International Urology and Nephrology (2019) 51:803–810
https://doi.org/10.1007/s11255-019-02128-y

UROLOGY - ORIGINAL PAPER

Uroprotective effect of ambroxol in cyclophosphamide-induced cystitis in mice

Elif Nur Barut[1] · Seckin Engin[1] · Burak Barut[2] · Cansu Kaya[3] · Gokcen Kerimoglu[3] · Arzu Ozel[2,4] · Mine Kadioglu[5]

Received: 18 January 2019 / Accepted: 11 March 2019 / Published online: 20 March 2019

Abstract

Purpose Hemorrhagic cystitis (HC) is defined as any types of acute or chronic inflammation of urinary bladder with several reasons. One of the most common causes of HC is cyclophosphamide (CYP), an effective antineoplastic agent, due to its urotoxic potential. Ambroxol (AMB) is a mucoactive drug that has been used for numerous respiratory diseases. Besides its mucolytic activity, AMB is a potent antioxidant and antiinflammatory agent that is becoming more attractive for the treatment of several oxidative/inflammatory disorders. The aim of this study was to evaluate the uroprotective potential of AMB in CYP-induced HC.

Method Male Balb/c mice were pretreated with AMB (30, 70, and 100 mg/kg) once a day for 3 consecutive days before HC induction with CYP (300 mg/kg). Mesna (30 mg/kg;i.p.), only drug in the management of CYP-induced HC, was administered 20 min before; 4 and 8 h after cystitis induction. The urinary bladders were harvested and evaluated in functional, biochemical, and histological studies.

Results CYP-induced HC markedly reduced acetylcholine (ACh)-induced contractions in detrusor strips and AMB at 100 mg/kg caused a significant increase in the responsiveness to ACh. Pretreatment with AMB prevented the elevation of malondialdehyde (MDA) and tumor necrosis factor-alpha (TNF-α) level, reduction of total glutathione (GSH) that induced by CYP. However, treatment with AMB did not improve the bladder weight and some histological parameters.

Conclusion These results suggest that AMB pretreatment could improve CYP-induced HC via antioxidant and antiinflammatory activities.

Keywords Cylophosphamide · Cystitis · Ambroxol · Antioxidant · Antiinflammatory · Mesna

Introduction

Cyclophosphamide (CYP), an alkylating antineoplastic agent, is an effective drug in the treatment of multiple malignancies, autoimmune disorders, and hematopoietic cell transplantation. CYP is converted to acrolein that is a highly toxic agent by hepatic microsomal enzymes [1]. Accumulation of acrolein in urinary bladder triggers reactive oxygen species (ROS) production in the urothelium by activating xanthine oxidase and aldehyde dehydrogenase to form peroxynitrite radicals and thus it destroys the urothelium, the underlying layer of detrusor smooth muscle (DSM), leading to hemorrhagic cystitis (HC) [2]. HC is a major dose-limiting clinical complication in patients receiving CYP treatment, defining as inflammation of urinary bladder accompanied with bladder wall edema, polymorphonuclear infiltration, and submucosal hemorrhage. Depending on the route of administration and dose of CYP given, the incidence of HC can vary [1]. The incidence can

✉ Mine Kadioglu
kadioglu20@gmail.com

1 Department of Pharmacology, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Turkey

2 Deparment of Biochemistry, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Turkey

3 Department of Histology and Embryology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

4 Drug and Pharmaceutical Technology Application and Research Center, Karadeniz Technical University, Trabzon, Turkey

5 Faculty of Medicine, Department of Pharmacology, Karadeniz Technical University, 61080, Trabzon, Turkey

reach up to 70% in patients receiving high dose CYP intravenously [3]. In this context, it is so crucial that prevention of CYP-induced HC in clinical practice to reduce chemotherapy-induced pain and hematuria.

Inflammation and ROS production induced by acrolein are considered to underly CYP-induced HC, supported by experimental studies reported that co-administration of antioxidants such as taurine, glutamine, *N*-acetylcysteine, glutathione, and seleno-L-methionine can reduce CYP-induced urotoxicity due to their efficacy in ameliorating oxidative bladder damage [1]. It has been shown that some preventive measures such as hyperhydration and mesna (2-mercaptoethane sulfonate sodium) can be effective at reducing the incidence of HC. Mesna is the only approved drug in the management of CYP-induced urotoxicity, acting by detoxifying of acrolein. But the most of side effects of CYP still occur dose-dependently with co-administration of mesna [4, 5]. In this regard, focusing on new therapeutic targets to prevent CYP-induced HC complications is urgently needed to increase patient compliance and drug safety.

Ambroxol (AMB, 2-amino-3,5-dibromo-*N*-[trans-4-hydroxycyclohexyl] benzylamine), a mucolytic agent, has been used in the treatment of respiratory disorders due to its mucocinetic and secretagogue activity [6]. After systemic administration, AMB is metabolized to dibromoanthranilic acid and glucronides. Also, it is excreted mainly by renal excretion and 5–6% of a dose is excreted unchanged in the urine [7, 8]. Recently, it has been shown to exhibit antioxidant and antiinflammatory properties in vivo and in vitro [4]. Gillissen et al. shown that AMB reduces ROS from polymorphonuclear and mononuclear cells [9]. Also, it's reported that AMB inhibited endotoxin-induced lipid peroxidation as well as its antiinflammatory function [9, 10]. It is well established that AMB was able to decrease the lipopolysaccharide-induced synthesis of cytokines, superoxide anion, and hydrogen peroxide production in rat alveolar macrophages [11]. Morever, certain studies have suggested that AMB diminishes pain-like behaviors in both inflammatory and neuropathic pain models in rats due to its sodium channel blocker activity [12–14].These wide ranges of AMB features suggest that it might be a new therapeutic candidate targeting especially inflammatory-based disorders.

