

The Protective Effects of N-acetylcysteine against 5-Fluorouracil Induced Intestinal Toxicity in Albino Rats

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Abstract

5-fluorouracil (5-FU) is a pyrimidine analogue widely used in the treatment of various malignancies. It belongs to the antimetabolites family that acts during the S-phase of the cell cycle thus it prevents DNA synthesis. N-acetylcysteine (NAC) is a nutritional complement that acts as antioxidant. The purpose of the current study is to investigate whether there is a protective role of N-acetylcysteine against intestinal toxicity induced by 5-fluorouracil in albino rats. Eighteen healthy adult male rats were distributed into 3 groups of 6 rats for each. Group A is a control group. Group B, rats injected with 5-FU (20 mg dissolved in 2ml normal saline per kilogram body weight intraperitoneally for 7 successive days. Group C, rats received N-acetylcysteine 200 mg per kilogram body weight 24 hour prior to 5-FU injections for 7 consecutive days. The animals were sacrificed one day after the last injection; specimens of the intestine (colon) tissue of the three groups were removed and prepared for light microscopic examination. Results showed an increase in the depth of the colonic crypts in group B rats as compared to the control group, mucinous degeneration of the intestinal mucosal cells along with necrosis, and inflammatory cells infiltration in the lamina propria. The appearance of the crypts is nearly normal in group C with reduction in the depth and normal columnar epithelium lining the crypts the study concluded that 5-FU seriously affects the structure of the intestinal tissue and pretreatment with NAC protects the intestinal tissue against the toxic effects provoked by 5-FU.

Keywords: N-acetylcysteine, 5-Fluorouracil, Intestinal, Toxicity, Rats.

التأثيرات المحمية لعقار ان اسيتايلسيستين ضد السمية المعوية التي يحدثها عقار خمسة فلورويوراسيل في الجرذان البيض

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الخلاصة

التأثيرات المحمية لعقار ان اسيتايلسيستين ضد السمية المعوية التي يحدثها عقار خمسة فلورويوراسيل في الجرذان البيض في هذه الدراسة ثمان عشرة من ذكور الجرذان البيض البالغة تتراوح اعمارها ما بين (٢,٥-٣) أشهر وأوزانها ما بين (٢٠٠-٢٥٠) غرام، تم تقسيمها الى ثلاث مجاميع متساوية مجموعة A مجموعة B ومجموعة C كل مجموعة تضم ٦ حيوانات. أعطيت المجموعة A محلول الملح القياسي فقط وهي مجموعة السيطرة، المجموعة B أعطيت عقار خمسة فلورويوراسيل (٢٠ ملليغرام لكل كيلو غرام من وزن الجسم) يوميا لمدة اسبوع، المجموعة الثالثة C أعطيت (٢٠٠ ملليغرام لكل كيلو غرام) من عقار ان اسيتايل سيستين بيوم واحد قبل اعطاء كل جرعة من عقار خمسة فلورويوراسيل و لمدة اسبوع. أظهر الفحص النسيجي للأمعاء في المجموعة الثانية B انحلال فجوي للخلايا الكأسية المبطنة للتجاويف الغذائية إضافة الى منطقة بورية للنخر محاطة بترسب الخلايا الألتهايبية أما في المجموعة الثالثة فقد لوحظ استعادة التركيب الطبيعي للخلايا المبطنة لتجاويف القولون الغذائية نسيجا طلايبا سليما في الغشاء المخاطي السطحي. نستنتج من هذه الدراسة ان عقار خمسة فلورويوراسيل يحدث تغييرات واضحة في نسيج الامعاء والتي يمكن تقليلها باستخدام عقار اسيتايلسيستين والذي يستخدم كمكمل غذائي لمرضى السرطان ويستخدمون العلاج الكيماوي.

الكلمات المفتاحية: ان اسيتايلسيستين، خمسة فلورويوراسيل، معوية، سمية، جرذان

Introduction

Chemotherapeutic drugs have been used worldwide for the treatment of a variety of neoplasm given as a single or combined treatment protocol ⁽¹⁾. 5-Fluorouracil (5-FU) is pyrimidine analogue that belongs to the family of antimetabolites.

It is S-phase specific drug which principally inhibits thymidine synthase (TS) enzyme resulting in a decreased DNA synthesis. Moreover, it interferes with RNA processing and protein synthesis ⁽²⁾. The cytotoxic effects of 5-FU might be exerted by the generation of reactive oxygen species (ROS), upward suggestion implies that stem cells of the

tumor may increase stemness of cancer cells causing 5-FU resistance ⁽³⁾.

