Urol Res (2010) 38:215–222
DOI 10.1007/s00240-009-0235-z

ORIGINAL PAPER

Taurine modulates neutrophil function but potentiates uropathogenic *E. coli* infection in the murine bladder

Claire Condron · Rowan G. Casey · Siobhan Kehoe · Deirdre Toomey · Tom Creagh · David J. Bouchier-Hayes

Received: 20 August 2009 / Accepted: 4 November 2009 / Published online: 26 November 2009

Abstract Eradication of a urinary tract infection (UTI) appears to be related to a number of innate host defence mechanisms and their interactions with invading bacteria. Recurrent UTIs (rUTIs) pose a difficult problem in that these bacteria use both host and bacterial factors to evade elimination. Neutrophil bactericidal function is depressed, both systemically and in urine, in patients with a history of recurrent UTI. Taurine is a semi-essential amino acid and is successful in preserving neutrophil bactericidal function in urine. Taurine may preserve neutrophil function at the urothelium and thus aid UTI resolution. Adult female (6 weeks old) C57Bl/6 mice were randomised into three groups: a saline gavage only control group, a saline gavage + *E. coli* group, and a taurine gavage + *E. coli* group [21 g/70 kg taurine in 0.9% normal saline (N/S) for 5 days]. Whilst taurine gavage pre-treatment resulted in increased serum neutrophils respiratory burst activity, at the urothelial–endothelial interface it caused higher colony forming units in the urine and a higher incidence of *E. coli* invasion in the bladder wall with no evidence of increased bladder wall neutrophils infiltration on MPO assay of histological assessment. Histologically there was also evidence of reduced bladder inflammation and urothelial cell apoptosis. In conclusion, taurine effectively increases neutrophils activity but given its anti-inflammatory properties, at the expense of decreased urothelial–endothelial activation thus preventing clearance of active *E. coli* infection in the bladder. Despite the negative results, this study demonstrates the importance of modulating interactions at the urothelial interface.

Keywords Urinary tract infection · *E. coli* · Taurine · Urothelium · Neutrophil · Inflammation

C. Condron · R. G. Casey · S. Kehoe · D. Toomey · D. J. Bouchier-Hayes
Department of Surgery, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland

T. Creagh
Department of Urology, Beaumont Hospital, Dublin 9, Ireland

R. G. Casey (✉)
Department of Urology, St. James's Hospital, James Street, Dublin 8, Ireland
e-mail: rowcasey@rcsi.ie

Introduction

Urinary tract infection (UTI) is a significant uropathogenic condition that accounts for a substantial amount of general practitioner/hospital visits and medical expenditure. *Escherichia coli* (*E. coli*) is the commonest organism and affects up to 40% of women in their lifetimes. Up to 25% of these may experience a recurrence within the first 6 months and many undergo multiple recurrent infections throughout their lives and it is this group of women with recurrent or persistent UTIs (rUTI) that pose the most difficult management problem [1, 2].

Eradication of a UTI appears to be related to a number of innate host defence mechanisms and their interactions with invading bacteria. Host defences include urinary pH, urothelial cell shedding, neutrophils activation and migration, cytokine production (IL-6) and a number of anti-adherence bladder-associated factors (Tamm Horsfall protein, secretory IgA, uromucoid) [3].

rUTIs pose a difficult problem in that these bacteria use both host factors (sequestering within host bladder urothelial cells) and bacterial factors (type-1 pilated *E. coli*) to evade elimination. Repeated courses of antibiotics may fail

to adequately treat recurrent infections due to disruption in first line defences, disturbed internal flora and creation of a continual imbalance in body processes especially increased bacterial adherence to mucosa and decreased bacterial killing by PMN [4].

We have previously demonstrated that neutrophil bactericidal function is depressed, both systemically and in the harsh environment of the urine, in patients with a history of recurrent UTI [5, 6]. This correlated with significant neutrophil cell death, both by apoptosis and necrosis. Furthermore, we have demonstrated that taurine is successful in down regulating neutrophils cell death and preserving bactericidal function in urine in vitro [6].

Taurine is a semi-essential beta-amino acid, the most abundant found in neutrophils, and has been shown to play a critical role in cellular metabolic pathways including osmoregulation and membrane stabilisation, which may be of particularly relevance in the hypertonic environment of the bladder. In addition, previous studies from our laboratory have shown that taurine can maintain neutrophils CD11b activity and respiratory burst even when this inflammatory cell is exposed to unfavourable conditions [7].

Therefore, it was hypothesised that taurine would preserve neutrophil function at the vasculo-urothelial interface and thus aid UTI resolution in vivo. Oral administration of taurine was assessed as a potential therapeutic option in a murine model of UTI.

