Evidence of Nonuniformity in Urothelium Barrier Function between the Upper Urinary Tract and Bladder

Nicholas A. Williams, Luke Barnard, Chris J. Allender,* Jenna L. Bowen, Mark Gumbleton, Tim Harrah, Aditya Raja and Hrishi B. Joshi

From the School of Pharmacy and Pharmaceutical Sciences, Cardiff University (NAW, LB, CJA, JLB, MG) and Department of Urology, University Hospital of Wales (AR, HBJ), Cardiff, Wales, United Kingdom, and Department of Research and Development, Boston Scientific, Urology and Women's Health Division (TH), Marlborough, Massachusetts

Purpose: We compared the relative permeability of upper urinary tract and bladder urothelium to mitomycin C.

Materials and Methods: Ex vivo porcine bladder, ureters and kidneys were dissected out and filled with 1 mg ml^{-1} mitomycin C. At 60 minutes the organs were emptied and excised tissue samples were sectioned parallel to the urothelium. Sectioned tissue was homogenized and extracted mitomycin C was quantified. Transurothelial permeation across the different urothelia was calculated by normalizing the total amount of drug extracted to the surface area of the tissue sample. Average mitomycin C concentrations at different tissue depths (concentration-depth profiles) were calculated by dividing the total amount of drug recovered by the total weight of tissue.

Results: Mitomycin C permeation across the ureteral urothelium was significantly greater than across the bladder and renal pelvis urothelium (9.07 vs 0.94 and 3.61 $\mu g\ cm^{-2}$, respectively). Concentrations of mitomycin C in the ureter and kidney were markedly higher than those achieved in the bladder at all tissue depths. Average urothelial mitomycin C concentrations were greater than 6.5-fold higher in the ureter and renal pelvis than in the bladder.

Conclusions: To our knowledge we report for the first time that the upper urinary tract and bladder show differing permeability to a single drug. Ex vivo porcine ureter is significantly more permeable to mitomycin C than bladder urothelium and consequently higher mitomycin C tissue concentrations can be achieved after topical application. Data in this study correlate with the theory that mammalian upper tract urothelium represents a different cell lineage than that of the bladder and it is innately more permeable to mitomycin C.

Key Words: urinary bladder, kidney, ureter, urothelium, mitomycin

Abbreviations and Acronyms

HPLC = high performance liquid chromatography

MMC = mitomycin C

RNU = radical nephroureterectomy

UP = uroplakin

UTUC = upper tract urothelial carcinoma

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* Correspondence: Room 0.45, School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Redwood Building, King Edward VII Ave., Cardiff, Wales, United Kingdom, CF10 3NB (telephone: +44 (0)29 208 75824; FAX: +44 (0)29 208 74149; e-mail: allendercj@cf.ac.uk).

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Urothelial carcinoma, the fourth most common tumor type, can develop in the lower or the upper urinary tract.[1] Bladder cancer accounts for 90% to 95% of all urothelial carcinomas while those originating in the upper tract represent 5% to 10%. Although rare, the incidence of UTUC has increased in the last 3 decades and is now about 2 cases per 100,000 person-years.[2] Due to restricted symptomology the disease is commonly advanced at diagnosis. Consequently prognosis is poor with an overall 5-year survival rate of less than 50%.[3] Although the urinary tract is lined by

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1 continuous urothelium, UTUC shows different pathological findings than those of the bladder. Most importantly UTUC is significantly more aggressive and invasive.[4]

Regardless of tumor location EAU (European Association of Urology) guidelines state that the gold standard treatment for UTUC is RNU.[1] In certain patients endoscopic management has emerged as a new treatment option and in 2009 it accounted for greater than 10% of all UTUC surgical interventions in England.[3] This conservative approach allows for preservation of the kidney while sparing the patient the complications and morbidity associated with major surgery.[1] Although to our knowledge no randomized, controlled trials comparing endoscopic management with RNU have been performed, a systematic review of oncologic outcomes suggested that in specific favorable low grade UTUC elective cases endoscopic treatment can yield effective oncologic control and renal preservation.[5] This is supported by comparable 5-year disease specific survival for immediate RNU and endoscopic management.[5,6] Unfortunately these benefits come at the expense of unfavorable tumor progression[5] with 1 group reporting recurrence in 68% of the cohort.[6]

