Effect of Cyclophosphamide on the Urinary Bladder of Adult Albino Rat and the Possible Protective Role of Quercetin with Mesna.

Thesis

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ABSTRACT

Key words: cyclophosphamide, quercetin, mesna, urinary bladder rat.

Cyclophosphamide is most commonly used as an anticancer and immunosuppressant drug. It is usually used for the treatment of chronic and acute leukemia, multiple myeloma, lymphomas, bone marrow transplantation, and some immune-related diseases. The urological side-effects of cyclophosphamide vary from transient irritative voiding symptoms, to life-threatening hemorrhagic cystitis. Bladder fibrosis, necrosis, contracture and vesicoureteric reflux were reported as cyclophosphamide side effects.

Although mercapto ethane sulfonate sodium (mesna) has been widely used as an agent against cyclophosphamide-induced cystitis, significant hemorrhagic cystitis can still occur.

Quercetin is a flavonoid with anti oxidant activity and has protective effects against oxidative stress.

The aim of this study was to evaluate the effect of cyclophosphamide on the urinary bladder of adult albino rat and the possible protective role of quercetin with mesna.

Sixty rats were used in this study divided into six groups (10 rats each).

Group (A): normal control group; the rats had no manipulations.

Group (B): sham control group; they were injected intraperitoneally with 1ml normal saline / day for 2 days.

Group (C): cyclophosphamide treated group; they were injected intraperitoneally with a single daily dose of 100mg/kg/day of cyclophosphamide for 2 days.

Group (D): cyclophosphamide and mesna treated group; they were injected with cyclophosphamide as in group (C) and 60 mg
/kg /day of mesna in three divided doses/day for 2 days intraperitoneally.

Group (E): cyclophosphamide and quercetin treated group; they were injected with cyclophosphamide as in group (C) and 60 mg /kg /day of quercetin in three divided doses/day for 2 days intraperitoneally.

Group (F): cyclophosphamide, mesna and quercetin treated group; they were injected with cyclophosphamide as in group (C) and mesna as in group (D) and quercetin as in group (E).

Each group was divided into two subgroups one (5rats) was sacrificed after 2 days and the other (5rats) was sacrificed after 2 weeks. Specimens from urinary bladder were taken and subjected to light microscopic examination. In group (C) examination revealed ulceration of mucosa, edema, hemorrhage and inflammatory cell infiltration (subgroup C1) and fibroses (subgroup C2). These pathological changes showed partial improvement in groups (D) and (E), but in group (F) they were reversed.
List of the contents

Page

LIST OF ABBREVIATIONS .......................................v

INTRODUCTION ......................................................1

AIM OF WORK .....................................................4

REVIEW OF LITERATURE .........................................5

MATERIAL AND METHODS .......................................23

RESULTS ......................................................................29

DISCUSSION ..........................................................92

SUMMARY AND CONCLUSION .................................103

REFERENCES ..........................................................108
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBB</td>
<td>Blood Brain Barrier initiates</td>
</tr>
<tr>
<td>Ca</td>
<td>calcium</td>
</tr>
<tr>
<td>CAT</td>
<td>Catalase Enzyme</td>
</tr>
<tr>
<td>cGMP</td>
<td>cyclic guanylate mono phosphate</td>
</tr>
<tr>
<td>C P</td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>GC</td>
<td>Guanylate Cyclase</td>
</tr>
<tr>
<td>GPx</td>
<td>Glutathione Peroxidase</td>
</tr>
<tr>
<td>H C</td>
<td>Hemorrhagic Cystitis</td>
</tr>
<tr>
<td>Hx &amp; E</td>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
</tr>
<tr>
<td>MESNa</td>
<td>Mercapto ethane sulfonate sodium</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide hydrogen</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>O2-</td>
<td>Superoxide</td>
</tr>
<tr>
<td>ONOO–</td>
<td>Peroxynitrite</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein Kinase C</td>
</tr>
<tr>
<td>Q</td>
<td>quercetin</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
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<tr>
<td>XO</td>
<td>Xanthine oxidase</td>
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Cyclophosphamide as an alkylating agent, is most commonly used as an anticancer and immunosuppressant. It is usually used for the treatment of chronic and acute leukemia, multiple myeloma, lymphomas, bone marrow transplantation, and some immune-related diseases such as rheumatoid arthritis (Dollery, 1999 and Xu et al., 2000).

