Original Article

Influence of granulocyte colony stimulating factor treatment on physiological indices in Wistar rats

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ABSTRACT

Background: Granulocyte colony stimulating factor (G-CSF) is used in clinical practice to mobilize neutrophils alone or in combination with chemotherapy. However, its influence in physiological indices has not been addressed well in certain animal models such as Wistar rats. **Aims:** To evaluate the single and combinatorial effects of G-CSF and cyclophosphamide (CTX) on physiological indices in Wistar rat. **Materials and Methods:** Naïve female Wistar rats were treated with subcutaneous injection of pharmaceutical benefits scheme, (5 µL/day/rat) G-CSF for 5 consecutive days and single intraperitoneal injection of CTX (4 mg/rat). Body weights were obtained daily. Rats were sacrificed 1-day after the last injection to obtain different organ weight and to analyze the physiological indices in plasma and the liver. **Results:** G-CSF alone induced increases in body weight, splenomegaly, white blood cells, platelets, and alanine aminotransferase (ALT) activity. It, however, decreased neutrophils and monocytes, aspartate aminotransferase (AST) activity, red blood cells and hemoglobin level. CTX alone induced decreases in body weight, white blood cells, neutrophils, red blood cells and hemoglobin level. It, however, increased spleen weight, lymphocytes, monocytes, ALT activity and AST. G-CSF + CTX induced increases in body weight, splenomegaly, lymphocytes and ALT. It, however, decreased white blood cells, platelets number, neutrophils, monocytes, red blood cells and hemoglobin level. **Conclusion:** Among different physiological indices, treatment with single or combinatorial G-CSF increases the total number of white blood cells in Wistar rats which need to be considered while using this model animal disease.

Key words: Alanine aminotransferase, aspartate aminotransferase, cyclophosphamide, granulocyte colony-stimulating factor

INTRODUCTION

Granulocyte colony-stimulating factor (G-CSF), is a growth factor produced by bone marrow, stimulates the granulopoiesis and increases the circulating polymorphonuclear leukocytes.[1] Receptors for G-CSF are present on precursors and mature neutrophilic granulocytes, monocytes, platelets and endothelial cells.[2] At the myeloid progenitor cell level, G-CSF stimulates the generation of neutrophil granulocyte precursors and regulates the survival of mature neutrophils by inhibition of their apoptosis. G-CSF is used clinically to treat chemotherapy-associated neutropenia and to mobilize hematopoietic stem cells for transplantation processes.[3]

Cyclophosphamide (CTX) is a common chemotherapeutic agent used clinically for the treatment of several human malignancies.[4] A common side-effect of CTX as a chemotherapy agent is significant neutropenia, which leads to reduction of the efficacy of the immune system and an increased risk of infection.[5] G-CSF substantially shortens the period of severe neutropenia that follows high-dose chemotherapy and autologous bone-marrow infusion by increasing the number of circulating progenitor cells and also stimulating granulopoiesis.[6] Cladribine and cytarabine, are another types of chemotherapies showed a significant effect when combined with G-CSF in the

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treatment of acute myeloid leukemia.[7] The effect of single and combinatorial treatment of G-CSF on the physiological indices has not been well studied in particular Wistar rats. In parallel studies, we have been using Wistar rats as a model of rheumatoid arthritis. Since recent studies showed promising beneficial effects of G-CSF induces mobilization of neutrophil for the treatment of rheumatoid arthritis.[3] In this study, we aimed to measure the influence of single and combinatorial treatment of CTX and G-CSF on the physiological indices during neutrophil mobilization.

MATERIALS AND METHODS

Animals

Female outbred Wistar rats, from 7 to 8 weeks old with average of 140–180 g obtained from VACSERA Institute (Cairo, Egypt) were housed in the animal house facility at Faculty of Science, Tanta University, Egypt. Rats were acclimatized at room temperature (22–25°C) with free water and food access throughout the experiment period.

Reagents

Recombinant human G-CSF (Neupogen)®: Formulated for clinical use and was purchased from United Pahrma Co., Egypt. The concentration of human granulocyte CSF was 300 µg/ml/vial. CTX monohydrate (CTX): And was purchased from Sigma-Aldrich Co., China. The concentration of CTX was reconstituted in pharmaceutical benefits scheme (PBS) and kept at −20°C until use.

