Original Article

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A histopathological evaluation of the effect of captopril on cyclophosphamide-induced hemorrhagic cystitis

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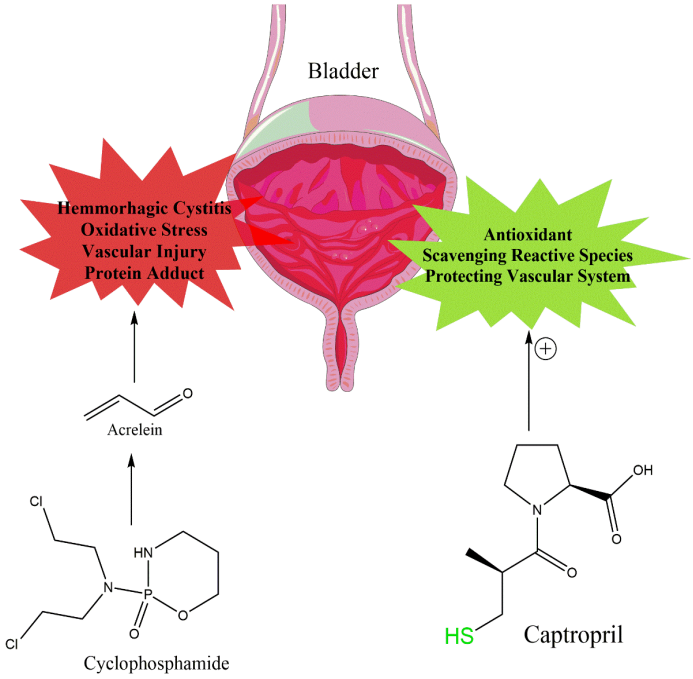
Graphical Abstract



A schematic representation of the effects of captopril against cyclophosphamide-induced hemorrhagic cystitis.

Abstract

Cyclophosphamide is one of the most prescribed antineoplastic agents. Hemorrhagic cystitis is one of the most common side effects of cyclophosphamide. Captopril is usually used as an anti-blood pressure agent. On the other hand, as a thiol group-containing agent, captopril has potent antioxidant activity. This

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study aims to evaluate the effect of captopril on cyclophosphamide-induced hemorrhagic cystitis in rats. Twenty-five male Sprague-Dawley rats were equally divided into five groups. Group I received saline (10 ml/kg, i.p.), Group II received a single dose of Cyclophosphamide (200 mg/kg, i.p), Groups III-V received cyclophosphamide and mesna (40 mg/kg, i.p.) or captopril (20 and 40 mg/kg, i.p.). Histopathological changes of bladder, liver, and kidney and lipid peroxidation and glutathione contents in the kidney and liver were monitored. Moreover, serum markers of organ injury were measured. It was found that captopril (20 and 40 mg/kg, i.p) and mesna (40 mg/kg, i.p), as the standard treatment of cyclophosphamide-induced hemorrhagic cystitis, conspicuously attenuated the macroscopic and microscopic damage in the bladder induced by cyclophosphamide. In contrast, mesna did not significantly improve histopathological changes. No significant difference in the serum level of liver and kidney injury biomarkers, tissue biomarkers of oxidative stress, and histopathological alteration were detected between control and cyclophosphamide-treated animals. On the other hand, both captopril and mesna alleviated histopathological bladder changes. Although the mechanism of action of captopril seems not to be mediated by alleviating oxidative stress, the present study results indicate that this drug could have a protective effect on cyclophosphamide-induced hemorrhagic cystitis.

Keywords: Captopril; Chemotherapy; Hemorrhagic cystitis; Oxidative stress

1. Introduction

Cyclophosphamide is a widely used cytostatic agent in chemotherapy regimens to treat non-Hodgkin lymphomas, various bone and soft tissue sarcomas, and some autoimmune diseases (1, 2). One of the most common side effects of cyclophosphamide, especially in children, is hemorrhagic cystitis (HC) (3). Cyclophosphamide-induced HC may include urothelial damage, bladder edema, ulceration, hemorrhage, and tissue necrosis (4, 5). Acrolein, a metabolite of cyclophosphamide, is suspected as the primary agent involved in HC (6). Besides, cyclophosphamide caused the epithelial injury in other organs besides the bladder (7). Several studies demonstrated that oxidative stress induced by Cyclophosphamide could damage DAN and organs, such as bladder, brain, lung, liver, and kidney (8-12). Inducible nitric oxide synthase(iNOS) induced nitric oxide (NO) production is the other important mechanism in cyclophosphamide-induced HC (13).