Materials and methods

UTI model

Adult female (6 weeks old) C57Bl/6 mice weighing 18–20 g (Charles River Institute, Margate, Kent, UK) were used for this study in a licensed biomedical facility. The mice were acclimatised for 1 week before experimentation and caged in groups of three or less in an air conditioned room at ambient temperature of 21–22°C and 50% relative humidity under a 12-h light–dark cycle. The animals were maintained on a purified chow pellet diet (W.M. Connolly & Sons Ltd, Kilkenny, Ireland) and water. The biomedical procedures used in this study were reviewed and approved by The Irish Department of Health and conformed to European legislatory requirements. All procedures were carried out under the supervision of a qualified biomedical technician. Animals were monitored daily for any adverse effects.

Mice were randomised into three groups: a saline gavage only control group, a saline gavage + *E. coli* group [also received 0.9% saline by gavage (0.5 ml) for 5 days], and a taurine gavage + *E. coli* group (which received 21 g/70 kg body weight of taurine in 0.9% normal saline by gavage for 5 days). This dose is equivalent to the clinical dose of taurine used in humans and as has previously been shown to be protective in a murine model of melanoma [8].

The UTI model was established by intravesical inoculation of a clinical isolate of 10^9 uropathogenic *E. coli* (UPEC) trans-urethrally into the bladder as previously described [9, 10]. The clinical isolate strain of UPEC AD 110 was obtained from ATCC (American Tissue Culture Collection).

Briefly, on day 6, the mice were weighed, anaesthetised with methoxyflurane ("Penthrane", Abbot Labs Ltd, Kent, UK—flow rate 50 ml/min maintenance). A sterile polyethylene catheter 0.61 mm external diameter, 0.28 cm internal diameter, 25 cm length was gently inserted, using lubricant (Instillagel, Bucks, UK) into the bladder through the urethra and the bladder emptied with urine being preserved for analysis. An inoculum of 25 µl containing 1×10^9 UPEC organisms in PBS was then infused into the bladder over 30 s via the same cannula. Vesicoureteral reflux was avoided by injecting a small volume of *E. coli* that would not cause significant distension of the empty bladder in adult female (6 weeks old) C57Bl/6 mice according to known maximal bladder capacity. Animals were cared for in an isolation chamber for 24 h and then killed.

Sample collection

Urine assessment

Urine was collected before intravesical inoculation and directly prior to killing. Bacterial growth was assessed, as colony forming units (CFUs), by plating serial dilutions of urine onto LB growth agar (Bio 101 Inc., Carlsbad, CA) plates and incubating at 37°C for 24 h. Urine in all groups was also assessed for urinary osmolarity.

Blood assessment

Blood was taken, under anaesthetic, into heparinised tubes (Starstedt, Wexford, Ireland) by cardiac puncture during killing for the assessment of neutrophil respiratory burst activity.

Reactive oxygen intermediate (ROI) generation (neutrophil respiratory burst activity)

Intracellular ROI generation by PMN was assessed using the BurstTest® kit (Becton–Dickinson, Mountain View, CA, USA). Briefly, 50 µl of whole mouse blood, taken during killing, was incubated with 10 µl of (phorbol myristate acetate) PMA at 37°C for 10 min. The fluorogenic probe dihydrorhodamine 123 was added for ex-vivo for a further 10 min incubation at 37°C. Red blood cells were hypotonically

lysed with lysing solution for 20 min; samples were centrifuged, washed and analysed flow cytometrically with λ_{em} = 520 nm.

ROI production is expressed as mean channel fluorescence (MCF) emitted by the cells. Neutrophils were gated on a dual parameter dot plot of FLS and SLS and this region was selected for analysis.

Bladder tissue assessment

Bladders were emptied and removed via midline lower abdominal incision and each organ was weighed and divided into three specimens for histological analysis of bladder inflammation and apoptosis, neutrophils infiltration using myeloperoxidase activity, and tissue culture for *E. coli*.

Tissue was either stored in formaldehyde or homogenised in 1 ml of cold buffer immediately using an electric homogeniser (T8.01 Netzgerät, IKA WERKE, Germany) on ice.

Bacterial enumeration in the bladder wall was assessed by plating serial dilutions of tissue homogenate onto LB growth agar (Bio 101 Inc., Carlsbad, CA) plates and incubating at 37°C for 24 h.

The remaining homogenates were frozen at 80°C for myeloperoxidase assay for assessment of neutrophils infiltration in bladder wall.

Bladder tissue analysis

Bladder inflammation

The bladder inflammatory index (BII) was used to objectively quantify inflammation on haematoxylin and eosin (H&E)-stained histological sections. The BII grades the severity of tissue oedema using a score of 0–3 as previously described [11]. This was performed by two blinded individuals experienced in microhistopathology techniques at magnification of 60×.

