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ORIGINAL PAPER

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Taurine protects against PMN dysfunction and death in urine

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Abstract The polymorphonuclear neutrophil (PMN) is the primary pro-inflammatory cell in the host response to bacterial infection and, as the first line of defence, is the principal cell responsible for the recognition, phagocytosis and killing of bacteria. PMN function is known to be defective in the urine. High osmolarity is physiologic in the urine and this hypertonic environment has been shown to compromise neutrophil function. In this study, PMN function was found to be suppressed in urine. This correlated with significant cell death, both by apoptosis and necrosis. The amino acid taurine down regulated PMN cell death and preserved function in the urine, suggesting taurine as a therapeutic option for urinary tract infection.

Keywords PMN · Respiratory burst · Apoptosis · Taurine · Urine

Introduction

Symptoms of urinary tract infection (UTI) and the tissue pathology associated with this common disease are caused by the host inflammatory response to the invading bacteria and not by the microbes per se. This response, however, is critical for host defence and relies on the so-called 'innate' immune system. Pyria, the presence of polymorphonuclear neutrophils (PMN) in the urine, is a classic hallmark of UTI, and PMN have been shown to accumulate in large numbers in the urine as early as 6 h following infection [1]. However the biological significance of PMN in the urine is controversial. Norden and Flynn reported that PMN of patients with UTI did not play a major role in excluding bacteria [2]. Other workers have reported that PMN are fundamental to host defence in experimental bladder infections [3, 4], and that PMN in the bladder mucosa ingested bacteria [5]. Furthermore PMN in the urine of patients with UTI have been shown to contain phagocytosed bacteria [6].

Susceptibility to recurrent UTI (rUTI) appears to be related to intrinsic host-bacteria interactions, especially increased bacterial adherence to mucosa and decreased bacterial killing by PMN. We previously demonstrated that people with a history of rUTI have a reduced PMN bactericidal function [7]. The ability of the PMN to function will also be dependent on the microenvironment in which the phagocyte must work. High osmolarity is physiological in the urine where sodium chloride and urea are the most important contributers. Impairment of PMN function in urine has been related to increased osmolarity and the concentration of dissolved inorganic salts [8], and furthermore, high osmolarity has been shown to be a greater suppressor of PMN function than pH [9]. Hypertonic urine has been shown to compromise PMN's chemotactic, migratory, adhesive, and bactericidal functions [10, 11]. Urinary constituents which may be of critical influence here include sodium chloride (NaCl) and urea. NaCl inhibits cellular function by energy exhaustion consistent with intracellular ATP depletion as the cell struggles to maintain homeostasis by redistributing energy to the sodium/potassium pump. Urea directly inhibits intracellular enzyme activity including NAPDH oxidase which is critical for superoxide production [12].

Cell death is a critical factor affecting the bactericidal capacity of the PMN, which may in turn be influenced by the tonicity of the fluid in which the cell must function. Recent focus on the physiology of cell death highlights the interplay between cell volume regulatory

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mechanisms and the cellular machinery leading to death of the cell. Dysfunction of cell volume regulation is associated with both necrotic and apoptotic cell death, which are coupled to cell shrinkage (apoptotic volume decrease: AVD) or cell swelling (necrotic volume increase: NVI). The characteristics of apoptotic cell death include cell shrinkage and rearrangement of the plasma membrane exposing phosphatidylserine residues at the extra-cellular surface [13]. In contrast, necrotic cell death is associated with membrane rupture as a result of uncontrolled cell swelling. Recently, the induction of AVD under normotonic conditions has been shown to be processed through the cellular machinery of the regulatory volume decrease (RVD) system. It is known that RVD is achieved by the parallel operation of Cl(−) and K(+) channels under hypotonic conditions. Both AVD and RVD were found to precede cytochrome c release, caspase-3 activation, DNA laddering, and ultrastructural alterations in three cell types after apoptotic insults with two distinct apoptosis inducers [14]. Interestingly, these authors also demonstrated that AVD was not prevented by a broad-spectrum caspase inhibitor, however, when AVD and RVD were prevented by blocking volume-regulatory Cl(−) or K(+) channels, the cells did not show succeeding apoptotic biochemical and morphological events and were rescued from death. Thus, it was concluded that AVD, caused by disordered cell volume regulation, is an early prerequisite for apoptotic events leading to cell death. This might suggest that PMN dysfunction in the urine is, as a consequence of cell death, due to the hyperosmolar environment and that modification of this process would be beneficial to bacterial clearance in the management of UTI.

