The effects of treatment with enriched bread on hematological parameters lymphocytes, monocytes, granulocytes of wistar rats

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Abstract

The aim of this research is to examine the effects of bread enriched with Fe (60 mg/kg) and folic acid (1.6 mg/kg), on the hematological parameters of blood: LYM (lymphocytes), MON (monocytes) and GRA (granulocytes) in male rats. It was found that the addition of iron and folic acid in bread leads to improvement of the studied parameters increases the number of lymphocytes, thus improves specific immunity because these cells are responsible for immune “memory” and consequently for faster and stronger response in the second encounter with the same antigen.

Key words: lymphocytes, monocytes, granulocytes, Fe, folic acid, fortification, bread

Introduction

Iron deficiency anemia still represents a huge global problem. According to World Health Organization (WHO) nationally representative surveys carried out in the period 1993-2005, 42% of pregnant women and 47% of preschool children worldwide have anemia (Black et al., 2008). If the most important indicators of nutrition considerer the level of anemia and lack of vitamin A, Macedonia is considered to be country with a medium to high risk of anemia, according to a report by UNICEF.

Anemia represents one of the most common pathological conditions in humans, often seen in developing countries, but it rarely appears in highly developed societies (Black et al., 2008). Iron is an essential element of all cells of the human body (Dzhekova-Stojkova et al., 1999). Main need for iron is the result of its necessity in the synthesis of hemoglobin, myoglobin and cytochrome; therefore this element is examined in the context of the anemia. The iron of animal food origin is better absorbed than that from plant foods (Brauni and Kermoar, 2005). In many developing countries, due to the economic status, the diet is consisted mainly of products of plant origin; from which iron is not assimilated in enough quantity. This is the reason why even in cases when nutrition support is sufficient, the majority of the population has a chronic iron deficiency. The result of such a chronic lack of effective iron in the human body is the appearance of different diseases. Grain naturally contains essential nutrients, but many of them are lost in the process of milling. Thus, if wheat contains about 33 mg/kg of iron before the milling process, after grinding amount is reduced to 11 mg/kg. With the addition of 20 mg/kg of iron in flour its natural qualities would be returned (Food and Nutrition Bulletin 2010). Fortification, as accepted technological process easy to realize by investing a little money, returns nutrients, and allows adding other vitamins and minerals.

Taking into consideration the scientific knowledge on the role of iron and folic acid on the health of people of all age groups, as well as the numerous hypotheses about their role in the prevention a number of diseases in humans, of particular interest to us was to determine whether to enrich flour as one of the most popular products with iron or other elements that lacking in the blood of the population in Macedonia.
Material and methods
Material in our study was peripheral blood of white laboratory rats weighing 150-270 g, total 16 in number. The experimental animals were divided as follows: the first group of animals (n=8) were fed with white bread; the second group of animals tested (n=8) were fed with enriched bread. As, a control the same animals on which the analysis were made before the start of treatment with white or enriched bread were used. Animals from all groups were on a diet for 21 days.

Bread making from enriched flour was as follow: to 10 kg of white type 500 we added 1 g of ELCOvit 2049E, product of German company Mühlenchemie, which is pre-mix of iron and folic acid (in 1 kg flour there are 60 mg iron and 1.6 mg folic acid). We also added 5 300 g water and 500 g yeast in the flour.

The biological material - peripheral blood of laboratory animals was taken twice, at the initial stage at the beginning of the experiment and at the end of the experiment. Blood was collected in plastic disposable tubes, which had anticoagulation substance heparin.

Analysis of blood parameters was performed on hematology analyzer (ABX Mikros). The results of the experimental research are represented as mean X ± SD and Standard deviation, while t-test was used to assess the variables between groups, the value of p<0.05 was considered statistically significant. For statistical evaluation of the results a computer program EXCEL and statistical package STATISTICA 7 were used.

Results and discussion
Table 1. Statistical analysis of hematological parameters (Note, significant deviations are indicated by *)

