PROTECTIVE EFFECT OF TAURINE AGAINST CYCLOPHOSPHAMIDE-INDUCED URINARY BLADDER TOXICITY IN RATS

Adel RA Abd-Allah,* Ali M Gado, Abdulhakeem A Al-Majed, Abdulaziz A Al-Yahya and Othman A Al-Shabanah

Department of Pharmacology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

SUMMARY

1. In the present study, the effect of taurine, on cyclophosphamide (CP)-induced urinary bladder toxicity was investigated.

2. Administration of a single dose of CP (150 mg/kg, i.p.) induced cystitis, as manifested by marked congestion, oedema and extravasation in rat urinary bladder, as well as a marked desquamative damage to the urothelium, severe inflammation in the lamina propria, focal erosions and polymorphonuclear leucocytes associated with occasional lymphocyte infiltration as determined by macroscopic and histopathological examination.

3. A significant decrease in the endogenous anti-oxidant compound glutathione and elevation of lipid peroxidation also resulted in rat urinary bladder tissue.

4. Cyclophosphamide-induced cystitis markedly affected the contractile function of the urinary bladder, as revealed by a significant inhibition of tissue responsiveness to acetylcholine (ACh) at different molar concentrations in vitro.

5. Conversely, pretreatment with taurine (1% in drinking water to reach a dose of 1 g/kg per day) for 7 days before and 1 day after CP injection produced a significant decrease in urinary bladder weight (oedema) and a marked decrease in vascular congestion and haemorrhage, as well as a profound improvement in histological structure. Moreover, taurine pretreatment resulted in a significant decrease in lipid peroxide in urinary bladder tissue and glutathione content was greatly restored.

6. Urinary bladder rings isolated from rats treated concurrently with taurine and CP showed a significant increase in their responsiveness to ACh compared with the CP group.

7. These results suggest that taurine offers a protective effect against CP-induced urinary bladder toxicity and may, therefore, decrease the limitation on its clinical application. These results merit extension and further investigation of the impact of taurine on CP antitumour activity.

Key words: cyclophosphamide, cystitis, lipid peroxidation, reduced glutathione, taurine.

INTRODUCTION

Cyclophosphamide (CP) is a drug with a wide spectrum of clinical uses that has been proved to be effective in the treatment of cancer and non-malignant disease states. However, this drug may induce acute inflammation of the urinary bladder if precautions are not taken. Moreover, CP has been shown to induce a severe haemorrhagic cystitis in laboratory animals, as well as in patients receiving the drug as a part of their treatment.

There have been many attempts to prevent CP-induced cystitis using pretreatments with different drugs, such as tachykinin and the neurokinin (NK1) receptor antagonist GR203040. This compound has been shown to reduce, in part, the magnitude of CP-induced cystitis.

Reactive oxygen species (ROS), such as the nitric oxide radical (·NO), are included in the pathogenesis of CP-induced cystitis. It has been also reported that increases in ROS, such as ·NO, lead to bladder oedema, inflammation and extravasation. Furthermore, Das et al. have reported that CP-induced testicular oxidative stress was manifested by a significant inhibition of peroxidase and catalase enzyme activities, as well as high levels of malondialdehyde (MDA) and conjugated dienes in the testis. These effects could be reversed by the coadministration of the anti-oxidant ascorbic acid. Moreover, Navarova et al. have used the anti-oxidant stobadine to protect mice against CP-induced oxidative stress. Based on these observations, it is clear that CP (and/or its metabolite acrolein) may stimulate the release of ROS, inducing inflammation and tissue damage. Anti-oxidants may ameliorate CP-induced inflammatory cystitis.

Taurine (2-amino ethane sulphonic acid) is an amino acid that differs from the more familiar amino acids in that class in being a sulphonic rather than a carboxylic amino acid and in being a β amino acid rather than an α amino acid. Taurine is synthesized in human liver tissue from cysteine and methionine. It exhibits polyvalent beneficial activities owing to its ubiquitous distribution in the body tissues and fluids of mammals. Taurine is known to be an anti-oxidant and a membrane-stabilizing agent. Parcell reviewed several reports of the clinical applications of taurine and concluded that taurine had beneficial effects in the treatment of a number of conditions, such as depression, fibromyalgia, arthritis,
interstitial cystitis, athletic injuries, congestive heart failure, diabetes, cancer and acquired immunodeficiency syndrome (AIDS). The low toxicological profile of taurine, combined with its promising therapeutic effects, warrant continued human clinical trials.18

In addition, taurine supplementation has been shown to restore kidney reduced glutathione (GSH) content and GSH peroxidase activity and to reduce platinum accumulation and MDA production in kidney tissue following cisplatin treatment.17 Taurine was also shown to protect against cisplatin-induced histopathological changes in the kidney.17 Therefore, the aim of the present study was to investigate the potential protective effect of taurine against CP-induced cystitis in rats.

