resultant infiltration of inflammatory cells at the inflamed site.108 Consistently, Chuang et al reported the increased expression of COX-2 and NF-κB following ketamine administration in rats.58,109 Further studies found that ketamine and its primary metabolites could directly initiate NF-κB translocation from the cell cytoplasm to the nucleus for COX-2 activation, whereas NF-κB inhibitor suppressed COX-2 expression.94 In addition, ketamine-induced inflammatory mediators, such as IL-6 and TNF-α, have been reported to enhance the expression of COX-2.110,111 Besides, considerable cross talk occurs between NOS and COX. NO synthesized by iNOS can stimulate the expression of COX-2 to increase the production of PGs.106 Thus, multiple signaling pathways are involved in inflammation in KC via activating the COX-2 enzyme, suggesting that COX-2 could be a promising target for KC treatment. Nonsteroidal anti-inflammatory drugs are used as the first-line treatment of KC and are believed to act via inhibiting COX-2.

3.1.3 | Immunoglobulin E-mediated inflammation

Immunoglobulin E (IgE) has been convincingly linked to the pathophysiology of acute allergic inflammation. However, a large body of evidence now suggests that IgE is also a key diver of the long-term pathophysiological changes associated with chronic inflammation.112,113 IgE is present at low concentrations in the blood of mice and humans but can be enhanced by a variety of environmental challenges as well as exposure to noxious xenobiotics, irritants, and venoms to promote inflammatory reactions.114 Ketamine has been demonstrated to be a noxious stimulus that can activate IgE in serum. In extensive studies of IgE, the levels of IgE were significantly higher in patients with KC than in normal controls.54,115 The patients who recently used ketamine displayed abnormally elevated IgE levels. However, the patients without recent ketamine use or termination of ketamine use had decreased IgE levels.115 Using immunohistochemical staining, Jhang reported that almost all patients with KC were positive for IgE in the lamina propria of the bladder. In contrast, no positive cases were found in the bladder of...
healthy controls. Further double immunohistochemical staining of tryptase and IgE showed the co-expression of mast cells and IgE.\textsuperscript{115} IgE-mediated hypersensitivity reaction to ketamine with elevated tryptase levels has been previously reported.\textsuperscript{116-118} Tryptase is the most abundant mediator stored in mast cells, and its release is the characteristic of mast cell degranulation.\textsuperscript{119} IgE crosses the epithelium and tightly binds to the mast cell surface through the high-affinity IgE receptor known as the Fc epsilon receptor. The binding of the antigen to these receptors stimulates mast cell degranulation and thus causes the release of chemical mediators from mast cells, such as tryptase, histamine, PGD2, and TNF. The chemical mediators secreted from mast cells can orchestrate the recruitment, tissue infiltration, and functional activation of circulating leukocytes, including eosinophils, basophils, and neutrophils, as well as monocytes, thus substantially increasing the diversity of cellular drivers of inflammation.\textsuperscript{112,120,121} Based on the aforementioned findings, IgE was suggested to play a key role in developing inflammation in the bladder of KC. IgE-neutralizing antibodies might help in relieving the inflammation in the bladders of patients with KC.

3.2 Lamina propria fibrosis

3.2.1 Transforming growth factor-β1-mediated fibrosis

The myofibroblasts are the key cellular mediators of fibrosis in multiple tissues, which, when activated, serve as primary collagen-producing cells.\textsuperscript{122,123} Myofibroblasts have been identified and enriched in the bladder lamina propria.\textsuperscript{124,125} Epithelial cells are reported to transdifferentiate into myofibroblasts via epithelial-mesenchymal transition (EMT) in several fibrosis models.\textsuperscript{126} Consistently, it has been recently reported that ketamine-induced bladder fibrosis involves EMT mediated by Transforming growth factor (TGF)-β1.\textsuperscript{126} Ketamine has been demonstrated to induce TGF-β1 expression in the urothelium of rats.\textsuperscript{127} In addition, TGF-β1 can also be produced by leukocyte lineages, including lymphocytes and macrophages. Inflammatory cells in lamina propria can be sources of TGF-β1 in the bladder of KC. Studies in a wide range of experimental models demonstrated that the canonical TGF-β1/activin-like kinase (ALK) 5/small mother against decapentaplegic (Smad) 2/3 pathway was critically involved in the pathogenesis of fibrosis in many tissues. TGF-β1 contacts with TGF-β receptors and leads to the recruitment and phosphorylation of ALK5, which propagates the signal to the nucleus through the phosphorylation of Smad2 and Smad3 transcription factors. The activation of Smad2 and Smad3 regulates the expression of ECM-related genes, including collagens,\textsuperscript{128,129} various proteoglycans,\textsuperscript{130-132} connective growth factors,\textsuperscript{133} and matrix metalloproteases (MMPs).\textsuperscript{134} In line with submucosal fibrosis, the upregulation of TGF-β1, as well as phosphorylated Smad 2 and Smad 3, was observed in the lamina propria from the bladders of patients with KC.\textsuperscript{18} Furthermore, Wang et al reported that the administration of TGF-β1 inhibitor inhibited ketamine-induced EMT and fibrosis in vitro and in vivo,\textsuperscript{127} suggesting the critical involvement of TGF-β1 in the bladder fibrosis of KC. The TGF-β1-mediated pathway can be an attractive therapeutic target for KC.

3.2.2 Chronic inflammation-mediated fibrosis

Some scientists suggested that fibrosis was the end result of chronic inflammation reactions.\textsuperscript{123} In the bladder with KC, histopathological examination revealed that submucous fibrosis developed in the late stage as a complication of inflammation,\textsuperscript{12,127} indicating that chronic inflammation contributes to the development of fibrosis in the bladder of KC. Chronic inflammation can lead to the release of a wide range of fibrotic factors to regulate fibrosis. For example, CC chemokine ligand (CCL)3 and CCL2 were identified as profibrotic mediators. Macrophages and epithelial cells are believed to be the key sources of CCL3. Studies showed that anti-CCL3 antibodies could significantly reduce the development of fibrosis.\textsuperscript{135,136} Similar results were obtained when CCL2 was neutralized, which was primarily secreted by monocytes, macrophages, and dendritic cells.\textsuperscript{123} IL-6 functions as a pro-inflammatory factor and as a profibrotic factor. It plays a crucial role in fibrosis via the signaling loop IL-6/ GP130/STAT3.\textsuperscript{137}

3.3 Lamina propria inflammation and fibrosis in bladder dysfunction

Lamina propria is the function center of the bladder, determining bladder compliance and serving as a communication center. The lamina propria is composed of an ECM containing several types of cells, including fibroblasts, adipocytes, interstitial cells, and myofibroblasts. In addition, the lamina propria contains a rich capillary network of afferent and efferent nerves.\textsuperscript{138} Thus, the inflammation and fibrosis of the lamina propria in the bladder with KC can significantly affect tissues and cells in the lamina propria and contribute to bladder dysfunction. Besides ketamine, a range of chemicals, including acetic acid, acrolein, CYP, zymosan, and (lipopolysaccharide) LPS, can support lamina propria inflammation- and fibrosis-mediated pathophysiology in bladder dysfunction. These chemicals were employed to develop animal models of chronic cystitis, which all revealed significant inflammation and fibrosis in the lamina propria with long-lasting
bladder hyperactivity and comprised compliance. In the lamina propria of these cystitis models, persistent inflammation can lead to the increased secretion of inflammatory mediators. These inflammatory mediators, including ATP, histamine, nerve growth factor (NGF), cytokines (TNF-α and IL-6), and those released from mast cells, are known to directly activate and sensitize afferent nerves, cause microvascular leakage, and impact BSM. For example, ATP release increases in the bladder of KC and IC/BPS, activating P2X2 and P2X3 receptors expressed in afferent nerves in the lamina propria. The enhanced activation of P2X2 and P2X3 receptors increases the excitability of these nerves, potentially contributing to overactivity, urgency, and pain. Mast cells are frequently observed in the lamina propria of bladder in patients with KC and IC/BPS. Mast cell-derived tryptase can cause microvascular leakage and stimulate protease-activated receptors, leading to inflammation and neuronal hyperexcitability. The increased release of inflammatory mediators under chronic conditions can further induce the development of fibrosis. Fibrosis has been recently demonstrated to play a prominent role in bladder compliance. Together, lamina propria inflammation and fibrosis play important roles in pathological and functional changes in the bladders of patients with KC.

4 | MICROVASCULAR INJURY

Evidence shows that microvascular is altered and injured in the bladders of patients with KC. Histological and cystoscopic studies on KC included qualitative descriptions of the features as increased vascular distribution/density, indicating angiogenesis. Angiogenesis is important for wound healing because it involves the growth of new capillaries to form granulation tissue. Compared with the normal control mice, ketamine-treated mice exhibited prominent granulation tissues with dense blood vessel distribution in the lamina propria. In addition, Lin et al, using immunohistochemical techniques and electron microscopy, showed the endothelial apoptotic and mesenchymal phenotypic changes in the microvascular in the bladder with KC.

4.1 | Microvascular angiogenesis

4.1.1 | Chronic inflammation in microvascular angiogenesis

Angiogenesis is suggested to be intrinsic to chronic inflammation, which can promote angiogenesis in a number of ways. Inflammatory cells such as lymphocytes, macrophages, mast cells, and monocytes can release numerous angiogenic factors including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), angiopoietin (Ang)-1, TGF-α, basic fibroblast growth factor (bFGF), and TNF-α, which can stimulate angiogenesis. Ketamine-induced inflammatory mediators such as COX-2, eNOS, and iNOS, have also been reported to play roles in vessel growth. COX-2 can promote the expression of angiogenic factor VEGF via p38 and c-Jun N-terminal kinase (JNK) activation pathways. eNOS and iNOS can contribute to the release of VEGF via the production of NO. Angiogenesis is attenuated when NO bioactivity is reduced. Furthermore, MMPs were expressed in the vessel wall with inflammation in the bladders of patients with KC, contributing to angiogenesis by degrading the basement membrane and ECM components, allowing endothelial cells to detach and migrate to new tissue, and by releasing ECM-bound proangiogenic factors such as VEGF. In addition, MMPs can trigger integrin signaling, thereby contributing to endothelial cell survival and proliferation. Together, the ketamine-induced persistent inflammation plays important roles in angiogenesis in the bladder.