<table>
<thead>
<tr>
<th></th>
<th>Control group &amp; Group fed with white bread after exp.</th>
<th>Control group &amp; Group fed with enriched bread after exp.</th>
<th>Group fed with white &amp; enriched bread after exp.</th>
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<tbody>
<tr>
<td>LYM (10⁹/L)</td>
<td>1.4±0.12: 1.5±0.15</td>
<td>1.5±0.07: 1.7±0.07</td>
<td>1.7±0.07: 1.5±0.15</td>
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<tr>
<td></td>
<td>p&lt;0.02*</td>
<td>p=0.000001*</td>
<td>p&lt;0.004*</td>
</tr>
<tr>
<td>MON (10⁹/L)</td>
<td>0.49±0.11: 0.56±0.1</td>
<td>0.6±0.1: 0.6±0.12</td>
<td>0.6±0.1: 0.56±0.1</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.002*</td>
<td>p=0.0000004*</td>
<td>p&lt;0.000007*</td>
</tr>
<tr>
<td>GRA (10⁹/L)</td>
<td>0.6±0.08: 0.7±0.13</td>
<td>0.6±0.09: 0.7±0.1</td>
<td>0.7±0.1: 0.7±0.13</td>
</tr>
<tr>
<td></td>
<td>n.s</td>
<td>p=0.02*</td>
<td>n.s.</td>
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There are five different types of leukocytes that can be divided into granulocytes and agranulocytes cells. In group of agranulocytes cells are lymphocytes and monocytes, while the group of granulocytes includes neutrophils, eosinophils and basophils. There are two main types of lymphocytes, B- vital part of the immune system, specifically the humoral immunity and T-cell which plays a central role in cell-mediated immunity (Alberts, 2005). Results presented in Table 1. showed that the number of lymphocytes LYM (10⁹/L) have identical values for the group fed with white bread and the group before the experiment (1.5 (10⁹/L)), and control group value is (1.4 (10⁹/L)) which shows a slight increase in the values for the group fed with white bread compared to the control group. In group fed with fortified bread, we noticed an increase of values (1.7 (10⁹/L)) compared to the group before the experiment. It can also be noted that the most significant differences exist in the second group.

Monocytes are strong phagocytic cells, they derive most macrophages after leaving
monocytes the bloodstream settle and grow in specific organs, such as Kupffer cells in the liver, then pneumofagocitite in the lungs and other (Alberts, 2005).
The values for the number of monocytes, MON (10⁹/L), showed minimal growth for the group fed with white bread (0.56 (10⁹/L) compared to the control group, (0.49 (10⁹/L)). Value of group fed with fortified bread is almost twice as high (1.06 (10⁹/L)) as the value of the group before the experiment (0.6 (10⁹/L)). From the presented results it can be concluded that the addition of iron and folic acid stimulates production of monocyte and leads to a significant increase in their number.

From all examined parameters only the number of granulocytes GRA (10⁹/L) have the same values for group fed with white and fortified bread (0.7 (10⁹/L)), and compared with the control group, (0.61 (10⁹/L)) and group before the experiment (0.6 (10⁹/L)) showed an insignificant increase.

From the results obtained in our previous research we found statistically significant difference in the values obtained for the total number of leukocytes for the group of rats fed with enriched bread (146.7 g/L compared with control (134.1 g/L) and the group fed with white bread (133.9 g/L) (Menkinoska and Gjorgoski, 2011)

In the consulted literature we found different data of hematological parameters which are mainly due to different procedures of making bread, the difference in the type and quantity of added iron in bread, different kind of enriched food or difference in whether before the experiment has been caused anemia of experimental animals or not. Reviewed literature of Sturza et al. (2009) showed increasing numbers of leukocytes for groups of experimental animals fed with enriched bread prepared by two different procedures, lactic acid method (with natural fermentation of batter without yeast at 30°C during the day) and bread with yeast (bi-phase method). In their research the numbers of leukocytes also increased in the case of animals fed with bread enriched with iron prepared by bi-phase method and reaches values of (13.88 (10⁹/L)). In the case of the group fed with bread prepared with lactic acid method leukocyte count was lower (10.6 (10⁹/L)). Same team for (LYM %) received a slight increase for all three tested groups, while the absolute number of lymphocytes noted essential increase. On the other side a group of authors by Gudumac et al. (2009) for the percentage of lymphocytes (LYM %) and total number of lymphocytes (LYM (10⁹/L)) recorded a significant increase after caused anemia for all three studied groups after a special diet enriched bread with iron and calcium.

The addition of iron and folic acid in bread increases the number of lymphocytes, thus improves specific immunity because these cells are responsible for immune "memory" for faster and stronger response in the second encounter with the same antigen.

Also when lymphocytes perform their function they soothe and cross in memory cells with long life expectancy. It is important here to mention that the use of enriched bread in the diet of experimental rats leads to increase in the number of monocytes that have a strong phagocytic ability.

Such changes in the formation of leukocytes and altered ratio of different types and subpopulations of leukocytes, is consistent with the large number of studies showing that different types alimony supplementary among other things, have the effect of leucocyte lineage hematopoiesis.

Conclusions

Based on the results we obtained in our study it can be concluded that the addition of iron and folic acid in white bread has no significant impact on the number of granulocytes but have significant effects on the number of monocytes and specific immune cells - lymphocytes. Because Macedonia is one of the developing countries and also faces the problem of the increasing number of anemic people of all age groups, it is necessary to
raise the public awareness of the problems that can arise from iron deficiency anemia and the importance of prevention using the fortified food. In this study it was found that using enriched food in experimental rats showed good results, improving examined biochemical parameters, which leads to increased animal defense mechanisms.

References
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