RESEARCHES REGARDING THE EVOLUTION OF LEUKOCYTES, THROMBOCYTES, LEUKOCYTE AND THROMBOCYTE PARAMETERS IN ACRYLAMIDE CHRONIC EXPOSURE

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Summary

The experimental researches on laboratory animals have proven the neurotoxic potential of acrylamide, translated into tremor, lack of coordination, ataxy, muscle weakness, increased bladder, loss of weight, severe tonic-clonic convulsions and other clinical signs of diffuse central excitement. Also, the acrylic amide is genotoxic, showing clastogenicity or interference with the chromosomal segregation. Moreover, the researches in the field have shown the carcinogenic potential of acrylamide for laboratory animals, the molecule of the toxic amide producing the increase of incidence of certain tumors, identified in different organs such as the thyroid, suprarenal glands, testicular mesothelioma and mammary glands. The present experiment aims to evaluate hematological modifications after the intake of acrylamide by thermally processed food consumption (French fries and toast) and the consumption of an acrylamide solution 0.1 µg/L, respectively in the case of laboratory animals. The chronic intoxication with acrylamide leads to a decrease of the number of thrombocytes and to an increase of the leukocyte parameters.

Key words: acrylamide, thermally processed foods, thrombocytes, leukocytes.

Introduction

Chemical compound with a structure characterized by the presence of two non-saturated centers, acrylamide owns a particular toxicological significance to contemporary medical world. Although the true dimension of long-term toxicity of this compound may not yet be assessed, the acrylamide aggression mark is highlighted at the level of liver, excretory and reproductive apparatus, central nervous system. At the same time, acrylamide is considered a highly carcinogenic, mutagenic and teratogenic compound (Awad et al., 1998; Boettcher et al., 2005).

The objectives of the present paper are as follows:

- monitoring of the hematological modifications after the intake of acrylamide by thermally processed food consumption (French fries and toast) in the case of laboratory animals;
- determination of the hematological modifications caused by the ad libitum consumption of an acrylamide solution 0.1 µg/L (the maximum limit related to the presence of acrylamide in the drinking water allowed by the European Union legislation);
- comparison of the impact determined by the chronic intoxication by the consumption of thermally processed food on the hematologic parameters with the effects resulted after the 90 days’ consumption of a acrylamide aqueous solution.
Material and methods

Hematologic analysis instruments

abc VET™ automatic hematology analyzer

Experimental model

All the experimental proceedings in this experiment were achieved according to the international ethic regulations and were approved by the Ethics Commission of the University of Medicine and Pharmacy “Gr. T. Popa” Iași.

A total number of 24 male rats, Wistar line, having ponderal weights comprised between 180 and 220 g, were divided into 4 groups each of 6 rats as follows: group 1 (reference group), group 2 (control group), group 3 (CP group) and respectively group 4 (PP group). The reference group was fed (at their discretion) with sliced white bread, corn, carrot and drinking water. The control group received the same diet as the reference group, except for the drinking water, which was replaced by an acrylamide solution 0.1 µg/L (the maximum allowed limit regarding the presence of acrylamide in the drinking water, according to the European Union legislation). For group 3 the food supply was made at two times: in the morning (8.00 o’clock), French fries at their discretion (under supervision, so that each animal benefits from this diet) and at 14.00 o’clock, sliced bread, carrot and corn beans. Throughout the day, the animals had drinking water available. The group 4’s diet (experimental group 2) was made of sliced white bread, toasted for 3 minutes into the electric toaster (8.00 o’clock) and the reference group’s diet (at 14.00 o’clock). The experiment was conducted during 90 days.

At the end of the experiment the animals were anesthetized with ketamine i.p. (75mg/kg). When the absence of the vital signs was observed (respiration, cord beats, reflexes) blood samples were collected from the retroorbital plex in view of performing hematologic analyses. After the collection, the blood samples meant for the hematologic test were treated with a disodium EDTA solution (1 mg NaEDTA/2 ml blood) and submitted to the analysis using the “abc Vet” hematology analyzer.

Table 1. Experimental model related to the intake of acrylamide-rich food

<table>
<thead>
<tr>
<th>Groups</th>
<th>Name</th>
<th>Acrylamide solution 0,1µg/L</th>
<th>H. S. with ACR*</th>
<th>H. S. with ACR**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Reference group</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group 2</td>
<td>Control group</td>
<td>Ad libitum</td>
<td>-</td>
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</tr>
<tr>
<td>Group 3</td>
<td>CP group</td>
<td>Ad libitum</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group 4</td>
<td>PP group</td>
<td>-</td>
<td>-</td>
<td>Ad libitum</td>
</tr>
</tbody>
</table>

ACR- acrylamide; HS with ACR* - French fries fried in sunflower oil; HS with ACR** - sliced toast

Results and discussions

a. Number of thrombocytes (number of blood platelets)

The thrombocytes are cytoplasmatic fragments without a nucleus, rich in granules, involved in hemostasis and the initiation of the tissue repair processes and vasoconstriction after the vascular injury and during the inflammatory processes, the platelet adherence and aggregation the result of which is the formation of the platelet thrombus which plugs the rupture in the small vessels walls.