The urological side-effects of cyclophosphamide vary from transient irritative voiding symptoms, including urinary frequency, dysuria, urgency, suprapubic discomfort, and strangury with microhematuria, to life-threatening hemorrhagic cystitis (Gray et al., 1986). Vesicoureteric reflux, bladder necrosis, fibrosis, contracture and as high as 4% mortality rate among patients with massive bladder hemorrhage have also been reported (Levine and Richie, 1989 and West, 1997).

Hemorrhagic cystitis (HC) is one of cyclophosphamide serious adverse effects. The incidence of this side-effect is related to dosage and can be as high as 75% in patients receiving a high intravenous cyclophosphamide dose (Traxer et al., 2001).

The urotoxicity of cyclophosphamide is not based on direct alkylating activity but on the formation of renally excreted 4-hydroxy metabolites, in particular acrolein that has...
INTRODUCTION

a destructive effect on the bladder wall (Gomes et al., 1995). Mercapto ethane sulfonate sodium (mesna) contains a sulfhydryl compound that binds acrolein within the urinary tract and detoxifies it; the resulting inert thio-ether dose not induce damage to the uroepithelium (Goren et al., 1997).

West (1997) reported that although mesna had been widely used as an agent against cyclophosphamide-induced cystitis, significant hemorrhagic cystitis, defined as an episode of symptomatic (burning, frequency, and dysuria), microscopic or macroscopic hematuria have been encountered clinically.

It has been shown that increasing nitric oxide (NO) production is involved in the toxicity of cyclophosphamide on the bladder. This toxicity is probably related to reactive nitrogen species (RNS), in particular a peroxynitrite (ONOO–), produced by reaction of nitric oxide with superoxide (O2-) which is associated with inflammation (Szabo, 1996 and Korkmaz et al., 2007).

The overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) during inflammation leads to considerable oxidant stress, cellular injury, and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage (Virag et al., 2003).
The antioxidative defense system in the body includes enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non-enzyme antioxidants such as carotenoids and flavonoids. These agents are key elements in reducing molecular damage caused by reactive oxygen and nitrogen species (Pietta, 2000 and Cuzzocrea and Reiter, 2001).

Flavonoids, which are available in common fruits and vegetables, inhibit the enzymes responsible for superoxide anion production such as xanthine oxidase (Hanasaki et al., 1994) and protein kinase C (Ursini et al., 1994).

Flavonoids have also been shown to inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase and NADH oxidase, all are involved in the generation of reactive oxygen species (Brown et al., 1998). The beneficial effects of flavonoids appear in various experimental models of inflammation (Rotelli et al., 2003).
AIM OF WORK

- The aim of this study was to evaluate the effect of cyclophosphamide on the urinary bladder of adult albino rat.
- The study also aimed to evaluate the possible protective role of quercetin with mesna against cyclophosphamide toxicity on the urinary bladder.
A-Morphology of the urinary bladder:-

The urinary bladder in rat is located in the posterior abdominal cavity at the midline of the body ventral to the colon. The urinary bladder can be divided into dorsal and ventral areas, as well as the blind dome which is referred to as the fundus or vertex (Frith et al., 1995).

The wall of the urinary bladder is organized in layers with the urothelium as the closest layer to the bladder lumen. Below the urothelium is the lamina propria layer. The outermost layer of the bladder is the detrusor smooth muscle, which consists of circular as well as longitudinal smooth muscle layers. The outer surface of the muscle coat is covered by the adventitia (Andersson and Arner, 2004).

