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RAPID COMMUNICATION

Effects of granulocyte-colony stimulating factor on peritoneal defense mechanisms and bacterial translocation after administration of systemic chemotherapy in rats

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Abstract

AIM: To investigate the effects of granulocyte-colony stimulating factor (G-CSF) on peritoneal defense mechanisms and bacterial translocation after systemic 5-Fluorouracil (5-FU) administration.

METHODS: Thirty Wistar albino rats were divided into three groups; the control, 5-FU and 5-FU + G-CSF groups. We measured bactericidal activity of the peritoneal fluid, phagocytic activity of polymorphonuclear leucocytes in the peritoneal fluid, total peritoneal cell counts and cell types of peritoneal washing fluid. Bacterial translocation was quantified by mesenteric lymph node, liver and spleen tissue cultures.

RESULTS: Systemic 5-FU reduced total peritoneal cell counts, neutrophils and macrophage numbers. It also altered bactericidal activity of the peritoneal fluid and phagocytic activity of polymorphonuclear leucocytes in the peritoneal fluid. 5-FU also caused significant increase in frequencies of bacterial translocation at the liver and mesenteric lymph nodes. G-CSF decreased bacterial translocation, it significantly enhanced bactericidal activity of the peritoneal fluid and phagocytic activity of polymorphonuclear leucocytes in the peritoneal fluid. It also increased total peritoneal cell counts, neutrophils and macrophage numbers.

CONCLUSION: Systemic 5-FU administration caused bacterial translocation, decreased the bactericidal activity of peritoneal fluid and phagocytic activity of polymorphonuclear leucocytes in the peritoneal fluid. G-CSF increased both bactericidal activity of the peritoneal fluid and phagocytic activity of polymorphonuclear leucocytes in the peritoneal fluid, and prevented the bacterial translocation. We conclude that intraperitoneal GCSF administration protects the effects of systemic 5-FU on peritoneal defense mechanisms.

Key words: Granulocyte-colony stimulating factor; 5-Fluorouracil; Bacterial translocation; Peritoneal defense mechanisms

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INTRODUCTION

Chemotherapy is one of the most important methods in the therapy of malignant diseases. It has been widely used and it is well known that systemic chemotherapy has immunosuppressive effects[1]. 5-Fluorouracil (5-FU) has side effects, including leucopoenia, stomatitis, appetite loss, diarrhea, and mild fever like other chemotherapy agents[2,3]. It has been reported that a high dose of 5-FU often induces cytotoxicity in intestinal tissue that results in ulceration, diarrhea, and bacterial translocation[4-8].

Bacterial translocation is one of the most important causes of sepsis and multiple organ failure. Intestinal mucosa has a barrier function that prevents the colonization of the bacteria and the passage of bacteria and their toxins from the intestinal system into the systemic circulation. It has been shown that bacterial translocation is also augmented by shock, mesenteric ischemia, thermal injury, malnutrition, obstructive jaundice, and intestinal obstruction[9-14].

Granulocyte-colony stimulating factor (G-CSF) is a cytokine that is used to reverse the neutropenia associated with cytotoxic chemotherapy bone marrow and haemopoietic stem cell transplantation[15]. The role of GM-

CSF in 5-FU induced immunosupression and bacterial translocation has been studied previously We aimed to determine the effects of G-CSF on peritoneal defense mechanisms in rats that were administered systemic 5-FU.

MATERIALS AND METHODS

Thirty male Wistar-Albino rats weighing 215g to 287 g were used in this study. The animals were obtained from the breeding unit of Suleyman Demirel University, School of Medicine; and "all of the guiding principles in the care and use of laboratory animals" were strictly adhered to throughout the entire study. The animals were randomly allocated into three groups, consisting of 10 animals in each.

Group I, the control group, received 4 mL/kg saline solution intravenously from the dorsal tail vein for three days. Group II, the systemic chemotherapy group, received 20 mg/kg per day 5-FU intravenously for three days. Group III, the systemic chemotherapy and G-CSF group, received 20 mg/kg per day 5-FU intravenously and 10 μg/kg G-CSF (Neupogen, Roche) intraperitoneally for three days.