K^+, Na^+ and Cl^-, as well as amino acids such as taurine, are known to participate in the volume regulatory responses of cells under hypo- and hypertonic shock [15]. Taurine is the most abundant free amino acid found in the cytosol of PMN, comprising up to 76% of the free amino acid pool [16]. It has previously been shown to play a critical role in osmoregulation and membrane stabilisation [17, 18]. Taurine supplementation restores senescent PMN function, preserving its antimicrobial capability [19]. Taurine also has important influences on PMN apoptosis. It has been shown to down regulate Fas mediated PMN apoptosis via a calcium-mediated pathway [20]. Furthermore, taurine blocks the induction of apoptosis following *Escherichia coli* ingestion through an anti-oxygen dependant mechanism [21].

It is known that urinary excretion of taurine is influenced by dietary supply and that extra taurine in the diet will significantly increase the amount excreted in the urine [22]. It was hypothesised, therefore, that taurine will preserve PMN bactericidal function in the urine by preventing cell death and thus up-regulating reactive oxygen intermediate (ROI) production. This paper assesses whether the compromised PMN function reported in urine is a result of hyperosmolar mediated PMN cell death and examines the ability of taurine to modulate the effects of the hyperosmolar environment found in urine.

Methods and materials

The addition of NaCl was used to modify the osmolarity of normal 0.9% saline as previously described [12]. Briefly NaCl was added to saline and the pH was kept constant at 7.4. Urine was collected from otherwise healthy premenopausal female patients ($n=22$, age 31 ± 2.2 years), with a history of recurrent UTI infections attending urology out patient clinics, and urine analysis carried out. Osmolarity was measured by the freeze thaw method using an osmometer (Fiske Associates, Mass.). The pH was assessed by pH meter (Radiometer, A/S) and urinalysis was carried out by dipstick (Multistix Bayer, Newberry, UK).

First morning void, following fluid consumption, and random midstream urine specimens were collected from healthy volunteers with no detectable abnormality in urinalysis. Any sediment was removed by centrifugation at $300\times g$ for 5 min. The samples were filter sterilised using 0.2 μm filters (Gelman, Mich., USA) and stored frozen at −80°C. The osmolarity of the samples was determined and a range of pooled urines from healthy volunteers in the same osmolarity range was used to represent the osmolarity found in rUTI patient urine. The addition of taurine did not significantly alter the osmolarity of the urine (Table 1). RPMI was used as a control medium.

PMN isolation

PMN were isolated from whole blood of healthy volunteers by dextran (Sigma) density sedimentation and Ficoll (Pharmica, Amersham Biosciences, UK) gradient centrifugation as previously described [20]. Cell concentration was adjusted to 2×10^6/ml and the cells were then incubated in polypropylene tubes (Starstedt) in urine of varying osmolarities ± taurine (Sigma) (1 mg/ml) for 1–6 h, in a humidified atmosphere of 5% CO_2 in air at 37°C. Preliminary studies had identified the optimal dose of taurine. PMN were gated on a dual parameter dot plot of forward light scatter (FLS) and

Table 1 Pooled urine + taurine 1 mg/ml. The addition of 1 mg/ml of taurine to the sterile urine samples had no significant effect on the osmolarity of the solution. Data is expressed as mosmol as determined by the freeze thaw method

	Urine (mosmol)	+ Taurine 1 mg/ml (mosmol)
Very concentrated	824	809
Mid range	654	635
Dilute	272	281
Very dilute	136	143
Control medium	334	351

side light scatter (SLS) and this region was selected for analysis.

Intracellular reactive oxygen intermediate

ROI generation by PMN was assessed by flow cytometry using the BurstTest Kit (Becton Dickinson) as per manufacture's instructions.

Cell surface CD 11b receptor expression

Receptor expression was assayed by staining cells with a fluorescently labelled antibody. Briefly, 100 µl of 2×10^6/ml PMN were incubated with 20 µl of PE-labelled anti-human CD11b or PE-labelled isotype control antibody on ice for 20 min in the dark. Samples were centrifuged, washed and analysed by flow cytometry with emission λ at 580 nm using CellQuest. Receptor density on the cell surface is expressed as mean channel fluorescence intensity of the cells.