4.1.2 | Other angiogenic factors in microvascular angiogenesis

According to the increase in blood vessel number, Shen et al reported that several angiogenesis-associated genes, such as cluster of differentiation (CD)31, amphiregulin (Areg), and collagen triple-helix repeat-containing 1 (Cthrc1), were differentially expressed after ketamine treatment, in accordance with the abundant formation of blood vessels in the bladder of KC. CD31 as a well-defined prognostic angiogenic marker expressed on the surface of endothelial cells and well established for the monitoring of vessel density; it was significantly upregulated in the bladder of KC. The upregulation of Cthrc1 can trigger Wnt signaling, which in turn regulates vascular endothelial growth factor (VEGF) and MMPs to induce angiogenesis. Areg can mediate TGF-β1 activation, which stimulates angiogenesis through Smad2/3-VEGF pathways. The upregulation of TGF-β1, as well as phosphorylated Smad 2/3 in the bladders of KC, may also participate in angiogenesis, besides regulating fibrosis.

4.2 | Endothelial apoptosis

4.2.1 | NMDAR in endothelial apoptosis

An in vitro study demonstrated that ketamine could time- and dose-dependently reduce the intracellular calcium levels and cause apoptosis. NMDAR is well known as a calcium channel, which can be blocked by ketamine. NMDARs have been reported as the mediator of ketamine-induced
apoptosis, the transient blockade of which triggers extensive apoptosis. NMDAR1 is expressed in the vascular endothelium in the bladder. Using immunofluorescent staining analysis, Lin et al reported the decreased expression of NMDAR1 in bladder vessels in patients with KC, which might downregulate the intracellular calcium levels. Meantime, ketamine in the blood can further block the calcium influx in endothelial cells by inhibiting NMDAR1. Based on these findings, NMDAR is proposed to mediate endothelial apoptosis in the bladders of patients with KC. However, the exact mechanism of NMDAR-mediated endothelial apoptosis needs further exploration.

4.2.2 | TNF-α in endothelial apoptosis

Another possible mechanism of endothelial apoptosis may be similar to apoptosis in the urothelium via oxidative stress induced by TNF-α. Long-term ketamine administration induces a high level of TNF-α, which has been shown to induce oxidative stress through endothelial mitochondrial and nicotinamide adenine dinucleotide phosphate oxidase. TNF-α induces oxidative stress through endothelial mitochondria principally at the ubiquinone site and damages the mitochondrial chain at complexes I, II, and III, consequently resulting in the increased production of oxygen radicals.

NADPH oxidase is another important source of oxidative stress. TNF-α can induce oxygen radicals in endothelial cells by stimulating NADPH oxidase expression and activating NADPH oxidase directly. In addition, TNF-α can bind to death receptor TNFRI and initiate an apoptotic signaling cascade. A more recent study reported that a redox enzyme p66Shc also regulated mitochondrial ROS generation and contributed to ketamine-induced apoptosis in endothelial cells.

4.3 | Microvascular fibrosis

Apoptosis has been identified as a potential initiator and propagator of fibrosis. Endothelial apoptosis induces endothelial-mesenchymal transition (EndoMT) via activating the TGF-β1 signaling pathway. EndoMT, as a crucial model of pathogenesis in tissue fibrosis, has been previously reported to be involved in vascular fibrosis in the bladder of KC. As mentioned earlier, TGF-β1 in the bladder of KC-mediated fibrosis in the lamina propria by inducing EMT, which is also believed to be involved in vascular fibrosis by activating EndoMT. Lin et al reported that ketamine increased and facilitated the expression of EndoMT markers, including fibroblast-specific protein 1 and α-smooth muscle actin (myofibroblast marker) in human bladder microvascular endothelial cells, indicating the activation of fibroblasts and myofibroblasts. Paracrine and autocrine regulators such as TGF-β1 and IL-6 derived from lymphocytes, macrophages, and other cellular sources cooperate to initiate and maintain myofibroblast activation. Therefore, EMT and EndoMT in the bladder with KC may affect each other via the secretion of these fibrotic regulators. In addition, chronic inflammation in the lamina propria may also contribute to the development of microvascular fibrosis via the release of fibrotic factors such as CCL2, CCL3, and IL-6.

4.4 | Microvascular injury initiate bladder dysfunction

Two distinct capillary networks, including muscular network and subepithelial network, in the successive layers of the human bladder wall have been distinguished. A number of studies demonstrated that the subepithelial capillary network was significantly changed in the bladders of patients with KC. However, little is known about the muscular network in the bladders of patients with KC, and further investigation is warranted. The subepithelial capillary network in the human bladder has been studied in detail by scanning electron microscopy, it is located close to the urothelium and surrounded by urothelial cells. It was suggested that the urothelial barrier function was dependent on an adequate supply of oxygen and nutrients from the blood. The capillaries in the bladder wall must be able to adapt to stretch and relaxation resulting from the filling and voiding cycles. However, ketamine can induce different signaling pathways to cause microvascular injury with enhanced apoptosis, fibrosis, and angiogenesis. Capillary fibrosis increases the fragility, which makes capillaries more susceptible to bleeding from the stretch. It is reasonable to assume that the subepithelial capillary network ineflectively performs its function in the bladders of patients with KC. Petechial hemorrhage and angiogenesis in the subepithelial cells of the bladder support this hypothesis. These changes may lead to compromised intrinsic microcirculation, resulting in bladder ischemia. Moderate ischemia causes bladder hyperactivity, whereas severe ischemia causes bladder underactivity. Ischemia can also lead to bladder fibrosis and diminished bladder capacity. Chu et al suggested that the symptoms of KC could be due to bladder ischemia. In addition, endothelial apoptosis contributes to increased microvascular permeability. Increased endothelial permeability leads to the leakage of large molecules and cells in the bladders of patients with KC. The leakage of larger molecules and cells can result in bladder inflammation, fibrosis, and disease progression of KC. Based on endothelial cell injury, Lin et al suggested that vessels in the bladder were the primary target sites that initiated the cascade of inflammatory reaction and fibrotic changes in the bladder with KC.
5  |  NEUROPATHOLOGICAL ALTERATIONS

Histopathological examinations of the bladder tissue from patients with KC showed neurogenesis with increased numbers of nerve fascicles and conspicuous perineuria. Jhang et al further reported neurogenic inflammation in the bladder with KC. Nerve fiber proliferation and inflammation in the suburothelium and muscular layer were reported in other forms of cystitis, such as IC/BPS, which caused high sensitivity to pain and pressure. The bladders of patients with KC, compared with patients with IC/BPS, displayed significantly increased nerve fibers and neuroinflammation, which were proposed as the plausible mechanisms of KC.

5.1  |  Neurogenesis

5.1.1  |  NMDAR-mediated neurogenesis

A number of studies reported that repeated or a single dose of ketamine administration modulated neurogenesis via blockade of the NMDAR. Consistently, NMDAR antagonists, such as Ro256981, MK-801, and CGP 37849, can stimulate neurogenesis, while NMDAR agonists, including N-methyl-D aspartic, have the reverse effect. NMDAR subunits are reported to be expressed in the urinary tract of humans and animals. Further research found them localized in neurons of the bladder, which were verified by the single-cell sequencing method.

One possible mechanism by which ketamine may induce neurogenesis in the bladder via blocking the NDMAR is the attenuation of the expression of transcription factors, such as forkhead box protein O (FOXO), cellular Jun (c-Jun), and cellular Fos (c-Fos). Ketamine inhibited the expression of these genes in BSM strips by inhibiting calcium-dependent signaling. The suppression of FOXO activities contributes to prolonging the life span of neurons involved in neurogenesis. The activation of FOXO leads to neuron cell death. C-Jun heterodimerizes with c-Fos to form the transcription factor activator protein (AP)-1. Peng et al reported that AP-1/c-Jun inhibited the formation of the neural ectoderm, and the ectopic expression of c-Jun is sufficient to inhibit neutralization. A second link between the blockade of NMDAR and induction of neurogenesis was offered by the NMDAR-nNOS pathway. Endogenous nNOS has been shown to regulate neurogenesis negatively. nNOS is mainly expressed in neurons and can be enhanced by NMDAR activation via the calcium influx that may activate the calcium-dependent pathway. A blockage of NMDAR by ketamine leads to a reduction of nNOS levels. This, in turn, results in the induction of neurogenesis. Shang et al reported that long-term ketamine administration decreased the expression of nNOS on the cavernous nerve. However, western blot (WB) results demonstrated no significant change in nNOS levels in the bladders of patients with KC compared with normal controls. Notably, previous reports of nNOS expression were tested by WB in the whole bladder, which did not represent the authentic expression of nNOS in the neurons. Further immunobiological studies are needed to examine the expression of nNOS localized in the nerves of the bladder in patients with KC.

5.1.2  |  Brain-derived neurotrophic factor-mediated neurogenesis

A recent study found that chronic ketamine users (at least four times each week) had an average twice the serum concentration of Brain-derived neurotrophic factor (BDNF) compared with the controls. BDNF has been extensively investigated because of its positive role in neurogenesis. BDNF mutant mice, which lacked dendritic expression and secretion of BDNF, exhibited an impaired differentiation of newborn neurons. BDNF functions by binding to tropomyosin receptor kinase B (TrkB), which is coupled with intracellular signaling cascades including MAPK/extracellular signal-regulated kinase (ERK) and phosphoinositide 3-kinase (PI3K)/Akt for neurogenesis. In addition, a recent study found that BDNF-activated neural stem cell (NSC) plasticity, proliferation of NSCs, and neurogenesis through nerve growth factor receptor (NGFR), the blockage of which reduced NSC plasticity, proliferation, and neurogenesis. The expansion of intense basal NGFR expression was observed in the bladders of patients with KC. BDNF and NGFR present in the bladder regulate bladder sensory afferent neural plasticity and are strongly linked to bladder hyperactivity and in some cases to pain. Taken together, the chronic use of ketamine resulting in the elevation of BDNF and NGFR could be associated with neurogenesis in the bladders of patients with KC.

5.2  |  Neuroinflammation

5.2.1  |  BDNF in neuroinflammation

As an important neurotrophic factor, BDNF plays an important role in angiogenesis. It also plays a crucial role in the occurrence and development of neuroinflammation. Binding BDNF to the TrkB receptor can induce the activation of the p38/JNK signaling pathway, which participates in inducing the expression of NF-κB. NF-κB, as one of the main factors of inflammatory activation, participates in releasing inflammatory cytokines, including IL-6, IL-1β, and TNF-α. Previous studies reported that the BDNF-TrkB-p38/JNK signaling...
signaling pathway was involved in neuroinflammation with CYP-induced cystitis. 207 The activation of p38/JNK, as well as an increased NF-κB level, was upregulated in the bladders of patients with KC. 51,53 indicating that the actions of the BDNF-TrkB-p38/JNK signaling pathway might be involved in the development of neuritis in the bladders of patients with KC. Interestingly, systemic knockdown of BDNF activity by the intraperitoneal injection of BDNF-neutralizing antibody reversed the inflammation and overactivity in the bladders of patients with cystitis. 208 Pinto et al also reported that the sequestration of BDNF using TrkB-lg2 intravenous or intravesical instillation in an inflammatory model improved the bladder function and decreased the expression of nociceptive markers in the afferent pathway. 209 Based on the aforementioned observations and the role of BDNF in neuroinflammation and neurogenesis, the sequestration of BDNF might be considered as a potential therapeutic strategy in KC.