Today mesna (2-mercaptoethane sulfonate), as the standard prevention for this side effect, can interact with toxic metabolites of Cyclophosphamide such as acrolein to produce inactive compounds (14). However, effective treatment of hemorrhagic cystitis is still a clinical concern. Captopril, an ACE inhibitor, is widely prescribed in the treatment of hypertension and heart failure. Captopril could have a protective effect on cyclophosphamide-induced genotoxicity by inactivating acrolein (15, 16) or reducing the hydroxyl radicals originating from the cyclophosphamide's metabolites through the existence of thiol group in its structure (17). Besides, earlier studies showed that treatment with ACE inhibitors could improve some disorders by reducing oxidative stress induced by Ang II (11, 18). In this study, captopril was used against cyclophosphamide-induced hemorrhagic cystitis compared with mesna as a standard treatment against this complication.

2. Material & methods

2.1. Animals

Male Sprague-Dawley rats (200-250 g) were purchased from the Shiraz University of Medical Sciences. The animals were maintained in controlled temperature (23±1 °C) and humidity (≈40-50%) under a 12-hour light/dark cycle. A standard rodent's food (RoyanFeed, Isfahan, Iran) and tap water were available ad libitum. Animals were acclimated for one week before experiments (19, 20). All experiments were carried out following a standard protocol approved by the animal ethics guidelines of the Shiraz University of Medical Sciences.

2.2. Experimental design

Animals were randomly divided into five groups (n=5/group). The group I received saline

(10 ml/kg, i.p.) and served as a control group. Group II received a single dose of cyclophosphamide (Endoxan®, Baxter Oncology Gmbh, Frankfurt, Germany) (200 mg/kg, i.p) Groups III-V received mesna (Uromitexan®, Baxter Oncology Gmbh, Frankfurt, Germany) (40 mg/kg, i.p.), captopril (Exir, Borujerd, Lorestan) (20 and 40 mg/kg, i.p.) 30 minutes and 7 hours after administration of cyclophosphamide, respectively. The experimental groups were designed based on previous studies (21-23).

Twenty-four hours after cyclophosphamide administration, animals were anesthetized (thiopental, 70 mg/kg, i.p), and blood samples were collected from the abdominal vena cava. Serum samples were prepared (3000 g, 10 min) and used for the determination of biochemical parameters, including the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), also blood urea nitrogen (BUN), and creatinine (Cr) (24-28). The bladder, kidney, and liver were carefully dissected, cleaned of extraneous tissues, and part of the tissues was immediately transferred to 10% formalin for histopathological assessments. A part of tissues was used for the examinations of oxidative stress markers (29).

2.3. Histopathological studies

Tissue samples were fixed in 10%buffered formalin for twenty-four hours. Subsequently, the samples were embedded in paraffin, cut into 5 µm sections, and stained with hematoxylin and eosin for histological examination (30-33). The macroscopic evaluation of the bladder was assessed based on previously described criteria (34). Edema was considered severe (3+) when fluid was seen externally and internally in the wall of the bladder, moderate (2+) when confined to the internal mucosa, mild (1+) when slight edema was observed, and absent (0). The bladders were also evaluated for hemorrhage and categorized into four categories, considering the presence of intravesical clots (3+), mucosal hematomas (2+), telangiectasia, or dilatation of the bladder vessels (1+), or normal aspect (0) (35, 36). Finally, these tissues assessed microscopically, which was scored as follows: normal epithelium and absence of inflammatory cell infiltration and ulceration (0), mild changes involving reduction of epithelial cells, flattening with submucosal edema, mild hemorrhage, and few ulcerations (1+), severe changes including mucosal erosions, inflammatory cell infiltration, fibrin deposition, hemorrhage, and multiple ulcerations (2+) (37-41). According to standard procedure, a part of the liver and kidney were prepared in the same way for histological examination.

2.4. Determination of lipid peroxidation

Lipid peroxidation was estimated as thiobarbituric acid reactive substances (TBARS) levels (26, 31, 42-44). Briefly, liver or kidney tissues were homogenized in buffered saline resulting in a 20% (w/v) whole homogenate. Then 800 µL of trichloroacetic acid (TCA; 28% w/v) was added to 400 µl of these mixtures. After centrifuging at 3000 g for 30 min, 600 µl of the supernatants were added to 150 µl of 2-thiobarbituric acid (TBA; 1% w/v), and the mixtures incubated for 15 minutes in a boiling water bath (30, 45-51). After adding 4 ml n-butanol to solutions, they were centrifuged, cooled, and absorbance was read at λ=532 nm by T80+ UV/VIS spectrophotometer (PG Instruments Limited®, Beijing, China) (52-58). The calibration curve of 1,1,3,3-tetraethoxypropane standard solutions was used to determine the concentrations of TBARS (59-63).