Apoptosis/necrosis

Annexin-V binds phosphatidylserine, which is flipped, to the outer leaflet of the cell membrane when a cell is apoptotic. This process is unique to cells undergoing apoptosis and is involved in the macrophage recognition of these apoptotic cells. Dual staining with propidium iodide facilitates the detection of necrotic cells or cells with compromised membranes. Briefly, 1 ml of 2×10^6/ml PMN was incubated in saline or urine of varying osmolarities ± taurine (1 mg/ml) for 6 h, in a humidified atmosphere of 5% CO_2 in air at 37°C. PMN apoptosis/necrosis was assessed using the TACS Apoptosis Detection Kit (R and D Systems Minneapolis, USA) according to the manufacturer's instructions.

Results

PMN function and cell death in saline

PMN apoptosis in saline

The apoptotic state of a cell is critical to cellular function and it is known that PMN lose effector function, including adhesion and phagocytosis, following the induction of apoptosis. The effect of a hyperosmolar environment on PMN cell death was determined by annexin V/PI staining and flow cytometry. Incubation of PMN for 6 h in hypertonic saline in the normal physiological range of human urine resulted in significantly up-regulated PMN apoptosis at all osmolarities studied compared to normal saline (NS: 0.9% = 290 mosmol) (NS: 8 ± 1.9 versus 475 mosmol: 19 ± 3.5, 600 mosmol: 21 ± 3.4, 900 mosmol: 23 ± 4.7, and 1,200 mosmol: 24 ± 8.3). Addition of 1 mg/ml taurine to the saline significantly reduced this apoptosis at 475 mosmol and 600 mosmol (475 mosmol: 10 ± 3, 600 mosmol: 16 ± 2.9, 900 mosmol: 19 ± 3.2, 1,200 mosmol: 19 ± 6.0) (Fig. 1).

PMN necrosis in saline

Incubation in hyperosmolar saline also significantly up-regulated PMN necrosis at 6 h (NS: 1.5 ± 0.28 versus 475 mosmol: 3.5 ± 0.5, 600 mosmol 6.25 ± 2.17, 900 mosmol 10.75 ± 3.06 and 1,200 mosmol: 26.5 ± 13.0). The addition of 1 mg/ml taurine to the saline significantly reduced PMN necrosis at 600 and 900 mosmol (NS: 0.75 ± 0.25 versus 475 mosmol: 3. ± 1.0, 600 mosmol: 2.75 ± 0.25, 900 mosmol 4.25 ± 0.75 and 1,200 mosmol 18.0 ± 5.5) (Fig. 2)

PMN bactericidal capacity

PMN generation of ROI in response to *E. coli* stimulation for 10 min was measured to assess cellular bactericidal capacity. Pre-incubation in hyperosmolar saline for 1 h significantly decreased PMN ROI production compared to normal saline (NS: 290 mosmol) (NS: 205 ± 39 versus 475 mosmol: 139 ± 3, 600 mosmol: 99 ± 5., 900 mosmol: 87 ± 2.5 and 1,200 mosmol 65 ± .5). Supplementing the saline with 1 mg/ml of taurine significantly improved ROI production in the hyperosmo-

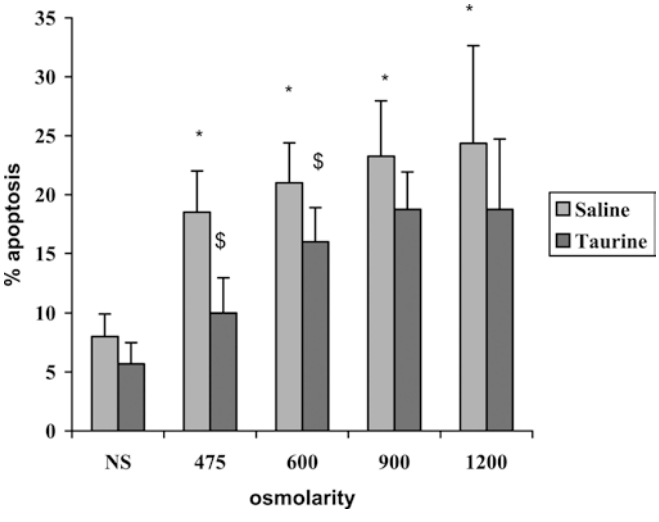

Fig. 1 PMN apoptosis in hypertonic saline. PMN apoptotic cell death was assessed following incubation in normal saline (osmolarity adjusted by the addition of NaCl) ± taurine 1 mg/ml for 6 h by measuring annexin V surface expression. Apoptosis is measured as a percentage of 10,000 cells acquired and expressed as mean + SEM ($n = 6$ individual experiments). Osmolarity is expressed as milliosmoles ($n = 6$ individual experiments). An *asterisk* denotes a significant difference from normal saline (290 mosmol) ($P < 0.05$, ANOVA, LSD post hoc) and *$* denotes a significant difference from equivalent osmolarity without taurine ($P < 0.05$, paired *t*-test)

