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Research Article

Effect of Selected Diets/Xanthone Derivatives on Morphotic Image of Bone Marrow and Other Tissues

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Abstract

Introduction: The diet as well as some xanthone derivatives can influence the processes occurring in the bone marrow, which is a hematopoietic organ and plays an important role in the immune system. Changes in the proportions of individual cell lines may lead to illnesses such as anemia or blood coagulation disorders.

Aim: The aim of the study is to identify the effect of selected xanthone derivatives and high-fat diet on the morphological construction of individual cell lines in the bone marrow (erythrocyte line, myeloblastic line, monocytic line, megakaryocytic line, lymphoid line).

Material and Methods: The studies were carried out on 4 groups of CD1 mice (n = 10): control (c), diabetes induced by a high-fat diet (f) and two derivatives of xanthone (gr. 1 and 2). Five-week-old animals received a standard or an enriched in fat diet for another 5 weeks. After decapitation of animals the bone marrow smear was prepared. The stained preparations were observed under a microscope and hematopoietic cells were counted.

Results: Based on the results and preliminary statistical treatment, it was found that there is a relationship between the diet used and changes in the proportion of cells of particular hematopoietic lines. The strongest changes were observed in the number of lymphocytes. The control group has statistically significantly more, compared to the group receiving the fat diet. It was also found that there is a relationship between the dietary xanthone derivatives used and the changes in the proportion of cells of particular hematopoietic lines.

Conclusion: The use of a high-fat diet and xanthone derivatives can have a significant impact on the change in the number of lymphocytes in the bone marrow.

Introduction

Diet and new xanthone drugs are of great importance for the body's health. Even slight changes in the proportion of individual nutrients and small doses of various potential drugs can cause changes in the functioning of the body. What we eat determines the proper formation of cells in the bone marrow. And the changes occurring there, are visible faster than in other parts of the body, because bone marrow is very sensitive and susceptibility to damaging factors. Negative effects of bone marrow damaging factors may contribute to aplasia, *i.e.* total destruction of hematopoietic tissue, resulting in suppression of blood morphology or hypoplasia - only partial destruction of hematopoietic tissue. The best-known factors that affect the processes of blood formation are: cobalt, benzene, vanadium, 5-fluorouracil or arsenic (III) [1-6]. Among dietary factors the best known are, among others, the influence of malnutrition on the process of hematopoietic formation and morphing picture of the bone marrow. As a result of malnutrition, there is anemia, and hence deficiencies of vitamins and minerals. These deficiencies are caused by disorders of physiological and biochemical processes. An important role in the process of hematopoiesis is played by vitamins B12, B6, C, folic acid, and minerals such as: Fe, Cu and Co [7]. The state of persistent malnutrition has a particular impact on erythropoiesis [6]. Additionally, an increase in bone marrow depletion was observed together with an increase in caloric restrictions [8,9]. Most researchers analyzing the impact of malnutrition on hematopoiesis focused on changes in the process of erythropoiesis. However, there are also studies on other hemopoietic pathways - granulocytopoiesis, and lymphocytopoiesis [10].

Another often raised issue is the impact of caloric restriction, without inducing malnutrition, on the aging process of the stem cell phenotype. It turns out that small caloric restriction delays the aging process of the stem cell phenotype, but at the same time leads to a decrease in the production of B cells [11,12]. Importantly, there are many studies analyzing changes in stem cells and the dynamics of hematopoietic cell proliferation processes depending on the age [13-15], but they do not take into account the nutritional status of the body.

The influence of protein-caloric malnutrition on the course of hematopoiesis is also of interest to scientists. Protein malnutrition can lead not only to the deficiency of all the morphotic elements of blood (pancytopenia) or leukopenia [15-17], but also to the complete inhibition of the hematopoietic process. Therefore, there are reasons for further research in this area, as the influence of all diet types on the morphotic picture of bone marrow is not yet known.

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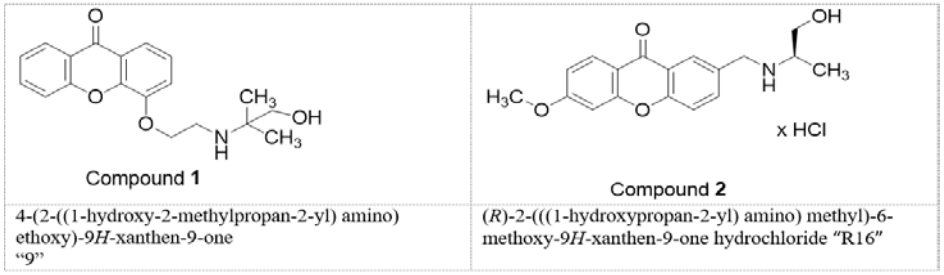

Figure 1: Chemical structures of the tested xanthone derivatives 1 and 2.