Also, bladder wall neutrophil infiltration was assessed as a measure of the inflammatory response. The number of PMNs per five high power fields (60×) was counted for each section.

Briefly, tissue specimens were fixed in formalin, and stained with H&E (Harris haematoxylin-acidified papanicolaou stain, Cellpath plc, Powys, UK).

Urothelial cell apoptosis

Further, paraffin-embedded bladder tissue was stained for urothelial cell apoptosis by the Tunel end labelling method using an in situ cell death detection kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions after tissue preparation. Only positively stained urothelial layer cells were counted and expressed as total cell number per high power field (400×). Five fields were reported per slide in a blinded fashion by a person experienced in immuno-histochemical techniques.

Bladder wall neutrophil infiltration

Myeloperoxidase is a haeme-containing enzyme that is found primarily in PMNs, and so can be used as an indirect measure of PMN infiltration into tissue. MPO was measured by spectrophotometer using *o*-diazidine spectrophotometric reduction method. One unit of MPO was defined as that degrading 1 μmol of peroxide/min at 25°C and calculated per gram of tissue using the following formula: (absorbance/10 min)/weight of tissue used/0.0113 = MPO/g of tissue and is expressed as mean ± SEM.

Results

Serum

Neutrophil bactericidal function (neutrophil respiratory burst) (N = 10)

Systemic neutrophil production of ROI in response to *E. coli* stimulation for 10 min was measured on blood samples, taken ex-vivo post-bladder *E. coli* instillation, to assess cellular bactericidal capacity. The taurine-treated animals had a significantly higher respiratory burst when compared to the saline-treated *E. coli* infection group (41 ± 4.1 MCF vs. 22 ± 2.0 MCF, $P = 0.001$, one-way ANOVA with post hoc testing) (Table 1).

Urine

Urine osmolarity post-treatment

There was no difference in urine osmolarity following saline or taurine gavage for 5 days (taurine 1,250 ± 0.019 mOsm vs. saline 1,243 ± 0.018 mOsm, $P = 0.66$, unpaired *t* test).

Bacterial growth in the urine (N = 9) (Table 2)

The presence of bacteria in the urine was assessed by plating the urine onto agar. The percentage of urine samples with *E. coli* in the taurine gavage *E. coli* group is significantly higher than the saline gavage *E. coli* group (78% infected vs. 44% infected). Similarly, the taurine groups had statistically higher numbers of CFUs present in the

Table 1 Tissue injury ($N = 10$ per group)

	Saline control	Taurine + *E. coli*	Saline + *E. coli*	*P* value
Serum neutrophil respiratory burst activity	4 ± 1.6 MCF	41 ± 4.1 MCF[£]	22 ± 2.0 MCF	0.001
Bladder tissue MPO activity (units/g)	2.23 ± 0.21	2.5 ± 0.18	3.56 ± 0.2[^]	<0.01
Tissue oedema (BII)	0.61 ± 0.03 (0.4–0.75)	1.28 ± 0.29 (0.82–1.42)	2.67 ± 0.21 (2.12–3.029)*	0.0036
Neutrophil infiltration	12 ± 2.6	36 ± 5.2[$]	137 ± 25	0.018
Bladder epithelial cell apoptosis	16 ± 1.3%	63.9 ± 3.49%[&]	32.6 ± 1.61%	0.0001

All statistical analysis with one-way ANOVA with post hoc testing

Bladder epithelial cell apoptosis assessed by Tunel staining. Positively stained cells were counted and expressed as a percentage of total cell number per high power field. Only the epithelial layer was assessed and five fields of vision are reported per slide

[£] Significant increase relative to saline + *E. coli* and saline control groups ($P < 0.001$)

[^] Significant increase in bladder tissue MPO activity in saline + *E. coli* groups which taurine pre-treatment reduced towards normal ($P < 0.01$)

* Saline + *E. coli* group has significantly more tissue oedema than the taurine and saline control groups ($P = 0.0036$). Tissue oedema was scored microscopically (×60) and is expressed as median (95% confidence intervals)

[$] Taurine treatment significantly decreased neutrophil infiltration into the bladder tissue compared to saline + *E. coli* treatment ([$]$P = 0.018$). The number of infiltrating neutrophils was counted in five high-powered fields per tissue section (×60)

[&] Significantly greater apoptosis at 24 h in the taurine group compared to the saline control and *E. coli*-treated group ($P = 0.0001$)

