A Rare Urachal Cyst in a Case of Ketamine-induced Cystitis Provides Mechanistic Insights

Elizabeth Kidger, Jens Stahlschmidt, Mary Garthwaite, Simon Fulford, Jennifer Southgate, and Simon C. Baker

OBJECTIVE To establish whether the urothelial ulceration observed in ketamine-induced cystitis is triggered by urinary or systemic factors. This was achieved with a rare case where an urachal cyst was found near the bladder dome in a patient undergoing cystectomy for unremitting pain following ketamine abuse.

METHODS Clinical investigations included cystoscopy, video urodynamic investigation, and computed tomography of the kidneys, ureters, and bladder. Histological staining was combined with immunoperoxidase labeling for markers of transitional epithelial differentiation.

RESULTS The urachus found near the dome of the bladder was observed to be a separate cyst, with no evidence of patency found during surgery or video urodynamic investigation. The urachus was lined by a mildly reactive metaplastic epithelium of mixed transitional and columnar morphologies. Evidence of widespread cytokeratin 13, basal $p75^{NTR}$, and sparse superficial uroplakin 3a immunoreactivity suggested the urachal epithelium was fundamentally transitional in nature. Near total loss of bladder urothelium was observed from regions in contact with urine, whereas the urachal epithelium (not exposed to urine) remained healthy.

CONCLUSION This study supports the hypothesis that urinary (and not systemic) factors are the main driver of urothelial ulceration in ketamine-induced cystitis. The most likely excreted factors responsible are ketamine and potentially its metabolites. This study reinforces the importance of complete cessation of ketamine use in patients with ketamine-induced cystitis. UROLOGY 90: 223.e1–223.e7, 2016. © 2016 Elsevier Inc.

Ketamine-induced cystitis (KIC) was first reported among recreational drug users in 2007.[1] Histological studies of KIC bladders describe extensive urothelial ulceration and marked chronic inflammatory changes with infiltrations of lymphocytes and variable number of eosinophils.[2-4] Oxley et al remarked that the urothelial atypia in KIC mimicked carcinoma in situ, with nuclear enlargement, increased expression of Ki67 and p53, and loss of cytokeratin 20 (CK20) noted on immunohistochemical analysis.[4] We previously reported histological findings on a series of 21 KIC bladder samples and observed neuritogenesis and peripheral nerve fascicle hyperplasia, resembling Morton's neuroma.[5] Changes in urothelial differentiation were also apparent, including suprabasal expansion of $p75^{NTR}$ expression that might indicate a dedifferentiation toward a more basal-like phenotype.[5]

The clinical syndrome includes extreme bladder pain, urinary frequency, and reduced bladder capacity, but the mechanism of pathogenesis is unknown (reviewed[6]). Chu and colleagues proposed several hypotheses, including: direct toxic damage to the urinary tract by ketamine and/or its metabolites; microvascular damage by ketamine and/or its metabolites; autoimmunity triggered by either circulating or urinary ketamine; and unrecognized bacteriuria.[7] To date, the question of whether urinary or circulating factors drive the pathology remains unanswered.

Ketamine is metabolized hepatically to norketamine by the cytochrome P450 enzyme CYP3A4 (and to a lesser extent CYP2B6 and CYP2B9)[8] as the first step in its detoxification. Further conversion of norketamine to dehydronorketamine and conjugated metabolites is also performed hepatically; however, the relative toxicity of these metabolites is currently unknown. Renal excretion is the primary route for ketamine/metabolites and they can be detected in urine as parent drug (2.3%), norketamine

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From the Jack Birch Unit of Molecular Carcinogenesis, Department of Biology, University of York, United Kingdom; the Urology Department, James Cook University Hospital, Middlesbrough, United Kingdom; and the Department of Pathology, St James's University Hospital, Leeds, United Kingdom

Address correspondence to: Simon Baker, Ph.D., Jack Birch Unit of Molecular Carcinogenesis, Department of Biology, University of York, United Kingdom. E-mail: simon.baker@york.ac.uk

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(1.6%), dehydronorketamine (16.2%), or conjugates (80%).[9] In addition, even after a single bolus administration, ketamine and its metabolites were detected in the urine for several days (ketamine, 5-11; norketamine 6-14; dehydronorketamine, 10),[10,11] leading to chronic urothelial exposure.

Here we report findings from analysis of a cystectomy performed in a single case of KIC, where a urachal cyst was identified on the bladder dome. This provided a unique opportunity to address the question of whether urinary or circulating factors drive the urothelial damage observed.