**METHODS**

**Chemicals**

Cyclophosphamide (Endoxan-Asta) was purchased from Asta Medica (Frankfurt am Main, Germany). Taurine was purchased from BDH Chemicals (Poole, England). Thiobarbituric acid (TBA) was obtained from Fluka (Buchs, Switzerland). All remaining chemicals were of the highest analytical grade available commercially.

**Animals**

Male Swiss albino rats, weighing 200–250 g, were obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University. Animals were maintained under standard conditions of temperature (24 ± 1°C) and 55 ± 5% relative humidity with a regular 12 h light–dark cycle and were allowed free access to standard laboratory food (Purina Chow, St Louis, MO, USA) and water.

**Experimental protocol**

Animals were divided randomly into four equal groups of 27 animals each (six animals were used for bladder ring preparation, 18 animals were used for biochemical analysis and three animals were used for histological examination). The first group received CP (150 mg/kg, i.p.)19 and the second group received both CP (150 mg/kg, i.p.) and taurine (1% in drinking water to reach a dose of 1 g/kg per day, p.o.)20 starting 7 days before CP and continuing throughout the duration of the experiment (24 h).21 The third group, the control group, was injected intraperitoneally with an equal volume of saline while being administered taurine (1% in drinking water 1 g/kg per day, p.o.).

Cyclophosphamide-induced cystitis was assessed 24 h after injection of CP.21 Animals were killed by cervical dislocation and the urinary bladder was quickly isolated, washed with saline, blotted dry on filter paper, examined macroscopically for the detection of extravasations, congestion, weighed to determine oedema and then 10% (w/v) homogenates of each sample were made from three pooled urinary bladders in ice-cold saline using a homogenizer (VWR Scientific, Danburg, CN, USA). Owing to the elasticity of urinary bladder tissue, homogenization was performed, on ice, with different speeds until a homogeneous tissue homogenate was obtained.

**Determination of lipid peroxides in urinary bladder homogenates**

Tissue levels of lipid peroxides were determined as thiobarbituric acid-reactive substances (TBARS).22 Tissue homogenates were prepared as mentioned above, then 0.1 mL homogenate was added to a tube containing 1.5 mL acetic acid (20%, pH 3.5), 0.2 mL sodium dodecyl sulphate (SDS; 8.1%), 1.5 mL TBA (0.8%) and 0.7 mL water; blanks were prepared by the addition of water instead of homogenate. The tubes were mixed and incubated in a water bath at 95°C for 60 min using glass balls as condensers. Then, all tubes were cooled to room temperature and centrifuged at 1000 g for 10 min. Absorbance of the supernatant was measured photometrically at 532 nm and concentrations, expressed as nmol MDA/g tissue, were determined against a standard curve of 1,1,3,3-tetraethoxypropylene (Sigma Chemical, St Louis, MO, USA).

**Determination of GSH content in urinary bladder tissue**

Tissue levels of acid-soluble thiols, mainly GSH, were determined colourimetrically at 412 nm.23 Briefly, 0.5 mL of the previously prepared homogenate was added to 0.5 mL of 5% trichloroacetic acid and, after centrifugation at 750 g for 5 min, the supernatant (200 μL) was added to a tube containing 1750 μL of 0.1 mol/L potassium phosphate buffer, pH 8, and 50 μL DTNB reagent. Tubes were mixed and the yellow colour developed was measured against a standard curve of GSH. The protein thiol (protein-SH) content was expressed as μmol/g tissue.

**Preparation of urinary bladder rings**

After animals had been killed by cervical dislocation, the lower abdomen was opened and the bladder exposed. The connective tissue and accompanying blood vessels were cut away and the bladder was cut into rings and placed in warm physiological salt solution (PSS). The composition of the PSS was (in g/L): NaCl 6.9; NaHCO3 2.1; KCl 0.35; MgSO4 0.15; KH2PO4 0.16; CaCl2 0.28; glucose 2.0. Rings were mounted horizontally between a clamp and a force transducer for measurement of isometric tension using a Statham transducer (Gould Instruments, Cleveland, OH, USA) in an organ bath filled with 10 mL PSS at 37°C and gassed with 95% O2–5% CO2.