Table 2 Urine bacterial growth

Treatment ($N = 9$)	*E. coli* positive culture (%)	Mean (CFU/ml) (range)
Taurine + *E. coli*	78% (7/9)	3.5×10^6* (2.55×10^2 to 13×10^6)
Saline + *E. coli*	44% (4/9)	1.2×10^6 (5.0×10^1 to 3.25×10^6)

Statistical analysis used was unpaired *t* testing with Welsh correction

Presence of bacteria in the urine was assessed by colony counting 24-h post-plating urine sample on agar. The incidence of infection is reported as a percentage of the group. There was no difference between the groups ($P = 0.1$, unpaired *t* testing)

The number of CFU is reported as the mean with maximum and minimum CFU for both groups

* Statistically significant higher number of bacteria present in the infected urine of the taurine group compared to the saline group ($P = 0.02$, unpaired *t* testing)

Table 3 Bladder tissue bacterial growth ($N = 10$)

Treatment ($N = 10$)	Positive bladder tissue for *E. coli* (%)	Mean (CFU/g) (range)
Taurine + *E. coli*	100%* (10/10)	2.8×10^6 (4×10^3 to 2.7×10^7)
Saline + *E. coli*	44% (4/9)	3.5×10^7 (6×10^3 to 10×10^7)

Statistical analysis performed using unpaired *t* testing with Welsh correction

Presence of bacteria in the bladder tissue homogenate was assessed by colony counting 24-h post-plating the sample on agar. The incidence of infection is reported as a percentage of the total group

There is no difference between the numbers of bacteria present in the bladder tissue of the infected animals

* Significantly higher incidence of bacterial infection in the bladder tissue of the taurine-treated group ($P < 0.05$, unpaired *t* testing). The number of CFU is reported as the mean with maximum and minimum CFU for both groups

urine of *E. coli*-infected animals compared to the number of CFU present in the urine of the *E. coli*-infected animals in the saline group (3.5 × 106 vs. 1.2 × 106 CFU/g, $P = 0.02$, unpaired *t* test with post hoc testing.) The control animals had no bacterial growth in their urine.

E. coli growth in urine (N = 3)

These data demonstrate that taurine treatment increases bacterial infection in the mice bladders. It is possible that taurine is having a direct effect on bacterial growth therefore *E. coli* proliferation in normal human urine supplemented with taurine at 1 and 2 mg/ml was assessed to determine if taurine promoted bacterial growth in urine as assessed by optical density of the culture at 24 h of three samples of urine samples from healthy individuals.

Taurine does not enhance *E. coli* growth in normal human urine (taurine 0.1250 ± 0.019 OD vs. control 0.1243 ± 0.018 OD, $P = 0.92$, unpaired *t* test). Furthermore, taurine supplementation does not alter *E. coli* growth in 100% nutrient broth (CM67, Oxid, Basingstoke, UK).

Bladder tissue analysis

Bacterial growth in the bladder tissue (N = 10) (Table 3)

There was a significantly higher incidence of bacterial infiltration in the bladder tissue samples of the taurine-treated *E. coli* group compared to the saline-treated *E. coli* group (100 vs. 44%, $P < 0.05$, unpaired *t* testing with post hoc analysis). There is no difference in the numbers of bacteria

present (CFUs) in the *E. coli*-infected tissue of either group (2.8 × 106 vs. 3.5 × 107 CFU/g) The control animals had no growth in their bladder tissue.

Tissue myeloperoxidase (Table 1)

Mice pre-treated with taurine for 5 days had significantly reduced neutrophil infiltration levels as assessed by tissue MPO. There was significantly higher myeloperoxidase content in the bladder tissue of the saline-treated *E. coli*-infected animals compared to control animals and the taurine animals with *E. coli* infection (control 2.43 ± 0.21 units/g, saline + *E. coli* 3.56 ± 0.2 units/g, taurine + *E. coli* 2.5 ± 0.18 units/g, $P < 0.05$, one-way ANOVA with post hoc testing).

Bladder inflammatory index (Table 1)

Bladder tissue oedema was significantly reduced in the taurine-treated tissue compared to the saline-treated and saline control animals (saline + *E. coli* 2.67 ± 0.21 vs. taurine 1.28 ± 0.29, $P = 0.0036$, one-way ANOVA with post hoc testing).

Neutrophil infiltration (Table 1)

Neutrophil infiltration was also assessed as a measure of the inflammatory response. The number of PMN per five high power fields (×60) was counted for each section. The taurine-treated group had significantly less neutrophils in the bladder tissue compared to the saline-treated group (36 ± 5.2 vs. 137 ± 25, $P = 0.018$, one-way ANOVA with post hoc testing) (Fig. 1a, b).