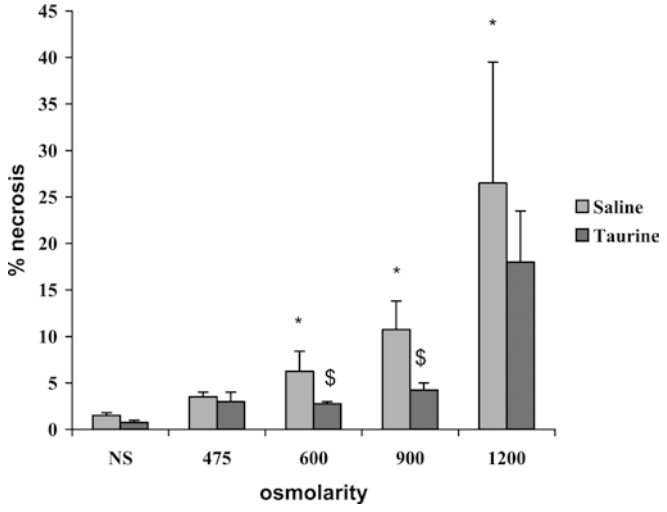

Fig. 2 PMN necrosis in hypertonic saline. PMN necrotic cell death was assessed following incubation in normal saline (osmolarity adjusted by the addition of NaCl) ± 1 mg/ml taurine for 6 h by measuring annexin V surface expression and PI staining of DNA. Necrosis is assessed as a percentage of 10,000 cells acquired and expressed as mean + SEM ($n = 6$ individual experiments). Osmolarity is expressed as milliosmolar. An *asterisk* denotes a significant difference from saline (290 mosmol) ($P < 0.05$, ANOVA, LSD post hoc) and *$* denotes a significant difference from equivalent osmolarity without taurine ($P < 0.05$, paired *t*-test)

lar environments of 600, 900 and 1,200 mosmol. (600 mosmol: 179 ± 6.5, 900 mosmol: 129 ± 9.5 and 1,200 mosmol: 120 ± 22.5) (Fig. 3)

PMN adhesion receptor expression

The ability of PMN to transmigrate out of blood, cross the epithelium and adhere to the bladder wall is facilitated by adhesion receptor CD11b on PMN and its ligand ICAM-1 [24]. PMN CD11b expression was assessed by flow cytometry following incubation in hyperosmolar saline for 1 h. Receptor expression was significantly down regulated by the two highest osmolarities of 900 and 1,200 mosmol compared to normal saline (NS: 504 ± 33 versus 475 mosmol: 486 ± 33, 600 mosmol: 347 ± 28, 900 mosmol: 260 ± 28.9 and 1,200 mosmol: 160 ± 40.8). Supplementing the saline with 1 mg/ml taurine preserved CD 11b expression at 900 mosmol (402 ± 28.8) but not in the very concentrated solution of 1,200 mosmol (Fig. 4).

Patient urinalysis

Urinalysis carried out on 22 patients with a history of rUTI showed no abnormalities of the urine. The median urine osmolarity of the morning void sample was 687 ± 43.3 mosmol with a maximum of 902 mosmol and a minimum of 293 mosmol. These figures are within the normal physiological range of human urine. A sample taken post 1 l of fluid consumption demonstrated that the kidneys were working to dilute the urine. pH analysis of the urine was also within the normal range (Table 2).

PMN function and cell death in urine

PMN apoptosis in urine

Having established that incubation in hypertonic saline resulted in PMN increased cell death and resultant