Eight hours after the last application, 10 mL of 12% sodium caseinate was administered to the abdominal cavity of the rats in order to achieve a high chemotactic gradient to the peritoneum. All animals were anaesthetized with 10 mg/kg ketamine hydrochloride and four mg/kg xylazine. Midline laparotomy was performed under sterile conditions after 16 h from the application of sodium caseinate. With a median incision of four cm, 10 mL of phosphate buffered saline (PBS) was administered to the abdomen and about 8 mL of this fluid was re-drawn. The following were evaluated from this peritoneal fluid; bactericidal activity, phagocytic activity of polymorphonuclear leucocytes (PMNL) and the count of peritoneal cavity cells (mainly neutrophil and macrophage). These procedures were carried out under sterile conditions.

The bactericidal activity was measured by the method described by Bergmann *et al*[16]. The peritoneal fluid collected by using PBS was centrifuged at 900 r/min for 15 min at 4℃. The sterility of this fluid was tested by culturing 0.1 mL in Mueller-Hinton broth. *Escherichia coli (E. coli)* K 12 was used to assess the antimicrobial activity. *E. coli* in Mueller-Hinton broth 0.1 mL was added to 0.9 mL of peritoneal fluid to give a final concentration of 102 organisms/ml. A sample of 0.1 mL of the inoculated fluid was spread over the surface of blood agar and incubated at 37℃. Colonies were counted after 24 h. For every sample of peritoneal fluid tested, a control culture was set up by inoculating 0.1 mL *E. coli* suspension into 0.9 mL saline to give the concentration of 10^2 organisms/mL. The results were presented as the percentage of bactericidal activity compared with the control, using the following formula; %PBA = 100 - (bacterial growth in sample/bacterial growth in control) × 100. Phagocytic activity was studied from the centrifuged peritoneal cavity cells as candidacidal assay[17]. The rate of phagocytosis was expressed as:

%Phagocytic activity = (number of dead Candida/300) × 100.

Haemocytometer was used for peripheral blood cell counts.

The sediment obtained from the centrifuged peritoneal fluid was used for peritoneal cell counts, which were were counted by a light microscope with a Thoma Zeiss cell counting chamber. To access the cell types, smears obtained from the sediment were stained with Giemsa. One hundred cells were counted and classified into four cell types as macrophages, lymphocytes, PMNL, and mast cells at a magnification of 1000 with a light microscope.

Tissue samples from the mesenteric lymph nodes, liver, spleen and caecum were removed. The sterile-scalpel method of homogenizing tissue was used for culturing in order to evaluate the bacterial translocation. Blood agar was used for culture and standard microbiological methods were followed.

Statistical analyses were undertaken using the Kruskall Wallis and Mann Whitney U tests, while *P*-values of less than 0.05 were accepted as significant.

RESULTS

Bactericidal activity of peritoneal fluid

The 5-FU administration significantly decreased the bactericidal activity of peritoneal fluid, while G-CSF improved it (Table 1). 5-FU decreased the phagocytic activity of polymorphonuclear leucocytes in the peritoneal fluid from 56.3 % to 36%, while G-CSF administration enhanced it to 42.6 % (Table 1).

Cell counts

The total cell counts in the peritoneal washing fluid and systemic WBC counts decreased after 5-FU administration but returned to normal levels after G-CSF administration (Table 1). G-CSF also significantly augmented the number of both neutrophil and macrophage numbers of the peritoneal fluid that had decreased with the 5-FU administration (Table 2).

Bacterial translocation

In the control group only one bacterial translocation on the mesenteric lymph nodes was detected. The 5-FU intervention caused a significant increase in the frequencies of bacterial translocation at the mesenteric lymph nodes and liver. G-CSF prevented the bacterial translocation when compared with 5-FU group (Table 3).

DISCUSSION

The results of the present study indicate that intravenous 5-FU administration has triggered the translocation of bacteria to the mesenteric lymph nodes and liver of rats, and has reduced both the peritoneal bactericidal and the phagocytic activity. However, G-CSF treatment has remarkably attenuated bacterial translocation and enhanced the peritoneal bactericidal and phagocytic activity.

