

POSSIBLE EFFECT OF ATORVASTATIN IN PREVENTING HEMORRHAGIC CYSTITIS INDUCED BY CYCLOPHOSPHAMIDE IN RATS

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ABSTRACT

Cyclophosphamide (CP) is an antineoplastic agent used for the treatment of many neoplastic and inflammatory diseases. Hemorrhagic cystitis is a frequent side effect of CP. Several studies show's that atorvastatin has important pleiotropic (anti-inflammatory and immunomodulatory) effects. The purpose of the study was to investigate the effect of atorvastatin in prevention from HC induced by CP. Thirty rats were divided into 5 main groups: Control, CP, CP and MESNA, CP and Atorvastatin, CP- MESNA and Atorvastatin. The bladder was collected and histopathological evaluated. Atorvastatin Show's significant reduction in HC scoring, MESNA still superior than Atorvastatin in prevention of HC, combination both Ator And MESNA show's no additive effect.

KEY WORDS: Atorvastatin-Cyclophosphamide-Hemorrhagic Cystitis Prevention-MESNA.

INTRODUCTION

Cyclophosphamide is an alkylating agent, used as monotherapy or in a combination with other chemotherapeutic agents for treatment of various neoplastic (e.g. breast cancer) and non-neoplastic diseases e.g. nephrotic syndrome in children⁽¹⁾. It's a prodrug that is hydroxylized by hepatic P450 to the active 4-hydroxy metabolites 3,5; phosphoramide mustard which is responsible for antitumor activity and acrolein (AC) which is responsible for the urotoxic effect of CP⁽²⁾.

Hemorrhagic cystitis (HC) is the main side-effect of CP and still encountered as an important dose-limiting problem. Mesna (2-mercaptoethane sulfonate) is the commonly used agent to protect against this side-effect. Although mesna has been widely used as an effective agent against CP-induced cystitis, significant HC, defined as an episode of symptoms (e.g., burning, frequency and dysuria), microscopic or macroscopic hematuria have still been encountered clinically⁽³⁾.

In the last decade, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have become the drug of choice for the treatment of dyslipidemia. Statins have been shown to be especially effective in reducing low-density lipoprotein cholesterol (LDL) and, to a lesser extent, triglyceride levels⁽⁴⁻⁵⁾. Statins' antiinflammatory effects have been proposed not only to reduce cardiovascular disease, but also to help improve other conditions that are not influenced by lipid levels, including rheumatoid arthritis, stroke⁽⁶⁻⁷⁾, graft rejection and infection⁽⁶⁻⁹⁾. In this research we evaluated the anti-inflammatory effect of statin against hemorrhagic cystitis induced by (CP) in rats and compared with conventional therapy.

MATERIAL AND METHOD

About 30 adult albinos Sprague Dawley rats, aging from 10-14 weeks with average weight 160-230 grams, bred in the animal house of the Pharmacy college Misurata university. Rats were caged every group in a cage. They were maintained under standard laboratory conditions at normal daily photoperiod (12 hr dark/12 hr light). Free water access was allowed. The rats were divided into 5 groups, 6 rats in each, the first group is control received normal saline intraperitoneal (IP), second group is received (CP) IP 200mg/kg, third group received mesna 80mg/kg IP 5 mint. Before (CP) injection, fourth group received atorvastatin daily orally for 1 week then received IP (CP), fifth group received atorvastatin daily orally for 1 week then mesna IP 5 mint. Before IP (CP), after 24 hours from (CP) injection all rats were sacrificed by over dose of diethyl ether, the urinary bladder was collected and fixed in 10% formalin for at least 24 Hours and processed routinely for histopathological examination. Finally, the tissue was infiltrated with paraffin. To make paraffin blocks then serially sectioned at 5µ thickness and were stained with hematoxylin and eosin (H&E). The histopathological study was performed in Misurata National institute of oncology, and the scores was given according to Gray's criteria (table 1).

Statistical analysis:

The data were collected, presented and analyzed using SPSS-PC (version 18) software. The histopathological changes were expressed as median and range. Comparisons between multiple groups of quantitative categories were done by Kruskal Wallis test followed by Mann-Whitney test to detect the difference between each two groups.