Histologically, the transitional epithelium of the normal urinary bladder of the rat consists of three distinct layers. The most superficial layer is composed of large cells sometimes referred to as umbrella cells, since they cover a number of smaller underlying cells. Scanning electron microscopy indicates that the surface cells are routinely pentagonal or hexagonal and are of similar size and shape. With transmission electron microscopy, these cells are shown to have a characteristic luminal asymmetric membrane and fusiform vesicles. The fusiform vesicles are believed to allow for
expansion of the urinary bladder as it fills with urine. Beneath the superficial layer is the intermediate cuboidal cell layer, and the third layer is a simple basal columnar layer. A mucin layer composed of proteoglycans present on the luminal surface of umbrella cells (Born et al., 2003).

Almost all of the vesical mucosa is attached only loosely to subjacent muscle; it folds when the bladder empties, and the folds are stretched flat as it fills. Over the trigone, immediately above and behind the internal urethral orifice, it is adherent to the subjacent muscle layer and is always smooth (Al-Motabagani, 2005).

The lamina propria consists mainly of collagenous connective tissue containing small blood vessels. The connective tissue of the superficial part of the lamina propria is dense and irregular in type while in the deeper part it is relatively loose (Al-Motabagani, 2005).

The muscularis appears to be formed of three interlaced layers; inner and outer thin longitudinal layers and a middle thick circular layer. As a result of intermingling of muscle fibers in these layers, the muscularis cannot be clearly separated into three well-defined strata (Fig.1). The muscle fibers are arranged into groups separated by loose connective tissue septa (Al-Motabagani, 2005).
(Fig.1) Normal histological picture of rat's urinary bladder.

(Al-Motabagani, 2005).
B- Cyclophosphamide:-

Cyclophosphamide is one of the currently available alkylating agents. It exerts cytotoxic effects via transfer of its alkyl group to various cellular constituents. Alkylation of DNA within the nucleus probably represents the major interaction that leads to cell death. The major site of alkylation within DNA is the N7 position of guanine which can result in miscoding through abnormal base pairing with thymine. Breakage in DNA strands appears to be of a major importance to the cytotoxic action of cyclophosphamide (Chabner et al., 2001 and Katzung, 2001).

Cyclophosphamide is a prodrug which is converted by oxidase enzymes in hepatic cytochrome P_{450} system to active metabolites. The main active metabolites are 4-hydroxy cyclophosphamide exists in equilibrium with aldophosphamide. These active metabolites are carried by the blood stream to tumor and normal tissues, where nonenzymatic cleavage of aldophosphamide to phosphoramide mustard and acrolein is carried out. The antineoplastic effect of cyclophosphamide is associated with phosphoramide mustard while acrolein is linked to its toxic side effects (Ludeman, 1999 and Kern and Kehrer, 2002).
Clinical uses of cyclophosphamide:-

Cyclophosphamide has a wide spectrum of clinical uses. It has been used in the treatment of malignant tumors like acute and chronic leukemias, multiple myeloma, lymphoma, cancer breast and neuroblastoma (Chabner et al., 2001 and Senthilkumar et al., 2006).

It is considered as immunosuppressant drug to prevent organ rejection as in cases of bone marrow transplantation. It has a role in the treatment of autoimmune diseases including Wegner’s granulomatosis, rheumatoid arthritis, nephritic syndrome in children and systemic lupus erythematosis (Chabner et al., 2001 and Manger et al., 2006).

Adverse effects of cyclophosphamide:-

Cyclophosphamide possesses wide spectrum of toxicities. The cellular mechanism of toxicity is mediated by an increase in free radicals through intracellular phosphoramid mustard and acrolein; the principle metabolites of cyclophosphamide (Ramu et al., 1995 and Lee et al., 1996).

Pulmonary toxicity is frequently reported and is manifested by cough; dyspnea and hypoxemia which if untreated may be