Rings were allowed to equilibrate for 30 min prior to experimentation under a resting load of 1 g.21 During this time, the bath solution was replaced every 5 min. Isometric tension was recorded by means of a Statham transducer connected to a physiograph (Narco Biosystems, Houston, TX, USA).

A concentration–response curve to acetylcholine (ACh) was constructed. Rings were exposed to different concentrations of ACh, ranging from 10–8 to 10–4 mol/L, in a non-cumulative manner. Exposure to a dose of ACh was maintained until the maximal response to that concentration was reached. Rings were then washed repeatedly with several changes of PSS and allowed to return to baseline before the addition of the next concentration of ACh.

**Histopathological examination**

Urinary bladders were collected, as detailed above, and fixed in 10% buffered formalin. The bladders were then processed and embedded in paraffin and 5 μm-thick sections cut and stained with haematoxylin and eosin. Tissue sections were examined microscopically, and histological changes were assessed.

**Table 1** Effect of taurine on cyclophosphamide-induced changes in urinary bladder contractile responses to acetylcholine

<table>
<thead>
<tr>
<th>Group</th>
<th>10–4</th>
<th>ACh (mol/L)</th>
<th>10–4</th>
<th>10–3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>48.5 ± 5.4</td>
<td>86.8 ± 7.9†</td>
<td>91.6 ± 11.1†</td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>15.95 ± 2.49*</td>
<td>42.85 ± 2.97*</td>
<td>48.3 ± 3.1*</td>
<td></td>
</tr>
<tr>
<td>Taurine</td>
<td>44.2 ± 4.9*</td>
<td>78 ± 7†</td>
<td>86.1 ± 6.2*</td>
<td></td>
</tr>
<tr>
<td>Taurine + CP</td>
<td>32.8 ± 3.0*</td>
<td>74.3 ± 10.2*</td>
<td>94.5 ± 10.1*</td>
<td></td>
</tr>
</tbody>
</table>

Data are the mean ± SEM (g tension/g tissue). Statistical analysis was performed using one-way ANOVA followed by the Tukey–Kramer test. *P < 0.05 compared with the corresponding CP group; †P < 0.05 compared with the corresponding 10–4 mol/L acetylcholine (ACh) group; ‡P < 0.05 compared with the corresponding 10–4 mol/L ACh group.
Protective effects of taurine

Then, tissues were embedded in paraffin wax and sections were cut at 5 μm and stained with haematoxylin–eosin or periodic acid–Schiff (PAS) stains using routine procedures. A histopathologist, who was unaware of the treatments, examined the coded slides using a light microscope and recorded and photographed the histopathological lesions.

Statistical analysis

Data are expressed as the mean±SEM. Statistical comparisons between different groups were performed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test to judge differences between groups. Significance was accepted at \( P < 0.05 \).

RESULTS

Effects of taurine on CP-induced changes in contractile responsiveness of isolated urinary bladder rings to ACh

The effects of CP and taurine on the responses of bladder rings to ACh are given in Table 1. The minimum and maximum tension responses of urinary bladder rings were recorded for ACh.

![Graph](image)

Fig. 1  Effect of taurine on cyclophosphamide (CP)-induced changes in the relative weight of the urinary bladder. Taurine (1% in drinking water) was given for 7 days before and during the experimental period with CP (150 mg/kg, i.p.; see text for details). (○), control; (■), CP alone; (□), taurine alone; (●), CP+taurine. Statistical analysis was performed using one-way ANOVA followed by the Tukey–Kramer test. *\( P < 0.05 \) compared with the control group; †\( P < 0.05 \) compared with the CP group.