Treatment injection

Wistar rats were treated with subcutaneous injection of G-CSF dissolved in PBS at a dose of 5 µg/rat for 5 consecutive days. CTX was injected intraperitoneally at 4 mg/rat. Rats were divided into four groups; untreated "rats injected with PBS," naïve rats treated with G-CSF for 5 consecutive days, naïve rats treated with chemotherapy CTX and naïve rats treated with G-CSF combined with CTX, (n = 5 rats/group).

Measurement of body weight changes

Rats of different experimental groups were weighed daily during experimentation till the end of the experiment. The change of the body weight was calculated and recorded as mean ± standard deviation (SD).

Measurement of organs relative weight changes

After dissection, kidneys, spleen and the liver were obtained, and the relative organs weight was determined according to the following formula:

$$\text{Relative weight} = \frac{\text{Organ weight}}{\text{Total body weight}} \times 100$$

The changes of rat weights were calculated and recorded as mean ± SD.

Complete blood count analysis

One day after the last injection, rats were bled from the orbital sinus to harvest peripheral blood. The total and differential percent and absolute number of neutrophils in peripheral blood was enumerated using an automated instrument for complete blood count (VetScan HM2™ Hematology System, Abaxis®, Union City, CA, USA). Prepared cells were resuspended in saline, and a drop of this mixture were examined and counted by hemocytometer. Triplicates of each aliquot were counted and the mean of cells counted was calculated according to the following formula:

$$\text{Total leukocytes count} = \text{mean number of leukocytes counted} \times \text{volume of counted samples} \times \text{dilution} \times 10^4.$$

The counted cells were presented as an absolute number of cells/gram.

Absolute number of cells where calculated using the following formula:

Percentage of cells × white blood cells number/100.

Statistical analysis

Results are presented as mean ± SD; all treatments effect was discussed in comparison to that in case of untreated. The statistical evaluation of all data was done by using one-way analysis of variance to check the effect of different treatment stimuli. $P \leq 0.05$ were considered as statistically significant.

RESULTS

Body weight

Rat body weight was measured through the 5 days of the experiment as shown in Table 1. Groups treated with G-CSF alone or G-CSF combined with CTX showed significant decreases in the body weight as compared to untreated rats. While group treated with CTX showed a stable body weight at the first 3 days and then started to show a sharp body weight loss in the last 2 days of treatment. However,

Table 1: Weight of Wistar rats for 5 days through the experiment after stimulation with G-CSF, CTX and combination of both

	Body weight			
Days	Normal	G-CSF	CTX	CTX+G-CSF
1	176.08±5.26	112.58±3.31	109.52±7.56	107.52±9.81
2	162.86±11.16	115.52±3.55	118±5.1	115.54±5.31
3	166.4±24.33	125.2±13.33	122.56±12.53	118.84±13.63
4	167.32±10.74	128.66±6.26	125.56±5.4	122.5±6.11
5	169.08±10.85	130.2±5.5	126.1±5.16	123.78±6.4

Each reading represents mean±SD (n=5 rats). The significance of difference in induced drugs was checked by the one-way ANNOVA test. G-CSF: Granulocyte colony stimulating factor, CTX: Cyclophosphamide, SD: Standard deviation

there was a gradual recovery with them throughout the 5 days period.

Organ weight

Treatment of rats with G-CSF alone or CTX alone induced decreases in the relative liver weight associated with splenomegaly when compared to untreated rats. Treatment of rats with CTX + G-CSF induced significant increases in the relative liver weight associated with significant splenomegaly when compared to untreated rats. While treatment with G-CSF alone, CTX alone or G-CSF + CTX showed no significant effects on kidney relative weight [Table 2].

Complete blood picture

Treatment of rats with G-CSF combined with CTX induced significant increases in the total number of white blood cells and relative lymphocytes when compared with untreated rats [Figure 1a and c]. In addition, treatment of rats with G-CSF alone also induced a significant increase in the total number of white blood cells, but it showed no difference in the relative lymphocytes when compared with untreated rats [Figure 1a and c].

Furthermore, treatment of rats with G-CSF alone and G-CSF combined with CTX showed a significant decrease in the differential count of neutrophils and monocytes [Figure 1b and d].

