Oxidant/Antioxidant Balance in Carnitine Supplemented Rats Exposed to Chronic Hypothermic Stress

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Abstract. Chronic stress is a major factor that contributes to several disorders. A common animal model, performed in order to evaluate the environmental experimentally-induced-stress is hypothermia. L-Carnitine is a natural substance required in the energetic metabolism of the mammals, which also has an antioxidant function. The aim of the present study was to evaluate the effect of chronic hypothermic stress on oxidant/antioxidant balance in the serum and tissues, (liver and muscle), on rats with and without carnitine supplementation. Rats were divided randomly into four groups of ten rats each: control group (I); hypothermic stress (II) - exposed to cold stress; supplemented with carnitine (III) supplemented with carnitine and exposed to hypothermic stress (IV). Our experimental results showed that chronic hypothermic stress increases the oxidative stress (OS) indicators in serum and tissues and decreased those of antioxidant (AO) defense. Carnitine supplementation in chronic hypothermic stress conditions had benefic effects on OS and AO defense indicators, this compound being likely to have favorable effects in an experimental hypothermic stress model.

Keywords: chronic hypothermic stress, oxidants/antioxidants, serum, tissues, carnitine

INTRODUCTION

Stress is one of the most debated scientific concepts in terms of definitions, terminology, theories, types and forms, body’s response, adaptive resources, methods of prevention and control. Stress-related research has been interested on the identification and characterization of tissue damage associated with various stressful conditions, understanding the mechanisms of response to stress and the implications of different therapeutic modalities. There are several types of stress: physical, environmental, psychosocial, professional, metabolic, psychosomatic, somatopsychic, psycho-emotional, prenatal, postnatal, cultural.

There are multiple animal models, mainly in rodents, that have evaluated the environmental experimentally induced stress. They depend on different stressful factors, such as: temperature fluctuations, noise, vibrations, radiation, pollution, gravity, immobilization/restraint stress, hypo-or hyperbaria, hypoxia and hyperoxia; oxidative stress, destruction of various parts of the brain, electrical shocks induced to the limbs, forced swimming, exercise, bleeding, neonatal isolation, social isolation, contact with the predator or its’ odor, exposure to a new, unknown, environment, hinlimb, changes related to the circadian rhythm, food, water and sleep deprivation, repeated intradermal shots with increased volumes of fluid, handling of the laboratory animals, their transport (Bhatia et al, 2011, Derevenco et al, 1992, Le Bars et al, 2008, Moberg and Mench, 2000, Sutanto and de Kloet, 1994,)

Hypothermia, as a therapeutic method, is recommended in the following situations: unconscious adult patients with spontaneous circulation after cardiac arrest, occurred outside
the hospital or in the hospital unit (Gupta et al., 2002), heart and brain surgery, offering protection from neurological implications of cerebral ischemia, occurred during surgery, traumatic brain injury (Gupta et al., 2002, Jiang et al., 2006), subarachnoid hemorrhage (Salerian, 2008), acute ischemic strokes, improving neurological damage (Bernard, 2004), transient hypoxic-ischemic damage in infants, spinal cord injury, as a protective agent against motor deficit, decreasing the number of destroyed neurons, effects that have been identified on animals (Wartenberg and Mayer, 2008), hepatic encephalopathy and epilepsy (Luscombe and Andrzejowski, 2006), control of fever (Polderman, 2008).

Carnitine is a quaternary ammonium compound, biosynthesised mainly in the liver and kidney, from the amino acids lysine and methionine. In humans, carnitine was detected in all cells and body fluids in different concentrations; more than 95% of total content of carnitine can be found in the skeletal muscle (Brass, 1995, Scholte, 2003, Stephens et al., 2007, Şiktar, 2009). The animal sources of carnitine are red meat and dairy products.

Other natural sources of carnitine are: nuts, pumpkin, sunflower and sesame seeds, the vegetables, beans, broccoli, garlic, fruits (apricots, bananas), corn, wheat germ, rice, etc. L-Carnitine is a natural substance required in the energetic metabolism of the mammals, but it also has an antioxidant role (Calò et al., 2006, Augustyniak et al., 2009, 2010).