5-FU has a half-life of approximately 10 minutes; just about 15-25% of the administered dose is excreted in urine ⁽⁴⁾.

5-FU is corrupted by the hepatic dihydropyridine dehydrogenase (DPD), which is the initial and rate limiting enzyme in 5-FU catabolism ⁽⁵⁾. It is used in the management of advanced colorectal cancer, breast cancer, carcinoma of the stomach and in ophthalmic surgery ⁽⁶⁾. Some adverse effects include stomatitis, mucositis and diarrhea ⁽⁷⁾.

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Received: 29/12 /2021

Accepted: 27/2 /2022

Extensive investigations have been conducted on the 5-FU induced hepatotoxicity, cardiotoxicity and neurotoxicity and pulmonary toxicity⁽⁸⁾. However, few accounts on the mechanism of intestinal toxicity caused by 5-FU. N-acetylcysteine (NAC) is the N-acetyl derivative of the amino acid L-cysteine, NAC is the drug of choice in acetaminophen overdose which is used frequently in self-poisoning⁽¹⁰⁾. It exhibits direct antioxidant effect through its free sulphhydryl (thio) group, NAC exerts an indirect antioxidant effect as a precursor of Glutathione⁽¹¹⁾. Formerly, there is some evidence supporting the use of N-acetylcysteine as an adjunctive therapy for COVID-19. Further randomized clinical trials studies are justified to establish the optimal dosage and path of administration, and to determine the efficiency and safety of N-acetylcysteine in the management of COVID-19⁽¹²⁾. The aim of present work is to assess the defensive role of NAC against damage induced by 5-FU in the colon of Albino rats.

Materials and Methods

Agreement from the Medical Research Ethics Committee in the College of Medicine, University of Mosul has been obtained. Lethal dose, pilot studies and related literatures were taken into account and the accurate doses of 5-FU and NAC were calculated^(13, 14). Eighteen adult healthy male Albino rats their age between (2.5-3 months) and their body weight ranges from 200 to 250 grams were obtained from the College of Veterinary Medicine, University of Mosul. The animals were housed in a standard condition. The body weight of each rat was recorded at the beginning of the experiment before the injection of 5-FU and recorded again at the end of the experiment just before killing of the animals. The animals were randomly divided into 3 groups (6 animals each). Group A (Control group): each animal of this group was given 2 ml /kg body weight /day of normal saline by intraperitoneal injection for 7 consecutive days and served as a control group. Group B (5-FU recipient group): each animal of this group was given 5-FU in a dose of 20mg/kg/day by intraperitoneal injection for 7 consecutive days. Group C (NAC+5-FU recipient group): each animal first received NAC in a dose of 200 mg/kg body weight by intraperitoneal injection as a single dose or for 7 days and 6 hours after each injection of NAC, each animal was given 5-FU in a dose 20mg/kg/day by the intraperitoneal route for 7 consecutive days.

One day after the last injection of the 3 groups, the animals were sacrificed and dissected under light ether to collect the colon specimens and were immediately fixed in 10% neutral buffered formalin solution for 24 hours. The histological sections were prepared according to Bancroft et.al, 1994⁽¹⁵⁾ in which small pieces of about 4-5 mm in thickness were cut from each colon and dehydrated

in ascending grades of ethanol then cleared by xylene and embedded. Serial sections of about 5 microns in thickness were obtained and stained with Harris Haematoxylin and Eosin stain and Masson's trichrome stain then examined microscopically.

Statistical analysis was performed by SPSS version 20 for windows software. Data were presented as mean \pm SD and were analyzed using one-way Analysis of Variance (ANOVA). The limit point for statistical significance was set at 0.05 thus ≤ 0.05 reflect a significant and > 0.05 reflect a nonsignificant value.

Results

The animals of the control group remained alive, active with good appetite whereas the treated group became less active and grouped at one place of the cage. Some rats had frequent diarrhea and loss of hair. The data of body weight were expressed as mean \pm SD. The substantial significant drop ($P=0.001$) of the animals' mean body weight was observed in the treated group (5-FU recipient group) compared with the control group)(Table 1).