2.5. Determination of glutathione content

The glutathione (GSH) level was determined using the Ellman reagent (5,5-dithiobis-2-nitrobenzoic acid; DTNB) as the reagent (64-70). Briefly, liver and kidney homogenate (4% w/v) were prepared in a buffer containing EDTA (0.2 M), 4 ml of distilled water, and 1 ml TCA (50% w/v) added to 5 ml of these suspensions and centrifuged (1000 g, 20 min, 4 °C). Then 1 ml of the supernatants was mixed with 4 ml of Tris buffer solution (0.4 M, pH = 8.9) and 0.1 ml of DTNB (0.01 M) (29, 71-75). Finally, samples were centrifuged, and the absorbance was measured at λ=412 nm using a T80+ UV/VIS spectrophotometer (PG Instruments Limited®, Beijing, China) (48, 67-69, 76, 77). GSH standard solutions' calibration curve was used to determine the GSH concentrations

Table 1. Effect of Mesna and Captopril on Cyclophosphamide-induced hemorrhagic cystitis in rats

	Macroscopic Evaluation	
	Edema	Hemorrhage
1:Control	0(0-0)	0(0-0)
2: Cyp (200 mg/kg)	3(2.5-3)	3(2-3)
3: Cyp+Mesna (40 mg/kg)	2(2-3) #	2(1.5-3) #
4: Cyp+Cap (20 mg/kg)	2(0.5-3) #	0(0-2) #
5: Cyp+Cap (40 mg/kg)	1(0.5-2) #	0(0-0.5) #
Groups	Significance between groups (P values)	
1-2	0.004	0.005
1-3	0.005	0.005
1-4	0.01	0.13
1-5	0.01	0.31
2-3	0.22	0.41
2-4	0.15	0.04
2-5	0.01	0.006
3-4	0.5	0.08
3-5	0.03	0.009

Cyp: Cyclophosphamide, Cap: Captopril
Data are presented as median and IQR (n=5).

(78-81).

2.6. Measurement of ALT, AST, ALP, BUN, and Cr in serum

Diagnostic kits obtained from Kimia Pajouhan® (Tehran, Iran) and XL 100 auto-analyzer (Erba Mannheim®, Mannheim, Germany) were used to measure the activities of AST, ALT, ALP, also BUN and Cr in serum (82-86).

2.7. Statistical Analysis

The histopathological observations were reported as medians, interquartile range (IQR), and the other data expressed as mean±SEM. The data analysis of these data was carried out by the Kruskal-Wallis test, followed by the Mann-Whitney analysis and one-way ANOVA, followed by the LSD test as the post-hoc. All the analyses were performed using IBM®SPSS® Statistics 16 software, and a value of $P < 0.05$ is considered statistically significant.

3. Results

For histopathological assessment, macroscopic and microscopic analyses of the bladders were carried out (Table 1 and Figure 1). Severe histological changes, including edema and hemorrhage compared, were detected in cyclophosphamide-treated animals compared with the control group (p=0.004 and p=0.005, respectively) (Table 1). Interestingly, significant edema and hemorrhage changes were detected in mesna-treated animals compared with the cyclophosphamide group (Table 1). On the other hand, captopril led to a significant decrease in edema and hemorrhage (p=0.01 and p=0.006, respectively) (Table 1). The lower dose of captopril (20 mg/kg) could significantly reduce the hemorrhage score (p=0.04) in cyclophosphamide-treated rats. In microscopic evaluation, the cyclophosphamide-treated group showed significant changes compared to the control (p=0.004) (Table 1). Administration of mesna presented significant improvements in microscopic abnormalities induced by cyclophosphamide. On the other hand, both doses of captopril (20 and 40mg/kg) decreased microscopic bladder tissue changes (p=0.04 and p=0.01, respectively) (Table 1). The comparison between the mesna and captopril group revealed a more significant captopril effect in alleviating cyclophosphamide-induced