The aim of the present study was to evaluate the effect of chronic hypothermic stress on oxidant/antioxidant balance in the serum and tissues, (liver and muscle), on rats with and without carnitine supplementation.

MATERIALS AND METHODS

The study was performed on adult male rats, Wistar breed, at the Department of Physiology from UMF "Iuliu Hațieganu", Cluj-Napoca, in the Laboratory of Experimental Physiology. The animal tests and experiments were allowed by the Bioethical Board of the UMF "Iuliu Hațieganu", Cluj-Napoca. The animals were caged in polycarbonate cages, at controlled temperature of 21-22°C, humidity (40-60%) and 12/12h light/dark cycle. Standard lab chow, and water were freely available.

Cold stress was applied to the animals for 3 hours daily, for 15 days long. The rats were placed in a cold room (ambient temperature 5°C), according to the literature data.

The animals were daily supplemented with L-Carnitine by oropharyngeal gavage, before exposure to stress, (Carnil 100 mg/ml, provided by Anfarm Hellas S.A. Pharmaceutical Industry Factory, Athens, Greece). Each animal received 100 mg/kg L-Carnitine, calculated according to daily dosage for humans. Rats were divided randomly into four groups of ten rats each: control group (I), hypothermic stress (II), supplemented with carnitine (III), supplemented with carnitine and exposed to hypothermic stress (IV). At the end of the experimental period, blood was collected from the retro orbital sinus, liver and gastrocnemius muscle were removed immediately. Euthanasia was induced according to the recommendation of the Bioethical Board of the University. Blood samples were immediately centrifuged at 4°C, then plasma was frozen at -20°C and then kept at deep freezer. Tissues were minced and homogenized and the supernatant was used to determine the level and activity of the oxidative stress (OS) indicators – MDA (Conti, 1991), (Reznick and Packer, 1994) and antioxidant (AO) system – DH (Janaszewska and Bartosz, 2002), SH (Hu, 1994), GSH (Hu, 1994).

All data are reported as the mean ± SD. Statistical analyses were performed by one-way analysis of variance ANOVA, followed by post hoc Tukey’s range test procedure, for pair-wise
comparisons. Pearson’s correlation was the test of choice, in order to assess the correlation between normally distributed variables. Statistical significance was at p<0.05. Statistical values were obtained using GraphPad Prism 5.0 software, and Microsoft EXCEL.

RESULTS AND DISCUSSIONS

Tab. 1

Statistical indicators for centrality and dispersion in serum

<table>
<thead>
<tr>
<th>Lot</th>
<th>MDA (nmoli/ml)</th>
<th>PC (nmoli/ml)</th>
<th>DH (inhib%)</th>
<th>SH (µmoli/ml)</th>
<th>GSH (nmoli/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MA ±SD</td>
<td>MA ±SD</td>
<td>MA ±SD</td>
<td>MA ±SD</td>
<td>MA ±SD</td>
</tr>
<tr>
<td>I</td>
<td>2.10 ±0.20</td>
<td>1.05 ±0.09</td>
<td>12.81 ±1.40</td>
<td>0.28 ±0.027</td>
<td>9.10 ±0.82</td>
</tr>
<tr>
<td>II</td>
<td>2.91a ±0.236</td>
<td>1.39a ±0.147</td>
<td>9.62a ±0.94</td>
<td>0.161 ±0.020</td>
<td>7.47a ±0.729</td>
</tr>
<tr>
<td>III</td>
<td>2.18 ±0.19</td>
<td>1.13 ±0.16</td>
<td>12.21 ±1.42</td>
<td>0.29 ±0.025</td>
<td>9.13 ±0.64</td>
</tr>
<tr>
<td>IV</td>
<td>2.42b ±0.098</td>
<td>1.21b ±0.15</td>
<td>11.26b ±1.37</td>
<td>0.29b ±0.028</td>
<td>9.31b ±0.80</td>
</tr>
</tbody>
</table>

Note: control group (I), hypothermic stress (II), supplemented with carnitine (III), supplemented with carnitine and exposed to hypothermic stress (IV). ANOVA test, p<0.05.  a= II vs I; b=IV vs II

The statistical analysis, performed on four groups, revealed that chronic hypothermic stress induced significant increases for OS indicators (MDA, PC) in serum of the hypothermic stress group (II) as compared to control group (I).