Xanthones (Greek xanthos - blonde) are a group of heterocyclic, tricyclic compounds dibenzo-γ-pyrone. They exhibit multidirectional biological effects. The xanthones have many derivatives and scientists still synthesize new compounds based on their basic structure [18,19]. Xanthones are characterized by a wide range of action in cardiovascular diseases, which are a serious problem. Some xanthones show antitumor activity through their effect on vascularization of cancer [20]. They also have an antibacterial and anti-inflammatory effect [21, 22].

There are no studies on the effect of xanthone derivatives on the bone marrow image. However, there are reports on the effect of xanthones on antitumor activity due to its preferential genotoxic, cytotoxic and cytostatic effects on proliferating cells [23]. There are also studies analyzing the impact of xanthone derivatives on the blood picture [24].

Design and synthesis of xanthone derivatives have been within our interest for many years - the tested compounds (Figure 1) 1 and 2 have been subject to former synthesis for their potential activity in the central nervous system [1] and circulatory system [2]. Both compounds exhibit aminoalkanol substituents, containing two-carbon linker between the amine and the hydroxyl group. Both compounds contain alkoxy substituent at the xanthone ring and it was interesting which of these two compounds might have a significant, beneficial additional impact on hematopoiesis.

Aim

The aim of this study was to examine the effect of a high-fat diet and diet with the addition of xanthone derivatives to morphological changes in the bone marrow.

Materials and Methods

The experimental study was conducted on CD-1 mice. It is a universal, multifunctional, safe and effective model. Animals were divided into groups (10 animals each). Each group will receive different diet/potential new drugs. The following groups were taken into account in the study: control group (Contr.), high-fat diet group (Fatty), Compound 1 - enriched diet, Compound 2 - enriched. Animal husbandry lasted 8 weeks. After this period mice were put to sleep with thiopental at a dose of 50 mg/kg. After the animals were put to sleep, femoral bones were isolated. The femoral bones were taken from the bone marrow. Marrow eruptions were performed using mouse plasma on a slide.

The colorization was performed using two pigments: Giemsy and May - Grunwald. Classical May - Grunwald staining, which stains granulocytes granules very well, exposes the polychroma of erythrocytes, but weakly stains cell nuclei. Giemsy staining, thanks to the use of Azur II, very well shows cell nuclei and Azuro - absorbent granules, but the hermaphroditic granules in granulocytes remain invulnerable.

Self-developed staining procedure:

- The dried smear was covered with 1 ml of May - Grunwald solution and left for 3 minutes.
- 1 ml of distilled water was added to the slide, mixed with the dye slightly swaying and left for 1 minute.
- Rinse quickly with distilled water.

The mixture was colored for 15 minutes with diluted Giemsy solution (1 ml of concentrated Giemsy solution and 20 ml of distilled water). The slide was rinsed with distilled water and the preparation was evaluated under immersion.

The analysis of the image included the places where the cells were properly colored, monolayered and did not overlap.

Colored preparations were observed under the OLYMPUS CX41 microscope, under 1000x a magnification. The image obtained under the microscope was photographed using a mounted camera, which allowed the camera to be viewed on a computer monitor. The hematopoietic cells were then counted and the results were presented in a summary table. The main cell systems were evaluated: red blood cell system, protein cell system and platelets.

Two femoral bones were isolated from each mouse and two smears were performed. The first 500 cells were counted from each smear, which gives 1000 cells per mouse. The results of observations for particular types of bone marrow cells were statistically analyzed using Statistical 12.0. The differences were evaluated using the Kruskal-Wallis test and statistical analysis of post-hoc Tukey's anova test for different N, assuming the level of statistical significance $P<0.05$.

Results

Based on the results and preliminary statistical treatment, it was found that there is a relationship between the diet used and changes in the proportion of cells of particular hematopoietic lines. The strongest changes were observed in the number of granulocytic line (Figure 2.1). The control group has statistically significantly less (30.71% ± 1.93%) compared to the group receiving the fat diet (33.28% ± 1.65%) $p=0.0253$. The smaller dependence but not statistical significance, was