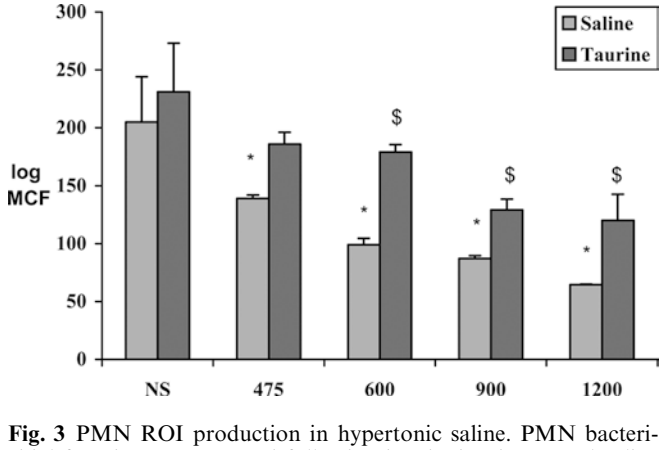

Fig. 3 PMN ROI production in hypertonic saline. PMN bactericidal function was assessed following incubation in normal saline (osmolarity adjusted by the addition of NaCl) ± 1 mg/ml for taurine 1 h by measuring ROI production in response to *Escherichia coli* (1×10^9) stimulation. ROI production is recorded as mean channel fluorescence emitted by the cells and expressed as mean + SEM ($n = 4$ individual experiments). An *asterisk* denotes a significant difference from normal saline ($P < 0.05$, ANOVA, LSD post hoc), and *$* denotes a significant difference from saline of equivalent osmolarity without taurine ($P < 0.05$, paired *t*-test)

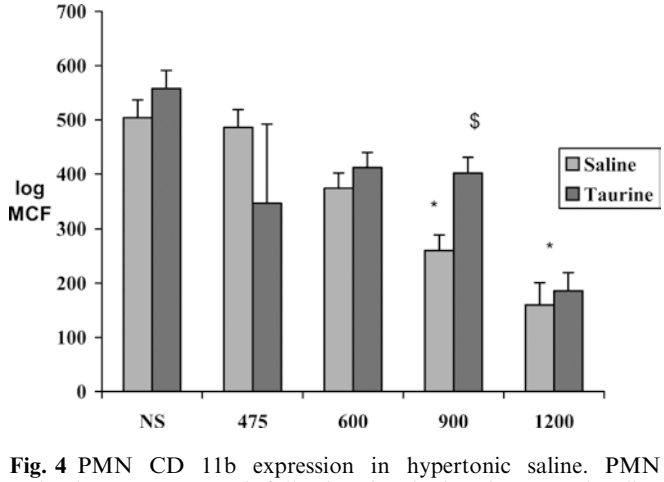

Fig. 4 PMN CD 11b expression in hypertonic saline. PMN activation was assessed following incubation in normal saline (osmolarity adjusted by the addition of NaCl) ± 1 mg/ml taurine for 1 h by measuring CD 11b adhesion receptor expression. CD11b expression is recorded as the mean channel fluorescence emitted by the cells and expressed as mean + SEM ($n = 4$ individual experiments). An *asterisk* denotes a significant difference from control ($P < 0.05$, ANOVA, LSD post hoc), *$* denotes a significant difference from saline of equivalent osmolarity without taurine ($P < 0.05$, paired *t*-test)

Table 2 Osmolarity and pH of patient morning first void urine samples and urine samples 1 h post 1 l fluid consumption expressed as mean ± SEM ($n = 20$). These samples are all within the normal range for human urine osmolarity and pH

Osmolarity at first void	Minimum	Maximum
687 ± 43.3	293 mosmol	902 mosmol
Osmolarity after 1 l fluid		
328 ± 54.7	66 mosmol	763 mosmol
pH of first void		
6.12 ± 0.1	5.7	6.8

dysfunction, PMN function and cell death were then examined in urine. Incubation in urine for 6 h significantly up-regulated PMN apoptosis compared to control medium in an osmolarity dependent manner (control: 20 ± 2.08% versus very concentrated 66.0 ± 6.86% midrange: 47.0 ± 1.94%, dilute: 37.0 ± 4.77%, very dilute: 30 ± 4.17%). The addition of taurine (1 mg/ml) to the urine significantly reduced apoptosis at each osmolarity studied (control: 16 ± 1.8% versus very concentrated 47.0 ± 10.46%, midrange: 37.0 ± 3.13%, dilute: 21 ± 3.67%, very dilute: 15 ± 3.9%) (Fig. 5).

PMN necrosis in urine

Incubation in urine for 6 h significantly up-regulated PMN necrosis compared to the controls in an osmolarity dependant manner (control: 2.33 ± 0.66% versus very concentrated 11.33 ± 1.86%, midrange: 15.0 ± 2.18%, dilute: 12.66 ± 1.76%, very dilute: 21.66 ± 1.45%) and again the addition of 1 mg/ml of taurine to the urine significantly attenuated this effect (control 3.0 ± 1.15% versus very concentrated 7.3 ± 1.45%, midrange 9.67 ± 1.76%, dilute 7.3 ± 0.88%, very dilute 17 ± 1.85%) (Fig. 6).