In addition, G-CSF alone and G-CSF + CTX treatments showed significant decreases in red blood cells, hemoglobin concentration and packed cell volume [Figure 2a, c and d] while platelets increased in number [Figure 2b].

Treatment of rats with CTX induced significant decreases in total white blood cells and neutrophil percent [Figure 1a and b] while significant increases occurred in relative lymphocytes and monocytes compared to untreated groups [Figure 2c and d]. In addition, CTX treatment showed decreases in red blood cells count, hemoglobin concentration and packed cell volume compared to untreated group [Figure 2a, c and d] while a significant increase occurred in platelets number [Figure 2b].

Table 2: Relative weight of different rats' organs injected with G-CSF, CTX and combination of both

	Relative organ weight			
Organ	**Normal**	**G-CSF**	**CTX**	**CTX+G-CSF**
Spleen	0.43±0.11	0.7±0.035	0.78±0.08	1.44±0.12
Kidney	0.42±0.17	0.585±0.071	0.67±0.0562	1.29±0.108
Liver	7.01±0.15	5.09±0.23	4.53±0.07	6.043±0.153

Each reading represents mean±SD (*n*=5 rats). The significance of difference in induced drugs was checked by the one-way ANNOVA test. G-CSF: Granulocyte colony stimulating factor, CTX: Cyclophosphamide, SD: Standard deviation

Liver function

Treated rats with G-CSF alone, CTX alone or CTX combined with G-CSF induced significant increase in alanine aminotransferase (ALT) activity when compared to untreated rats. In addition, treatment of rats with G-CSF alone induced significant decrease in aspartate