5.2.2 | Inflammatory cells in neuroinflammation

Another possible mechanism in the genesis and development of neuroinflammation in the bladder with KC may be linked with the infiltration of inflammatory cells in the lamina propria and muscular layer, such as mast cells. An increased number of tryptase-positive mast cells were found in the bladder tissue from patients with KC. 214 Mast cells are commonly found in the close vicinity of substance P (SP)-positive fibers. 146,210 Once activated, mast cells undergo degranulation to release inflammatory and nociceptive mediators, such as histamine, tryptase, TNF-α, IL-6, IL-8, and PGs. 211,212 These mediators under chronic conditions can promote neuritis and stimulate the secretion of neurotransmitters that, in turn, activate mast cells. For example, histamine plays a key role in neuroinflammation, and mast cells residing in the bladder are a primary source of histamine. Once released, histamine induces the release of inflammatory mediators, such as SP and calcitonin gene-related peptide (CGRP), from adjacent neurons, which, in turn, can induce adjacent mast cell activation and degranulation, further leading to inflammation and neuronal hyperexcitability. 213 The severity of neuroinflammation induced by intravenous SP or LPS is greatly reduced in the bladder in mice deficient in mast cells, 214 suggesting an important role for mast cells in neuroinflammation in the bladder.

5.3 | Neurogenesis and neuroinflammation in bladder dysfunction

Neuroinflammation and neurogenesis are associated with increased afferent nerve excitability and sensitization of primary afferent neurons as shown in a number of animal cystitis models. 146 These neurological changes with alterations in the expression and function of K+ and Na+ ion channels, which govern neuronal excitability, have been widely reported, including Kv1.4, Nav1.8, and Nav1.9. 215-218 In addition, neuroinflammation-induced excessive release of SP, CGRP, and neurokinin (NKA) with an increased number of nerve fibers also play roles in afferent nerve hyperexcitability. CGRP can enhance tetrodotoxin (TTX)-resistant currents. 219 SP has been implicated in the pathophysiology of pain and can increase the number of action potentials. 220 SP and NKA both can activate neurokinin receptors to influence the excitability of C-fibers in the bladder by lowering the threshold for triggering an action potential. 217,220 In human bladder tissue from patients with IC/BPS, the number of SP nerve fibers significantly increased in the bladder wall. 146 The function of excessive SP and the increased number of SP nerve fibers might explain the pathophysiology of urgency, frequency, and painful nature of IC/BPS. However, increased NGFR-positive nerve fibers were present in the bladder wall in the human bladder tissue from patients with KC. 17 Since the upregulation of NGF in the bladder wall of rodents resulted in bladder hyperexcitability, reduced bladder capacity increased voiding frequency and increased the amplitude of non-voiding contraction. 221 The function of the increased NGFR-positive nerve fibers might explain the pathophysiology of KC. Furthermore, on inflammation, the cells in the bladder can synthesize and release different factors that can further influence neuroinflammation and neurogenesis and contribute to symptoms, such as ATP, SP, NO, PGs, NGF, cytokines, and others. 146 For example, ATP can be released by urothelial cells, afferent cells, and efferent cells and can act on nearby sensory fibers resulting in altered sensations or changes in bladder reflexes. 222 Together, neuropathological alterations in the bladder of KC can contribute to urgency, increased voiding frequency, and pain.

6 | BSM ABNORMALITIES

The smooth muscle is the main component of the urinary bladder wall. The relaxation and contraction of urinary BSM allow the bladder to store and void urine. Pathological and functional abnormalities of BSM have a direct role in the development of LUTS. Histological research on patients with KC typically showed degeneration of smooth muscle with collagen deposition. 12,30 Urodynamic studies revealed a reduced bladder capacity and compliance, sometimes combined with detrusor overactivity. 8,11,223 Recent studies demonstrated that ketamine-induced BSM abnormalities via various signaling pathways, which played a crucial role in the development of KC.
6.1 | Inhibition of BSM generation

In cultured smooth muscle cells (SMCs), ketamine induces dose-dependent inhibitory effects on cell generation. Among the MAPK family, ERK1/2 is one of the well-known signal transduction pathways for SMC differentiation and proliferation. Besides, Akt, a serine/threonine protein kinase, was found to be activated via the PI3K pathway; it also plays roles in SMC differentiation and proliferation. Ketamine was shown to attenuate PI3K, Akt, and ERK1/2 phosphorylation in SMCs. PDGF-BB is a potent mitogen for bladder SMCs, which can increase DNA synthesis in a PI3K/Akt-dependent manner. However, ketamine can significantly reverse PDGF-BB-induced PI3K/Akt phosphorylation and suppress SMC generation.

In addition, ketamine can affect SMC differentiation and proliferation by the inhibition of Cav1.2. Calcium influx through Cav1.2 activates calcium-dependent signaling proteins, which propagate the signal into the nucleus to regulate transcription factors such as cAMP response element-binding protein (CREB), myocyte enhancer factor, and nuclear factor of activated T-cell protein. c-Fos and c-Jun are well-documented immediate early genes (IEGs) in response to Cav1.2 activation. IEGs and transcription factors modulated by Cav1.2 signaling are important for cell survival, proliferation, and differentiation. Cav1.2 blockers, nifedipine and ketamine, were both found to inhibit BSM cell generation via the inhibition of these IEGs and transcription factors. Also, heterozygous Cav1.2 mice displayed smaller bladders with the degeneration of BSM, further indicating that the inhibition of Cav1.2 signaling was an important pathway in BSM degeneration in the bladders of patients with KC.

6.2 | BSM inflammation and fibrosis

Ketamine has been indicated to induce the direct expression and deposition of ECM-related proteins, such as collagen I, collagen II, and fibronectin, in cultured human SMCs, consequently increasing the distribution and accumulation of ECM components between BSM cells in the bladder. Another possible mechanism of ketamine-induced fibrosis might be related to persistent inflammation in the muscular layer and lamina propria. Ketamine has been reported to directly induce inflammatory cytokines, such as TGF-β1 and COX-2, in cultured SMCs, which can modulate the inflammatory response and regulate fibrosis. Using immunostaining analysis, Lin et al reported the expression of multiple inflammatory mediators, such as COX-2 and iNOS, in the muscular layer of the bladder in patients with KC, which were involved in the stimulation of inflammatory cytokines and chemokines and the recruitment of inflammatory cells. Infiltrated inflammatory cells are commonly found in the muscular layer, contributing to BSM fibrosis via the secretion of fibrotic factors. In addition, inflammatory and fibrotic factors, such as IL-6, TGF-β1, CCL2, and CCL3, derived from lymphocytes, macrophages, mast cells, and other cellular sources in the lamina propria and microvasculature may also cooperate to initiate and maintain the inflammation and fibrosis in the muscular layer.

6.3 | BSM dysfunction

A previous in vitro study showed that ketamine could dose-dependently attenuate vascular SMC contraction induced by adrenaline, noradrenaline, angiotensin II, vasopressin, and KCl. In addition, ketamine has also been demonstrated to be a potent bronchodilator that relaxes the airway smooth muscle. Furthermore, a single anesthetic dose of ketamine has been found to significantly decrease the carbachol-induced contractile response of rat smooth muscle strips. A previous study found that ketamine suppressed signaling pathways mediated by muscarinic receptors heterologously expressed in Xenopus oocytes, which partially explained the mechanism of the inhibition of smooth muscle contraction in response to cholinergic stimulation with ketamine. However, we recently reported that ketamine and its metabolite nor-ketamine could dose-dependently inhibit BSM contraction in response to electrical field stimulation (EFS), high K, carbachol, and α, β-meATP. Purinergic and cholinergic signaling and high K levels all stimulate smooth muscle contraction by initiating an increase in the intracellular calcium concentration. Some studies suggested that ketamine inhibited smooth muscle contraction by regulating the intracellular calcium level in SMCs.

6.3.1 | Cav1.2 in BSM dysfunction

We recently found that ketamine and nor-ketamine were novel antagonists of the L-type calcium channel (LTCC). Hence, they could inhibit BSM contraction in response to EFS, purinergic, cholinergic, and depolarization signaling, independent of NMDAR. Cav1.2 is one of the LTCCs expressed in BSM cells. More importantly, Cav1.2 was demonstrated to be essential for urinary BSM contractility. Mouse BSM deficient in Cav1.2 lacked contraction in response to carbachol and high K. Heterozygous Cav1.2 inactivation in smooth muscle indicated bladder overactivity with reduced bladder volume, contractile pressure, and compliance, recapitulating KC in humans. Ketamine/Nor-ketamine is present at a concentration of tens of μg/mL in the plasma of ketamine abusers. Furthermore, ketamine/nor-ketamine is mostly eliminated by renal excretion into the urine, and a high concentration is found in the urine of ketamine abusers.
Ketamine is a lipophilic drug that easily crosses epithelial cells via passive diffusion, which may partially inhibit Cav1.2 on BSM cells, thereby impacting BSM function.

6.3.2 | Purinergic signaling in BSM dysfunction

Enhanced ATP release and upregulation of the P2X1 receptor may also contribute to BSM dysfunction in the bladder of KC. P2X1 receptors located on the BSM cells mediate bladder contraction. ATP can lead to the rapid engagement of the P2X1 receptor on BSM cells. The P2X1 receptor-mediated Na\(^+\) and Ca\(^{2+}\) influx in BSM cells creates an excitatory junction potential. However, it is the subsequent opening of voltage-gated calcium channels and calcium-induced calcium release from intracellular stores, making the concentrations of intracellular necessary for smooth muscle contractions. ATP and P2X1 receptor upregulation can enhance spontaneous calcium activity in BSM, which is associated with bladder overactivity. The immunostaining analysis showed that the P2X1 receptor in BSM of the bladder in patients with KC was significantly upregulated. In addition, the P2X1-mediated contraction force also increased. Enhanced purinergic signaling with increased P2X1-mediated contraction force might explain the failure of anticholinergic agents to relieve the symptoms induced by chronic ketamine abuse.