**Fig. 2**  (a) Urinary bladder section from a control animal showing a normal mucosa (arrow 1) and muscular coat with fluid in the lumen (arrow 2). Haematoxylin and eosin stain; original magnification ×100. (b) Urinary bladder section from a cyclophosphamide (CP)-treated animal showing oedema, congestion (arrow 1), petechial haemorrhage (arrow 2) and inflammation in the lamina propria (arrow 3) with proteinaceous material infiltrating into the lumen as well as desquamative epithelium (arrow 4). Haematoxylin and eosin stain; original magnification ×200. (c) Urinary bladder section from a CP-treated animal showing oedema, congestion, polymorphonuclear leucocytes and occasional erythrocytes (arrow 1), lymphocyte (arrow 2) and plasma cell (arrow 3) infiltration with acute and chronic inflammation (periodic acid–Schiff stain; original magnification ×400). (d) Urinary bladder section from rats treated with taurine followed by CP showing mild oedema (arrow 1) and mild residual non-specific inflammation with almost normal structure (haematoxylin and eosin stain; original magnification ×100).
concentrations ranging from $10^{-3}$ to $10^{-5}$ mol/L. The responses to these concentrations showed statistically significant changes but responses to lower or higher concentrations of ACh did not differ significantly between groups (Table 1). Mean responses of control urinary bladder rings to $10^{-3}$, $10^{-4}$ and $10^{-5}$ mol/L ACh were 48.5 ± 5.4, 86.8 ± 7.9 and 91.6 ± 11.1 g tension/g tissue, respectively. Treatment with CP significantly decreased the responsiveness of tissues rings towards ACh compared with the control group. Thus, mean responses to $10^{-3}$, $10^{-4}$ and $10^{-5}$ mol/L ACh in the CP-treated group were 15.95 ± 2.49, 42.85 ± 2.97 and 48.3 ± 3.0 g tension/g tissue, respectively. Treatment of rats with taurine did not significantly alter the responses of isolated urinary bladder rings to ACh compared with control, as shown in Table 1. Urinary bladder rings isolated from rats treated concurrently with taurine and CP showed a significant increase in responsiveness to ACh compared with the CP group. Mean response to $10^{-2}$, $10^{-3}$ and $10^{-4}$ mol/L ACh in the taurine and CP group were 32.8 ± 3.0, 74.3 ± 10.2 and 94.5 ± 10.1 g tension/g tissue, respectively. Meanwhile, the responses to ACh of rings isolated from rats treated with both taurine and CP were not significantly different from the responses of the control group (Table 1).

Effect of taurine on CP-induced changes in weight and macroscopic findings of the urinary bladder, as well as histopathological examination

Figure 1 shows the effect of oral supplementation of taurine on CP-induced changes in urinary bladder weight. A single injection of CP induced acute urinary bladder damage. This was manifested by a significant increase in urinary bladder weight as a ratio of bodyweight, reaching a more than twofold increase with marked vascular congestion and extravasation. In addition, histological examination revealed that CP induced extensive inflammation of the lining urothelium, petechial haemorrhage in the lamina propria and infiltration of erythrocytes, desquamative epithelium and plasma cells, as well as polymorphonuclear leucocytes (PMN) and occasional lymphocytes, with focal erosions of the urinary bladder (Fig. 2b,c). Pretreatment with taurine (1% in drinking water reaching a dose of 1 g/kg per day) caused a significant decrease in urinary bladder weight (oedema; Fig. 1) and markedly decreased vascular congestion and haemorrhage and resulted in a profound improvement in the histological structure of the urinary bladder (Fig. 2d). Taurine alone did not produce any changes in the normal structure of the urinary bladder (data not shown).

Effect of taurine on CP-induced changes in glutathione content and lipid peroxides in urinary bladder homogenates

Figures 3, 4 show the effects of oral supplementation with taurine on CP-induced changes in lipid peroxides, measured as MDA, and glutathione content in urinary bladder homogenates. A single injection of CP induced acute urinary bladder damage, as manifested by a more than fourfold increase in lipid peroxides. In addition, there was a significant decrease in glutathione content by 59.96%. Pretreatment with taurine ameliorated CP-induced cystitis, as indicated by a significant decrease in lipid peroxides and a marked restoration of glutathione content.

DISCUSSION

It is known that CP can produce haemorrhagic cystitis. In the present study, treatment with CP produced marked congestion, oedema and extravasations in rat urinary bladder. Histopathological examinations also showed petechial haemorrhage and an inflammatory reaction in the lamina propria of the urinary bladder associated with proteinaceous material into the lumen. Polymorphonuclear leucocytes with occasional lymphocyte infiltration and focal erosions were also observed in urinary bladders from CP-treated rats. These results are consistent with those of previous studies reporting that CP caused excessive protein extravasation, vascular congestion and oedema of the bladder and extensive leucocyte production of NO. Increased ROS formation, such as urinary levels of NO metabolites, has also been observed in other animal models of chemical cystitis, Ribeiro et al. stated that...
CP produced cellular infiltration and damage of the urothelium, marked inducible nitric oxide synthase (iNOS) expression with increases in the urinary excretion of NO metabolites indicating a fundamental role for ROS in the pathogenesis of CP-induced cystitis. In addition, CP produced a decrease in the endogenous anti-oxidant compound glutathione and an elevation of lipid peroxidation in urinary bladder.42,43 These findings are in agreement with those of the present study, in which there was a significant depletion of GSH pool and a significant increase in lipid peroxides (MDA) in urinary bladder homogenates after a single CP injection. Similarly, Davis and Kuttan33 reported that CP produced a marked decrease in urinary bladder GSH content.