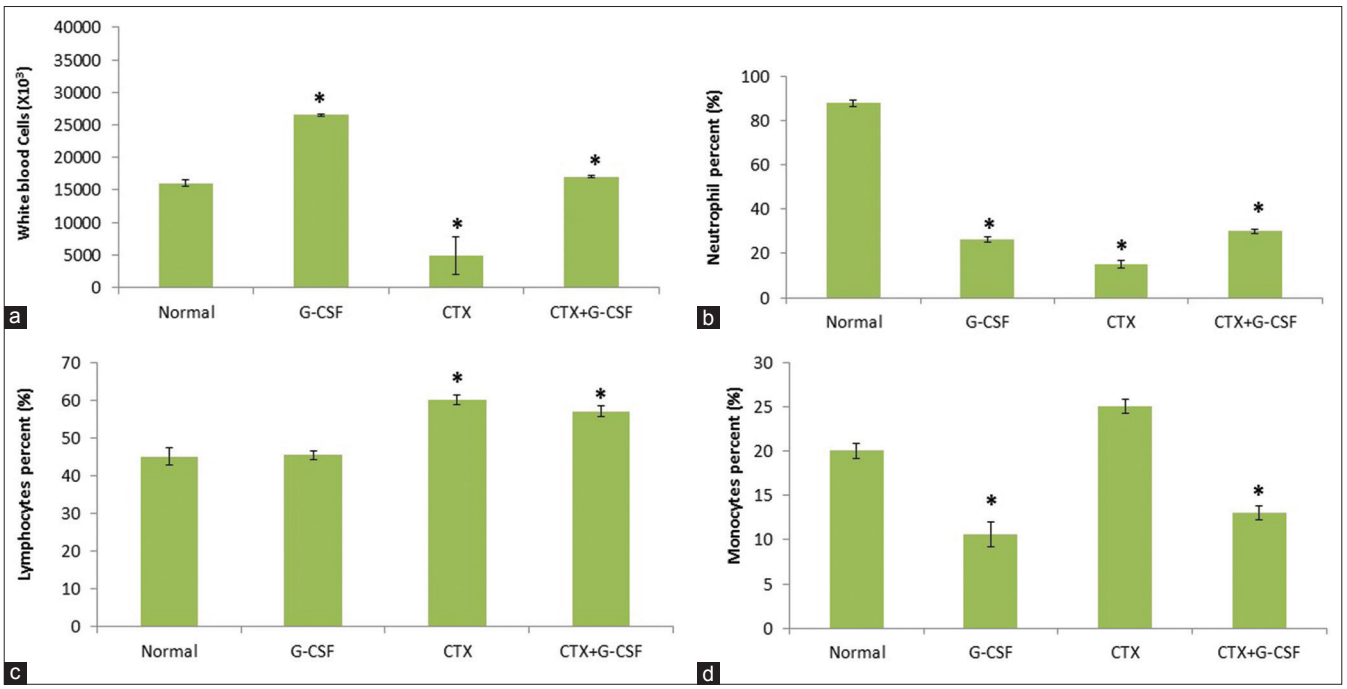

Figure 1: White blood cells and differential count in Wistar rats treated with granulocyte colony stimulating factor (G-CSF), cyclophosphamide (CTX) and CTX + G-CSF. (a) Total number of white blood cells, (b) neutrophils (c) lymphocytes and (d) monocytes. Data were presented as mean ± standard deviation animals/group. From *n* = 5 animals/group. Analysis of variance was done. *$P \leq 0.05$ as compared to normal rats

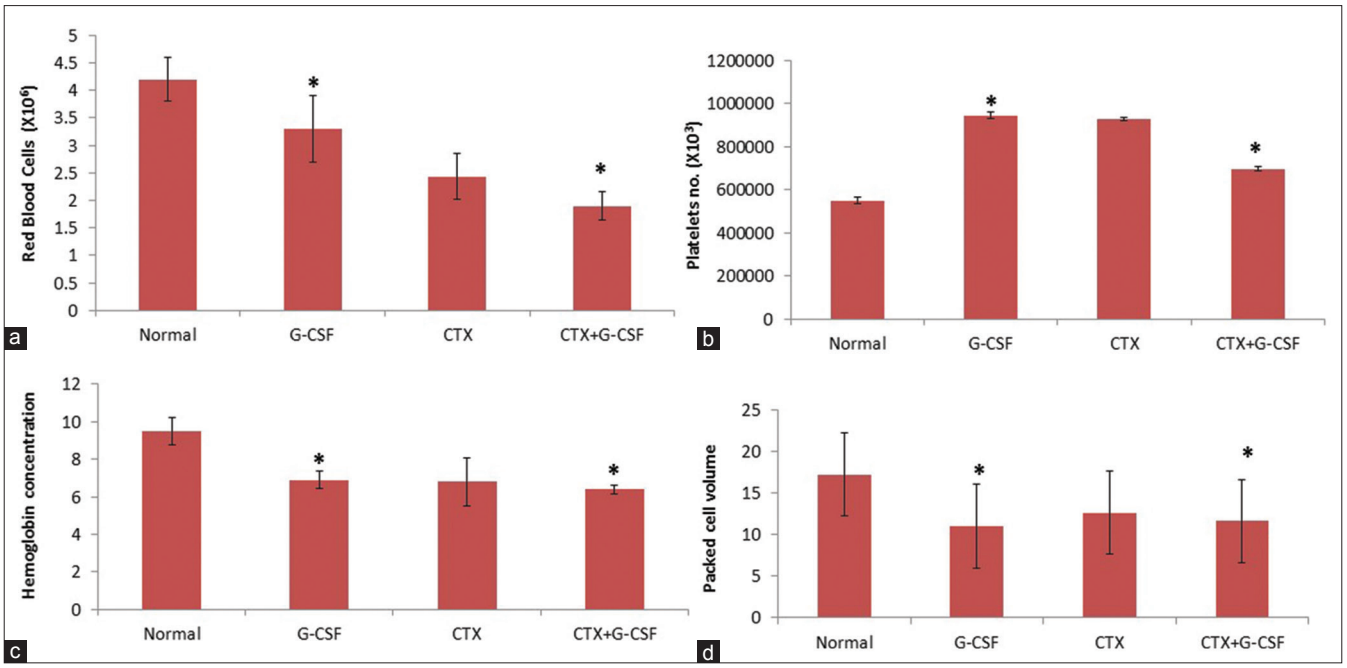

Figure 2: Red blood cells count in Wistar rats treated with granulocyte colony stimulating factor (G-CSF), cyclophosphamide (CTX) and CTX + G-CSF. (a) Total number of red blood cells, (b) platelets (c) hemoglobin and (d) packed cell volume PCV. Data were presented as mean ± standard deviation animals/group. From $n = 5$ animals/group. Analysis of variance was done. *$P \leq 0.05$ as compared to normal rats

aminotransferase (AST) activity while treatment with CTX alone induced significant increase in AST level when compared to untreated group [Figure 3].