6.4 | BSM abnormalities in bladder dysfunction

The primary function of BSM is to contract during urination to push the urine out of the bladder. BSM contraction is predominantly regulated by acetylcholine and ATP, which bind to muscarinic receptors M2/3 and purinergic receptor P2X1, respectively. Muscarinic and/or purinergic stimulation initiates extracellular calcium entry through the Cav1.2 channel, which plays an essential role in bladder function. Ketamine has antagonist activity on muscarinic receptors and Cav1.2, which may partially inhibit muscarinic receptors and Cav1.2 activities in vivo. Direct intravesical infusion of ketamine in the bladder of mice significantly increases voiding frequency and decreases peak bladder pressure. In addition, the use of Cav1.2 antagonists, such as nifedipine and felodipine, has a great association with LUTS. Similar to ketamine, the intravesical infusion of nifedipine also decreases voiding interval and peak bladder pressure. Consistently, the heterozygous inactivation of Cav1.2 in the smooth muscle indicated bladder overactivity with reduced bladder volume, contractile pressure, and compliance. The P2X1 receptor is highly expressed in BSM cells. The magnitude of P2X1-mediated force generation is believed to be species dependent, with a relatively small contribution in humans. However, P2X1-mediated contraction can account for up to 65% of the total force under pathological conditions such as in patients with overactive bladder, partial bladder outlet obstruction, and diabetic bladder dysfunction.

A common finding in these patients is elevated ATP release and/or altered P2X receptor expression, which was also observed in patients with KC. Therefore, the dysregulation of P2X1-mediated purinergic signaling in BSM plays an important role in bladder dysfunction.

In addition, BSM degeneration and fibrosis, which reduce the BSM content, also play important roles in bladder function. The most often used method to evaluate the BSM content is the morphometric analysis of the muscle layer and calculation of the collagen to BSM (C/M) ratio. Several studies investigated the correlation between the C/M ratio and urodynamic parameters. Landau et al showed that dysfunctional bladders were significantly less compliant compared with the normal ones, and these bladders had increased collagen deposition and C/M ratio, which were consistent with the findings in the bladders of patients with KC. Such histological changes were reflected by abnormal low bladder capacity and decreased bladder compliance, and these could be indicators for a loss of bladder wall elasticity. Previous studies demonstrated that increased detrusor collagen deposition led to a reduction of bladder elasticity and compliance. However, a bladder with smoother muscle content would be expected to have more elastic properties and higher compliance. EGCG or hyaluronan treatment reversed the C/M ratio and restored bladder storage function in the bladders of KC, indicating that the loss of BSM content was associated with bladder dysfunction in the bladders of patients with KC.

7 | CONCLUSIONS

The pathogenesis of KC is complicated and involves many tissue injuries, including urothelium disruption, microvascular injury, neurogenic alterations, BSM abnormalities, and lamina propria fibrosis and inflammation. Various ketamine-induced signaling pathways are involved in these tissue injuries (Figure 1). Some of these signaling pathways can be regulated by ketamine directly. In contrast, others can be regulated by ketamine-induced oxidative stress and persistent inflammation, which can recurrently lead to tissue injuries and contribute to the pathogenesis of KC. Recent studies have greatly improved the knowledge of the pathogenesis of this clinical condition. However, further investigation is needed to determine the precise pathways of oxidative stress and inflammation involved. Enhanced knowledge of the role and function of these signaling pathways may allow for a better understanding of the
Proposed pathways for tissue injuries in the bladder of ketamine cystitis. Ketamine has detrimental effects on urothelial cells by induction of APF, ATP, and oxidative stress. APF can activate P38/MAPK and inhibit Akt signaling pathways to suppress urothelial cell proliferation and junction protein expression. ATP and oxidative stress can initiate apoptotic signaling pathways to enhance urothelial cell apoptosis abnormally. Besides, oxidative stress can disrupt tight and adherent junctions by modulating GSH/GSSH homeostasis, rearranging the actin cytoskeleton, and initiating secondary oxidation products. These detrimental effects of ketamine on the urothelium can affect the production and secretion of GAGs and uroplakins, consequently leading to urothelium disruption. In the lamina propria, ketamine initiates the upregulation of NOS and COX-2 to produce large amounts of NO and PGs, respectively, both of which can promote the inflammatory response. In addition, ketamine can activate IgE, which triggers inflammation via stimulating inflammatory cells to release inflammatory cytokines. Fibrosis is another important pathogenesis in the lamina propria of the bladder in patients with KC, which is controlled by EMT mediated by TGF-β1. Other cytokines and chemokines, such as IL-6, CCL3, and CCL2, from infiltrated inflammatory cells and other cellular sources may also participate in fibrosis. Ketamine also contributes to microvascular injury via inducing microvascular angiogenesis, fibrosis, and endothelial cell apoptosis. Ketamine induces endothelial cell apoptosis via inhibiting NMDAR expression and activity to downregulate the intracellular calcium level and by upregulating TNF-α to activate death receptor and stimulate oxidative stress. Endothelial cell apoptosis and ketamine can further initiate microvascular fibrosis via EndoMT mediated by the activation of the TGF-β1 signaling pathway. Ketamine can activate angiogenesis-associated genes, such as Areg and Chthc1, to stimulate microvascular angiogenesis. A chronic inflammatory response also contributes to angiogenesis via numerous angiogenic factors including PDGF, VEGF, Ang-1, TGF-α, bFGF, and TNF-α. Ketamine has effects on neurogenesis via the regulation of NMDAR- and BDNF-dependent signaling. In addition, the activation and upregulation of BDNF may also play a role in the development of neuroinflammation via NF-κB. Furthermore, inflammatory cells secrete cytokines and chemokines, such as histamine, tryptase, TNF-α, IL-6, and PGs, which also promote neuritis in the bladders of patients with KC. In the muscular layer, ketamine affects BSM cell generation by attenuating PI3K/Akt and MAPK/ERK1/2 phosphorylation and Cav1.2-dependent calcium signaling. Ketamine also contributes to inflammation and fibrosis in the muscular layer by inducing inflammatory and fibrotic mediators from BSM and other tissues. Furthermore, the inactivation of Cav1.2 and muscarinic receptor signaling and the induction of purinergic signaling by ketamine are associated with BSM dysfunction.
pathophysiology of this disease and for the identification of novel pharmacologic targets for KC.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS
Conceptualization: H. Chen and X. Xie; Investigation and Analysis: H. Chen, X. Xie, J. Liang, R. Huang, C. Luo, J. Yang, H. Xing, L. Zhou, H. Qiao and E. Ergu; Writing—original draft: H. Chen and X. Xie; Writing—review & editing: H. Chen and X. Xie; Supervision: H. Chen; Project administration: H. Chen; Funding acquisition: H. Chen.

REFERENCES
1. Domino EF. Taming the ketamine tiger. 1965. Anesthesiology. 2010;113(3):678-684.
2. Lankenum SE, Clatts MC. Drug injection practices among high-risk youths: the first shot of ketamine. J Urban Health. 2004;81(2):232-248.
3. Kalsi SS, Wood DM, Durgan PI. The epidemiology and patterns of acute and chronic toxicity associated with recreational ketamine use. Emerg Health Threats J. 2011;15(4):7107.
4. Li Y, Shi J, Yang BF, et al. Ketamine-induced ventricular structural, sympathetic and electrophysiological remodelling: pathological consequences and protective effects of metoprolol. Br J Pharmacol. 2012;165(6):1748-1756.
5. Wei YB, Yang JR, Yin Z, Guo Q, Liang BL, Zhou KQ. Genitourinary toxicity of ketamine. Hong Kong Med J. 2013;9(4):341-348.
6. Cheung TT, Poon RT, Chan AC, Lo CM. Education and Imaging. Hepatobiliary and pancreatic: cholangiopathy in ketamine user—an emerging new condition. J Gastroenterol Hepatol. 2014;29(9):1663.
7. Bokor G, Anderson PD. Ketamine: an update on its abuse. J Pharm Pract. 2014;27(6):582-586.
8. Rajandram R, Ong TA, Razack AH, MacIver B, Zeidel ML, Yu W. Intact urothelial barrier function in a mouse model of ketamine-induced voiding dysfunction. Am J Physiol Renal Physiol. 2016;310(9):F885-F894.
9. Shahani R, Streutker C, Dickson B, Stewart RJ. Ketamine-associated ulcerative cystitis: a new clinical entity. Urology. 2007;69(5):810-812.
10. Chu PS, Ma WK, Wong SC, et al. The destruction of the lower urinary tract by ketamine abuse: a new syndrome? BJU Int. 2008;102(11):1616-1622.
11. Tsai YC, Kuo HC. Ketamine cystitis: its urological impact and management. Urol Sci. 2013;26(3):153-157.
12. Jhang JF, Hsu YH, Kuo HC. Possible pathophysiology of ketamine-related cystitis and associated treatment strategies. Int J Urol. 2015;22(9):816-825.
13. Lee CL, Jiang YH, Kuo HC. Increased apoptosis and suburothelial inflammation in patients with ketamine-related cystitis: a comparison with non-ulcerative interstitial cystitis and controls. BJU Int. 2013;112(8):1156-1162.
14. Liu KM, Chuang SM, Long CY, et al. Ketamine-induced ulcerative cystitis and bladder apoptosis involve oxidative stress mediated by mitochondria and the endoplasmic reticulum. Am J Physiol Renal Physiol. 2015;309(4):F318-F331.
15. Lee YL, Lin KL, Chuang SM, et al. Elucidating mechanisms of bladder repair after hyaluronan instillation in ketamine-induced ulcerative cystitis in animal model. Am J Pathol. 2017;187(9):1945-1959.
16. Duan Q, Wu T, Yi X, Liu L, Yan J, Lu Z. Changes to the bladder epithelial barrier are associated with ketamine-induced cystitis. Exp Ther Med. 2017;14(4):2757-2762.
17. Baker SC, Stahlschmidt J, Oxley J, et al. Nerve hyperplasia: a unique feature of ketamine cystitis. Acta Neuropathol Commun. 2013;1:64.
18. Kim A, Yu HY, Heo J, et al. Mesenchymal stem cells protect against the tissue fibrosis of ketamine-induced cystitis in rat bladder. Sci Rep. 2016;6:30881.
19. Huang CJ, Lee FK, Chen SK, Chien CC, Wu ST, Wang YC. Clinical significance of interleukin-6 and inducible nitric oxide synthase in ketamine-induced cystitis. Int J Mol Med. 2018;41(2):836-844.
20. Chen H, Vandorpe DH, Xie X, Alper SL, Zeidel ML, Yu W. Disruption of Cav1.2-mediated signaling is a pathway for ketamine-induced pathology. Nat Commun. 2020;11(1):4328.
21. Gu D, Huang J, Yin Y, Shan Z, Zheng S, Wu P. Long-term ketamine abuse induces cystitis in rats by impairing the bladder epithelial barrier. Mol Biol Rep. 2014;41(11):7313-7322.
22. Hurst RE, Greenwood-Van Meerveld B, Wisniewski AB, et al. Increased bladder permeability in interstitial cystitis/painful bladder syndrome. Transl Androl Urol. 2015;4(5):563-571.
23. Wang Q, Wu Q, Wang J, et al. Ketamine analog methoxetamine induced inflammation and dysfunction of bladder in rats. Int J Mol Sci. 2017;18(1):117.
24. Shen CH, Wang ST, Wang SC, et al. Ketamine-induced bladder dysfunction is associated with extracellular matrix accumulation and impairment of calcium signaling in a mouse model. Mol Med Rep. 2019;19(4):2716-2728.
25. Hurst RE, Rhodes SW, Adamson PB, Parsons CL, Roy JB. Functional and structural characteristics of the glycosaminoglycans of the bladder luminal surface. J Urol. 1987;138(2):433-437.
26. Hurst RE, Zebrowski R. Identification of proteoglycans present at high density on bovine and human bladder luminal surface. J Urol. 1994;152(3 Pt 1):1641-1645.
27. Hurst RE, Roy JB, Min KW, et al. A deficit of chondroitin sulfate proteoglycans on the bladder uroepithelium in interstitial cystitis. Urology. 1996;48(5):817-821.
28. Hurst RE, Roy JB, Parsons CL, Hurst SN. The role of glycosaminoglycans in normal bladder physiology and the pathophysiology of interstitial cystitis. In: Sant GR, ed. Interstitial Cystitis. Philadelphia, PA: Lippincott-Raven; 1997:93-100.
29. Ho CC, Pezham H, Praveen S, et al. Ketamine-associated ulcerative cystitis: a case report and literature review. Malays J Med Sci. 2010;17(2):61-65.
30. Lin HC, Lee HS, Chieueh TS, et al. Histopathological assessment of inflammation and expression of inflammatory markers in patients with ketamine-induced cystitis. Mol Med Rep. 2015;11(4):2421-2428.
31. Kidger E, Stahlschmidt J, Garthwaite M, Fulford S, Southgate J, Baker SC. A rare urachal cyst in a case of ketamine-induced cystitis provides mechanistic insights. Urology. 2016;90:223.e1-223.e7.
32. Chen CH, Lee MH, Chen YC, Lin MF. Ketamine-snorting associated cystitis. *J Formos Med Assoc.* 2011;110(12):787-791.