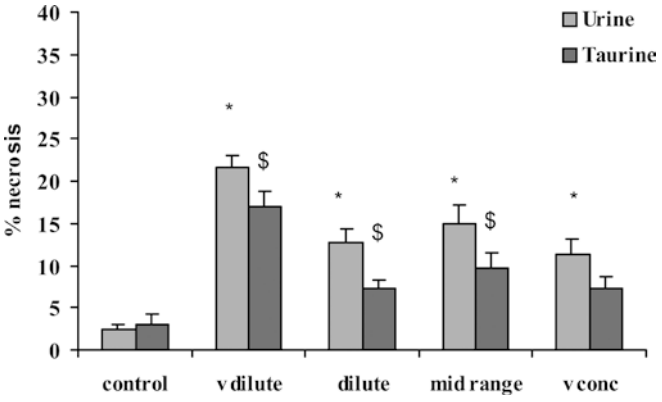

Fig. 6 PMN necrosis in urine. PMN necrotic cell death was assessed following incubation in pooled human urine ± 1 mg/ml taurine for 6 h by measuring annexin V surface expression and propidium iodide staining of DNA. Necrosis is measured as a percentage of 10,000 cells acquired and expressed as mean + SEM ($n = 6$ individual experiments). An *asterisk* denotes a significant difference from control medium ($P < 0.05$, ANOVA, LSD post hoc), *$* denotes a significant difference from equivalent osmolarity without taurine. ($P < 0.05$, paired *t*-test)

PMN bactericidal capacity in urine

PMN production of ROI in response to *E. coli* stimulation for 10 min was measured to assess cellular bactericidal capacity. Pre-incubation in hyperosmolar urine for 1 h significantly decreased PMN ROI production compared to the control medium (very concentrated 297 ± 6 MCF, midrange 375.5 ± 34.9 MCF versus control 701 ± 40.8 MCF). Supplementing the urine with 1 mg/ml taurine significantly improved ROI production in the hyperosmolar environments (very concentrated 466 ± 36.4 MCF, midrange: 535 ± 62.2 MCF) (Fig. 7).

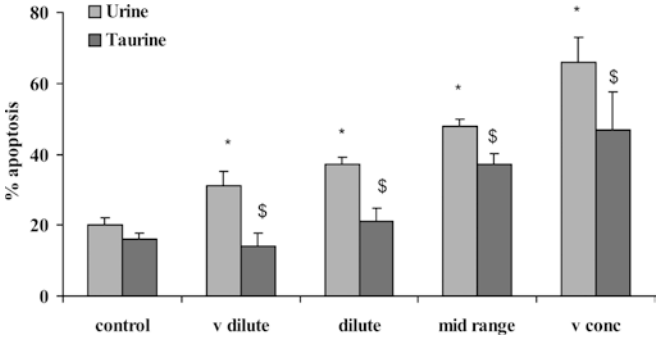

Fig. 5 Neutrophil apoptotic cell death was assessed following incubation in pooled human urine from healthy volunteers ± taurine 1 mg/ml for 6 h by measuring annexin V surface expression. Apoptosis is assessed as a percentage of 10,000 cells acquired and expressed as mean + SEM ($n = 6$ individual experiments). An *asterisk* denotes a significant difference from control medium, *$* denotes a significant difference from the equivalent osmolarity without taurine. ($P < 0.05$, paired *t*-test). Refer to Table 2 for the details of the milliosmolarity for the different urine groups