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(Table 1) The histopathology criteria of evaluation

Histological parameter	Normal (0)	Mild (+1)	Moderate (+2)	Sever (+3)
Inflammation	No inflammation	Less than 14 inflammatory cells/field(×40)	more than 14 inflammatory cells/field(×40)	More than 30 inflammatory cells/field(×40)
Hemorrhage	No hemorrhage	With telangiectasia	With mucosal hematoma	With intravesical clots
Tissue injury (hyperplasia of the basal cells layer)	1 layer of basal cells	2 layer of basal cells	More than 2 layer of basal cells
Epithelial ulceration	6 layer of transitional cells (no more)	3 layers of transitional cells	1-2 layers of transitional cells	transitional cells were reduced to zero
Epithelial necrosis	No necrosis	Local necrosis in some epithelial cells in a limited area	Spread necrosis in several layers of epithelium in a limited area	Spread necrosis in several layers of epithelium and along the urinary tract including transitional epithelium and connective tissue
Hydropic degeneration	No hydropic degeneration	Some small drops of water in some cells but cells was survived(these cells had nuclei)	Large drops in many cells leading to cell death (these cells did not have nuclei)	Many drops of water were accumulated to form one large drop of water
Vascular congestion	No vascular congestion	It has seen some red blood cells within the blood vessels	Large amount of red blood cells within the blood vessels without extravasation of these cells to the neighboring connective tissue	Large amount of red blood cells within the blood vessels with extravasation of these cells to the neighboring connective tissue

RESULTS AND DISCUSSION

The histopathological data shows the highest score for rats that receive CP in compared to control group that receive normal saline indicating that the hemorrhagic cystitis will occurred in CP dose 200mg/kg which is going with many literatures using the same dose of CP (table 2).

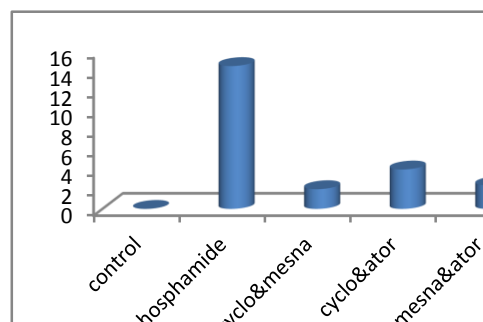
(Table 2) Median, and range values of histopathological scoring of different groups

Studied Group	N	Median	Minimum	Maximum
Control	6	0	0	0
Cyclophosphamide	6	14.5	8	16
Cyclo.+ Mesna	6	2	1	3
Cyclo + Ator.	6	4	3	5
Cyclo + Mesna + Ator	6	2.5	2	3

Mesna is the conventional therapy for hemorrhagic cystitis that might rise during therapy with CP, our experiment shows that, rats received CP and mesna show significant reduction in the total histopathological scoring compaired with rats that received CP alone (figure 1).

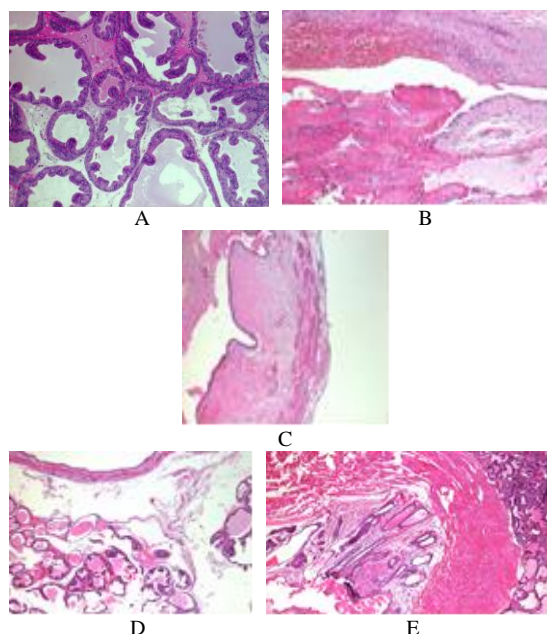
Atorvastatin is a lipophilic statin used as testing drug in this experiment for possibility of protective effect against hemorrhagic cystitis, our data shows that, rats that received atorvastatin and CP significantly

reduce the total histopathological scoring compared with rats that received CP alone (figure 1, 2), the combination of atorvastatin and mesna shows no additive effect in prevention of hemorrhagic cystitis that induced by CP.



(Figure 1) Median values of histopathological scoring of different groups

Francis M and his fellow, 2013 concluded that Simvastatin was effective at ameliorating the negative urodynamic changes and inflammation in the bladder after CP administration and is a potential therapy for preventing side effects in patients undergoing this chemotherapy. this result was in accordance with our result⁽¹⁰⁾. Another study confirmed this observed correlation between anti-inflammatory effect of statins and HC, and it was in accordance with present work result⁽¹¹⁾.



(Figure 2) photomicrograph sections of groups (H&E stainx40) (A) group1 control received normal saline (B) group2 received CP (C) group3 received mesna then CP (D) group4 received atorvastatin then CP (E) group5 received atorvastatin and mesna then CP.

CONCLUSION

The atorvastatin is significantly reduce the severity of inflammation which is most probably due to the anti-inflammatory effect that have been proposed as pleotropic effect of statin , the combination of both mesna and atorvastatin produced no additive effect in protecting from hemorrhagic cystitis that induced by cyclophosphamide.

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