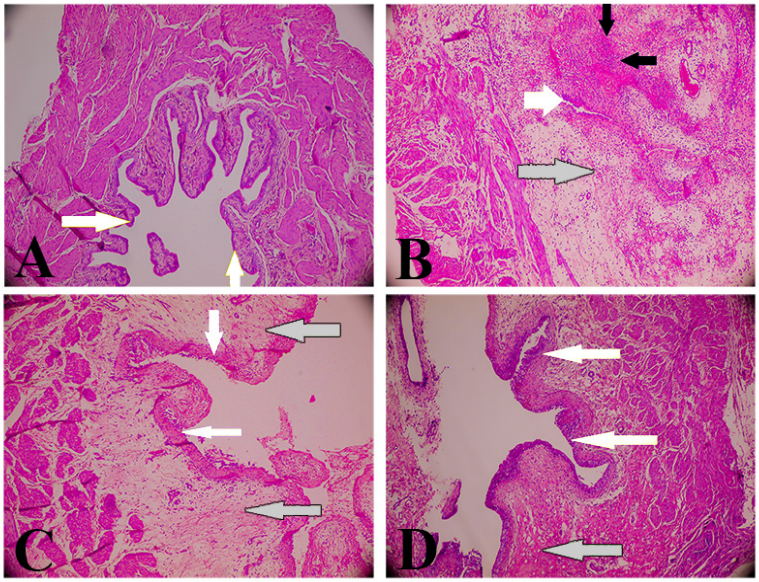

Figure 1. Effect of the Captopril and Mesna on microscopic histopathological changes in the bladder tissue of cyclophosphamide-treated rats. Hematoxylin–Eosin (HE) stain and original magnification (magnification ×200) A: The bladder section of the control group shows normal appearance with the absence of edema or hemorrhage. B: The bladder section of cyclophosphamide (200 /kg i.p.) treated rats shows severe hemorrhagic cystitis with severe edema and epithelial necrosis. C: The bladder section of the rats treated with Mesna (40 mg/kg i.p.) shows insufficient improvement in hemorrhagic cystitis induced by Cyclophosphamide. D: The bladder section of the rats treated with Captopril (40 mg/kg i.p.) shows the near-normal architecture. The grades of tissue histopathological changes are represented in Table 1. White, gray, and black arrows indicate mucosal changes, edema, and necrosis, respectively.

hemorrhagic changes (Table 1).

Figure 1 showed the control group had the typical architecture of the bladder. The severe hemorrhagic cystitis with denuded epithelium, severe submucosal edema, transmural hemorrhage, and fibrin deposition were observed in the cyclophosphamide group (Figure 1). The treatment with mesna did not significantly improve the microscopic damage induced by cyclophosphamide, while Captopril significantly attenuated these injuries (Figures 1C and 1D). Histopathological evaluation and comparisons of liver and kidney specimens were made between the experimental groups, and no significant changes were observed (data not shown).

Cyclophosphamide, mesna, and captopril administration caused no significant change in AST, ALT, and ALP levels (Table 2). However, a significant increase in BUN and Cr levels were detected in cyclophosphamide-administered animals versus the control group (p=0.003) (Table 2). It was found that captopril treatment in both doses

Table 2. Effect of mesna and captopril treatment on serum biochemical parameters in cyclophosphamide-treated rats.

	ALT(IU/l)	AST(IU/l)	ALP(IU/l)	BUN(mg/dl)	Cr(mg/dl)
Control	44.78±4.78	177.46±15.18	742.5±101.51	24.74±1.82	0.44±0.03
Cyp (200mg/kg)	41.80±6.38	170.14±14.40	561.8±30.75	54.52±14.13[a]	0.58±0.08
Cyp+ mesna (40mg/kg)	43.30±7.08	160.52±19.84	656±58.60	35.90±5.33	0.51±0.06
Cyp+ Cap (20mg/kg)	36.72±3.83	158.36±15.47	708.8±29.44	29.40±2.83[b]	0.37±0.06[c]
Cyp+ Cap (40mg/kg)	41.42±3.05	163.66±7.13	672.2±56.39	25.28±1.67[d]	0.49±0.07

Cyp: Cyclophosphamide, Cap: Captopril, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, BUN: Blood Urea Nitrogen, Cr: CreatinineData are presented as mean±SEM (n=5). [a]p value=0.003 compared with control group, [b]p value=0.01 compared with Cyp alone group, [c]p value=0.01 compared with Cyp alone group, [d]p value=0.003 compared with Cyp alone group.

Table 3. Oxidative stress markers in the liver and kidney tissues of cyclophosphamide-treated rats.

	Liver		Kidney	
	TBARS (nmol/ml)	GSH (nmol/ml)	TBARS (nmol/ml)	GSH (nmol/ml)
Control	1.17±0.12	11.88±1.48	1.49±0.11	6.93±1.43
Cyp (200mg/kg)	1.20±0.06	10.12±1.86	1.43±0.16	4.93±0.72
Cyp+Mesna (40mg/kg)	1.20±0.10	11.84±1.58	1.48±0.10	6.27±1.27
Cyp+Cap (20mg/kg)	1.24±0.13	11.40±2.76	1.50±0.14	4.53±0.39
Cyp+Cap (40mg/kg)	1.29±0.07	11.73±1.74	1.41±0.14	5.61±0.78

Cyp: Cyclophosphamide, Cap: Captopril, TBARS: Thiobarbituric acid reactive substances, GSH: Glutathione Data are presented as mean±SEM. (n=5).