Microscopic findings

The control group showed normal mucosal lining with intact surface epithelium & closely packed simple straight tubular intestinal glands (colonic crypts) in the lamina propria (Figure.1). The mucosal surface epithelium and the epithelial lining of the crypts were formed of simple columnar cells with oval nuclei basally located & numerous goblet cells (Figure 2). The group which received 5-FU showed increasing in the depth of the crypts with vascular degeneration in the serosa (Figure 3), mucinous degeneration of the goblet cells, which is nearly obliterating the lumen of the crypts with inflammatory cells in the lamina propria (Figure 4), desquamation of the surface epithelium lining the mucosa with some necrosis (Figure 5). The group which received NAC then 5-FU showed nearly normal appearance of the crypts which are covered by columnar epithelium with only few mononuclear cells infiltration between the goblets (Figure 6).

Table 1. The mean changes of the body weights at the beginning and the end of the experiment

	Groups	N	Body weight (Mean \pm SD) at the beginning of the experiment	Body weight (Mean \pm SD) at the end of the experiment	Statistical significance among the groups	P-values
A	Control	6	152.00 \pm 16.43	155.50 \pm 17.70	A vs. B=(VHS) A vs. C = (NS)	0.001** 0.1
B	5-FU	6	160.50 \pm 69.72	106.60 \pm 39.39	B vs. A = (S) B vs. C = (NS)	0.02 0.4
C	NAC+5-FU	6	185.00 \pm 21.67	159.20 \pm 28.10	C vs. A = (NS) C vs. B = (NS)	0.6 0.3

SD= Standard deviation; S=Significant ($P \leq 0.05$); NS=Non-significant ($P > 0.05$); VHS= very high significant ($P < 0.01$); vs. =versus; A=control group; B=5-FU recipient group; C= NAC+5-FU group.

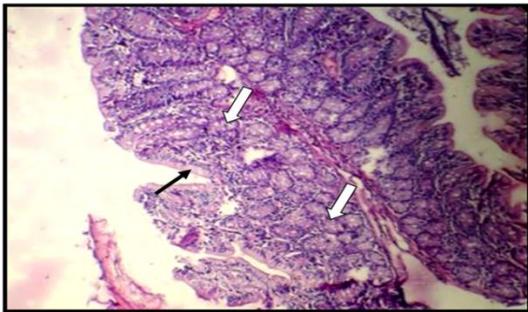


Figure 1. Micrograph of the colon of group A (control groups) showing surface epithelium (black arrows), intestinal glands in the lamina propria (white arrows) (H&E X 100).

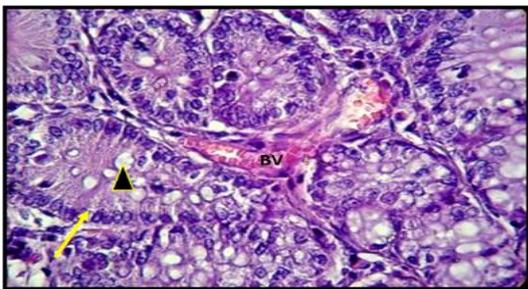


Figure 2. Micrograph of colon of group A (control groups) showing intestinal glands lined by simple columnar cells with basal oval nuclei (black arrows) and goblet cells in between them (arrow heads), A blood vessel in the lamina propria (BV) (H&E X 400).

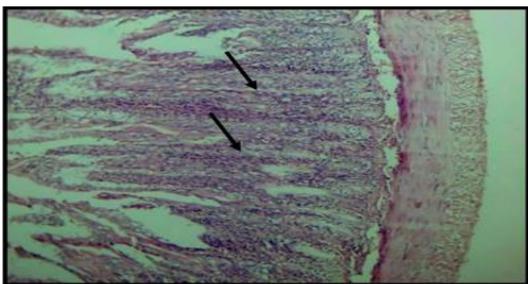


Figure 3. Micrograph of colon of group B showing elongation of colonic crypts occupying most of the mucosa (black arrows) (H&E X 40).

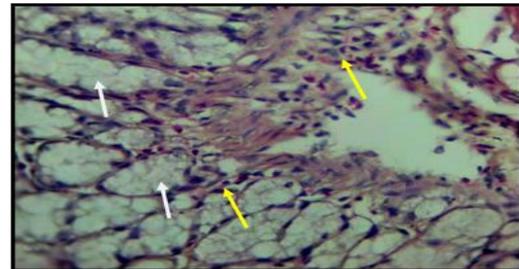


Figure 4. Micrograph of colon of group B showing mucinous degeneration of goblet cells nearly obliterating the colonic crypts (white arrows), inflammatory cells infiltration in the lamia propria (yellow arrows) (H&E X150).