DISCUSSION

Few studies have addressed the influence of physiological indices induced by G-CSF in animal disease models such as Wistar rats. CTX is also used as a single or in combination with other drugs to treat inflammation diseases. Given that these rats are used in several disease models, in particular inflammatory diseases, the present study aimed to evaluate the effect of single or combinatorial treatment with G-CSF and CTX on certain physiological indices in Wistar rat model by measuring the changes in body weight, different organs weight, complete blood picture and liver function.

We found that rats received G-CSF treatment showed increases in the body weight. These data are in contrast with data reported by[8] on the effect of treatment with G-CSF. While rats received treatment with CTX showed a stable body weight at the first 3 days then started a sharp body weight loss in the last 2 days of treatment as compared to untreated rats, however, there was a gradual recovery with them throughout the 5 days period.

Groups received treatment with G-CSF or CTX showed significant decreases in the relative liver weight associated with splenomegaly, which might explain the increase in body weight. These data are consistent with data reported by[9] on the effect of G-CSF on Wistar rats. Interestingly, concomitant treatment of rats with G-CSF and CTX resulted in normal body weight, indicating to the beneficial effect of G-CSF to overcome the toxic effect of CTX.

Treatment of rats with CTX alone showed a significant decrease in the relative liver weight but a slight change in spleen weight. These data are consistent with data reported by[10] on the effect of CTX in Wistar rats. Treatment with G-CSF alone, CTX alone or G-CSF + CTX showed no significant difference in kidney weight. When compared to liver and kidney, spleen weight was highly increased by treatment with CTX + G-CSF as compared to rest of treatments. These data indicate that the increase in the body weight could be due to at least in part to the increase in the relative weight of the liver and spleen.

A major differences on the effect of treatment occurred in the blood cells count. We found that rats received treatment with G-CSF showed an increase in white blood cells count and decrease in both neutrophils and monocyte count compared to untreated rat cell count. These data are consistent with data reported by[11] on the effect of G-CSF. We also found that rats received treatment with CTX induced sharp decrease in both in the total number of white blood cells as well as in the relative number of neutrophils. This treatment, however, induced decreases in the relative number of lymphocytes and monocytes. These data are consistent with data reported by[12] on the effect of CTX in Wistar rat. Administration of G-CSF after CTX did not alter the effect of the later.

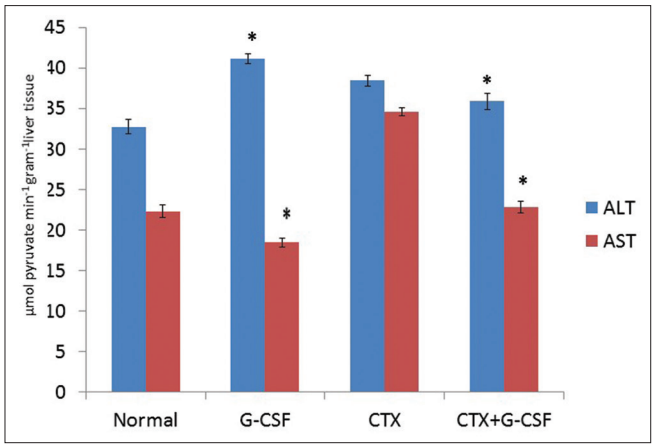

Figure 3: Alanine aminotransferase and aspartate aminotransferase activity (µM/min/g wet weight tissue) in liver of rats after administration of induced drugs stimuli. Each reading represents mean ± standard deviation (n = 5 rats). Analysis of variance was done. *$P \leq 0.05$ compared to normal rats

Red blood cells count and hemoglobin percentage decreased while platelets number increased in G-CSF, CTX and G-CSF + CTX treated groups. By evaluating the liver function, we found that CTX treatment associates with dysregulation in liver function, as shown by increases in AST and ALT activities. These effects were ameliorated after treatment with G-CSF, indicating to its protective effect against CTX-induced toxicity.[13]

Taking our data together on the effect of G-CSF and/or CTX on the body weight, relative organs weights, complete blood picture and liver functions, it could be suggested that Wistar rats are respond to CTX induced toxicity which can be ameliorated by G-CSF treatment. These data might be useful when these rats are used in preclinical disease models.

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