Bladder urothelial cell apoptosis (Table 1)

24-h post-*E. coli* infection in the saline treated animals had significantly less urothelial cell apoptosis per HPF than the taurine-treated *E. coli* infected group. The surface urothelial layer had been shed and the underlying urothelial cells proliferate to replace this discarded barrier (Fig. 2a, b).

The taurine-treated *E. coli* infected group had significantly delayed urothelial cell apoptosis at 24 h resulting in less urothelial cell exfoliation at 24 h (taurine + *E. coli* 63.9 ± 3.49% vs. saline + *E. coli* 32.6 ± 1.61%, $P = 0.0001$, one-way ANOVA with post hoc testing).

Discussion

Bladder mucosal defence networks are characterised by a multifaceted interplay between urothelial, inflammatory and immune cells that works to eradicate bacterial

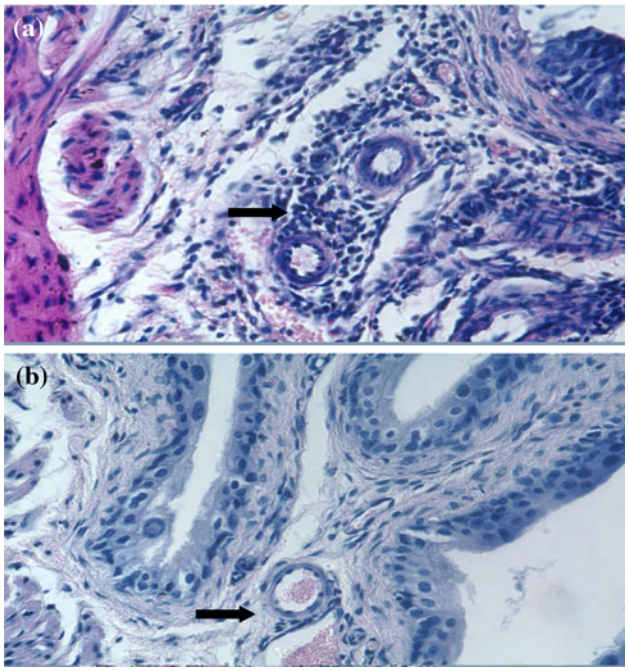

Fig. 1 Neutrophil infiltrate. The number of infiltrating neutrophils was counted in five high-powered fields per tissue section (×60). Neutrophils can clearly be seen migrating from the microvessel into the bladder tissue in the saline-treated group (**a**). The number of neutrophils is significantly reduced in the bladder tissue of the taurine-treated group (**b**). Tissue architecture is also preserved by taurine treatment

pathogens encountered in the endothelial–urothelial environment. Neutrophil tissue infiltration has been shown to be critical for bacterial clearance in murine models of UTI [12, 13]. *E. coli* stimulates urothelial cells to produce ICAM-1 and stimulate neutrophils CD11b in order to promote neutrophils migration across the urothelial barrier [14]. This demonstrates that the bacterial/urothelial interface interactions play a key role in induction of neutrophils migration during mucosal infection and show the necessity of host-derived chemotactic factors and cell adhesion events.

As expected in this study, taurine treatment significantly up-regulated peripheral blood neutrophil bactericidal function compared to saline-treated animals and this has been demonstrated in a number of other studies [15, 16].

However, taurine failed to prevent or down-regulate bladder wall and urinary infection. Although taurine did up-regulated peripheral blood neutrophil function, taurine significantly decreased neutrophil infiltration into the bladder tissue (reduced myeloperoxidase activity in the bladder tissue and tissue staining for infiltrating neutrophils) and tissue oedema (a marker of inflammation). These data demonstrate that taurine prevents the inflammatory response to infection that occurs at the endothelial cell-target organ tissue interface which in this model was bladder mucosa. Taurine functions include: up-regulation of endogenous nitric