Moreover, in the present study, CP-induced cystitis markedly affected the contractile function of the urinary bladder, manifested by the responsiveness of urinary bladder tissues to ACh at different molar concentrations, indicating extensive urinary bladder damage, which is consistent with the results of the study of Alfieri et al.,40 who reported that CP-induced cystitis is characterized by evident urothelial damage, oedema, extensive white blood cell infiltrates and vascular congestion of the bladder.

The mechanism of CP-induced cystitis remains controversial. Inci et al.32 have examined the effect of inflammation on rat urinary bladder-derived relaxant factor in the coxial bioassay system. The authors concluded that CP-induced bladder inflammation did not alter the synthesis and/or release of the bladder-derived relaxing factor, which is neither a cyclo-oxygenase product nor NO, but the altered contractility may be due to inflammation-induced oedema. This result may explain the relationship between urothelial integrity and bladder contractility. Conversely, another study conducted by Oter et al.31 reported that NO produced by iNOS is an important mediator in the pathogenesis of CP-induced cystitis. Furthermore, Manesh and Kuttan44 showed that ROS play a major role in CP-induced pathogenesis with regard to either urothelial integrity or bladder motility because sulphur-containing compounds could ameliorate the CP-induced bladder damage and function. Another concept was adopted by Qiao and Vizzard,34 who illustrated that CP-induced cystitis is accompanied by upregulation of either the expression or phosphorylation of tyrosine kinase receptors in rat micturation pathways. Qiao and Vizzard34 attributed this effect to the expression of neurotropic factors in the inflamed urinary bladder because the interaction of such neurotropic factors and tyrosine kinase receptors may play a unique role in decreased urinary tract plasticity with CP-induced cystitis. Nevertheless, Hu et al.35 showed that cyclo-oxygenase 2 and the products of its metabolic pathways alter the micturation reflex with CP-induced cystitis.

Furthermore, previous studies have shown that CP toxicity is associated with oxidative cell damage;12 thus, therapeutic strategies aim to limit free radical-mediated urinary bladder injury.36 This is why we examined the possible protective effect of the potent anti-oxidant taunine against acute CP-induced cystitis. The anti-oxidant properties of taunine have been demonstrated previously because it has been shown to have a strong ability to decrease diabetes-induced lipid peroxidation in the kidney,37 lens,38 retina,38 liver and pancreas.40 Moreover, taunine stabilizes cell membranes, regulates osmotic pressure in the cell, maintains homeostasis of intracellular ions, inhibits phosphorylation of membrane proteins and prevents lipid peroxidation.40 Taunine is also an intra- and extracellular calcium regulator.41

The evidence obtained from the present study indicates that pretreatment with taunine ameliorates CP-induced cystitis because it leads to a reduction in the macroscopic changes induced by CP, such as haemorrhage and oedema, as well as a profound improvement in the histological structure and shape. Taunine also offers marked protection against extensive CP-induced inflammation. Furthermore, taunine leads to restoration of GSH content in rat urinary bladder and pretreatment with taunine leads to a significant decrease in lipid peroxidation induced by CP treatment. With regard to the contractile function of the urinary bladder, this was markedly improved when taunine administration preceded CP injection.

Many studies have focused on the detoxification function of taunine and have demonstrated that taunine reacts with and neutralizes ROS, which are generated during an oxidative cell burst.42,43 Timbrell et al.44 reported that individuals who are taunine deficient may become more susceptible to tissue damage by xenobiotic agents, such as aldehydes, chlorine and certain amines. Furthermore, animal studies have demonstrated the ability of taunine to make a complex with or neutralize the toxic effects of carbon tetrachloride and retinal.44,45 Taunine was found to significantly inhibit intestinal endotoxin translocation and subsequently decrease hepatic injury from these substances.46,47

In summary, the results of the present study suggest that CP-induced cystitis is related to oxidative stress and ROS formation owing to depletion of GSH and/or inflammation. Taunine, which was useful for the amelioration of the toxicity of other models of oxidative stress including hepatitis, liver ailments and the complications of diabetes,48,49 can also protect the structure and function of the urinary bladder against CP-induced oxidative stress. Further investigations are needed to explore other possible mechanism(s) of the protective action of taunine, as well as its possible effect(s) on the antitumour activity of CP.

REFERENCES