In the present study, we aimed to evaluate the possible protective effect of AMB on CYP-induced oxidative/inflammatory damage in mice urinary bladder and CYP-induced changes on contractile function of DSM.

Materials and methods

Chemicals

Cyclophosphamide (CYP) (Endoxan® 500 mg vial, Eczacıbaşı Baxter, Turkey) and mesna (Uromitexan® 400 mg, Eczacıbaşı Baxter, Turkey) were used. Acetylcholine (ACh) and ambroxol (AMB) were purchased from Sigma (St. Louis, MO, USA). In addition, TNF-α (R&D Systems, USA) and glutathione (Cayman Chemical, USA) commercial kits were used for biochemical analysis. CYP, mesna, and AMB were dissolved or diluted in 0.9% sterile saline.

Animals

We used 108 male Balb/c mice weighing 25–40 g and all mice were between 3 and 4 months of age in this study. They were kept in the temperature and humidity-controlled (24 ± 1 °C) rooms with food and water given ad libitum.

CYP-induced HC induction and dose treatment schedule

Mice were randomly divided into six groups of 18 animals each: (1) untreated control group, (2) CYP (300 mg/kg)-treated group, (3) CYP + Mesna (30 mg/kg)-treated group, (4) CYP + AMB30 (30 mg/kg) treated group, (5) CYP + AMB70 (70 mg/kg) treated group, and (6) CYP + AMB100 (100 mg/kg) treated group. In the untreated control group, mice were injected with equivalent volumes of saline depending on experimental protocol. In group 2, mice were pretreated with saline once a day for 3 consecutive days, followed by CYP (300 mg/kg) administration 30 min after the last dose of saline on the third day. In group 3, mice were treated with Mesna 20 min before and 4 and 8 h after cystitis induction with a single dose of CYP (300 mg/kg). In groups 4, 5 and 6; mice were pretreated with AMB once a day for 3 consecutive days, followed by CYP (300 mg/kg) administration 30 min after the last dose of AMB on the third day. All drugs were injected intraperitoneally. Doses of drugs and protocol were selected depending on previous studies with some modifications [15–18].

24 h after CYP administration, animals were killed by cervical dislocation and the bladder was quickly removed for the contractility studies and histologic evaluation. For the biochemical studies bladder tissues were frozen immediately in liquid nitrogen and stored at − 80 °C until use.

Contractility studies

The urinary bladder was surgically removed from the mice placed in Krebs Henseleit solution (NaCl 118 mM; KCl 4.8 mM; $MgSO_4.6H_2O$ 1.2 mM; KH_2PO_4 1.2 mM; $NaHCO_3$ 25 mM; glucose 11.7 mM; $CaCl_2.2H_2O$ 2.5 mM). The bladder was carefully dissected from the adherent tissues and DSM strips (4–5 mm long and 2–3 mm wide) were prepared. DSM strips were then placed in water-jacketed organ baths containing Krebs Henseleit solution at 37 °C and bubbled with 95% O_2 and 5% CO_2 throughout the experiments. To measure tissue contractility, one end of the strip was attached to an isometric force transducer (MAY FDT10A) connected to data acquisition and recording system (BIOPAC MP 100).

The strips were placed under 1 g load resting tension and allowed to equilibrate for 60 min, with while replacing the bath solution every 20 min. After the equilibration period, to test the effect of AMB pretreatment on CYP-induced changes in contractile function of DSM, cumulative concentration response to ACh (10^{-8}–10^{-3} M) and potassium chloride (KCl; 60 mM)-induced contraction were evaluated in DSM obtained from all groups. The data were expressed as mg contraction.

Biochemical analysis

Preparation of bladder homogenates

Each frozen bladder tissue was homogenized in 5–10 ml cold phosphate buffer saline (PBS, pH 6–7) containing 1 mM EDTA per gram tissue. Then the samples were centrifuged at 4 °C, 10,000×*g* for 15 min. The supernatants which were used for glutathione (GSH) assay were deproteinated according to the manufacturer's instructions at commercial available kit (GSH assay kit, Cayman Chemical #703002, USA).

Lipid peroxidation assay

Tissue levels of peroxides were determined as thiobarbituric acid reactive substances (TBARS) described by Stocks & Dormandy in 1971. In the protocol, 1 ml of 15% TCA in 0.25 M HCl was added to 10% tissue homogenate and centrifuged at 2000 rpm for 15 min. 1.2 ml of supernatants were mixed with 0.6 ml 37% TBA in 0.25 M HCl and held in a boiling water bath for 15 min after cooling under the tape water. The absorbance of supernatant was measured photometrically at 532 nm using Multiskan^{Go} microplate spectrophotometer. TBARS levels were calculated using 1,1,3,3-tetraethoxypropane as the standard and expressed as nmol MDA per g protein.