33. Lai Y, Wu S, Ni L, et al. Ketamine-associated urinary tract dysfunction: an underrecognized clinical entity. *Urol Int.* 2012;89(1):93-96.

34. Meng E. Ketamine cystitis—A long-lasting burning issue in Taiwan. *Urol Sci.* 2015;26(3):158-159.

35. Ou YL, Liu CY, Cha TL, Wu ST, Tsao CW. Complete reversal of bladder uroplakin III gene expression by mitochondrial stress. *Am J Pathol.* 2000;151(5):961-972.

36. Matuszewski MA, Tupikowski K, Dolowy Ł, Szymańska B, Dembowski J, Zdrojowy R. Uroplakin and their potential applications in urology. *Cent European J Urol.* 2016;69(3):252-257.

37. Hu P, Deng FM, Liang FX, et al. Ablation of uroplakin III gene results in small urothelial plaques, urothelial leakage, and vesicoureteral reflux. *J Cell Biol.* 2000;151(5):961-972.

38. Baker SC, Shabir S, Georgopoulos NT, Southgate J. Ketamine-induced apoptosis in normal human urothelial cells: A direct, N-methyl-d-aspartate receptor-independent pathway characterized by mitochondrial stress. *Am J Pathol.* 2016;186(5):1267-1275.

39. Sun TT, Liang FX, Wu XR. Uroplakins as markers of urothelial differentiation. *Adv Exp Med Biol.* 1999;462:7-18.

40. Kątnik-Prastowska I, Lis J, Matejuk A. Glycosylation of uroplakins. Implications for bladder physiopathology. *Glycoconjug J.* 2014;31(9):623-636.

41. Acharya P, Beckel J, Ruiz WG, et al. Distribution of the tight junction proteins ZO-1, occludin, and claudin-4, -8, and -12 in bladder epithelium. *Am J Physiol Renal Physiol.* 2004;287(2):F305-318.

42. Rickard A, Dorokhov N, Ryerse J, Klumpp DJ, McHowat J. Characterization of tight junction proteins in cultured human urothelial cells. *Vitro Cell Dev Biol Anim.* 2008;44(7):261-267.

43. Lee JD, Lee MH. Decreased expression of zona occludens-1 and occludin in the bladder urothelium of patients with interstitial cystitis/painful bladder syndrome. *J Formos Med Assoc.* 2014;113(1):17-22.

44. Hartsock A, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta.* 2008;1788(3):660-669.

45. de Beco S, Gueudry C, Amblard F, Cosoy S. Endocytosis is required for E-cadherin redistribution at mature adherens junctions. *Proc Natl Acad Sci U S A.* 2009;106(17):7010-7015.

46. Zhang CO, Wang JY, Koch KR, Keay S. Regulation of tight junction proteins and bladder epithelial paracellular permeability by an antiproliferative factor from patients with interstitial cystitis/painful bladder syndrome. *J Urol.* 2005;174(6):2382-2387.

47. Rashid HH, Reeder JE, O’Connell MJ, Zhang CO, Messing EM, Keay SK. Interstitial cystitis antiproliferative factor (APF) as a cell-cycle modulator. *BMC Urol.* 2004;6(4):3.

48. Keay S, Leitzell S, Ochzrin A, Clements G, Zhan M, Johnson D. A mouse model for interstitial cystitis/painful bladder syndrome based on APF inhibition of bladder epithelial repair: a pilot study. *BMC Urol.* 2012;12:17.

49. Kim J, Keay SK, Freeman MR. Heparin-binding epidermal growth factor-like growth factor functionally antagonizes interstitial cystitis antiproliferative factor via mitogen-activated protein kinase pathway activation. *BJU Int.* 2009;103(4):541-546.

50. Peerapen P, Thongboonkerd V. p38 MAPK mediates calcium oxalate crystal-induced tight junction disruption in distal renal tubular epithelial cells. *Sci Rep.* 2013;3:1041.

51. Hills CE, Jin T, Siamantouras E, Liu IK, Jefféron KP, Squires PE. ‘Special k’ and a loss of cell-to-cell adhesion in proximal tubule-derived epithelial cells: modulation of the adherens junction complex by ketamine. *PLoS One.* 2013;8(8):e71819.

52. Yu L, Gan X, Liu X, An R. Calcium oxalate crystals induces tight junction disruption in distal renal tubular epithelial cells by activating ROS/Akt/p38 MAPK signaling pathway. *Ren Fail.* 2017;39(1):440-451.

53. Xi XJ, Zeng JJ, Lu Y, et al. Extracellular vesicles enhance oxidative stress through P38/NF-kB pathway in ketamine-induced ulcerative cystitis. *J Cell Mol Med.* 2020;24(13):7609-7624.

54. Conrads TP, Tocci GM, Hood BL, et al. CKAP4/p63 is a receptor for the frizzled-8 protein-related antiproliferative factor from interstitial cystitis patients. *J Biol Chem.* 2006;281(49):37836-37843.

55. Zhang J, Planey SL, Ceballos C, Stevens SM Jr, Keay SK, Zacharias DA. Identification of CKAP4/p63 as a major substrate of the palmitoyl acyltransferase DHHC2, a putative tumor suppressor, using a novel proteomics method. *Mol Cell Proteomics.* 2008;7(7):1378-1388.

56. Tuffy KM, Planey SL. Cytoskeleton-associated protein 4: functions beyond the endoplasmic reticulum in physiology and disease. *ISRN Cell Biol.* 2012;4:1-11. https://doi.org/10.5402/2012/142313

57. Han X, Fink MP, Yang R, Delude RL. Increased iNOS activity results in small urothelial plaques, urothelial leakage, and vesicoureteral reflux. *BMC Anesthesiol.* 2017;23(1):263.

58. Weckmann K, Labermaier C, Asara JM, Müller MB, Turck CW. Oxidative stress- induced disruption of epithelial and endothelial tight junctions antiproliferative factor via mitogen-activated protein kinase pathway activation. *BJU Int.* 2009;103(4):541-546.

59. Fanning AS, Jameson BJ, Jesaitis LA, Anderson JM. The tight junction. *Biol Med.* 2003;7(3):247-256.

60. Fanning AS, Jameson BJ, Jesaitis LA, Anderson JM. The tight junction. *Biol Med.* 2003;7(3):247-256.

61. Rao RK, Basuroy S, Rao VU, Karnaky KJ Jr, Gupta A. Tyrosine phosphorylation and dissociation of occludin-ZO-1 and E-cadherin-beta-catenin complexes from the cytoskeleton by oxidative stress. *Biochem J.* 2002;368(Pt 2):471-481.

62. Rao R. Oxidative stress- induced disruption of epithelial and endothelial tight junctions. *Front Biosci.* 2008;13:7-210,7226.

63. Mn P, Kit S, Kitts DD. The role of nitric oxide in regulating intestinal redox status and intestinal epithelial cell functionality. *Int J Mol Sci.* 2019;20(7):1755.

64. Rao RK, Basuroy S, Rao VU, Karnaky KJ Jr, Gupta A. Tyrosine phosphorylation and dissociation of occludin-ZO-1 and E-cadherin-beta-catenin complexes from the cytoskeleton by oxidative stress. *Biochem J.* 2002;368(Pt 2):471-481.

65. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal.* 2014;20(7):1126-1167.

66. Madara JL. Intestinal absorptive cell tight junctions are linked to cytochrome. *Am J Physiol.* 1987;253(1 Pt 1):C171-C175.

67. Fanning AS, Jameson BJ, Jesaitis LA, Anderson JM. The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *J Biol Chem.* 1998;273(45):29745-29753.

68. Xu Q, Huff LP, Fujii M, Griendling KK. Redox regulation of the actin cytoskeleton and its role in the vascular system. *Free Radic Biol Med.* 2017;109:84-107.

69. Shan Z, Wei L, Yu S, et al. Ketamine induces reactive oxygen species and enhances autophagy in SV-HUC-1 human uroepithelial cells. *J Cell Physiol.* 2019;234(3):2778-2787.