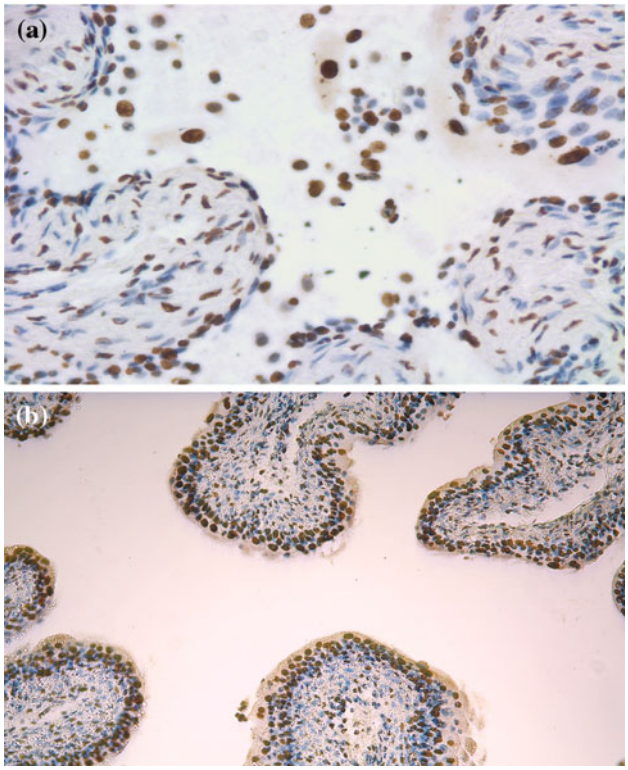

Fig. 2 Bladder epithelial shedding. The number of apoptotic cells was counted in five high-powered fields per tissue section and expressed as a percentage of total cells number (×400). Apoptotic epithelial cells can be seen detached from the epithelial barrier in the saline-treated group (**a**). The number of apoptotic cells is significantly increased in the taurine-treated group with delayed shedding evident (**b**)

oxide [17] (an endothelial protectant), inhibition of inducible nitric oxide synthase [18], anti-oxidant properties and preservation of cell–cell gap junctions [19] and thus serves to suppress excess inflammation, oxidative stress and apoptosis following a variety of stresses (ischaemic [20], sepsis [21] and metabolic (glucose [16]). This results in less recruitment and transmigration of activated neutrophils across the endothelial–urothelial cell barrier into the urine.

It is well established that urothelial cells are the first host barrier to come into contact with microbial invaders and as a result these cells have a vital role to play in innate host defence. The luminal surface of the bladder is normally covered by extremely long-lived (average life span 50–200 days) [22] highly differentiated superficial facet (umbrella) cells. Filamentous type-1 pili on *E. coli* contain an adhesion molecule (FimH) which binds to mannose glycoprotein receptors on the bladder luminal surface. FimH-mediated attachment of UPEC in the bladders of mice results in rapid loss of this superficial facet cell layer via apoptosis as a protective mechanism [23]. Six hours after inoculation, exfoliation of bacteria-laden superficial facet cells occurs, exposing underlying, less differentiated intermediate cells [24]. When apoptosis is blocked with a pan-caspase inhibitor, exfoliation is delayed and this in turn has been shown to significantly delay clearance of UPEC [24].

In this murine model of UTI there were significantly more bacteria present in the urine and significantly higher bladder tissue *E. coli* infiltration in the taurine-treated animals compared to the saline-treated group. The taurine-treated group in this study showed significantly delayed shedding of apoptotic bladder epithelial cells compared to the saline-treated group, which, at 24 h, exposed the intermediate cells. This result is consistent with recent data that suggested taurine may prevent apoptosis in epithelial cells [25] possibly through attenuation of DNA fragmentation [26]. Indeed, it has been demonstrated that the anti-inflammatory properties of taurine protects the urothelium following a variety of insults [27–30].

However, it may also be that *E. coli* which have penetrated the superficial urothelial layer has formed foci of intracellular "bacterial factories" allowing bacterial multiplication [31] and which are protected from innate immunity in the acute phase until the bacteria "permit" apoptotic shedding and thus spread within the bladder and to within intermediate layers of the exfoliated mucosa [32]. The mechanism of this is unclear and further experimental work needs to be carried out using immunofluorescence and confocal/electron microscopy techniques to examine for bacterial reservoirs in bladder tissue.

FimH adhesin also stimulates the host-cell signalling cascades and causes rearrangement of the cellular cytoskeleton thus allowing internalisation of *E. coli*. Once bacteria subvert innate responses of urothelial cell apoptosis and shedding, they invade superficial bladder cells, and mature into biofilms creating pod-like bulges on the bladder surface surrounded by a protective film of uroplakin (a transmembrane urothelial protein). This explains how bladder infections may persist in the face of robust host defences and systemic antibiotic treatment [33].

Furthermore, 100% of the taurine-treated animals had *E. coli* within the bladder walls and a larger number of CFUs within the urine compared to saline + *E. coli* animals. Even the taurine + *E. coli* group that had cleared the infection and had sterile urine displayed *E. coli* infiltrated bladder tissue.

Our study demonstrates that animals can have sterile urine but yet have bacterial infiltration in the bladder tissue. These findings indicate that *E. coli* can establish a reservoir in bladder tissue that may permit intracellular proliferation. This invasion allows the bacterium to exploit the intracellular environment and affords the chance to multiply unhindered by antibiotics or host defence.

In this present study, delayed urothelial cell apoptosis and reduced epithelial cell shedding correlated with increased incidence of persistent bladder infection.