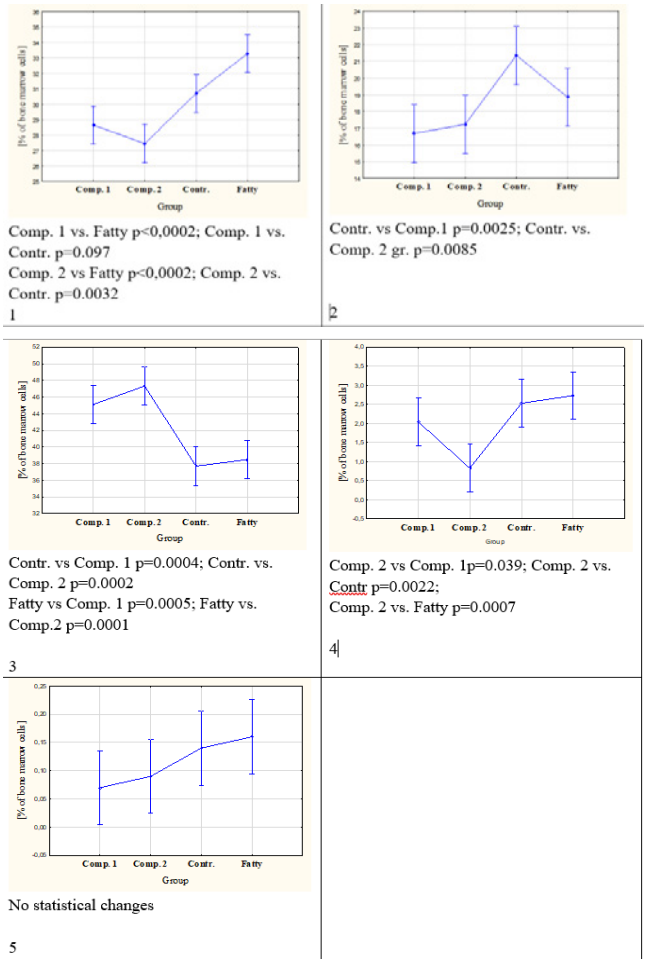

Figure 2: Effect of diet and xanthone derivatives on individual cell lines in the bone marrow. 1). Granulocytic line. 2). Lymphoid line. 3). Erythrocytic line. 4). Monocytic line. 5). Megakaryocytic line.

noted in the case of the lymphocytic line (Figure 2.2). The control group is characterized by a higher content of lymphocytic cells (21,37 ± 1,84) than the group on a high-fat diet (18,86 ± 2,45) p=0,0843. The content of cells in the remaining hematopoietic lines (erythropoietic (Figure 2.3), monocytic (Figure 2.4), megakaryocytic (Figure 2.5)) did not change significantly depending on the diet used. Based on the results and preliminary statistical treatment, it was found that there is a relationship between used diet and xanthone derivatives and changes in the proportion of cells of particular hematopoietic lines. It was noted that both xanthone compounds 1 and 2 had sometimes different activity on bone marrow cells. The strong influence of both xanthone derivatives was noted especially on erythrocytic line (Figure 2.3) where these compounds increased percentage of this line to the similar level to that caused by fatty and control diet (p<0.002).

In case of granulocytic and lymphocytic line both examined xanthone compounds 1 and 2 decreased the percentage of marrow cells (Figure 2.1 and 2.2). In granulocytic line compound 2 decreased statistically percentage of bone cells compared to both control group but compound 1 only to fatty diet. In these cases, p was <0.004 (Figure 2.1). In lymphocytic line (Figure1.2) both tested xanthone derivatives decrease percentage of bone marrow cells compared only to control

