THE MOLECULAR MECHANISM OF BLADDER REGENERATION AFTER HYALURONIC ACID TREATMENT IN KETAMINE-INDUCED ULCERATIVE CYSTITIS RAT MODEL

Hypothesis / aims of study
Ketamine-induced ulcerative cystitis (KIC) is a new entity occurred in those long-term ketamine abusers. However, the exact pathophysiology of KIC remains unclear. We hypothesize that the composition of bladder urothelial layer and the expression of hyaluronan metabolizing enzymes and receptors are altered after ketamine. Hyaluronic acid (HA) treatment may eliminate KIC associated bladder dysfunction.

Study design, materials and methods
In vivo study, we develop ketamine-associated ulcerative cystitis model by intraperitoneally injecting ketamine in rat. Female Sprague-Dawley (SD) rats were divided into four groups, including control group, ketamine injection group, ketamine injection and rest group, and ketamine plus HA treatment group. Cystometry (CMG) and micturition frequency/volume studies were recorded for bladder function. Western blot and Immunofluorescence analysis were carried out to examine the protein expression and distribution of urothelial keratin (keratin 20, 13, 5 or 17), proliferation (Ki67), antiproliferation (ARF), differentiationin (uroplakin 1-like receptor 1A), and junction (cadherin). Moreover, Real time-quantitative-PCR was performed to evaluate for hyaluronidas 1-4 (HYALs 1-4 and PH20), hyaluronan synthases (HAS1-3), and HA receptors (CD44, RHAMM, and TLR-4).

Results
The ketamine-treated group displayed bladder hyperactivity and decreased bladder capacity. These bladder dysfunctions were accompanied by increases in the expressions of COX-2, TGF-β1, fibronectin and type I collagen in the protein levels. KIC also accompanied with the disruptions of urothelial layer (Uroplakin III), E-cadherin and tight-junction-associated proteins (Claudin-4 and ZO-1). On the contrary, ketamine treatment combined with HA intravesical instillation attenuated these bladder barrier damage and interstitial fibrosis. HA treatment also increase urothelial proliferation (Ki67) and improve bladder regeneration. The RT-PCR data demonstrated that the expression of HAS (HAS 1-3), HYALs (HYAL1-4, PH20) and HARS (CD44, RHAMM, and TLR-4) involved in HA biosynthesis are altered in bladder after ketamine and HA treatment.

Interpretation of results
Bladder urothelial glycosaminoglycan (GAG) layer cover the umbrella cells, including hyaluronic acid (hyaluronan, HA), chondroitin sulfate, and heparan sulfate. GAG layer replenishment therapy is widely accepted as therapy for patients with bladder pain syndrome/interstitial cystitis (BPS/IC) who have poor or inadequate response to conventional therapy. The ketamine-treated group displayed bladder hyperactivity and decreased bladder capacity through disruptions of urothelial layer and damages to urothelial barrier functions. HA can function as a negative regulator in inflammatory activation and protector of cells against ketamine-induced damages. HA-modulated cell proliferation and migration through direct interactions with HA receptors are important for proper tissue remodeling and regeneration in these KIC bladders.

Concluding message
In this bladder KIC rat model, HA modulate inflammatory responses, enhance cell differentiation, and improve urothelial repair. HA can improve the bladder regeneration through enhanced hyaluronan synthases and restore bladder urothelial barrier damage.

Disclosures
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