METHODS

Clinical Investigations

Preliminary investigations included blood tests: full blood count, urea and electrolytes, and liver function tests (including γ-glutamyl transpeptidase). Urinalysis was performed by microscopy, culture, and sensitivity, with additional cytological analysis. The urinary tract was imaged by ultrasound scan, which demonstrated a normal upper urinary tract (data not shown), and computed tomography of the kidneys, ureters, and bladder. The bladder was further studied by cystoscopy and video urodynamic investigation (with a filling rate of 25 mL/min).

Ethical Approval

The study was approved by the Northern and Yorkshire NHS Research Ethics Committee (project 10/H0903/43).

Histology

The anterior and posterior segments of the bladder were fixed in 10% formalin for 5 days before being further dissected and embedded into paraffin wax. Five-micron sections were dewaxed in xylene and rehydrated through ethanol to water before staining with Mayer's hematoxylin and eosin.

An Alcian Blue-Periodic acid Schiff stain was performed to reveal mucin production by urachal epithelial cells. Briefly, slides were stained in 1% (w/v) Alcian Blue 8GX (Sigma) solution made in 3% (v/v) acetic acid (pH 2.5) for 10 minutes. Slides were digested in 1% (w/v) Diastase (Sigma) solution in water for 30 seconds to remove glycogen. Slides were differentiated in 0.1% (v/v) periodic acid solution for 5 minutes followed by Schiff's reagent (1% paraosaniline HCl and 4% sodium metabisulphite in 0.25 M hydrochloric acid, Sigma) for 15 minutes with washes between. Slides were counterstained in Gill's hematoxylin (0.6 % [w/v] hematoxylin, 0.06 % [w/v] sodium iodate, 5.28 % [w/v] aluminum sulphate in aqueous solution; Sigma). All slides were dehydrated and mounted in DPX (CellPath).

Immunohistochemistry

Immunohistochemical analysis was performed using a standard immunoperoxidase technique and included positive and negative specificity controls. Blocking steps were included to neutralize endogeneous peroxidase and avidin-binding activities. Heat-mediated antigen retrieval was performed by boiling for 10 minutes in 10 mM citric acid buffer (pH 6) to enhance detection of mouse monoclonal antibodies raised against cytokeratin 13 (clone IC7, Abnova) and uroplakin 3a (clone AU1, Progen). Following heat-mediated antigen retrieval, signal amplification was required for $p75^{NTR}$ (clone 7F10, Novocastra) using the ImmPRESS Excel staining kit (Vector Laboratories) according to the manufacturer's instructions. Sections were counterstained with Mayer's hematoxylin, dehydrated and mounted in DPX.

RESULTS

In this study we report a 30-year-old male with an 8-year history of ketamine abuse peaking at several grams per day. Urinary frequency was debilitating, with a painful urge to void every 30 minutes and persisting through the night. The full blood count and urea and electrolytes analysis did not reveal any abnormality. All the liver function markers were in the normal range apart from γ-glutamyl transpeptidase. Over a 5-year period, the patient's γ-glutamyl transpeptidase activity was monitored at each clinic he attended and was elevated when he reported using ketamine (mean 172.5 U/L, standard deviation 54.9, n = 4) and normal when he abstained (mean 47.6 U/L, standard deviation 10.7, n = 5, reference range 1-78 U/L). Urinalysis detected scattered squamous epithelial and urothelial cells with no significant bacteriuria. An ultrasound scan demonstrated a normal upper urinary tract. A video urodynamic investigation recorded a severe urge to void at 40 mL on bladder filling (25 mL/min) and bilateral vesicoureteric reflux was present.

Computed tomography of the kidneys, ureters, and bladder demonstrated a markedly contracted bladder with calcification in the bladder wall (Fig. 1A). The patient was considered to have end-stage bladder disease that was unlikely to improve with medical therapy and was treated surgically by subtotal cystectomy and substitution cystoplasty. At cystoscopy, there was calcified debris at the base of the bladder, with further calcified material adherent to the mucosa in the dome of the bladder. The bladder was noted to be shrunken and grossly abnormal, with the perivesicular fat firmly adhered to the outside of the bladder wall (Fig. 1B). There was obvious inflammation, with extensive bleeding from the luminal surface of the bladder apparent on dissection (Fig. 1C).

Histologically, there was a proliferation of small vessels and evidence of calcification toward the luminal aspect of the bladder wall, with chronic inflammation present (Fig. 2A). There was widespread loss of urothelium throughout both the anterior and posterior surfaces of the bladder (Fig. 2A) and where retained, urothelial cells were scanty (Fig. 2B), except where partially protected by the local bladder structure. Several von Brunn's nests were noted (Fig. 2B); these represent invaginations of the urothelium into the lamina propria that are continuous with the luminal