Determination of total glutathione (GSH) content in urinary bladder homogenates

Tissue levels of acid-soluble thiols, mainly GSH, were determined colorimetrically at 412 nm using a commercial available kit (GSH assay kit, Cayman Chemical, USA). The assay was performed according to the manufacturer's instructions and the protein thiol content was expressed as μgmol/g tissue.

Determination of tumor necrosis factor (TNF)-α in urinary bladder homogenates

Level of TNF-α was determined by ELISA technique in urinary bladder homogenate using a commercial available kit (Mouse TNF-α HS Quantikine ELISA Kit, R&D system, USA) in accordance with the manufacturer's instructions and TNF-α content was expressed as pg/mL.

Histopathological analysis

For histopathological analyses, mice bladders were fixed with 10% formaldehyde, processed with graded alcohols, embedded in paraffin. Five micrometer sections were cut from paraffin blocks using a rotary microtome (RM 2255; Leica Instruments, Nussloch, Germany) and basic staining method hematoxylin and eosin (H&E) was applied. Slides were observed by light microscope (Olympus BX51; Olympus Co., Tokyo, Japan) fitted with a digital camera (Olympus DP 71 Olympus Co.,Tokyo, Japan). The severity of mucosal edema, leukocyte infiltration, mucosal abrasion, and hemorrhage scored in ten fields of each slide. The histologist was blinded to the groups [19].

Mucosal edema was evaluated under ×200 magnification. No edema was scored as 0, minimal edema (no change in connective tissue thickness) as 1, moderate edema (connective tissue thickness increased by < twofold) as 2, and severe edema connective tissue thickness increased by (> twofold) as 3.

Leukocyte infiltration was evaluated in mucosa under ×400 magnification. No extravascular leukocytes were scored as 0, > 20 leukocytes as 1, 20–45 leukocytes as 2, and > 45 leukocytes as 3.

Mucosal hemorrhage and mucosal abrasion were evaluated under ×100 magnification. The presence of hemorrhage and mucosal abrasion was scored as 1, and no change was scored as 0.

The total score of all fields of view for mucosal edema, leukocyte infiltration, mucosal abrasion, and hemorrhage divided by the maximum possible score and then multiplied by 100. Bladder hypertrophy was measured by the ratio of bladder weight (mg) to body weight (g).

Statistical analysis

All data were represented as the mean ± SEM or mean ± SD. Statistical analyses were performed using SPSS software (version 22; IBM SPSS Statistics, Armonk, NY, USA) and GraphPad Prism (Version 5.01; Graphpad Software, San Diego, CA, USA). The differences between groups were compared with ANOVA, followed by Fisher's least significant difference (LSD) post-hoc test for biochemical analysis. For contractility studies, one-way ANOVA followed by

Dunnett's t-test used to evaluate differences among groups. Histopathological score was analyzed by using the one-way ANOVA followed by Bonferroni's test to determine significant differences. $p<0.05$ was considered to be statistically significant.

Results

AMB pretreatment does not effect CYP-induced changes in urinary bladder weight

In CYP-treated group, the ratio of bladder weight to body weight was significantly increased compared to untreated control group (mean 2.26 ± 0.26 vs 0.92 ± 0.07 mg/g, respectively). As shown in Fig. 1, pretreatment with AMB failed to decrease the CYP -induced increment of bladder weight.

Contractility studies

Effect of AMB pretreatment on CYP-induced changes in contractile function of DSM

To investigate the effect of AMB on CYP-induced bladder dysfunction on bladder contraction, *in vitro* contractility studies were performed on DSM. Cumulative ACh (10^{-8}–10^{-3} M)-evoked contractile response significantly decreased ($p<0.05$) in DSM strips obtained from CYP-treated group compared with the untreated control group. Pretreatment with AMB at 100 mg/kg caused a significant increase in the responsiveness to ACh compared with the CYP-treated group (Fig. 2a). KCl (60 mM)-induced contraction was also evaluated in DSM strips from all groups. There was no significant difference in KCl-induced contractile response among the groups (Fig. 2b).

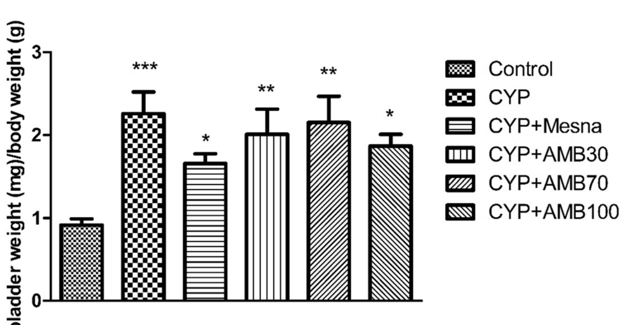

Fig. 1 Effect of AMB pretreatment on the weight of the CYP-treated urinary bladder. Bladder weight was expressed as ratio of bladder weight (mg) to body weight (g) ($n=6$). $*p<0.05$, $**p<0.01$, $***p<0.001$ significantly different from control group

Biochemical analysis

AMB pretreatment ameliorates CYP-induced changes in lipid peroxides in urinary bladder

To test whether AMB pretreatment alters the oxidative damage in CYP-induced cystitis, changes on the lipid peroxidation end-product, MDA level, were investigated. Bladder MDA level nearly tripled in CYP-treated group compared with the untreated control group ($p<0.05$). As presented in Fig. 3, pretreatment with AMB at 70 mg/kg and 100 mg/kg significantly prevented the elevation of MDA level as mesna treated group.