70. Huang LK, Wang JH, Shen SH, Lin AT, Chang CY. Evaluation of the extent of ketamine-induced uropathy: the role of CT urography. *Postgrad Med J.* 2014;90(1062):185-190.
69. Cui L, Jiang X, Zhang C, et al. Ketamine induces endoplasmic reticulum stress in rats and SV-HUC-1 human uroepithelial cells by activating NLRP3/TXNIP aix. *Biosci Rep*. 2019;39(10):BSR20190595.

70. Ryter SW, Kim HP, Hoetzl A, et al. Mechanisms of cell death in oxidative stress. *Antioxid Redox Signal*. 2007;9(1):49-89.

71. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim Biophys Acta*. 2016;1863(12):2977-2992.

72. Nakagawa T, Zhu H, Morishima N, et al. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. *Nature*. 2000;403(6765):98-103.

73. Yoneda T, Imazumi K, Oono K, et al. Activation of caspase-12, an endoplasmic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J Biol Chem*. 2001;276(17):13935-13940.

74. Wieder T, Essmann F, Prokop A, et al. Activation of caspase-8 in drug-induced apoptosis of B-lymphoid cells is independent of CD95/Fas receptor-ligand interaction and occurs downstream of caspase-3. *Blood*. 2001;97(5):1378-1387.

75. Kantari C, Walczak H. Caspase-8 and bid: caught in the act between death receptors and mitochondria. *Biochim Biophys Acta*. 2011;1813(4):558-563.

76. Costantini P, Bruye JM, Castedo M, et al. Pre-processed caspase-9 contained in mitochondria participates in apoptosis. *Cell Death Differ*. 2002;9(1):82-88.

77. Rao RV, Hermel E, Castro-Obregon S, et al. Coupling endoplasmic reticulum stress to the cell death program. Mechanism of caspase activation. *J Biol Chem*. 2001;276(36):33869-33874.

78. Görlach A, Klappa P, Kietzmann T. The endoplasmic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2- dependent mechanism in response to the ER stress. *J Biol Chem*. 2010;1863(12):2977-2992.

79. Cui L, Jiang X, Zhang C, et al. Ketamine induces endoplasmic reticulum stress in rats and SV-HUC-1 human uroepithelial cells by activating NLRP3/TXNIP aix. *Biosci Rep*. 2019;39(10):BSR20190595.

80. Gorlach A, Klappa P, Kietzmann T. The endoplasmic reticulum: folding, calcium homeostasis, signaling, and redox control. *Antioxid Redox Signal*. 2006;8(9-10):1391-1418.

81. Bahar E, Kim H, Yoon H. ER stress-mediated signaling: action potential and Ca(2+) as key players. *Int J Mol Sci*. 2016;17(9):1558.

82. Kale J, Osterlund EJ, Andrews DW. BCL-2 family proteins: changing partners in the dance towards death. *Cell Death Differ*. 2002;9(1):46-54.

83. Aronsson P, Vesela R, Johnsson M, et al. Inhibition of nitric oxide synthase expression in human bladder cancer and its relation to angiogenesis. *Urol Res*. 2003;31(4):232-235.

84. Giglio D, Ryberg AT, To K, Delbro GS, Tobin G. Altered muscarinic receptor subtype expression and functional responses in cyclophosphamide induced cystitis in rats. *Auton Neurosci*. 2005;122(1–2):9-20.

85. Lin Z, Chen S, Ye C, Zhu S. Nitric oxide synthase immunoreactivity in human bladder carcinoma. *Mol Pathol*. 2001;54(4):248-252.

86. García-Aranda MI, Gonzalez-Padilla JE, Gómez-Castro CZ, et al. Anti-inflammatory effect and inhibition of nitric oxide production by targeting COXs and iNOS enzymes with the 1,2-diphenylbenzimidazole pharmacophore. *Bioorg Med Chem*. 2020;28(9):115427.

87. Shochina M, Fellig Y, Sughrayer M, et al. Nitric oxide synthase expression of cyclooxygenase-2 are enhanced by ketamine-induced ulcerative cystitis in rat bladder. *Am J Pathol*. 2015;185(8):2269-2285.

88. Yuan T, Yang T, Chen H, et al. New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis. *Redox Biol*. 2019;20:247-260.

89. Sharma JN, Al- Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*. 2005;12(3):443-452.

90. Korhonen R, Lahti A, Kankaanranta H, Moilanen E. Nitric oxide in inflammatory reactions. *Inflammopharmacology*. 2005;12(3):443-452.
106. Cuzzocrea S, Salvemini D. Molecular mechanisms involved in the reciprocal regulation of cyclooxygenase and nitric oxide synthase enzymes. *Kidney Int.* 2007;71(4):290-297.

107. Park JM, Yang T, Arend LJ, Smart AM, Scherhammer JB, Briggs JP. Cyclooxygenase-2 is expressed in bladder during fetal development and stimulated by outlet obstruction. *Am J Physiol. 1997;273(4):F538-F544.*

108. Aoki T, Narumiya S. Prostaglandins and chronic inflammation. *Trends Pharmacol Sci.* 2012;33(6):304-311.

109. Chuang SM, Lu JH, Lin KL, et al. Epigenetic regulation of interleukin-1beta, interleukin-6 and NF-κB in human colorectal cancer. *J Biol Chem.* 2001;276(20):17058-17062.

110. Wiseman OJ, Fowler CJ, Landon DN. The role of the human bladder lamina propria myofibroblast. *BJU Int.* 2003;91(1):89-93.

111. Yu W, Zeidel ML, Hill WG. Cellular expression profile for interstitial cells of cajal— a cell often misidentified as myocyte or myofibroblast. *PLoS One.* 2012;7(11):e48897.

112. Galli SJ, Serrano-Candelas E, Molina-Molina GJ, Martín M. IgE-related chronic diseases and Anti-IgE-based treatments. *Nat Med.* 2003;9(4):528-536.

113. Wiseman OJ, Fowler CJ, Landon DN. The role of the human bladder lamina propria myofibroblast. *BJU Int.* 2003;91(1):89-93.

114. Hayes MD, Ward S, Crawford G, et al. Inflammation-induced NF-κB expression in vascular smooth muscle. *Biochem J.* 1995;310(Pt. 1):73-81.

115. Chen Y, Blom IE, Sa S, Goldschmeding R, Abraham DJ, Leask A. CTGF expression in mesangial cells: involvement of SMADs, MAP kinase, and PKC. *Kidney Int.* 2002;62(4):1149-1159.

116. Smith RE, Strieter RM, Phan SH, et al. Production and function of TGF-β1 and basic fibroblast growth factor in human lung fibroblasts. *J Biol Chem.* 2008;283(12):7844-7852.

117. Smith RE, Strieter RM, Phan SH, et al. Production and function of murine macrophage inflammatory protein-1 alpha in bleomycin-induced lung injury. *J Immunol.* 1994;153(10):4704-4712.

118. Smith RE, Strieter RM, Zhang K, et al. Role of C-C chemokines in fibrotic lung disease. *J Leukoc Biol.* 1995;57(5):782-787.

119. O’Donoghue RJ, Knight DA, Richards CD, et al. Genetic partitioning of interleukin-6 signalling in mice dissociates Stat3 from Smad3-mediated lung fibrosis. *EMBO Mol Med.* 2012;4(9):939-951.

120. Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest.* 2003;112(12):1776-1784.

121. Wang J, Chen Y, Gu D, et al. Ketamine-induced bladder fibrosis involves epithelial-to-mesenchymal transition mediated by transforming growth factor-β1. *Am J Physiol Renal Physiol.* 2017;313(4):F961-F972.

122. Verrecchia F, Chu ML, Mauviel A. Identification of novel TGF-β1/Smad gene targets in dermal fibroblasts using a combined cDNA microarray/promoter transactivation approach. *J Biol Chem.* 2001;276(20):17058-17062.

123. Verrecchia F, Vindevoghel L, Lechleider RJ, Uitto J, Roberts AB, Mauviel A. Smad3/4 interactions control transcriptional responses to TGF-beta in a promoter-specific manner. *Oncogene.* 2001;20(26):3332-3340.

124. Schönherr E, Järveläinen HT, Sandell LJ, Wight TN. Effects of platelet-derived growth factor and transforming growth factor-beta 1 on the synthesis of a large versican-like chondroitin sulfate proteoglycan by arterial smooth muscle cells. *J Biol Chem.* 1991;266(26):17640-17647.

125. Romaris M, Bassols A, David G. Effect of transforming growth factor-beta 1 and basic fibroblast growth factor on the expression of cell surface proteoglycans in human lung fibroblasts. Enhanced glycanation and fibronectin-binding of CD44 proteoglycan, and down-regulation of glypican. *Biochem J.* 1995;310(Pt. 1):73-81.

126. Waller RA, Kalluri R. Chronic prostatitis induces bladder hypersensitivity and sensitizes bladder afferents in the mouse. *J Urol.* 2016;196(3):892-901.

127. Grundy L, Daly DM, Chapple C, Grundy D, Chess-Williams R. TRPV1 enhances the afferent response to P2X receptor activation in the mouse urinary bladder. *Sci Rep.* 2018;8(1):197.

128. Ryu CM, Shin JH, Yu HY, et al. N-acetylcysteine prevents bladder tissue fibrosis in a lipopolysaccharide-induced cystitis rat model. *Sci Rep.* 2019;9(1):8134.

129. Cheng JK, Ji RR. Intracellular signaling in primary sensory neurons and persistent pain. *Neurochem Res.* 2008;33(10):1970-1978.

130. Botha J, Shortridge EM, Won D, et al. Human sensory neurons: membrane properties and sensitization by inflammatory mediators. *Pain.* 2014;155(9):1861-1870.
144. Sun Y, Keay S, De Deyne PG, Chai TC. Augmented stretch activated adenosine triphosphate release from bladder uroepithelial cells in patients with interstitial cystitis. *J Urol*. 2001;166(5):1951-1956.

145. Sun Y, Chai TC. Augmented extracellular ATP signaling in bladder uroepithelial cells from patients with interstitial cystitis. *Am J Physiol Cell Physiol*. 2006;290(1):C27-34.

146. Birder LA, Kullmann FA. Role of neurogenic inflammation in local communication in the visceral mucosa. *Semin Immunopathol*. 2018;40(3):261-279.

147. Li J, Xiong J, Yang B, et al. Endothelial cell apoptosis induces TGF-β signaling-dependent host endothelial-mesenchymal transition to promote transplant arteriosclerosis. *Am J Transplant*. 2015;15(12):3095-3111.