95% of recurrent UTIs are thought to be discrete infections caused by retrograde urethral ascent of bacteria from the faecal flora. Many of the UTIs currently classified as recurrent infections might be persistent infections which have acquired the ability to invade uroepithelial cells and proliferate internally. Recognising bacterial persistence is important because the inappropriate use or dosing of antimicrobials can confer transferable resistance and result in multi-drug resistant strains and thus poorer bacteriological and clinical outcomes.

Antibiotics have been chosen for their ability to kill the infectious organism with maximal efficacy in the urine rendering urine sterile within hours of the first dose; however, the findings of this study suggest that tissue penetration is an important factor to be considered in the choice of antibiotic for rUTI.

The data from our work question the use of sterile urine as an appropriate measure of bacterial eradication in rUTI. A bladder biopsy under cytoscopy may aid in the detection of bacterial reservoirs in bladder epithelium in treatment of unresolved rUTI and thus permit administration of pharmacological agents systemically or intra-vesically [34, 35] that may allow normal or enhanced shedding of urothelium and thus exposure or elimination of sub-mucosal bacterial reservoirs.

In conclusion, this work demonstrates that despite augmentation of neutrophil function, bladder cell exfoliation is critical for bacterial clearance from the bladder. This shedding of superficial urothelial cells may be viewed as a critical host defence mechanism to shed invading bacteria. Oral taurine supplementation, whilst augmenting neutrophil function, failed to prevent infection of the urothelium by bacteria and seems to disrupt a vital bladder response mechanism to invading bacteria probably through dampening endothelial cell–urothelial interactions.

Acknowledgments This work was funded by The Dept of Surgical Research, Beaumont Hospital and The Royal College of Surgeons in Ireland.

References

1. Hooton TM, Stamm WE (1997) Diagnosis and treatment of uncomplicated urinary tract infection. Infect Dis Clin North Am 11:511–581
2. Foxman B (1990) Recurring urinary tract infection: incidence and risk factors. Am J Public Health 80:331–333
3. Mulvey MA, Schilling JD, Martinez JJ, Hultgren SJ (2000) Bad Bugs and beleaguered bladders; interplay between uropathogenic Escherichia coli and innate host defences. Proc Natl Acad Sci USA 97(16):8747–9348
4. Shahin RD, Engberg I, Hagberg L, Svanororg-Eden C (1997) Neutrophil recruitment correlated with LPS responsiveness in local gram-negative infection. J Immunol 138:3475–3480
5. Condron C, Toomey D, Casey RG, Shaffii M, Creagh T, Bouchier-Hayes D (2003) Neutrophil bactericidal function in patients with recurrent urinary tract infections. Urol Res 31:329–334
6. Condron C, Toomey D, Casey RG, Creagh T, Bouchier-Hayes D (2004) Taurine protects against PMN dysfunction and death in urine. Urol Res 32(5):338–345
7. Wang JH, Redmond HP, Watson RW, Condron C, Bouchier-Hayes D (1996) The beneficial effect of taurine on the prevention of human endothelial cell death. Shock 6(5):331–338
8. Finnegan N, Toomey D, Condron C, Redmond HP, Da Costa M, Bouchier-Hayes DJ (2002) Potentiation of the therapeutic index of interleukin-2 immunotherapy by combination with taurine in a murine tumour model. Ir J Med Sci 171(2):85–88
9. Hof H, Christen A, Hacker J (1986) Comparative therapeutic activities of ciprofloxacin, amoxicillin, ceftriaxone and co-trimoxazole in a new model of experimental infection with *Escherichia coli*. Infection 14(4):190–194
10. Lanne B, Olsson BM, Jovall PA, Angström J, Linder H, Marklund BI, Bergström J, Karlsson KA (1995) Glycoconjugate receptors for P-fimbriated *Escherichia coli* in the mouse. An animal model of urinary tract infection. J Biol Chem 270(15):9017–9025
11. Jerde TJ, Bjorling DE, Steinberg H, Warner T, Saban R (2000) Determination of mouse bladder inflammatory response to *E. coli* lipopolysaccharide. Urol Res 28(4):269–273
12. Stamey TA (1975) The role of introital enterobacteria in recurrent urinary infections. J Urol 109:467
13. Haraoka M, Hang L, Frendeus B, Giodaly G, Burdick M, Strieter R, Svanborg C (1999) Neutrophil recruitment and resistance to mucosal infection with uropathogenic *Escherichia coli*. J Infect Dis 180:1220–1229
14. Agace WW, Patarroyo M, Svensson M, Carlemalm E, Svanborg C (1995) Escherichia coli induces transuroepithelial neutrophils migration by an intercellular adhesion molecule-1 dependent mechanism. Infect Immun 63:4054–4062
15. Robb WB, Condron C, Moriarty M, Walsh TN, Bouchier-Hayes DJ (2009) Taurine attenuates radiation-induced lung fibrosis in C57/Bl6 fibrosis prone mice. Ir J Med Sci 16 [Epub ahead of print]
16. Casey RG, Gang C, Joyce M, Bouchier-Hayes DJ (2007) Taurine attenuates acute hyperglycaemia-induced endothelial cell apoptosis, leucocyte-endothelial cell interactions and cardiac dysfunction. J Vasc Res 44(1):31
17. Fennessy FM, Moneley DS, Wang JH, Kelly CJ, Bouchier-Hayes DJ (2003) Taurine and vitamin C modify monocyte and endothelial dysfunction in young smokers. Circulation 28, 107(3):410–415
18. Park E, Schuller-Levis G, Quinn MR (1995) Taurine chloramines inhibits production of nitric oxide and TNF-α in activated RAW 264.7 cells by mechanisms that involve transcriptional and translational events. J Immunol 154:4778–4784
19. Gordon RE, Heller RF, Del Valle JR, Heller RF (1989) Membrane perturbations and mediation of gap junction formation in response to Taurine treatment in normal and injured alveolar epithelia. Exp Lung Res 15(6):895–908
20. Barry MC, Kelly CJ, Abdih H, Watson RW, Stapleton P, Sheehan SJ, Redmond HP, Hayes DB (1997) Differential effects of lower limb revascularisation on organ injury and the role of the amino acid taurine. Eur J Vasc Endovasc Surg 13(2):193–201
21. Erdem A, Sevgili AM, Akbiyik F, Atilla P, Cakar N, Balkanci ZD, Iskit AB, Guc MO (2008) The effect of taurine on mesenteric blood flow and organ injury in sepsis. Amino Acids 35(2):403–410 [Epub 2007 Dec 28]
22. Levi PE, Cooper EH, Anderson CK, Williams RE (1969) Analysis of DNA content nuclear size and cell proliferation in transitional cell carcinoma in man. Cancer 23:1074
23. Mulvey MA, Schilling JD, Martinez JJ, Hultgren SJ (2000) Bad bugs and beleaguered bladders: interplay between uropathogenic