AMB pretreatment improves CYP-induced reduction of GSH content in urinary bladder

To investigate whether AMB pretreatment affects the antioxidant defense system in the CYP-induced bladder damage, GSH level was determined. CYP treatment caused a significant decreased in GSH level compared to untreated control group ($p<0.01$). As shown in Fig. 4, pretreatment with mesna and the lowest dose of AMB (30 mg/kg) significantly prevented the reduction in GSH level ($p<0.001$, $p<0.01$).

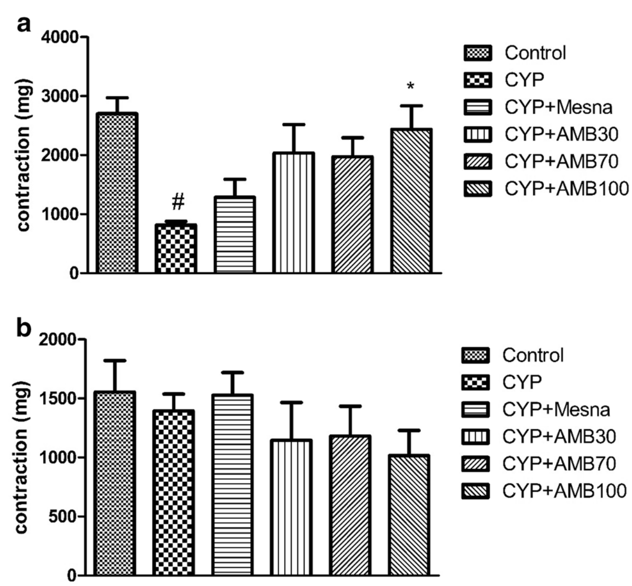

Fig. 2 **a** Effect of AMB pretreatment on CYP-induced changes in urinary bladder contractile response to cumulative ACh (10^{-8}–10^{-3} M). **b** Effect of AMB pretreatment on CYP-induced changes in urinary bladder contractile response to KCl (60 mM). The data are presented as the percentage of the maximum response in the control strips and values are presented as mean ± SEM. ($n=4$–6) $^{\#}p<0.05$ significantly different from control group. $*p<0.05$ significantly different from CYP-treated group

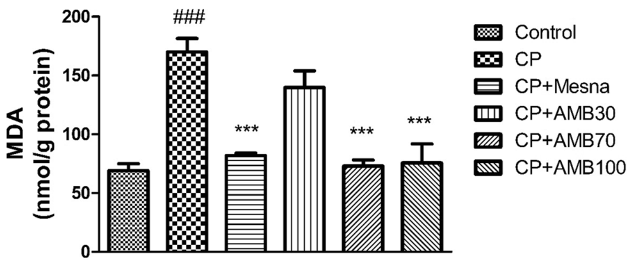

Fig. 3 Effect of AMB pretreatment on MDA level in urinary bladders from CYP-treated mice. Data are expressed as mean ± SEM ($n = 3$). ###$p < 0.05$ significantly different from untreated control group. ***$p < 0.05$ significantly different from CYP-treated group

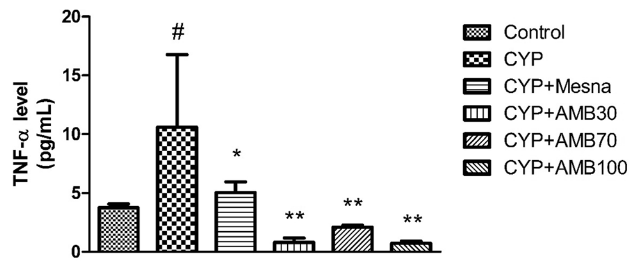

Fig. 5 AMB pretreatment decreased CYP-induced increment of TNF-α level in urinary bladder. Data are expressed as mean ± SEM; ($n = 5$). **$p < 0.01$, *$p < 0.05$ compared with the CYP group, #$p < 0.05$ compared with the control group

AMB pretreatment prevented CYP-induced increment of TNF-α level in urinary bladder

To investigate the effect of AMB pretreatment on inflammatory processes which were induced by CYP treatment in urinary bladder, changes on the TNF-α level were determined. CYP treatment induced a significant increase on TNF-α level. Mesna and AMB pretreatment at all doses remarkably prevented the increment of TNF-α level (Fig. 5, $p < 0.01$, $p < 0.05$).

Effects of AMB pretreatment on CYP-induced bladder pathological changes

To examine the protective effect of AMB treatment on cytoarchitectural changes in CYP-induced cystitis, alterations of the mucosal edema, hemorrhage, leukocyte infiltration, and mucosal abrasion parameters were analyzed. Whereas there was no change hemorrhage, edema, and other parameters in the control group; severe hemorrhage, edema, mucosal abrasion, and leukocyte infiltration were observed in the CYP-treated group ($p < 0.001$). Although mesna treatment caused a significant decrease in the severity of mucosal edema, hemorrhage, and leukocyte infiltration, no changes was observed in mucosal abrasion compared to CYP-treated group ($p < 0.01$, $p < 0.001$). Morever, only CYP + AMB70 exhibited a significant reduction in mucosal hemorrhage ($p < 0.05$). Also mucosal abrasion and leukocyte infiltration tend to decrease in CYP + AMB70 group, but there was no significant difference when compared to CYP group (Fig. 6, Table 1).