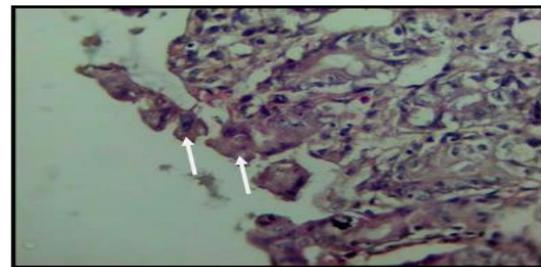


Figure 5. Micrograph of colon of group B showing desquamation of the surface epithelium lining the colonic mucosa (white arrows) (H&E X150).

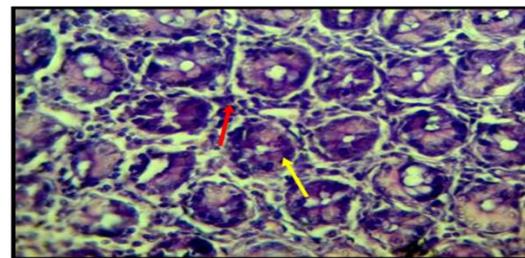


Figure 6. Micrograph of colon of group C showing the columnar epithelium lining the colonic crypts (white arrows) with few mononuclear cells infiltration in between the crypts (black arrows) (H&EX150).

Discussion

In the present study, the structural changes in the colon induced by 5-FU, as degenerative changes with elongation of the colonic crypts which are lined by degenerated goblet cells observed in the 5-FU recipient group could be attributed to the epithelial damage induced by oxidative stress provoked by 5-FU which contribute to intestinal mucositis, in addition to the alteration of epithelial function mediated by chronic inflammatory cells as mast cells ⁽¹⁶⁾. The mucinous degeneration of the goblet cells might be a consequence of stem cells damage, which suppresses the renewal of goblet cells ⁽¹⁷⁾. The inflammatory cells infiltration observed in the mucosa and submucosa of colon induced by 5-FU is similar to the intestinal mucositis which precedes gut dysbiosis in the mouse model following the exposure to irradiation ⁽¹⁸⁾. Previous studies suggested that 5-FU might increase the release of pro-inflammatory cytokines like prostaglandins, interleukins and tumor necrosis factors and it also increases the release of 5-hydroxytryptamine (5-HT) from chromaffin cells of the intestinal mucosa ⁽¹⁹⁾. Desquamation and necrosis of the epithelium lining the colon observed in 5-FU recipient group could be attributed to stromal edema and mucosal damage mediated by oxidative damage similar observation previously noticed in the rats treated with Methotrexate ⁽²⁰⁾. Partial occlusion of the lumen of colonic crypts observed in the 5-FU recipient group might be due to proliferation of epithelium lining the crypts, such finding is in agreement with those reported in the intestinal mucositis induced by chemotherapeutic drugs due to inflammation in the mucosa arising from stem cell apoptosis and disturbed cellular renewal and maturation processes ⁽²¹⁾. The appearance of nearly normal epithelial lining the colonic crypts with few mononuclear infiltration in group C indicates the defensive effect of NAC against the colonic mucosal damage induced by 5-FU. This is in agreement with what has been reported when dietary supplement with NAC can alleviate colitis induced by acetic acid in a pig model ⁽²²⁾. Such antioxidant role of NAC has been reported by Shahripour et.al, 2014 ⁽²³⁾, who observed a protective action of NAC & improved the clinical status in chronic neurological disorders and similarly the damaged intestinal mucosa induced by 5-FU in rats was markedly minimized by the administration of curcumin probably by the same antioxidant mechanism as that of N-acetylcysteine ⁽²⁴⁾.

Conclusion

The use of 5-FU for the treatment of some tumors seriously affects the structure of the intestinal tissue causing increase in the depth of the colonic crypts, mucinous degeneration of the intestinal mucosal cells along with necrosis, and

inflammatory cells infiltration. Pretreatment with NAC protects the intestinal tissue against the toxic effects provoked by 5FU. Thus, NAC may be considered as a useful dietary supplement for patients taking antineoplastic drugs like 5-FU.

Acknowledgment

We would like to precise our deepest appreciation to all staff members in the Department of Anatomy in the Mosul College of Medicine for their support. A special thanks to all the staff of College of Veterinary Medicine for their aid in the histopathological assessment of the sections.

Conflict of Interest

The author declares that there are no conflicts of interest regarding the publication of this manuscript.

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