For the reference group, the analysis of the thrombocytes number has shown values within the limits of the reference range (minimum value 364 x 10^3/mm³, average value 402.25 x 10^3/mm³, maximum value 459 x 10^3/mm³) (fig. 1).

In comparison with the reference group’s values, the only group which presents a significant decrease from a statistic perspective (which is yet situated within the limits of the reference range) is the control group, to which the acrylamide solution 0.1 µg/L was supplied ad libitum. The average value of the platelets number is of 315.4 x 10^3/mm³ (p<0.05 in comparison with the reference group). The groups exposed to acrylamide intake by consumption of food present a slight decrease of the thrombocytes number, however not significant from a statistic
perspective (p>0.05 in comparison with the reference group).

![Fig. 1. Number of thrombocytes](image1)

**b. Mean platelet volume (MPV)**

The mean platelet volume shows the size uniformity of the thrombocyte population, being useful in the differential diagnosis of thrombocytopenia.

The reference group presents an average MPV value of 7.95 μm$^3$ and a dispersion of the values which is given by a standard deviation of 0.6244 and a relative standard deviation of 7.8% (fig. 2). In comparison with the reference group, the other three groups do not show a significant variation from a statistic perspective. Thus, the control group shows values comprised between 7.1 and 9.5 μm$^3$, with an average value of 8.4 μm$^3$ and a standard deviation of 1.0392, while the group which was exposed to the acrylamide intake by consumption of French fries, holds an average value of 8.08 μm$^3$ (SD – 0.4438, RSD – 0.0549, p>0.05 in comparison with the reference group). For the PP group the values of the mean platelet volume were comprised within the limits of the reference range, having an average value of 7.96 μm$^3$, almost identical to that of the reference group.

Therefore, the acrylamide intake during the 90 days did not lead to any modification of the mean platelet volume.

**c. Number of leukocytes (number of white blood cells, WBC)**

Leukocytes or the white blood cells (WBC) are mediators of inflammations and the immune response. There are different types of leukocytes which normally appear in the blood: neutrophils (PMN), eosinophils (*in Romanian “EO”*), Basophiles (BASO), lymphocytes (LYMPH) and monocytes (MONO). Leukocytes are divided in two main groups: granulocytes and nongranulocytes. Granulocytes are named as such due to the distinct granulations’ presence in the cytoplasm and three granulocytes types can be identified according to the coloring affinities on the Wright colored blood smear: neutrophils, eosinophils and basophiles. These cells are also called polymorphonuclear leukocytes due to the multilobular nucleus. The non-granulocytes which consist of lymphocytes and monocytes do not generally contain distinct cytoplasmatic granulations and have a non-lobular nucleus, also being named mononuclear leukocytes (Befus, 2004; Thomas, 1998; Watts, 2004).

![Fig. 2. Mean platelet volume](image2)

The hematologic test of the blood samples taken from the reference group has shown values situated on the lower side of the reference range, having an average value of 7.9 x 10$^3$/mm$^3$ and a dispersion which is given by a standard deviation of 0.7071 (fig. 3). The groups which were fed with acrylamide-rich food (CP and PP groups) do not show significant variations from a statistic perspective, in comparison with the reference group. However, for the control group higher values have been obtained in comparison with the reference group (p<0.05), values situated at the middle of the reference range. The values are characterized by the average value of 9.08 x 10$^3$/mm$^3$ and a standard deviation of 0.5675.
The increase of the leukocytes number is present in case of pathologic conditions such as microbial infectious diseases, tissue necrosis, presence of tumors, increased uremia, but the values obtained for the control group do not raise such issues.

d. Lymphocytes (LYM)

The lymphocytes represent a heterogeneous cellular population which is different according to the origin, life time, location on the level of the lymphoid organs and function. Although certain morphologic characteristics such as: size, granularity, the nuclear-cytoplasmatic relationship differentiate the lymphocyte populations between each other, they do not offer any information related to their type and function. Lymphocytes are items which intervene in a direct and specific manner in the immune reactions of defense against microorganisms.

For the reference group, the results of the hematologic test reveal values comprised within the reference range (1.2 – 3.2), with an average of 1.6 and a standard deviation of 0.4546. The rest of the experimental groups do not show significant variations of the lymphocytes number (p>0.05 in comparison with the reference group). If the group which was exposed to the acrylamide intoxication by the ad libitum consumption of acrylamide solution has an average lymphocytes value of 1.05 and a dispersion of the values which is given by a standard deviation of 0.3511, the values for the group of animals which was fed with French fries is situated within the 1.5 – 1.9 range, with an average value of 1.76 and a standard deviation of 0.5019 (fig. 4).