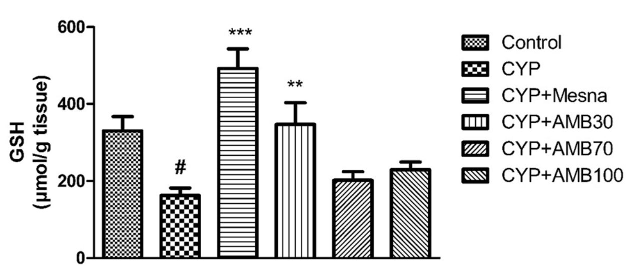

Fig. 4 AMB pretreatment improves CYP-induced reduction of GSH content in urinary bladder. Data are expressed as mean ± SEM; ($n = 5$). ***$p < 0.001$, **$p < 0.01$ compared with the CYP group, #$p < 0.01$ compared with the control group

Discussion

CYP is an oxazaphosphorine alkylating agent that was approved in 1958 for the treatment of solid tumors, lymphoma, leukemia etc. Over the years, it is also used for non-malignant diseases such as rheumatoid arthritis and systemic lupus erythematosis [20, 21]. Despite its proven efficacy in numerous conditions, CYP has several side effects and the major one that restricts clinical use is HC [22]. There are numerous reports about the molecular mechanisms of CYP-induced HC, indicating that during hepatic metabolism of CYP, a highly urotoxic metabolite called as acrolein is generated then accumulate in the bladder [2]. The exact urotoxic mechanism of CYP metabolite, acrolein, is still unclear, however, oxidative stress and inflammation are considered to be implicated in the pathogenesis of CYP-related HC [22]. CYP causes over-production of ROS species dramatically which induces transcription factors of pro-inflammatory cytokine such as TNF-α and interleukin-1beta (IL-1β) [4, 23]. These factors contribute to harmful reaction in the bladder urothelium and lead to ulceration, hematuria, severe pain, and irritative voiding symptoms [2, 21]. There are numerous reports showing that antioxidants and anti-inflammatory agents may eliminate its urotoxicity leading to improve the tolerability of CYP [1, 24].

Mesna is a potent uroprotective agent that can directly bind to acrolein and efficiently detoxify it [25]. However, numerous studies showed that prophylaxis with mesna is not fully sufficient for blocking acrolein urotoxic effects and it reduces the incidence of cystitis only about 5% in the clinic [5, 26, 27]. Also, it causes allergic reactions in some

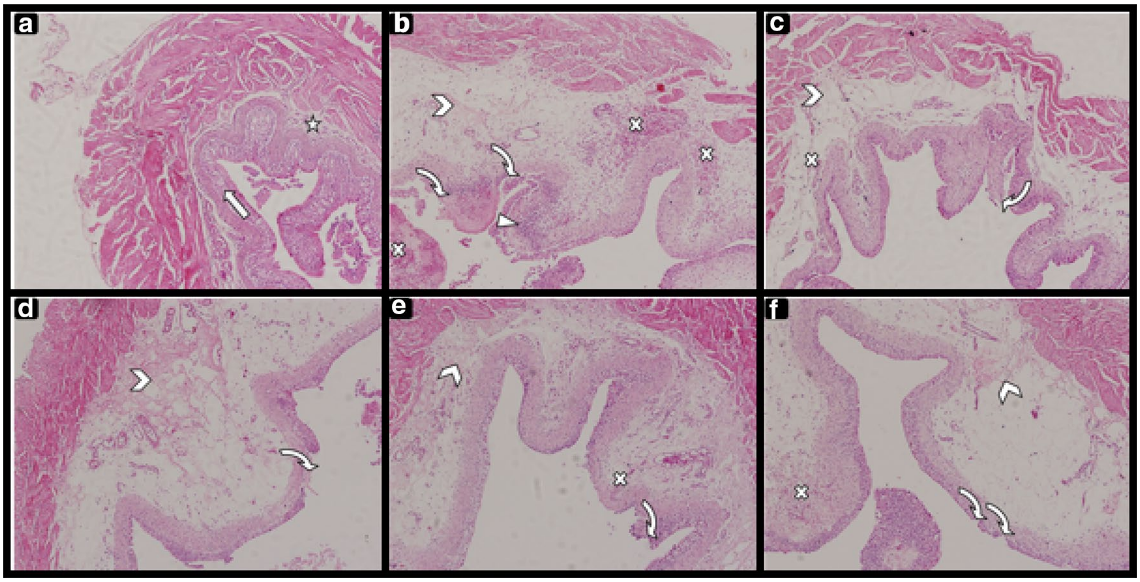

Fig. 6 Representative photographs of bladder sections obtained from all groups. Control group (**a**) showing normal histoarchitecture of bladder with intact epitelium ( ) ve lamina propia (☆). CYP group (**b**) showing severe mucosal abrasion ( ), marked hemorrhage ( ) and edema ( ). CYP + Mesna group (**c**) showing significantly decreased hemorrhage ( ), edema ( ) and mucosal abrasion ( ). CYP + AMB30 (**d**) still showing marked mucosal abrasion ( ) and edema ( ). CYP + AMB70 (**e**) showing mucosal abrasion ( ), edema ( ) and significantly decreased hemorrhage ( ). CYP + AMB100 (**f**) still showing marked mucosal abrasion ( ), hemorrhage ( ) and severe edema ( ). (H&E; × 100)