148. D’Andrea MR, Saban MR, Nguyen NB, Andrade-Gordon P, Saban R. Expression of protease-activated receptor-1,-2,-3, and -4 in control and experimentally inflamed mouse bladder. *Am J Pathol*. 2003;162(3):907-923.

149. Saban R, D’Andrea MR, Andrade-Gordon P, et al. Mandatory role of proteinase-activated receptor 1 in experimental bladder inflammation. *BMC Physiol*. 2007;7:4.

150. Cheng F, Birder LA, Kullmann FA, et al. Layer-dependent role of collagen recruitment during loading of the rat bladder wall. *Biomech Model Mechanobiol*. 2018;17(2):403-417.

151. Meng E, Wu ST, Cha TL, Sun GH, Yu DS, Chang SY. A murder of young bladders: Ketamine-associated cystitis. *Urol Sci*. 2013;24(4):113-116.

152. Honnegowda TM, Kumar P, Udupa EGP, Kumar S, Kumar U, Rao P. Role of angiogenesis and angiogenic factors in acute and chronic wound healing. *Plast Aesthet Res*. 2015;2:243-249.

153. Shen CH, Wang SC, Wang ST, et al. Evaluation of urinary bladder fibrogenesis in a mouse model of long-term ketamine injection. *Mol Med Rep*. 2016;14(3):1880-1890.

154. Lin CC, Lin AT, Yang AH, Chen KK. Microvascular injury in ketamine-induced bladder dysfunction. *PLoS One*. 2016;11(8):e0160578.

155. Szade A, Grochot-Przezczek A, Florczyk U, Jozkowicz A, Dulak J. Cellular and molecular mechanisms of inflammation-induced angiogenesis. *IUBMB Life*. 2015;67(3):145-159.

156. Ribatti D, Crivellato E. Immune cells and angiogenesis. *J Cell Mol Med*. 2009;13(9A):2822-2833.

157. Wu G, Luo J, Rana JS, Laham R, Sellke FW, Li J. Involvement of COX-2 in VEGF-induced angiogenesis via P38 and JNK pathways in vascular endothelial cells. *Cardiovasc Res*. 2006;69(2):512-519.

158. Cooke JP, Losordo DW. Nitric oxide and angiogenesis. *Circulation*. 2002;105(18):2133-2135.

159. Namba T, Koihe H, Murakami K, et al. Angiogenesis induced by endothelial nitric oxide synthase gene through vascular endothelial growth factor expression in a rat hindlimb ischemia model. *Circulation*. 2003;108(18):2250-2257.

160. Rundhag JE. Matrix metalloproteinases and angiogenesis. *J Cell Mol Med*. 2005;9(2):267-285.

161. Rundhag JE. Matrix metalloproteinases, angiogenesis, and cancer: commentary re: A. C. Lockhart et al.,Matrix metalloproteinases angiogenesis and cancer: commentary re: A. C. Lockhart. *Clin Cancer Res*. 2003;9(2):551-554.

162. Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. *J Clin Invest*. 1999;103(9):1237-1241.

163. Schlüter A, Weller P, Kanaan O, et al. CD31 and VEGF are prognostic biomarkers in early-stage, but not in late-stage, laryngeal squamous cell carcinoma. *BMC Cancer*. 2018;18(1):272.

164. Ma MZ, Zhuang C, Yang XM, et al. CTHRC1 acts as a prognostic factor and promotes invasiveness of gastrointestinal stromal tumors by activating Wnt/PCP-Rho signaling. *Neoplasia*. 2014;16(6):265-278.e13.

165. Olsen JJ, Pohl SO, Deshmukh A, et al. The role of Wnt signalling in angiogenesis. *Clin Biochem Rev*. 2017;38(3):131-142.

166. Minutti CM, Modak RV, Macdonald F, et al. A macrophage-pericyte axis directs tissue restoration via amphiuregulin-induced transforming growth factor beta activation. *Immunity*. 2019;50(3):645-654.e6.

167. Goumans MJ, Valdimarsdottir G, Itoh S, Rosendahl A, Siders P, ten Dijke P. Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. *EMBO J*. 2002;21(7):1743-1753.

168. Wang X, Ma W, Han S, et al. TGF-β participates choroid neovascularization through Smad2/3-VEGF/TNF-α signaling in mice with Laser-induced wet age-related macular degeneration. *Sci Rep*. 2017;7(1):9672.

169. 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. *Crit Care Med*. 2005;33(5):1044-1049.

170. Takadera T, Ishida A, Ohyashiki T. Ketamine-induced apoptosis in cultured rat cortical neurons. *Toxicol Appl Pharmacol*. 2006;210(1-2):100-107.

171. Wang C, Sadovova N, Hotchkiss C, et al. Blockade of N-methyl-D-aspartate receptors by ketamine produces loss of postnatal day 3 monkey frontal cortical neurons in culture. *Toxicol Sci*. 2006;91(1):192-201.

172. Ikonomidou C, Bosch F, Miksa M, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science*. 1999;283(5398):70-74.

173. Chen X, Andresen BT, Hill M, Zhang J, Booth F, Zhang C. Role of reactive oxygen species in tumor necrosis factor-alpha induced endothelial dysfunction. *Curr Hypertens Rev*. 2008;4(4):245-255.

174. Schulze-Osthoff K, Bakker AC, Vanhaesebroeck B, Beyaert R, Jacob WA, Fiers W. Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. *J Biol Chem*. 1992;267(8):5317-5323.

175. Liu Y, Schubert DR. The specificity of neuroprotection by antioxidants. *J Biomed Sci*. 2009;16(1):98.

176. Zhou X, Liu J, Yang S, Su Y, Meng Z, Hu Y. Ketamine ameliorates hypoxia-induced endothelial injury in human umbilical vein endothelial cells. *Clinics (Sao Paulo)*. 2020;75:e1865.

177. Johnson A, DiPietro LA. Apoptosis and angiogenesis: an evolving mechanism for fibrosis. *FASEB J*. 2013;27(10):3893-3901.

178. Ruiz-Ortega M, Rodríguez-Vita J, Sanchez-Lopez E, Carvajal G, Egido J. TGF-beta signaling in vascular fibrosis. *Cardiovasc Res*. 2007;74(2):196-206.

179. Miodoński AJ, Litwin JA. Microvascular architecture of the human urinary bladder wall: a corrosion casting study. *Anat Rec*. 1999;254(3):375-381.

180. Congiu T, Radice R, Raspanti M, Reguzzoni M. The 3D structure of the human urinary bladder mucosa: a scanning electron microscopy study. *J Submicrosc Cytol Pathol*. 2004;36(1):45-53.
181. Andersson KE, Boedtkjer DB, Forman A. The link between vascular dysfunction, bladder ischemia, and aging bladder dysfunction. Ther Adv Urol. 2017;9(1):11-27.

182. Azadzoi KM, Tarcan T, Kozlowski R, Krane RJ, Siroky MB. Overactivity and structural changes in the chronically ischemic bladder. J Urol. 1999;162(5):1768-1778.

183. Azadzoi KM, Tarcan T, Siroky MB, Krane RJ. Atherosclerosis-induced chronic ischemia causes bladder fibrosis and noncompliance in the rabbit. J Urol. 1999;161(5):1626-1635.

184. Meegan JE, Shaver CM, Putz ND, et al. Cell-free hemoglobin increases inflammation, lung apoptosis, and microvascular permeability in murine polymicrobial sepsis. PLoS One. 2020;15(2):e0228727.

185. Christmas TJ, Rode J, Chapple CR, Milroy EJ, Turner-Warwick RT. Nerve fibre proliferation in interstitial cystitis. Virchows Arch A Pathol Anat Histopathol. 1990;416(5):447-451.

186. Dong C, Rovnaghi CR, Anand KJ. Ketamine alters the neurogenesis of rat cortical neural stem progenitor cells via the PI3K/Akt-p27 signaling pathway. Birth Defects Res B Dev Reprod Toxicol. 2014;101(5):355-363.

187. Dong C, Rovnaghi CR, Anand KJ. Ketamine alters the neurogenesis of rat cortical neural stem progenitor cells. Crit Care Med. 2012;40(8):2407-2416.

188. Cameron HA, McEwen BS, Gould E. Regulation of adult neurogenesis by excitatory input and NMDA receptor activation in the dentate gyrus. J Neurosci. 1995;15(6):4687-4692.

189. Hu M, Sun YJ, Zhou QG, et al. Cell-free hemoglobin increases inflammation, lung apoptosis, and microvascular permeability in murine polymicrobial sepsis. PLoS One. 2020;15(2):e0228727.

190. Molinuevo JJ, Lang AE, Marsden CD, et al. The role of non-motor symptoms in Parkinson's disease. Mov Disord. 2011;215(1):143-154.

191. Packer MA, Stasiv Y, Benraiss A, et al. Nitric oxide negatively regulates mammalian adult neurogenesis. Proc Natl Acad Sci U S A. 2003;100(16):9566-9571.

192. Luo CX, Lin YH, Qian XD, et al. Interaction of nNOS with PSD-95 negatively controls regenerative repair after stroke. J Neurosci. 2014;34(40):13535-13548.

193. Shang HS, Wu YN, Liao CH, Chiuhe TS, Lin YF, Chiang HS. Long-term administration of ketamine induces erectile dysfunction by decreasing neuronal nitric oxide synthase on cavernous nerve and increasing corporal smooth muscle cell apoptosis in rats. Oncotarget. 2016;8(43):73670-73683.

194. Ricci V, Martinotti G, Gelfo F, et al. Chronic ketamine use increases serum levels of brain-derived neurotrophic factor. Psychopharmacology. 2011;215(1):143-148.

195. Waterhouse EG, An JJ, Orefice LL, et al. BDNF promotes differentiation and maturation of adult-born neurons through GABAergic transmission. J Neurosci. 2012;32(41):14318-14330.

196. Murray PS, Holmes PV. An overview of brain-derived neurotrophic factor and implications for excitotoxic vulnerability in the hippocampus. Int J Pept. 2011;2011:654085.

197. Bhattacharri P, Cosacak MI, Mashkaryan V, et al. Neuron-glial interaction through Serotonin-BDNF-NGFR axis enables regenerative neurogenesis in Alzheimer’s model of adult zebrafish brain. PLoS Biol. 2020;18(1):e3000585.

198. Cruz CD. Neurotrophins in bladder function: what do we know and where do we go from here? Neurourol Urodyn. 2014;33(1):39-45.

199. White AO, Kramár EA, López AJ, et al. BDNF rescues Baf53b-dependent synaptic plasticity and cocaine-associated memory in the nucleus accumbens. Nat Commun. 2016;7:11725.