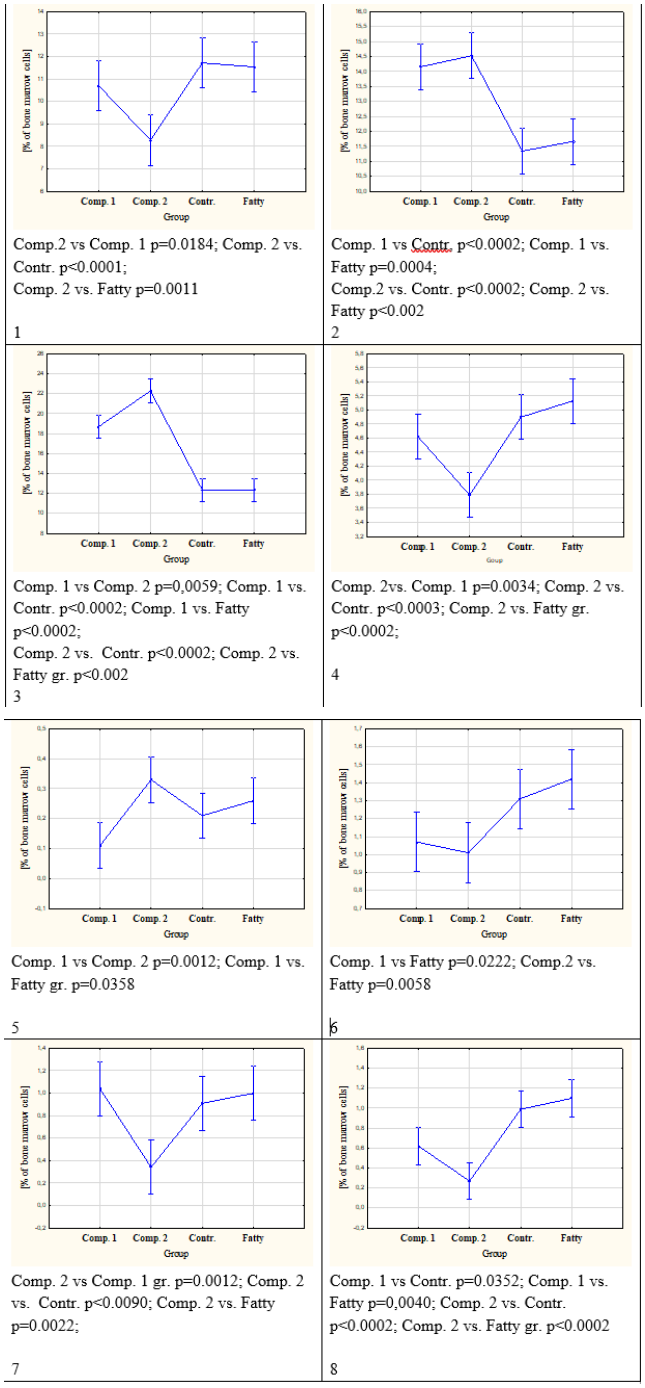

Figure 3: Influence of potential diabetic drugs on the proportions of cells of individual hematopoietic lines. 1). Basophilic erythrocytes. 2). Neutrophilic erythrocytes. 3). Acidophilic erythrocytes. 4). Neutrophilic myelocytes. 5). Eosinophilic metamielocytes. 6). Segmented eosinophils. 7). Promonocyte. 8). Monocytes.

group (p<0.009).

In case of monocytic line (Figure 2.4) statistical influence on percentage of bone marrow cells was observed only for derivative 2 in comparison to control and fatty diet (p<0.003). This compound also showed statistical difference compared to xanthone compound

1 ($p<0.04$). For megacaryocytic line (Figure 2.5) statistical difference was not observed between type of diet and used compounds.

More interesting changes in bone marrow cells were observed in particular stages of development of the tested cell lines.

The effect of the two compounds on the cell proportions of individual hematopoietic lines was compared. It was shown that there is a relationship between the used potential drugs and changes in the cell proportions. In the case of basophilic erythrocytes (Figure 3.1), compound 2 statistically reduced their numbers, compared to both control groups C and F and compound 1 ($p<0.02$). A similar effect was observed for neutrophilic myelocytes (Figure 3.4) and promonocytes (Figure 3.7). In these cell groups, the compound 2 statistically reduced their population. Compound 2 also showed a statistical difference compared to compound 1 ($p<0.004$).

A strong effect of both xanthone derivatives (1 and 2) on basophilic (Figure 3.2) and eosinophilic erythrocytes (Figure 3.3) was also observed. These compounds increased the percentage of these cells, compared to groups using the fat and control diet. In addition, a statistical difference was observed between compound 2 and 1 in the case of basophils ($p <0006$).

Both tested compounds statistically reduced the percentage of segmented eosinophils (Figure 3.6) and monocytes (Figure 3.8) compared to group F, and in the case of monocytes also compared with group C ($p <0.04$). Xanthone derivative 1 reduces the eosinophilic metamielocyte population (Figure 3.5) compared to groups of compounds 2 and F. A statistical difference was also observed between compound 1 and compound 2 ($p <0.002$).

Discussion

The study considered the influence of a high-fat diet and two xanthone derivatives on bone marrow morphology.

In this study, xanthone derivatives added to the feed proved to have an effect on the morphotic picture of the bone marrow. Effect on granulocytic, erythroblastic, monocyte, myeloblast and lymphocyte lines has been shown. The total number of individual hematopoietic lines in the marrow was examined in relation to the other groups. It turned out that the groups receiving xanthone derivatives together with their diet are characterized by increased cell production in the erythroblastic line in comparison with the other groups. In order to find the cause of this phenomenon, it is worthwhile to look at the results of an experiment, according to which the studied xanthone stimulated apoptosis [25]. The breakdown of red blood cells in the laboratory blood test manifests itself in an increase in the percentage of reticulocytes, because the body gives a signal to the bone marrow to produce more erythrocytes when they disintegrate and there are too few of them. This is in accordance with the results of this experiment.