Table 1 Histopathological changes in mice urinary bladders

Groups	Histological parameters			
	Mucosal edema	Leukocyte infiltration	Mucosal hemorrhage	Mucosal abrasion
Control	7.99 ± 3.80***	8.66 ± 2.98***	12.00 ± 4.47***	12.00 ± 4.47***
CYP	66.66 ± 16.63	63.66 ± 16.36	68.75 ± 15.53	61.12 ± 21.47
CYP + Mesna	25.00 ± 5.06***	33.89 ± 22.84**	44.00 ± 11.40*	38.33 ± 18.35
CYP + AMB30	57.32 ± 17.22	50.66 ± 10.90	56.00 ± 19.49	58.00 ± 22.80
CYP + AMB70	64.16 ± 6.31	46.66 ± 8.16	36.67 ± 5.77*	37.50 ± 15.00
CYP + AMB100	59.44 ± 2.51	53.33 ± 11.35	70.00 ± 14.14	66.67 ± 10.33

Data are expressed as mean ± SD (n = 3–10). *p < 0.05, **p < 0.01, ***p < 0.001 compared with the CYP group

cases [28]. Thus, there is a growing interest in developing the potential alternative therapeutic agent especially those with a potential antioxidant and antiinflammatory activity for CYP-induced HC.

AMB, a metabolite of bromhexine, has been widely prescribed to treat respiratory diseases associated with increased mucus production. Besides its mucolytic action, antiinflammatory and antioxidant properties of AMB has been described in vivo and in vitro [4].

In view of these considerations, we investigated the possible protective effect of AMB on CYP-indued oxidative/inflammatory damage and changes in contractile function in mice urinary bladder.

It's known that in CYP-induced HC, detrusor muscle contractions by muscarinic receptor agonist were significantly reduced [29, 30]. In accordance with previous works, we observed that CYP treatment caused a significant decrease in ACh-induced cholinergic contraction. Although mesna did not alter the reduction in the ACh-induced cholinergic contractions, pretreatment with AMB at 100 mg/kg restored ACh-induced contraction impaired by CYP. However, no changes were noticed in KCl-induced contraction regardless of treatment.

MDA is known as an end-product of lipid peroxidation process and used as a marker of cellular oxidative damage [4, 31]. CYP treatment caused a significant increase in MDA production in previous studies. The production of ROS induced by CYP treatment is responsible for the elevation of MDA level [32–35]. We have shown that the administration of 300 mg/kg dose of CYP resulted in development of oxidative damage in urinary bladder tissue. Pretreatment with mesna and AMB at 70 mg/kg and 100 mg/kg attenuated oxidative damage in urinary bladder, evidenced by decreased MDA level. In agreement with our results, Zhi et al., also demonstrated that AMB treatment decreased the MDA content which were enhanced in paraquat-induced pulmonary fibrosis [36].

GSH that plays a crucial role in the defense toward oxidative damage catalyzes the reduction of hydrogen peroxide to oxygen and water, and blocks the generation of hydroxyl radicals that are highly toxic ROS [20, 37]. In previous studies, it's established that GSH content is reduced in CYP-induced bladder damage [28, 32, 38]. Also our results showed that CYP treatment significantly reduced GSH content in mice urinary bladder as in the literature. Pretreatment with AMB at 30 mg/kg markedly increased the GSH content compared with CYP group.

Most of studies demonstrated that inflammatory mediators and several cytokines have a crucial role in chemotherapy-induced HC pathogenesis [39–41]. It is suggested that CYP-induced cystitis caused a sudden and dramatic increase in mRNA level of the major proinflammatory cytokine, TNF-α [42]. After understanding the participation of TNF-α pathway in CYP-induced damage, recent studies focus on antiinflammatory therapies for the treatment of CYP-induced cystitis [24, 43]. In our study, we observed that CYP treatment augmented TNF-α level dramatically compared to control group. In addition, AMB treatment at all doses markedly decreased TNF-α level as mesna treated group compared with CYP group. In agreement with our results, Su et al. reported that in a murine model of acute lung injury AMB treatment decreased TNF-α level in bronchoalveolar lavage fluid through its antiinflammatory potential [15]. Although antiinflammatory mechanism of AMB is yet incompletely understood, Ferretti et al. suggested that AMB can inhibit the phospho-diesterases which block the production of proinflammatory cytokines [44]. Additionally, AMB could also inhibit the activation of proinflammatory transcription factor, nuclear factor kappa B (NFκB) [45].

CYP-induced HC caused pathological alterations in mice urinary bladders compatible with previous works due to activation of oxidative and inflammatory mechanisms [28, 46]. Mesna treatment improved CYP-induced bladder damage histologically, but AMB pretreatment did not provide remarkable improvement compared with mesna-therapy. Only AMB treatment at 70 mg/kg markedly reduced CYP-induced mucosal hemorrhage.

To the best of our knowledge, this is the first study showing that AMB pretreatment ameliorates oxidative/inflammatory damage and improves detrusor contractility in the urinary bladder of CYP-treated mice. Although further studies are required to elucidate the exact mechanism of uroprotective effects of AMB treatment, our promising results obviously showed us AMB might be favorable uroprotective drug candidate for the effective treatment of CYP-induced HC.