In an attempt to decrease recurrence after endoscopic management the postoperative administration of adjuvant topical chemotherapy with agents such as MMC[6–11] and immunotherapy with bacillus Calmette-Guérin[12] has been reported. The rationale behind this stems from the established efficacy of these agents in treating bladder cancer.[13,14] However, the efficacy of topical chemotherapy in UTUC is not proven. The poor quality of the studies, that is small, retrospective series with limited followup and no control arm, has prevented results from demonstrating unequivocal benefit.[1,5] If topical drug delivery is to be beneficial in decreasing the recurrence of UTUC, efficacious concentrations of drug must be achieved in the target tissue.[5]

Currently the accepted dogma is that urothelial permeability is consistent throughout the urinary tract. This is largely based on the assumption that histologically the urothelium is unchanged in the upper and the lower urinary tract.[15] To our knowledge no group to date has investigated the relative permeability of bladder, ureter and renal pelvis urothelium. However, evidence suggests that despite apparent histological homology protein expression on the surface of urothelial umbrella cells is not consistent.[16,17]

Therefore, given the important role of umbrella cells in maintaining barrier function, we hypothesized that this may give rise to varying transurothelial permeation at these distinct locations. In this study we compared the relative permeability of upper urinary tract urothelium and bladder urothelium to MMC.

MATERIALS AND METHODS

Mitomycin C

Topical Instillation in Isolated Porcine Bladder, Ureter and Kidney. En bloc porcine urinary tracts from pigs weighing 70 to 90 kg were obtained fresh from a local abattoir within 5 minutes of sacrifice and immediately immersed in cold oxygenated Krebs buffer. Working in a shallow bed of Krebs buffer the excess perivesical fat was trimmed, and the bladder, ureters and kidneys were dissected out. Ureters (about 10 cm) were dissected out with approximately 2 cm remaining attached to the bladder and kidney. Organs were rinsed with saline to remove residual urine and filled with MMC solution (1 mg ml^{-1} in normal saline, that is MMC 40 mg powder for solution for injection, ProStrakan, Galashiels, United Kingdom) using a 5Fr 70 cm open-ended ureteral catheter (Cook Medical, Bloomington, Indiana). The bladder, kidney and ureter were filled through the urethra, ureteral orifice and directly into the ureter, respectively. Since the volume of the renal pelvis is variable, preexperimental test instillations with methylene blue (1 mg ml^{-1} in normal saline) were done to ensure adequate contact with the renal pelvis urothelium.

After instillation the entry orifices were sutured and the organs were submerged in oxygenated Krebs buffer maintained at 37C in a waterbath for 60 minutes. Four experiments were performed, each representing a different ex vivo porcine urinary tract. Time from tissue recovery to start of the experiment was approximately 30 minutes.

Distribution in Bladder, Ureter and Kidney Wall. Following 60-minute instillation the organs were removed, emptied and opened by a single vertical incision. To remove surface adsorbed drug the urothelium was thoroughly rinsed with saline. Tissue samples from areas of drug contact (observed due to purple staining conferred by MMC) were excised and surface area was measured. Tissue samples were immediately snap frozen between 2 metal plates using liquid nitrogen and fixed to cork mounts with Tissue-Tek® CRYO-OCT Compound. Tissue was sectioned using a CM3050 S cryostat (Leica Microsystems, Buckinghamshire, United Kingdom). The time between experiment end and freezing was less than 2 minutes.

Samples were serially sectioned parallel to the urothelial surface at 50 μm and sections were collected in preweighed 1.5 ml Eppendorf tubes. Two 50 μm tissue sections between 0 and 100 μm were grouped for analysis as were the 2, 50 μm sections between 100 and 200 μm. Groups of 6, 50 μm tissue sections between 200 and 1,400 μm, and groups of 12, 50 μm tissue sections between 1,400 and 7,400 μm were also grouped. For all groups the tissues sections were weighed and homogenized in a Precellys®24 homogenizer. Drug was extracted in 1 ml of mobile phase for 24 hours with 10-minute sonication per sample. Samples were then centrifuged at 7,000 rpm at 2,680 × gravity and supernatant was isolated for analysis by HPLC.

Average drug concentrations at different tissue depths were calculated by dividing the total amount of drug recovered by the total weight of tissue. Transurothelial permeation was calculated by normalizing the total