The peritoneal defense mechanisms are activated with the irritation of the peritoneum by chemical agents or bacteria[18] and aim to remove the bacteria from the peritoneum or to localize the infective material. Bacteria

Table 1 Effects of 5-FU and 5-FU + G-CSF administration on peritoneal defense mechanisms and systemic WBC counts. Figures are expressed as median (range)

	Control (*n*: 10)	5-FU (*n*: 10)	5-FU + G-CSF (*n*: 10)
Bactericidal activity of peritoneal fluid MIC_{90} (bacteria/mL)	17.90 (14.1-24.6)	9.40 (6.2-13.8)[a]	19.20 (14.3-26.8)
Phagocytic activity of PMNL	56.3 (48.3-62.6)	36 (25-51.6)[a]	43.5 (37-58.6)
Cell counts in peritoneal fluid	2000 (1500-2400)	950 (600-1200)[a]	1380 (800-1900)
Systemic WBC Counts	9000 (4000-14000)	2100 (1100-2600)[a]	3400 (2200-4600)

[a]$P < 0.05$ *vs* control and 5-FU + G-CSF groups.

Table 2 Cell types in peritoneal fluid. Values are presented as median (range)

Groups	*n*	Neutrophil/mm^3	Macrophage/mm^3
Control	10	88 (80-94)	14 (1-20)
5-FU	10	68 (62-92)[a]	8 (6-12)[a]
5-FU + G-CSF	10	80 (73-87)	12 (6-15)

[a]$P < 0.05$ *vs* control and 5-FU + G-CSF groups.

Table 3 Bacterial translocation of the groups

	Control	5-FU	5-FU + G-CSF
Spleen	0	0	0
Liver	0	2	0
Mesenteric lymph node	1	5	0

via the lymphatic system can easily pass into the systemic circulation and cause bacteriemia and septicemia[19]. Another possible pathway of septicemia is bacterial translocation defined as the passage of viable bacteria from the gastrointestinal tract through the epithelial mucosa into the lamina propria and then to the mesenteric lymph node and possibly other organs[20], which may be important in immunosuppressive hosts.

Although 5-FU is a widely used antineoplastic agent, the cytotoxicity of 5-FU is not limited to tumor tissue. Hematopoietic cells and normal epithelial cells of the gastrointestinal tract are susceptible to 5-FU induced cytotoxicity producing severe leukopenia and intestinal toxicity leading to lethal translocation of intestinal microflora[21-24]. In general our findings are in line with previous studies that have reported that systemic 5-FU administration causes epithelial barrier dysfunction of the rat intestine[25], and causes bacterial translocation[26].

G-CSF is a pleitropic cytokine that has specific effects on the proliferation, differentiation, and activation of neutrophils and enhances their phagocytic and bactericidal activity[27]. The attenuation of bacterial translocation by G-CSF administration may be related to several mechanisms. G-CSF has some qualitative and quantitative effects on peritoneal defense cells (e.g. macrophages, polymorphonuclear leukocytes) and this may facilitate the removing of the bacteria translocated in mesenteric lymph nodes. It was shown in a previous report that the decreased numbers of viable pneumococci were recovered from tracheobronchial lymph nodes from the G-CSF-treated splenectomized mice and the sham-operated mice *vs* saline solution-treated controls[28]. It is well known that bacterial products and/or secondary inflammatory mediators induced by an infection are the potent stimulators for the production of G-CSF[29]. The beneficial effects of G-CSF on chemotaxis, neutrophil, phagocytosis, and bactericidal activities have also been demonstrated in some studies[27,30].

In this study, we found that systemic 5-FU administration also led to significant changes in the total peritoneal cell counts and cell types. The most affected cell type was macrophage. On the other hand, any significant differences in total cell counts and cell types could not be detected in the 5-FU + G-CSF group. We concluded that G-CSF increased the total peritoneal cell counts, neutrophil and macrophage counts when compared with the systemic 5-FU group. It has been reported that G-CSF administration increases the number of peritoneal exudates neutrophils, and the bactericidal activity of them, but does not affect the phagocytic activity of peritoneal exudates neutrophils[31]. However, G-CSF increases the number of circulating neutrophils and enhances their functions[27,32,33].

In conclusion, the systemic 5-FU administration caused bacterial translocation, decreased the bactericidal activity of peritoneal fluid, phagocytic activity of polymorphonuclear leucocytes in the peritoneal fluid and G-CSF increased both peritoneal and phagocytic activity of peritoneal fluid and prevented bacterial translocation. We conclude that intraperitoneal GCSF administration protects the effects of systemic 5-FU on peritoneal defense mechanisms.

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