Escherichia coli and innate host defences. Proc Natl Acad Sci USA 97:8829–8835
24. Mulvey MA, Lopez-Boado YS, Wilson CL, Roth R, Parks WC, Heuser J, Hultgren SJ (1998) Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. Science 282:1061–1066
25. Chen F, Chen C (2002) An experimental research of taurine on H_2O_2-induced bovine lens epithelial cell apoptosis. Chung Hua Yen Ko Tsa Chih. 36(4):272–274
26. Hizoh I, Haller C (2002) Radiocontrast-induced renal tubular cell apoptosis: hypertonic versus oxidative stress. Invest Radiol 37(8):428–434
27. Sener G, Sehirli O, Ipci Y, Cetinel S, Cikler E, Gedik N, Alican I (2005) Protective effects of taurine against nicotine-induced oxidative damage of rat urinary bladder and kidney. Pharmacology 74:37–44
28. Abd-Allah AR, Gado AM, Al-Majed AA, Al-Yahya AA, Al-Shabanah OA (2005) Protective effect of taurine against cyclophosphamide-induced urinary bladder toxicity in rats. Clin Exp Pharmacol Physiol 32:167–172
29. Zeybek A, Saglam B, Cikler E, Cetinel S, Ercan F, Sener G (2007) Taurine ameliorates stress-induced degeneration of the urinary bladder. Acta Histochem 109:208–214
30. Zeybek A, Saglam B, Cikler E, Cetinel S, Ercan F, Sener G (2006) Protective effects of taurine on protamine sulphate induced bladder damage. World J Urol 24:438–444
31. Martinez JJ, Hultgren SJ (2002) Requirement of Rho-family GTPases in the invasion of Type 1-piliated uropathogenic Escherichia coli. Cell Microbiol 4(1):19–28
32. Mulvey MA, Schilling JD, Hultgren SJ (2001) Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. Infect Immun 69:4572–4579
33. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ (2003) Intracellular bacterial biofilm-like pods in urinary tract infections. Science 301:105–107
34. Gorman SP, McCafferty DF, Woolfson AD, Jones DS (1987) Reduced adherence of micro-organisms to human mucosal epithelial cells following treatment with taurolin, a novel antimicrobial agent. J Appl Bacteriol 62:315–320
35. Nagl M, Pfausler B, Schmutzhard E, Fille M, Gottardi W (1998) Tolerance and bacterial action of *N*-chlorotaurine in a urinary tract infection by an omniresistant *Pseudomonas aeruginosa*. Zentralbl Bakteriol 288:217–223