THE INFLUENCE OF THE EXPERIMENTAL STRESS ON HOMOCYSTEINE PLASMA LEVELS IN RAT

Elena Albu¹, Cristina Filip², Nina Zamosteanu², Irina M. Jaba¹, Nastasia Gheorghita², Luminita Jerca², O. C. Mungiu²

¹ Department of Pharmacology - Algesiology, Faculty of Medicine and Pharmacy, “Grigore T. Popa” University of Medicine and Pharmacy Iaşi
² Department of Biochemistry, Faculty of Medicine and Pharmacy, “Grigore T. Popa” University of Medicine and Pharmacy Iaşi

Abstract. Nowadays it is generally accepted that in the aging processes an important role is played by stress. The main mechanism through which stress is involved in the aging processes seems to be the reactive species’ generation. On the other hand, it is also known that hyperhomocysteinemia is a risk factor in cardiovascular diseases, as it disturbs the normal endothelium functions and generates thrombosis. The mechanism through which homocysteine triggers these effects is not yet clarified, but it is believed that a possible explanation is the reactive species’ involvement. In our work we have studied the influence of experimentally induced stress on homocysteine levels, in rats. Experimental stress was induced by reversing the normal day/night cycle (1800 – 600 light). The activities of intracellular superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant status (TAS) were measured in order to establish if stress by itself generates reactive species. The plasmatic concentrations of homocysteine were also measured. Our data show an increase in SOD activity, simultaneously with the decrease in GPx activity. The total antioxidant status and homocysteine levels have registered no significant changes. In conclusion, subacute stress activated the antioxidant defense systems, but did not influence the total antioxidant capacity and homocysteine levels, in rats.

Keywords: hyperhomocysteinemia, stress, total antioxidant status

Introduction

Nowadays it is generally accepted that in the aging processes an important role is played by stress. The main mechanism through which stress is involved in the aging processes seems to be the reactive species’ generation.

On cellular level, the oxidative stress is equivalent to significant decreases of the cells’ capacity of detoxifying reactive species through reduction reactions [1]. Cells can normally defend themselves against reactive species using enzymatic and non-enzymatic systems. Superoxide-dismutase, catalase, glutathione peroxidase and peroxiredoxine are efficiently used by cells to annihilate the reactive oxygen species (ROS). Among small molecules, ascorbic acid (vitamin C), tocopherol (vitamin E) and glutathione play important roles as intracellular antioxidant structures. By contrast, the extracellular space has a much lower antioxidant capacity, uric acid being perhaps the most important antioxidant molecule.

The effects of oxidative stress depend on its intensity, as such cells can overcome mild stress and return to normal state, or under higher or prolonged stress they can trigger apoptosis or even cellular necrosis [2].
On the other hand, it is also known that hyperhomocysteinemia is a risk factor in cardiovascular diseases as it disturbs the normal endothelium functions and generates thrombosis even at slightly increased concentrations. [3-5].

The mechanism through which homocysteine triggers these effects is not yet clarified, but it is believed that a possible explanation is the reactive species’ involvement.

In our work we have studied the influence of experimentally induced stress on homocysteine levels, in rats.

The experimental stress was induced by reversing the normal day/night cycle (18:00–6:00 light). The activities of intracellular superoxide dismutase (SOD), glutathione peroxidase (GPx) and serum total antioxidant status (TAS) were measured in order to establish if stress by itself generates reactive species. Plasmatic concentrations of homocysteine were also measured in order to establish if there is a direct correlation between these two risk parameters: stress and hyperhomocysteinemia.

Our data show an increase in SOD activity, simultaneously with the decrease of GPx activity. The total antioxidant status and homocysteine levels have registered no significant changes. In conclusion, subacute stress activated the antioxidant defense systems, but did not influence the total antioxidant capacity and homocysteine levels, in rats.

**Material and Method**

We have worked on 10 Wistar adult rats weighting 150-200 g. Stress was induced to all animals in the two series by reversing the normal day/night cycle (18:00–6:00 light) for 15 days. The two series received different food regimens as follows:

**Series I** received food without folic acid and vitamin B₁₂ supply. The food for series I consisted of: apple 5g/100g animal, bread 10g/100g animal, barley 10g/100g animal, for all 15 days.

**Series II** received standard animal food consisting of: folic acid 0.5 mg/kg b.w. and vitamin B₁₂ 10 mg/kg b.w. for all 15 days.

Blood samples were collected before and after the 15 days of induced stress in order to estimate the oxidative stress as well as homocysteine levels.

Superoxide dismutase and glutathione peroxidase activity were determined in red blood cells using the Randox test for manual assay. The total antioxidant status was determined in plasma also through Randox manual tests. Total homocysteine was determined in plasma using the Dyazime test.

**Results and Discussions**

Homocysteine levels determined before and after 15 days of induced stress are presented in table I.

<table>
<thead>
<tr>
<th>Homocysteine plasma concentration (μM/ml)</th>
<th>Before stress exposure</th>
<th>After 15 days of stress exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series I (low vitamins food + induced stress)</td>
<td>18.21</td>
<td>16.72</td>
</tr>
<tr>
<td>Series II (standard vitamins food + induced stress)</td>
<td>16.80</td>
<td>19.39</td>
</tr>
</tbody>
</table>

**Table I.** Homocysteine concentrations determined in rats’ plasma before and after 15 days of stress exposure

Homocysteine plasma levels determined after 15 day of induced stress were not significantly modified, as compared with the initial moment. The series II presented a mild (not statistically significant) increase in homocysteine level, despite the fact that it received standard vitamins food. It is known that vitamin supplementation is one way of decreasing homocysteine levels in humans, and we would have expected homocysteine levels to remain the same as in the beginning of the experiment. Our data show that there is no correlation between stress and homocysteine levels. The determined antioxidant status in rats’ plasma is presented in table II.

After 15 day of stress exposure there were no significant changes in TAS levels in series I, meanwhile in series II there was a small TAS decrease. We suppose that subacute stress did not affect the total antioxidant capacity.

The superoxide dismutase and glutathione peroxidase activities, determined in red blood cells, are presented in table III and IV, respectively.

The registered data show that in both series I and II, SOD activity significantly increased as a consequence of stress exposure. We consider that...
stress exposure generates higher amounts of reactive species, which triggered SOD activity to rise as response to their presence.

The obtained data show that, in both series, I and II, SOD activity significantly decreased as a consequence of stress exposure.

It is known that glutathione peroxidase is an enzyme which uses glutathione as main cofactor for its activity. In this regard, we assume that the decrease of glutathione peroxidase activity is a consequence of the diminished amount of glutathione. Glutathione is a small molecule which detoxifies the H₂O₂ relying on its SH group. The increased amount of reactive species, generated by stress exposure, consumed the cellular deposits of glutathione, causing an important decrease of its concentration and of GPx activity.

Our data show a general activation of the systems involved in antioxidant defense. The fact that TAS remains almost unchanged, indicates that stress exposure does not exhaust the intra- and extracellular antioxidant capacity against reactive species.

**Conclusions**

The results described above indicate that stress exposure does not determine an increase in homocysteine levels. Even under conditions when certain systems in antioxidant defense are activated, the total antioxidant capacity does not suffer major changes.

Standard vitamins’ foods regimens seem to have no influence in homocysteine levels.

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The influence of the experimental stress on homocysteine plasma levels in rat

References


