Special K: Once the fun is over an EMT arrives for the bladder

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Running head: Ketamine causes epithelial transition in urothelium

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The first clinical case series on ketamine cystitis was published in 2007, and was followed by world-wide reports of this painful bladder syndrome occurring with high frequency among recreational users at night clubs, where ketamine had become a popular dance drug (2, 13). These patients were young, yet the extent of bladder damage they presented with was often extreme and included symptoms of severe dysuria, frequency, urgency, gross hematuria and severe ulcerative cystitis. Their bladders were contracted, with inflammation and epithelial denudation evident. About 50% of patients with ketamine cystitis also had hydronephrosis and impaired kidney function. In the worst cases, functional capacities are reduced to 50 ml or less and augmentation enterocystoplasty has been used to restore partial function (7). The National Institute for Drug Abuse reports that 1 - 2% of U.S. 10-12th graders and over 2.3 million Americans older than 12 have abused ketamine. According to the United Nations World Drug Report, ketamine abuse has spread to become a global phenomenon during the past 20 years, and is particularly prevalent in some areas of Western Europe and Southeast Asia.

While ketamine has serious effects on multiple organ systems it is possible that the profound effects on the bladder arise because the kidney concentrates both the drug and its metabolite norketamine, to high levels in the urine (11). Thus the bladder is uniquely exposed from all sides. The mechanisms behind the deleterious effects of ketamine on the bladder are being investigated with a view to understanding the causes underlying the pathology of suburothelial inflammation (9) and increased urothelial apoptosis (6). Potential hypotheses, have included an autoimmune inflammatory response (5), microvascular toxicity causing ischemia and fibrosis (8), neuronal degeneration and hyperplasia and direct damage to urothelial barrier function (3).

Since the urothelium is in direct contact with ketamine in the urine, and has been shown to suffer a loss of cell differentiation markers like ZO-1 and claudin 4 and to become more apoptotic, it has been the focus of several investigations (1, 10). There is evidence that ketamine causes the urothelium to become leaky (3) and the sequelae from an injury of this type could include inflammation, pain and bladder hypersensitivity. However, Rajandrum et.al. found that 12 weeks of daily injections of ketamine in mice, while increasing voiding frequency and reducing voided volumes, had no effect on urothelial structure or permeability (12), suggesting that ketamine does not disrupt urothelial barrier function directly. Others expressed caution in reaching this conclusion (14) and certainly from an experimental perspective, time of exposure, frequency of exposure and dosage are certain to be critical to the observable outcomes and disease progression.
In this issue we have an intriguing new contribution to the ketamine story. Wang et al. report that in rats exposed to daily ketamine for sixteen weeks, the urothelium begins to undergo phenoconversion to a more mesenchymal state. This epithelial to mesenchymal transition (EMT), has several classic molecular hallmarks and Wang et al. demonstrate convincingly that a number of these including downregulation of E-cadherin, upregulation of mesenchymal markers vimentin and fibroblast specific protein (FSP-1), colocalization of both epithelial and mesenchymal markers in a subset of urothelium-associated cells and upregulation of TGFβ1 expression in urothelium are all present. They further supported their in vivo molecular data by demonstrating many of the same features occurring in an immortalized human urothelial cell line (SV-HUC-1) in culture. Furthermore, the rats treated with ketamine exhibited higher voiding frequency by urine spotting assay on filter paper and by cystometry (continuous intrabladder pressure recordings during filling), and higher numbers of non-voiding contractions – a hallmark of bladder instability. Importantly, they show that rats given both ketamine and a TGFβ1 receptor inhibitor, were essentially protected from these functional and molecular effects, thus strongly implicating a TGFβ1-mediated process.

What are the possible consequences of having uroepithelial cells undergo reprogramming to differentiate into fibroblasts? Fibrosis is a complex process which occurs as a normal part of tissue healing following an injury, however, it leads to serious organ damage when it becomes dissociated from the original stimulus and results in inappropriate and large scale deposition of matrix. Fibroblasts, the primary matrix-producing cell, are normally quiescent, however, in pathologic settings they assume the phenotypic characteristics of highly activated myofibroblasts (4). These cells arise from existing resident populations in stroma, from recruitment of bone marrow derived stem cells and, it is now thought, from epithelia that undergo EMT. EMT results in a new pool of mesenchymal cells which possess two key properties, an ability to migrate and an ability to synthesize interstitial matrix. EMT could therefore result in two mutually reinforcing and deleterious outcomes for the bladder. First, expansion of a pool of activated matrix-depositing stromal fibroblasts and secondly, a loss of epithelial cells leading to potential impairment of barrier function and conceivably a loss or dysregulation of mechanosensory signaling during bladder filling. The urothelium is now known to be a dynamic sensor of stretch and to respond by releasing neurotransmitter-like signals in the form of ATP, acetylcholine and NO. These are thought to have paracrine effects on underlying sensory afferents. EMT could thus alter normal intercellular communication and lead to neurogenic effects.

This provocative study raises, several important questions. How does ketamine lead to the initiation of EMT? Is the EMT program directly stimulated by ketamine, or is EMT pursuant
to an already established fibrotic and/or inflammatory process? Is it possible that urodynamic changes to bladder function i.e. increased frequency or altered contractility are responsible for signals that lead to epithelial reprogramming?

While we await more research to provide some of these answers, the knowledge that EMT is involved at some point in the pathophysiological cascade, suggests that we may be able to consider interventions, for example by targeting TGFβ1 or its receptor.

References:


