EFFECTS OF GRANULOCYTE-COLONY STIMULATING FACTOR ON BONE MARROW MORPHOLOGY FOLLOWING CYCLOPHOSPHAMIDE INDUCED NEUTROPENIA IN RATS

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Abstract

Granulocyte-colony stimulating factor is a glycoprotein that stimulates synthesis of granulocytes, especially of neutrophiles. It can be used to correct myelosupression associated with long-term chemotherapy or in the treatment of neutropenia. The aim of our study was to assess the effects of G-CSF on bone marrow after cyclophosphamide induced neutropenia in rats. The study was conducted on 24 female Wistar rats divided in 3 experimental groups; the control group, group of cyclophoshamide treated animals and the group of animals that were treated with Granulocyte-colony stimulating factor after neutropenia induction with cyclophosphamide. Cytological exam of bone marrow aspirates and histological exam from sternal bone marrow were realized using routine techniques. Examination of the aspirates taken from the femoral bone marrow and of the histological sections taken from the sternum showed a dramatic reduction in the number of myeloid precursors in individuals of group 2 which have been subjected to cyclophosphamide-induced myelosuppression, while the administration of G-CSF to the individuals of group 3 induced marked proliferation of the myeloid precursor cells, correcting the myelosuppressive effect of the cyclophosphamide In conclusion, G-CSF can be used for the stimulation and mobilization of myeloid progenitor cells from the bone marrow.

Keywords*: myelosuppression, neutrophil, growth factor*

Granulocyte-colony stimulating factor (G-CSF) is aglycoprotein that belongs to the family of colony-stimulating factors, along with macrophage colony stimulating factor and granulocyte macrophage colony stimulating factor (Cetean *et al.*, 2015). G-CSF is secreted by fibroblasts and endothelial cells from the bone marrow, but also by cells of the immune system like monocytes.

As a major role, granulocyte-colony stimulating factor regulates the differentiation of hematopoietic cells in bone marrow. It has been also shown that G-CSF accelerates leukocyte and neutrophil recovery after high-dose chemotherapy. In the present time, high-dose chemotherapy is still associated with prolonged periods of myelosuppression and absolute leukopenia that can last for 8 to 10 days (Peters, 1993). G-CSF has also a great role in the dendritic cell activation, cells with a great importance in the immune response initiation (Cetean *et al.*, 2015).

The production of this growth factor is stimulated mainly by bacterial lipopolysaccharides, so it is secreted mainly in bacteria induced inflammation, but also in other conditions like sterile inflammation (Saba *et al.*, 2002, Peters *et al.*, 1993).

G-CSF acts by binding to his specific receptor on responsive cells which are represented by variety of myeloid progenitor cells.

The most important role of the G-CSFs is the synthesis of granulocytes, which includes neutrophils, eosinophils, and basophils. These cells are essential in the immune response. The most sensible granulocytes to the action of G-CSF are the neutrophils, the growth factor

stimulates the production, mobilization and survival of these cells (Cetean, *et al.*, 2015).

G-CSF is already used in human medicine to minimize chemotherapy-induced myelosuppression and it's also used in veterinary medicine in the treatment of clinical neutropenia (Fernandez *et al.*, 2007).

Cyclophosphamide is an alkylating agent of the family of oxazaphosphorines, which works by adding an alkyl group to the guanine from the structure of DNA and blocks DNA replication by interchain and intrachain cross-linking. It is a synthetic antineoplastic drug with various life-threatening side effects (Murali and Kuttan, 2015).

Cyclophosphamide is used for the treatment of various neoplastic processes (eg, lymphoma, leukaemia, Langerhans cell histiocytosis, intracranial tumors like: astrocytoma, glioblastoma, meningioma) and in various autoimmune diseases due to the strong immunosuppressant action (*eg*. lupus erythematosus, rheumatoid arthritis) (Murali and Kuttan, 2015).

One of the most important side effect of cyclophosphamide administration is neutropenia, which may predispose patients to infections with opportunistic bacterial and fungal agents (Martin *et al.*, 1997; Hellmich *et al.*, 1999).

The aim of our study was to assess the effects of G-CSF on bone marrow after cyclophosphamide induced neutropenia in rats.

Materials and methods

The study was conducted on 24 female Wistar rats (5 months old), with an average weight of 250–300 grams, that were divided in 3 experimental groups. The animals were purchased from the Laboratory animal facility of the "Iuliu Hatieganu" University of Medicine and Pharmacy of Cluj-Napoca, Romania and the *in vivo* study was realized in the same place.

During the study, all the animals were kept in special cages, in an artificially illuminated room (12 h dark/12 h light cycle), at a temperature of 22–23 °C and at 50%–60% humidity. Standard pelleted diet and water *ad libitum* were administered. All the experiments were approved by the Ethical Committee on Animal Welfare of "Iuliu Hatieganu" University and complying with Guidelines in the Use of Animals in Toxicology.

Experimental design: the animals were randomly divided in 3 experimental groups (n=8), the first one was represented by the control group (group1); the second one by the cyclophoshamide treated group (induced neutropenia) (group 2) and the third one by the cyclophoshamide and G-CSF treated group (group 3).

Animals from the control group did not receive any treatment.

To induce neutropenia (group 2 and 3), the cyclophoshamide was administered in a unique dose of 50 mg/kg intraperitoneally.

Animals from group three received 30 μg/kg G-CSF (Filgastrim) administered subcutaneously starting from 48 hours after cyclophosphamide administration, 11 days once a day.

At the end of the experimental period (14 days after initiation), the animals were killed by cervical dislocation and immediately necropsied.

For the histological study, the sternum and the femur of the animals were fixed in 10% buffered neutral formalin, decalcified in a mix of 8% formic and 8% clorhidric acid for 24 hours and embedded in paraffin.

Sections were made at 4 micrometers and the slides were stained by Haematoxiline–Eosine (HE) method.

The slides were examined under a BX51 Olympus microscope and images taken with an Olympus UC 30 digital camera.

Sections were examined by an independent observer blinded to the experimental

protocol.

Cytological exam was also performed; aspirates were realized from the femoral bone marrow and stained by Wright-Giemsa method.

The sections and aspirates were assessed for their cellularity on a 6-point scale (0–5), with 0 indicating no particles present, 1 hypocellular, 2 low but normal, 3 normal, 4 high but normal and 5 hypercellular (Teg *et a.l*, 1999) and the data were analyzed statistically using ANOVA two way test. Standard deviation was also calculated. The myeloid/erythroid ratio was also assessed.

Results and discussion

All animals survived the experiment. No relevant gross lesions were observed during necropsy.

Microscopically we evaluated bone marrow sections by assessing cellularity, adipose tissue distribution, the number of megakaryocytes and the erythroid/myeloid ratio.

There were significant differences between individuals of the three experimental groups regarding the evaluated parameters.

Animals from the control group presented normal histological and cytological features regarding cellularity, the number and distribution of megakaryocytes. Also the erythroid/ myeloid ratio was normal, showing a mild myeloid predominance.

On sections from the cyclophosphamide treated group (group 2) there is a dramatic reduction in the number of myeloid precursors and the number of megakaryocytes and a proportional increase in the number of erythroid precursors, the myeloid/erythroid ratio showing marked erythroid predominance. The amount of adipose tissue was also reduced.

Animals of group three presented a marked increase in cellularity and in the number of myeloid precursors, with evident myeloid predominance.

Table 1

Cellularity and myeloid/erythroid ratio assessment (mean ±SD)

Group	Group 1	Group 2	Group 3
Cellularity	5,14 ± 0,69	4,28 ± 0,75	5,71 ± 0,48
Myeloid/erythroid ratio	Mild myeloid predominance	Marked erythroid predominance	Marked myeloid predominance

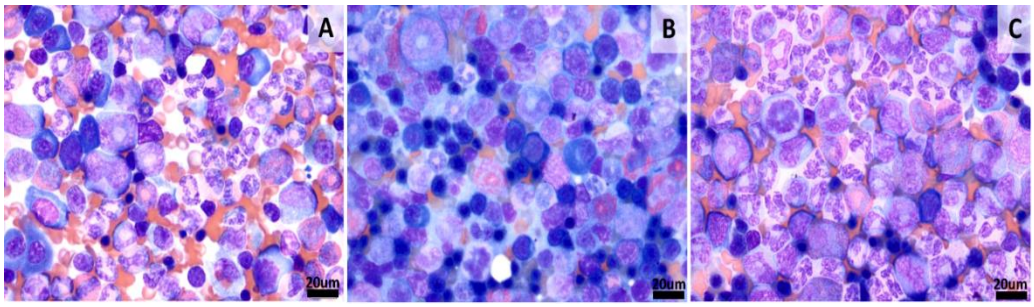

Fig.1. Cytological exam of bone marrow aspirate from different experimental groups. (A) Control group, normal cellularity and myeloid/erythroid ratio; (B) Group 2, high cellularity, marked erythroid predimonance; (C) Group 3, high cellularity, marked myeloid predominance; Wright-Giemsa x1000, Scale bar=20 µm.

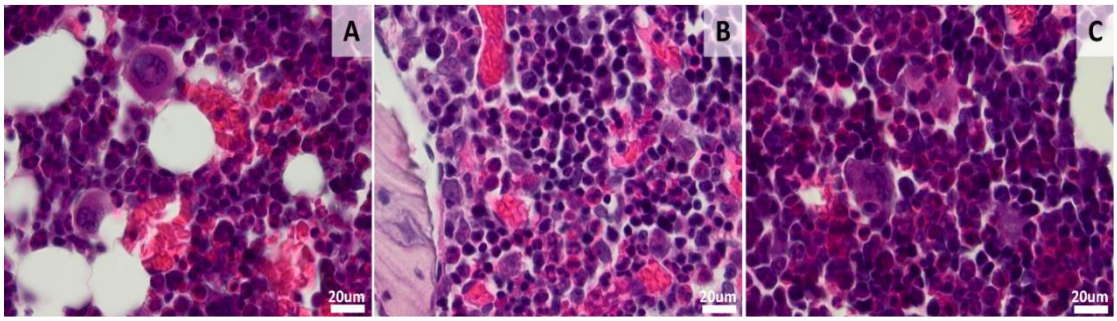

Fig. 2.Histology of sternal bone marrow sections from different experimental groups.(A) Control group, normal myeloid/erythroid ratio, adipose tissue distribution and megakaryocyte number and morphology; (B) Group 2, decreased cellularity and adipose tissue content, marked erythroid predimonance; (C) Group 3, increased cellularity, decreased adipose tissue content, marked myeloid predominance; HE x1000, Scale bar=20 µm

The main side effect of the majority of anticancer drugs is myelosuppression, which limit their use in the usual therapeutic dose and can reduce the frequency of administration. Death during an episode of severe myelosuppression is usually due to either bleeding or due to septic processes. The proliferation of hematopoietic progenitor cells is controlled by different growth factors, the production of neutrophilic granulocytes being stimulated by G-CSF (Lemoli and D'Addio , 2008).

Examination of the aspirates taken from the femoral bone marrow and of the histological sections taken from the sternum showed a dramatic reduction in the number of myeloid precursors in individuals of group 2 which have been subjected to cyclophosphamide-induced myelosuppression, while the administration of Filgastrim to the individuals of group 3 induced marked proliferation of the myeloid precursor cells, correcting the myelosuppressive effect of the cyclophosphamide.

Previous studies showed that G-CSF administration can correct cyclophosphamide induced neutropenia in animal models or in human patients (Teg *et al.*, 1999; Murali and Kuttan, 2015). In human medicine, this drug is used in a series of diseases like non neutropenic patients infections (pneumonia); infertility; several neurological disturbances; therapy of acute myocardial infarction; regenerative medicine (skeletal muscle) (Cetean *et al.*, 2015).

Although the assessment of the effects of granulocyte colony stimulating factor on animals that suffered treatment with cyclophosphamide was semi quantitative, we were able to highlight the main changes and benefits induced by this growth factor.

Conclusions

Filgastrim has remarkable effects in the treatment of cyclophosphamide induced myelosuppression.

G-CSF can be used for the stimulation and mobilization of hematopoetic stem cells from the bone marrow.

The use of G-CSF in veterinary medicine can be a very important therapeutical method in the recovery of bone marrow after chemotherapy, to reduce the duration of neutropenia, and in other conditions like bacterial infections, neurological diseases or muscle regeneration.

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