Although the immune function plays an important role in health and development of diseases, research on the effect of xanthone derivatives on the immune system is limited. One of the few studies on this subject was to assess the immunomodulatory activity of xanthone (α-mangostin) derivative on lymphocyte line in human Peripheral Blood Mononuclear Cells (PBMCs) [26]. These cells consist of lymphocytes and monocytes. The results showed that α -mangostin had no significant effect on immune cells. In this study, however, the effect of xanthone derivatives on lymphocytes and monocytes was demonstrated. Therefore, there are premises of carrying out more research in this area.

The topic of the influence of xanthone derivatives on the line of myeloblasts has not been a source of interest for scientists so far and the results of this study should be confirmed. The effect of the high-fat diet on the morphing of the bone marrow was also checked. High-fat diets are still very popular. Fatty diet may cause a decrease in blood glucose [27]. It is used by people who want to reduce their weight. However, this diet is considered to be harmful to health. It is based on the consumption of high amounts of fats, including animal fats, which leads to the excessive consumption of cholesterol, but also to deficiencies of various vitamins and minerals. In addition, the lack of carbohydrates in the diet is inadvisable, as it leads to a series of adverse metabolic changes [28].

There are very few studies analyzing the effect of a high-fat diet on bone morphology. The results of one of them suggest that the high-fat diet increases the growth of melanoma cells in the bone marrow by inducing osteopontin and interleukin 6 [29]. The result of further research on this subject is data that led us to the conclusion that a high-fat diet induces leukocytosis and neutrophilia suggesting changes in the modulation of the hematopoiesis system [30]. Leukocytosis suggests an increased number of leukocytes (white blood cells) in the peripheral blood morphology, and thus lymphocytes. In this study, however, a statistically lower number of lymphocytes were observed in the high-fat diet group compared to the control group. This may be due to the fact that, in addition to bone marrow, lymphocytes are also produced in the thymus, lymph nodes, spleen and lymph nodes of the mucous membranes.

However, there is a study confirming the results of this experiment. In the study, the addition of animal fat had a definite effect on the proportional composition of the bone marrow cells. In the erythroid precursors, adding fat to the diet resulted in a statistically significant increase in the percentage of these cells. The reverse effect was considered for the lymphoid cell line, where the high-fat diet reduced the number of lymphocytes compared to the control diet [5]. In our study, we focused on the bone marrow picture, because it seems to accurately depict possible changes in the process of blood formation, which can then be verified by means of a blood picture. However, in the case of a factor, such as diet, knowledge is incomplete and such studies are necessary.

The studied compounds (1 and 2) affect the number of cells of individual homeopathic lines to varying degrees, causing an increase or decrease in their population. The group of compounds 2 is characterized by a reduced number of basophilic erythrocytes, compared to group 1. Too small number of basophilic erythrocytes may be unfavorable for health, as they synthesize hemoglobin, which takes part in oxygen transport from lungs to tissues as oxyhemoglobin, transporting CO_2 from tissues to lungs. In such cases, aplastic anemia is possible [31]. Moreover, decreased numbers were observed in neutrophilic myelocytes, promonocytes and monocytes. Too low level of neutrophil lymphocytes causes a decrease in the level of neutrophils, which can contribute to agranulocytosis, *i.e.* the complete lack of neutrophilic granulocytes. In such cases, the susceptibility to infections increases rapidly [32]. Decreased levels of promonocytes

and monocytes indicate impaired immune system. Monocytes are involved in the regulation of immunoglobulin biosynthesis and antibacterial and antiparasitic reactions [31].

On the other hand, the group applying compound 2 has a statistically higher number of acidophilic erythrocytes, eosinophilic metamyelocytes than the group applying the potential drug 9. Eosinophilic metamyelocytes are a precursor of eosinophilic granulocyte, belonging to immune system cells, which play the essential role in the control of parasites and allergic reactions [33].

An interesting phenomenon is the differences in the subsequent stages of development of the erythrocytic line, especially between basophils and eosinophils. Perhaps this is due to the effect of xanthone derivatives on the length of cell maturation at individual development stages. There are premises for more studies are needed, however the compound 1 seems to be more promising than 2. In this study, many new and interesting phenomena have been observed that have not yet been described. However, these innovative studies need confirmation, which should be an incentive for scientists to take up the subject.

Declaration

All human and used research was approved by the relevant ethics committee and then applied in accordance with the therapeutic standards and methods in the 1964 Helsinki Declaration and its subsequent amendments.

All institutions and agreements regarding health care and the use of laboratory animals were followed.

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