Acknowledgements This study was supported by the Scientific Research Project Coordination Unit of Karadeniz Technical University (Grant Number: THD-2018-7356). The authors are grateful to Asst. Prof. Mahmoud Abudayyak for helping to determine the antioxidant assays.

Compliance with ethical standards

Ethical approval The experimental protocols were approved by Karadeniz Technical University the Institutional Animal Ethical Committee (2016/29). All animal studies were performed according to the Guide for the Care and Use of Laboratory Animals.

References

1. Ribeiro RA, Lima-Junior RCYP, Leite CAVG et al (2012) Chemotherapy-induced hemorrhagic cystitis: pathogenesis, pharmacological approaches and new insights. J Exp Integr Med 2(2):95–112
2. Haldar S, Dru C, Bhowmick NA (2014) Mechanisms of hemorrhagic cystitis. Am J Clin Exp Urol 2(3):199–208
3. Shepherd JD, Pringle LE, Barnett MJ et al (1991) Mesna versus hyperhydration for the prevention of cyclophosphamide-induced hemorrhagic cystitis in bone marrow transplantation. J Clin Oncol 9(11):2016–2020
4. Keles I, Bozkurt MF, Cemek M et al (2014) Prevention of cyclophosphamide-induced hemorrhagic cystitis by resveratrol. A comperative experimental study with mesna. Int Urol Nephrol 46(12):2301–2310
5. Bhatia K, Ahmad F, Rashid H et al (2008) Protective effect of S-allylcysteine against cyclophosphamide-induced bladder hemorrhagic cystitis in mice. Food Chem Toxicol 46(11):3368–3374
6. Zhang SJ, Jiang JX, Ren QQ et al (2016) Ambroxol inhalation ameliorates LPS-induced airway inflammation and mucus secretion through the extracellular signal-regulated kinase 1/2 signaling pathway. Eur J Pharmacol 15(775):138–148
7. Rupali R, Dhot KS, Ilango KB et al (2012) Pharmacokinetic studies of ambroxol hydrochloride microspheres in rats after oral administration. IJRPC 2(2):280–288
8. Jauch R, Bozler G, Hammer R et al (1978) Ambroxol: Studies of metabolism in man and quantitative determination in biological samples. Arzneimittelforschung 28(5a):904–911
9. Gillissen A, Bartling A, Schoen S et al (1997) Antioxidant function of ambroxol in mononuclear and polymorphonuclear cells in vitro. Lung 175(4):235–242
10. Nowak D, Pierscinski G, Drzewoski J (1995) Ambroxol inhibits doxorubicin-induced lipid peroxidation in heart of mice. Free Radic Biol Med 19(5):659–663
11. Jang YY, Song JH, Shin YK et al (2003) Depressant effects of ambroxol and erdosteine on cytokine synthesis, granule enzyme release, and free radical production in rat alveolar macrophages activated by lipopolysaccharide. Pharmacol Toxicol 92(4):173–179
12. Gaida W, Klinder K, Arndt K et al (2005) Ambroxol, a Nav1.8-preferring Na(+) channel blocker, effectively suppresses pain symptoms in animal models of chronic, neuropathic and inflammatory pain. Neuropharmacology 49(8):1220–1227