200. Wei P, Zheng Q, Liu H, et al. Nicotine-induced neuroprotection against cognitive dysfunction after partial hepatectomy involves activation of BDNF/TrkB signaling pathway and inhibition of NF-κB signaling pathway in aged rats. Nicotine Tob Res. 2018;20(4):515-522.

201. Luo H, Xiang Y, Qu X, et al. Apelin-13 suppresses neuroinflammation against cognitive deficit in a streptozotocin-induced rat model of Alzheimer’s disease through activation of BDNF-TrkB signaling pathway. Front Pharmacol. 2019;10:395.

202. Ding H, Chen J, Su M, et al. BDNF promotes activation of astrocytes and microglia contributing to neuroinflammation and mechanical allodynia in cyclophosphamide-induced cystitis. J Neuroinflammation. 2020;17(1):19.

203. Xia CM, Gulick MA, Yu SJ, et al. Up-regulation of brain-derived neurotrophic factor in primary afferent pathway regulates colon-to-bladder cross-sensitization in rat. J Neuroinflammation. 2012;9:30.

204. Pinto R, Frias B, Allen S, et al. Sequestration of brain derived nerve factor by intravenous delivery of TrkB-Ig2 reduces bladder overactivity and noxious input in animals with chronic cystitis. Neuroscience. 2010;166(3):907-916.

205. Pang X, Marchand J, Sant GR, Kream RM, Theoharides TC. Increased number of substance P positive nerve fibres in interstitial cystitis. Br J Urol. 1995;75(6):744-750.

206. Sant GR, Theoharides TC. The role of the mast cell in interstitial cystitis. Urol Clin North Am. 1994;21(1):41-53.

207. Cao J, Papadopoulou N, Kempuraj D, et al. Human mast cells express corticotropin-releasing hormone (CRH) receptors and CRH signaling leads to selective secretion of vascular endothelial growth factor. J Immunol. 2005;174(12):7665-7675.

208. Rosa AC, Fantozzi R. The role of histamine in neurogenic inflammation. Br J Pharmacol. 2013;170(1):38-45.

209. Bjorling DE, Jerde TJ, Zine MJ, Busser BW, Saban MR, Saban RT. Nerve fibre proliferation in interstitial cystitis. J Urol. 1995;154(6):269-276.

210. Sant GR, Theoharides TC. The role of the mast cell in interstitial cystitis. Urol Clin North Am. 1994;21(1):41-53.

211. Cao J, Papadopoulou N, Kempuraj D, et al. Human mast cells express corticotropin-releasing hormone (CRH) receptors and CRH signaling leads to selective secretion of vascular endothelial growth factor. J Immunol. 2005;174(12):7665-7675.

212. Rosa AC, Fantozzi R. The role of histamine in neurogenic inflammation. Br J Pharmacol. 2013;170(1):38-45.

213. Bjorling DE, Jerde TJ, Zine MJ, Busser BW, Saban MR, Saban RT. Nerve fibre proliferation in interstitial cystitis. J Urol. 1995;154(6):269-276.

214. Sant GR, Theoharides TC. The role of the mast cell in interstitial cystitis. Urol Clin North Am. 1994;21(1):41-53.

215. Cao J, Papadopoulou N, Kempuraj D, et al. Human mast cells express corticotropin-releasing hormone (CRH) receptors and CRH signaling leads to selective secretion of vascular endothelial growth factor. J Immunol. 2005;174(12):7665-7675.

216. Rosa AC, Fantozzi R. The role of histamine in neurogenic inflammation. Br J Pharmacol. 2013;170(1):38-45.

217. Bjorling DE, Jerde TJ, Zine MJ, Busser BW, Saban MR, Saban RT. Nerve fibre proliferation in interstitial cystitis. J Urol. 1995;154(6):269-276.

218. Sant GR, Theoharides TC. The role of the mast cell in interstitial cystitis. Urol Clin North Am. 1994;21(1):41-53.

219. Cao J, Papadopoulou N, Kempuraj D, et al. Human mast cells express corticotropin-releasing hormone (CRH) receptors and CRH signaling leads to selective secretion of vascular endothelial growth factor. J Immunol. 2005;174(12):7665-7675.

220. Rosa AC, Fantozzi R. The role of histamine in neurogenic inflammation. Br J Pharmacol. 2013;170(1):38-45.

221. Bjorling DE, Jerde TJ, Zine MJ, Busser BW, Saban MR, Saban RT. Nerve fibre proliferation in interstitial cystitis. J Urol. 1995;154(6):269-276.
219. Natura G, von Banchet GS, Schaible HG. Calcitonin gene-related peptide enhances TTX-resistant sodium currents in cultured dorsal root ganglion neurons from adult rats. Pain. 2005;116(3):194-204.
220. de Groat WC, Yoshimura N. Mechanisms underlying the recovery of lower urinary tract function following spinal cord injury. Prog Brain Res. 2006;152:59-84.
221. Zvara P, Vizzard MA. Exogenous overexpression of nerve growth factor in the urinary bladder produces bladder overactivity and altered micturition circuitry in the lumbarosacral spinal cord. BMC Physiol. 2007;28(9).
222. Pandita RK, Andersson KE. Intravesical adenosine triphosphate stimulates the micturition reflex in awake, freely moving rats. J Urol. 2002;168(3):1230-1234.
223. Zeng J, Lai H, Zheng D, et al. Effective treatment of ketamine-associated cystitis with botulinum toxin type A injection combined with bladder hydrodistention. J Int Med Res. 2017;45(2):792-797.
224. Chang Y, Li JY, Jayakumar T, et al. Ketamine, a clinically used anesthetic, inhibits vascular smooth muscle cell proliferation via PP2A-activated PI3K/Akt/ERK inhibition. Int J Mol Sci. 2017;18(12):2545.
225. Adam RM, Roth JA, Cheng HL, et al. Signaling through PI3K/Akt mediates stretch and PDGF-BB-dependent DNA synthesis in bladder smooth muscle cells. J Urol. 2003;169(6):2388-2393.
226. Morgan JI, Curran T. Role of ion flux in the control of c-fos expression. Nature. 1986;322(6079):552-555.
227. Cruzalegui FH, Hardingham GE, Bading H. c-Jun functions as a calcium-regulated transcriptional activator in the absence of JNK/SAPK1 activation. EMBO J. 1999;18(5):1335-1344.
228. Araya R, Liberona JL, Cárdenas JC, et al. Dihydropyridine receptor-mediated, slow calcium signal in skeletal muscle cells. J Biol Chem. 2003;278(15):13560-13567.
229. Altura BM, Altura BT, Carella A. Effects of ketamine on vascular smooth muscle relaxation. Br J Pharmacol. 1980;67(2):257-267.
230. Lundy PM, Gowdey CW, Colhoun EH. Tracheal smooth muscle relaxant effect of ketamine. Br J Anaesth. 1974;46(5):333-336.
231. Hirshman CA, Downes H, Farboud A, Bergman NA. Ketamine block of bronchospasm in experimental canine asthma. Br J Anaesth. 1979;51(8):713-718.
232. Ceran C, Pampal A, Goktas O, Pampal HK, Olmez E. Commonly used intravenous anesthetics decrease bladder contractility: an in vitro study of the effects of propofol, ketamine, and midazolam on the rat bladder. Indian J Urol. 2010;26(3):364-368.
233. Durieux ME. Inhibition by ketamine of muscarinic acetylcholine receptor function. Anesth Analg. 1995;81(1):57-62.
234. Hirota K, Zsigmond EK, Matsuki A, Rabito SF. Topical ketamine inhibits albumin extravasation in chemical peritonitis in rats. Acta Anaesthesiol Scand. 1995;39(2):174-178.
235. Akata T, Izumi K, Nakashima M. Mechanisms of direct inhibitory action of ketamine on vascular smooth muscle in mesenteric resistance arteries. Anesthesiology. 2001;95(2):452-462.
236. Wegener JW, Schulla V, Lee TS, et al. An essential role of Cav1.2 L-type calcium channel for urinary bladder function. FASEB J. 2004;18(10):1159-1161.
237. Keiser M, Hasan M, Oswald S. Affinity of ketamine to clinically relevant transporters. Mol Pharm. 2018;15(1):326-331.
238. Jonkman K, Dahan A, van de Donk T, Aarts L, Niesters M, van Velzen M. Ketamine for pain. F1000Res. 2017;6:1711.
239. McCarthy CJ, Marangos C, Fry CH, Ikeda Y. ATP transients accompany spontaneous contractions in isolated guinea-pig detrusor smooth muscle. Exp Physiol. 2019;104(11):1717-1725.
240. Meng E, Chang HY, Chang SY, Yu DS, Cha TL. Involvement of purinergic neurotransmission in ketamine induced bladder dysfunction. J Urol. 2011;186(3):1134-1141.
241. Hao Y, Wang L, Chen H, et al. Targetable purinergic receptors P2Y12 and A2b antagonistically regulate bladder function. JCI Insight. 2019;4(16).e122112.
242. Andersson KE, Arner A. Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol Rev. 2004;84(3):935-986.
243. Elhebir ES, Hughes JD, Hulmi SC. Calcium antagonists use and its association with lower urinary tract symptoms: a cross-sectional study. PLoS One. 2013;8(6):e66708.
244. Salmon M, Khan AH, Syed Sulaiman SA, Khan JH, Hussain K, Shehzadi N. Effect of calcium channel blockers on lower urinary tract symptoms: a systematic review. Biomed Res Int. 2017;2017:4269875.
245. Yu W, Sun X, Robson SC, Hill WG. Extracellular UDP enhances P2X-mediated bladder smooth muscle contractility via P2Y(6) activation of the phospholipase C/inositol trisphosphate pathway. FASEB J. 2013;27(5):1895-1903.
246. Bellucci CHS, Ribeiro WO, Hemerly TS, et al. Increased detrusor collagen is associated with detrusor overactivity and decreased bladder compliance in men with benign prostatic obstruction. Prostate Int. 2017;5(2):70-74.
247. Landau EH, Jayanthi VR, Churchill BM, et al. Loss of elasticity in dysfunctional bladders: urodynamics and histochemical correlation. J Urol. 1994;152(2 Pt 2):702-705.
248. Ohnishi N, Kishima Y, Hashimoto K, et al. [Morphometric study of low compliant bladder]. Hinyokika Kiyo. 1994;40(8):657-661.
249. Inui E, Ochiai A, Naya Y, Ukimura O, Kojima M. Comparative morphometric study of bladder detrusor between patients with benign prostatic hyperplasia and controls. J Urol. 1999;161(3):827-830.

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