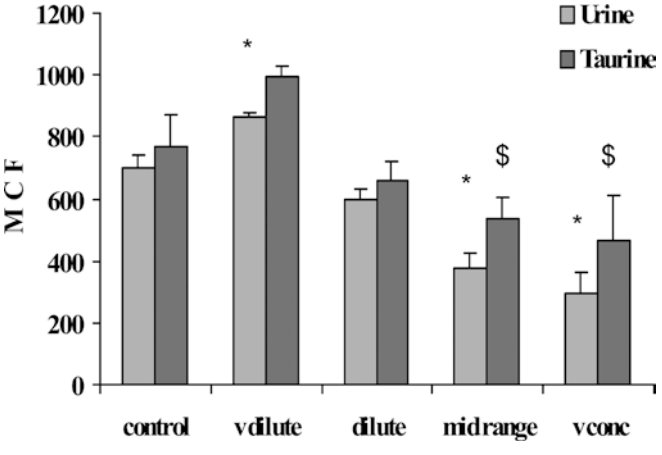

Fig. 7 PMN ROI production in urine. PMN bactericidal function is assessed following incubation in urine ± taurine 1 mg/ml for 1 h by measuring ROI production in response to *E. coli* (1×10^9) stimulation. ROI production is recorded as mean channel fluorescence emitted by the cells and expressed as mean + SEM ($n = 6$ individual experiments). An *asterisk* denotes a significant difference from control medium $P < 0.05$, ANOVA, LSD post hoc), and *$* denotes a significant difference from urine of equivalent osmolarity ($P < 0.05$, paired *t*-test)

PMN adhesion receptor expression in urine

PMN CD11b expression was assessed by flow cytometry following incubation in hyperosmolar urine for 1 h. Receptor expression was significantly down regulated by the highest osmolarity compared to controls (very concentrated 182 ± 52.5 MCF versus control 328 ± 30.1 MCF). The midrange or dilute urine had no effect on the receptor expression compared to the controls, however, the hypo-osmolar urine significantly up-regulated receptor expression. Addition of 1 mg/ml taurine to the urine had no effect on CD11b expression (Fig. 8).

Discussion

PMN are the first line of defence in the innate immune system, which has been shown to be vital to the resolution of UTI. High osmolarity is physiological in the urine and this creates a harsh environment known to compromise PMN bactericidal function. Our work confirms previous evidence that PMN function is disrupted in the urine. A relationship between urine osmolarity and PMN death is clearly identified. Incubation in urine significantly up-regulated PMN cell death, which resulted in impaired PMN bactericidal function. Increasing urine osmolarity augmented this effect and cells underwent rapid cell death with resultant dysfunction in hyperosmolar urine. As expected, hypo-osmolar urine mediated cell death by necrosis consistent with membrane rupture, whereas concentrated urine caused death by apoptosis. PMN cell death was significantly elevated by incubation in either hypertonic saline or sterile urine. Taurine prevented cell death in both urine and saline defending against hyperosmolar shock mediated by NaCl and urea. The amino acid taurine maintained PMN function and prevented cell death in the harsh environment of high osmolarity in vitro.

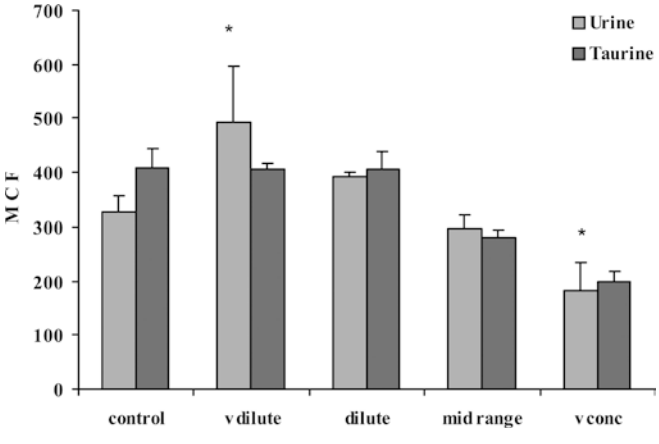

Fig. 8 PMN CD11b adhesion receptor expression in urine. PMN activation was assessed following incubation in pooled human urine $\pm$ 1 mg/ml taurine for 1 h by measuring CD 11b adhesion receptor expression. CD11b expression is recorded as mean channel fluorescence emitted by the cells and expressed as mean + SEM ($n = 6$ individual experiments). An *asterisk* denotes a significant difference from control medium, ($P < 0.05$, ANOVA, LSD post hoc)

This prevention of cell death may be attributed to several events. Taurine has been shown to regulate osmotic pressure in the cell and maintain homeostasis of intracellular ions [17]. Taurine stabilises cell membranes by inhibiting phosphorylation of membrane proteins and preventing lipid peroxidation [15]. Recently hyperosmotic exposure has been shown to induce trafficking of the death factor Fas/CD95 to the plasma membrane followed by activation of caspase-3 and 8 in cells [24]. This hyperosmotic Fas targeting to the plasma membrane was dose-dependently diminished by taurine, which the authors suggested was due to an augmentation of volume regulatory increase. It has also recently been demonstrated that stimulation of the Fas receptor leads to a release of taurine, which immediately precedes apoptotic cell shrinkage in lymphocytes [25]. We have previously demonstrated that exogenous taurine prevents Fas mediated apoptosis in PMN [7]. Taurine, as the most abundant amino acid found in the cytosol of PMN, is known to participate in volume regulation, and may facilitate the volume regulatory mechanism in these stressed cells thus protecting against cell death.