13. Hama AT, Plum AW, Sagen J (2010) Antinociceptive effect of ambroxol in rats with neuropathic spinal cord injury pain. Pharmacol Biochem Behav 97(2):249–255
14. Leffler A, Reckzeh J, Nau C (2010) Block of sensory neuronal Na + channels by the secreolytic ambroxol is associated with an interaction with local anesthetic binding sites. Eur J Pharmacol 630(1–3):19–28
15. Su X, Wang L, Song Y et al (2004) Inhibition of inflammatory responses by ambroxol, a mucolytic agent, in a murine model of acute lung injury induced by lipopolysaccharide. Intensive Care Med 30(1):133–140
16. Nowak D, Pietras T, Antczak A et al (1993) Ambroxol inhibits endotoxin-induced lipid peroxidation in mice. Pol J Pharmacol 45(3):317–322
17. Song J, Liu L, Li L et al (2014) Protective effects of lipoic acid and mesna on cyclophosphamide-induced haemorrhagic cystitis in mice. Cell Biochem Funct 32(2):125–132
18. de Oliveira MG, Monica FZ, Calmasini FB et al (2018) Deletion or pharmacological blockade of TLR4 confers protection against cyclophosphamide-induced mouse cystitis. Am J Physiol Renal Physiol 315(3):460–468
19. Donmez MI, Inci K, Zeybek ND et al (2016) The early histological effects of intravesical instillation of platelet-rich plasma in cystitis models. Int Neurourol J 20(3):188–196
20. Eltantawy FM, Sobh MAA, EL-Waseef AM et al (2018) Protective effect of Spirulina against cyclophosphamide-induced urotoxicity in mice. Egypt J Basic Appl Sci 5(3):191–196
21. Korkmaz A, Topal T, Oter S (2007) Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of reactive oxygen and nitrogen species as well as PARP activation. Cell Biol Toxicol 23(5):303–312
22. Matz EL, Hsieh MH (2017) Review of advances in uroprotective agents for cyclophosphamide- and ifosfamide-induced hemorrhagic cystitis. Urology 100:16–19
23. Lima MV, Ferreira FV, Macedo FY et al (2007) Histological changes in bladders of patients submitted to ifosfamide chemotherapy even with mesna prophylaxis. Cancer Chemother Pharmacol 59(5):643–650
24. Dantas AC, Batista-Júnior FF, Macedo LF et al (2010) Protective effect of simvastatin in the cyclophosphamide-induced hemorrhagic cystitis in rats. Acta Cir Bras 25(1):43–46
25. Siu LL, Moore MJ (1998) Use of mesna to prevent ifosfamide-induced urotoxicity. Support Care Cancer 6(2):144–154
26. Etlik O, Tomur A, Deveci S et al (1997) Comparison of the uroprotective efficacy of mesna and HBO treatments in cyclophosphamide-induced hemorrhagic cystitis. J Urol 158(6):2296–2299
27. Morais MM, Belarmino-Filho JN, Brito GA et al (1998) Pharmacological and histopathological study of cyclophosphamide-induced hemorrhagic cystitis—comparison of the effects of dexamethasone and Mesna. Braz J Med Biol Res 32(10):1211–1215
28. Eser N, Gocmen C, Erdogan S et al (2012) Effect of silymarin on bladder overactivity in cyclophosphamide-induced cystitis rat model. Phytomedicine 19(8–9):840–845
29. Liu M, Shen S, Kendig DM et al (2015) Inhibition of NMDAR reduces bladder hypertrophy and improves bladder function in cyclophosphamide induced cystitis. J Urol 193(5):1676–1683
30. Giglio D, Ryberg AT, To K et al (2005) Altered muscarinic receptor subtype expression and functional responses in cyclophosphamide induced cystitis in rats. Auton Neurosci 122(1–2):9–20
31. Yurdakul T, Kulaksizoglu H, Piskin MM et al (2010) Combination antioxidant effect of a-tocoferol and erdosteine in ischemia–reperfusion injury in rat model. Int Urol Nephrol 42:647–655
32. Jamshidzadeh A, Niknahad H, Azarpira N et al (2009) Effect of lycopene on cyclophosphamide induced hemorrhagic cystitis in rats. Iran J Med Sci 34(1):46–52
33. Abo-Salem OM (2013) Uroprotective effect of pentoxifylline in cyclophosphamide-induced hemorrhagic cystitis in rats. J Biochem Mol Toxicol 27(7):343–350
34. Abraham P, Isaac B, Ramamoorthy H et al (2011) Oral glutamine attenuates cyclophosphamide-induced oxidative stress in the bladder but does not prevent hemorrhagic cystitis in rats. J Med Toxicol 7(2):118–124
35. Abd-Allah Adel RA, Gado AM, Al-Majed AA et al (2004) Protective effcet of taurine against cyclophoshamide-induced urinary bladder toxicity in rats. Clin Exp Pharmacol Physiol 32(3):167–172
36. Zhi QM, Yang LT, Sun HC (2011) Protective effect of ambroxol against paraquat-induced pulmonary fibrosis in rats. Intern Med 50(18):1879–1887
37. Iynem Alkan H, Alademir AZ, Obek C et al (2004) The effect of prostate cancer and antianrogenic therapy on lipid peroxidation and antioxidant systems. Int Urol Nephrol 36(1):57–62
38. Al-Yahya AA, Al-Majed AA, Gado AM et al (2009) Acacia Senegal gum exudate offers protection against cyclophosphamide-induced urinary bladder cytotoxicity. Oxid Med Cell Longev 2(4):207–213
39. Souza-Fiho MV, Lima MV, Pompeu MM et al (1997) Involvement of nitric oxide in the pathogenesis of cyclophosphamide-induced hemorrhagic cystitis. Am J Pathol 150(1):247–256
40. Ribeiro RA, Freitas HC, Campos MC et al (2002) Tumor necrosis factor-alpha and interleukin-1beta mediate the production of nitric oxide involved in the pathogenesis of ifosfamide induced hemorrhagic cystitis in mice. J Urol 167(5):2229–2234
41. Oter S, Korkmaz A, Oztas E et al (2004) Inducible nitric oxide synthase inhibition in cyclophosphamide induced hemorrhagic cystitis in rats. Urol Res 32(3):185–189
42. Malley SE, Vizzard MA (2002) Changes in urinary bladder cytokine mRNA and protein after cyclophosphamide-induced cystitis. Physiol Genom 9(1):5–13
43. Sakura M, Masuda H, Matsuoka Y et al (2009) Rolipram, a specific type-4 phosphodiesterase inhibitor, inhibits cyclophosphamide-induced haemorrhagic cystitis in rats. BJU Int 103(2):264–269
44. Ferretti C, Coppi G, Blengio M et al (1992) Inhibitory effect of theophylline, theophylline-7-acetic acid, ambroxol and ambroxol-theophylline-7-acetate on rat lung cAMP phosphodiesterase isoenzymes. Int J Tissue React 14(1):31–36
45. Yamaya M, Nishimura H, Nadine LK et al (2014) Ambroxol inhibits rhinovirus infection in primary cultures of human tracheal epithelial cells. Arch Pharm Res 37(4):520–529
46. Ge B, Yang D, Wu X et al (2018) Cytoprotective effects of glycyrrhetinic acid liposome against cyclophosphamide-induced cystitis through inhibiting inflammatory stress. Int Immunopharmacol 54:139–144