(20 and 40 mg/kg) significantly decreased the elevated level of BUN (p=0.01and p=0.003, respectively) (Table 2), while mesna did not change these parameters (Table 2).

It was found that the administration of cyclophosphamide, mesna, and captopril caused no significant changes in TBARS and GSH levels in the liver and kidney tissues (Table 3).

4. Discussion

Cyclophosphamide-induced hemorrhagic cystitis is a common and severe clinical complication (ref). Mesna is used as a standard treatment for cyclophosphamide-induced hemorrhagic cystitis. However, all aspects of this complication are not alleviated with mesna, especially in patients with malignancies which are usually poly-medicated.

The current study found that captopril mitigated hemorrhagic cystitis development induced by cyclophosphamide, as evidenced by pathologic assessments.

Inconsistent with the present results, the previous studies reported that edema and hemorrhage in the bladder were increased after cyclophosphamide administration (22, 87). Cyclophosphamide-treated rats also presented severe submucosal edema, hemorrhage, and epithelial denudation in microscopic evaluation of the bladder compared with controls (22, 88, 89).

Acrolein is the suspected cytotoxic metabolite of the cyclophosphamide. Acrolein is formed by hepatic microsomal enzymes (1). The production of various inflammatory mediators, including cytokines, free radicals, and nitric oxide, plays an essential role in the signaling pathway of acrolein-induced hemorrhagic cystitis (8, 90). The activation of the mentioned pathways leads to marked inflammatory signs, edema, and hemorrhage (8, 90). The involvement of oxidative stress in acrolein-induced hemorrhagic cystitis is well-established (91). Several intracellular targets, including biomembranes (lipid peroxidation), DNA, and proteins, are affected by acrolein-induced oxidative stress (91). Acrolein is a reactive carbonyl species with the simultaneous existence of two reactive sites in a conjugated C=C-C=O system and readily reacts with many biological nucleophiles such as proteins. Many proteins are progressively affected by acrolein due to the presence of sulfhydryl groups in their structures. These events could contribute to bladder structure anomalies induced by acrolein (15, 16).

Although mesna (40mg/kg, i.p) showed insufficient improvement in hemorrhagic cystitis induced by cyclophosphamide in the current study, previous investigations reported that other routes of mesna administration of Mesna could almost abolish the macroscopic and microscopic alterations in the cyclophosphamide-injected rats (22). A statistically significant decrease was observed in the scores for edema, hemorrhage, inflammation, and mucosal ulceration in the group receiving mesna 20 min before cyclophosphamide injection and at 4 and 8 h after cyclophosphamide injection at a dose of 21.5 or30 mg/kg (i.p) in some investigations (88, 89). Some studies showed that sulfur-containing compounds diminished histopathological changes of the bladder tissue (92-95). In the current study, we found that captopril post-treatment revealed more significant protective effects than mesna. This effect could be an advantage for captopril treatment.

Several mechanisms could be proposed for the cytoprotection provided by captopril. Compounds with thiol-containing groups as powerful nucleophiles could be good candidates for acrolein scavenging. Hence, captopril's acrolein-trapping capability could contribute to its protective effects. Some studies revealed that captopril increased enzymatic and non-enzymatic antioxidant defense mechanisms in various tissues (96). Moreover, captopril could attenuate oxidative/nitrosative stress and nitric oxide production by decreasing Ang II levels. Ang II plays an activator of nicotinamide-adenine dinucleotide phosphate oxidase (NADPH oxidase) (97, 98). NADPH oxidase plays an essential role in ROS formation and oxidative stress (97, 98).

It has been found that cyclophosphamide could also damage hepatocytes in earlier studies (99). Renal injury is also another well-known adverse effect of cyclophosphamide (100-102). Some studies indicated that captopril markedly attenuated histopathological changes induced by nephrotoxic and hepatotoxic compounds (100-102). An essential finding of the current study was the protective effects of captopril on cyclophosphamide-induced hepatic and renal injury (Table 2). Interestingly, these effects were not found by mesna administration. Based on these data, captopril could have a superiority on mesna treatment in cyclophosphamide receiving patients.

Our findings indicate that captopril could have a protective effect on cyclophosphamide-induced hemorrhagic cystitis. Further studies could confirm the administration of captopril against hemorrhagic cystitis in clinical settings.

Conflict of Interest

None declared.

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