Taurine is also a calcium regulator, and we have previously demonstrated that decreased intracellular calcium leads to PMN cell death, an effect prevented by taurine supplementation [7]. We have also previously demonstrated that taurine up-regulates ROI production in vitro [26]. In this saline model, a hypertonic environment significantly decreased PMN ROI production in response to a bacterial stimulus, an effect statistically augmented by supplementation with taurine. The higher osmolarity saline also significantly disrupted adhesion receptor expression, however, taurine had a limited effect in preserving receptor expression. In keeping with previous reports, this study demonstrated that hyperosmolar urine significantly decreased PMN function. CD 11b adhesion receptor expression and ROI production in response to a bacterial stimulus was significantly down regulated following incubation in hyperosmolar urine. Taurine preserved bactericidal function by maintaining ROI production in urine. However, it has a limited effect on the preservation of CD11b expression, effective only in the midrange urine.

Conversely, incubation in hypo-osmolar urine with an osmolarity lower than the control medium significantly up-regulated adhesion/activation marker expression and ROI production in control PMN. This finding is in agreement with Edashige et al. who reported that hypotonic shock primed PMN for enhanced superoxide generation and tyrosyl phosphorylation of cellular proteins [27]. Current clinical practice in the management of UTI is to recommend fluid intake to improve the clearance of bacteria by increased voiding. These results suggest that in addition to the recognised value of increased voiding which removes bacteria, urine dilution would improve PMN bactericidal function in infected urine. This study demonstrated that the optimal condi-

tions for PMN anti-bacterial function in the urine are, in fact, hypo-osmolar (maximal at 132 mosmol), with the complement receptor CD11b and reactive oxygen production significantly elevated compared to controls at this osmolarity. However, necrosis was also evelated in this environment. Diluting the urine may up-regulate PMN bactericidal function as well as clearing bacteria by increased voiding. To have a beneficial effect, it would be necessary to maintain the urine very dilute, and the use of a dipstick would allow patients to monitor the osmolarity of their urine.

These studies reveal the critical role of osmolarity in the process of PMN cell death by either apoptosis or necrosis in urine. Urine from healthy volunteers had a threefold more detrimental effect on PMN apoptosis than saline of similar osmolarity, and indeed urine of ideal osmolarity (272 mosmol≈plasma) caused significantly elevated apoptosis and necrosis. These data indicate the presence of pro-apoptotic agents in urine which play a major role in the PMN demise. The urine may in fact act as an immunomodulator. Healthy PMN recruited to the urine are eliminated rather than activated, preventing tissue damage. Recent results from a murine study demonstrated that PMN migration across the epithelial layer is IL-8 receptor dependant and that abrogation of this effect, by blocking the IL-8 receptor, is disastrous for tissue integrity. PMN become trapped in the epithelium and disintegrate causing tissue damage [28]. There has been controversy in the literature on the biological significance of the urinary PMN. Earlier studies in the gut suggested that mucosal surfaces are the ''graveyards'' where effete PMN go to die [29]. The present study suggests that urine from healthy volunteers contains agents that actively induce PMN death and thus act as a vital part of the anti-inflammatory process, removing these potentially auto-injurious cells quickly and safely. However during infection the urine contains pro-inflammatory factors that promote PMN activation and prolong survival. Il-8 and IL-6 are present in larger quantities during UTI, and IL-6 levels have been shown to correlate with the severity of disease [30, 31]. Controlled cell death is as essential to the maintenance of homeostasis and the survival of a healthy individual as cell proliferation. The urine is a harsh environment for cells to survive and function, this, however, may in fact be by design. The urine maybe the final resting place where PMN that have ingested bacteria go to die and be cleared safely from the body.

In conclusion, high osmolarity is detrimental to PMN survival and function in the urine. Increased fluid intake will reduce the osmolarity of the urine, and maintaining it at a range of 130–280 mosmol will significantly prevent cell death and augment bactericidal function. It is known that extra taurine in the diet will significantly increase the amount of taurine excreted in the urine and thus modulate the harsh environment in which the PMN must work. Hence by altering the patient's urine osmolarity and amino acid content it may be possible to maintain PMN bactericidal function in vivo.

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