Monocytes (MON)

Monocytes are the largest cells in the blood; they are part of the mononuclear phagocytic system/reticuloendothelial system composed of monocytes, macrophages and their bone marrow forerunners. The monocytes are released into the blood and after a short circulation time, they migrate to different tissues, at random or in a specific manner, as a response to the various chemotactical factors. In the tissues, as a response to various soluble stimuli, they differentiate into tissue macrophages, having characteristic morphologic and functional qualities, a process which was named activation and which is reversible (‘deactivation’). The mononuclear phagocytic system’s cells are very primitive from a philogenetic perspective, no animal being able to live without them. They fulfill a wide variety of essential functions in the organism, including the removal of strange particles and senescent cells, dead or damaged, adjustment of other cells’ functions, processing and submittal of antigens in immune reactions, participation into various inflammatory reactions, destruction of bacteria and tumoral cells. Monocytes and macrophages produce numerous bioactive products: enzymes, complement factors, coagulation factors, reactive oxygen and azote species, angiogenesis factors, connection proteins (transferrin, transcobalamin II, fibronectin, apolipoprotein E), bioactive lipids (derivatives of the arachidonic acid), chemotactical factors, cytokines and growing factors (IFN α and γ, IL 1,3,6,8,10,12, FGF, PDGF, TNF, M-CSF).
For the reference group, the hematologic tests reveal an average monocytes value of 0.325, with a standard deviation of 0.1538 and a relative standard deviation of 5%. In comparison with the reference group, the control group presents a slight decrease of the monocytes number (minimum value – 0.2; average value – 0.28; maximum value 0.3), however not significant from a statistic perspective (p>0.05 in comparison with the reference group) (fig. 5). The values of the control group are below the lower limit of the reference range (0.3 – 0.8). For the group which was fed with French fries, the results are characterized by an average value of 0.3 and a standard deviation of 0.4082, while for the group which was fed with toast it presents an average value of 0.275, situated outside the reference range.

**f. Neutrophils (polymorphonuclear neutrophil granulocytes, GRA)**

They represent the most numerous type of leukocytes, with a major role in the organism’s primary anti-infectious defense, by the microorganisms’ phagocyte character and digestion. Their faulty activity may lead to lesions of the normal tissues of the organism by the release of enzymes and pyrogenous agents. Upon the occurrence of the infection, chemotactical agents are produced and they determine the migration of the neutrophils to the infection spot and the activation of their defensive functions, with the transformation into phagocyte of the respective agent, followed by the release of granules in the phagocytosis vesicle and the destruction of the infectious agent. This effect is frequently associated with the increase of the neutrophils production and release into the bone marrow.

The neutrophils values for the reference group are comprised between the 1.6 – 1.8 range and are characterized by an average value of 1.7 and a standard deviation of 0.0816 (fig. 6). The control group, exposed to the intoxication by consumption of acrylamide solution 0.5 μg/L, presents higher values, comprised within the 1.6 – 2.6 range and characterized by a dispersion which is given by a standard deviation equal to 0.4394. However, the increase of the neutrophils values for this group is not significant from a statistic perspective (p>0.05 in comparison with the reference group). The group which was fed with French fries presents a higher dispersion of the values, represented by a standard deviation equal to 0.6814. The variation of the values is in this case too insignificant from a statistic perspective, as compared to the reference group. Finally, the group which was fed with toast presents the lowest average value (1.375). However, the decrease is not important from a statistic perspective.

**Fig. 5. Monocytes values**

**Fig. 6. Granulocytes values**

**Conclusions**

1. In terms of the thrombocytes number, the only group which shows a significant decrease from a statistic perspective is the control group, to which the 0.5 μg/L acrylamide solution was supplied ad libitum. The groups exposed to acrylamide intake by consumption of food present a slight decrease of the thrombocytes number, however not significant from a statistic perspective.
2. In the case of the mean platelet volume, the groups exposed to the chronic acrylamide concentration do not show any significant variation from a statistic perspective. Therefore, the acrylamide intake during the 90 days did not lead to the modification of the mean platelet volume.

3. The groups which were fed with acrylamide-rich food (French fries and toast) do not show any significant variations from a statistic perspective in comparison with the reference group in regard to the number of leukocytes. However, for the control group higher values were obtained, in comparison with the reference group.

4. In the case of lymphocytes, monocytes and neutrophils, both the control group and the groups which were fed with French fries and toast do not show any value modification, significant from a statistic perspective, as compared to the reference group.

Acknowledgement:
This paper was supported by the project PERFORM-ERA "Postdoctoral Performance for Integration in the European Research Area" (ID-57649), financed by the European Social Fund and the Romanian Government.

References