The antioxidant defense indicators (DH, SH, GSH) in the serum of chronic hypothermic stress were lower than in controls. Carnitine supplementation in chronic hypothermic stress conditions (group IV) induced significant changes by diminishing the OS indicators and increasing AO defense indicators as compared to hypothermic stress group (II) (Tab 1).

Tab. 2

Correlation indicators for O/AO balance in serum, at the end of the experiment (n=40)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pearson Correlation Coefficient</th>
<th>p</th>
<th>Parameters</th>
<th>Pearson Correlation Coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA PC</td>
<td>0.57***</td>
<td>&lt;0.0001</td>
<td>PC DH</td>
<td>-0.57***</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MDA DH</td>
<td>-0.66***</td>
<td>&lt;0.0001</td>
<td>PC SH</td>
<td>-0.56***</td>
<td>0.0001</td>
</tr>
<tr>
<td>MDA SH</td>
<td>-0.71***</td>
<td>&lt;0.0001</td>
<td>PC GSH</td>
<td>-0.45**</td>
<td>0.003</td>
</tr>
<tr>
<td>MDA GSH</td>
<td>-0.51***</td>
<td>0.0006</td>
<td>DH SH</td>
<td>0.57***</td>
<td>0.0001</td>
</tr>
<tr>
<td>SH GSH</td>
<td>0.71***</td>
<td>&lt;0.0001</td>
<td>DH GSH</td>
<td>0.45***</td>
<td>0.003</td>
</tr>
</tbody>
</table>

* weak correlation,** acceptable correlation,*** good correlation,**** very good correlation (Colton Scale)

There were negative correlations between OS and AO defense indicators (Tab. 2).
The chronic hypothermic stress induced increases of the OS and decreases in the AO defence indicators (DH and GSH) in the studied tissues (liver and muscle). In terms of thiol groups, they showed a significant decrease in muscle only. Carnitine supplementation revealed protective effects in chronic hypothermic stress by reducing the level of tissues MDA, whereas PC was lower only in the liver. Carnitine supplementation increased the levels of AO defense indicators of the chronic hypothermic stress, as it follows: DH in both examined tissues, while SH and GSH showed significant changes in the muscle only (Tab 4).
There were positive correlations between the liver OS indicators, but negative correlations between OS and AO defense indicators (DH and GSH). The AO defense indicators (DH and GSH) showed a positive correlation (Tab 5).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pearson Correlation Coefficient</th>
<th>p</th>
<th>Parameters</th>
<th>Pearson Correlation Coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA PC</td>
<td>0.39**</td>
<td>0.057</td>
<td>PC DH</td>
<td>-0.70***</td>
<td>0.0001</td>
</tr>
<tr>
<td>MDA DH</td>
<td>-0.76****</td>
<td>&lt;0.0001</td>
<td>PC SH</td>
<td>-0.41***</td>
<td>0.04</td>
</tr>
<tr>
<td>MDA SH</td>
<td>-0.68***</td>
<td>0.0002</td>
<td>PC GSH</td>
<td>-0.84****</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MDA GSH</td>
<td>-0.63***</td>
<td>0.0007</td>
<td>DH SH</td>
<td>-0.61***</td>
<td>0.001</td>
</tr>
<tr>
<td>SH GSH</td>
<td>0.59***</td>
<td>0.002</td>
<td>DH GSH</td>
<td>0.84****</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* weak correlation, ** acceptable correlation, *** good correlation, **** very good correlation (Colton Scale)

Pearson correlation coefficient showed that, there were negative correlations between the OS and AO defense indicators. The AO defense indicators were positively correlated one to each other (Tab 6). ROS increases lipid peroxidation, influences membrane solubility and the membrane enzyme function. In the literature, many results show that exposure to cold induces OS in different tissues, but there are also contradictory opinions related to this subject (Alva, 2010).

Sometimes, the differences depend on the species, the tissue that is examined, the duration of exposure to cold. Generally, short periods of exposure to hypothermic stress are not able to induce tissue oxidative alterations (Venditti, 2010). The mechanism that makes hypothermia to induce oxidative modifications of plasma proteins is not completely understood.

Serum biochemical changes in terms of hypothermic stress are relatively few in the literature. The presented data have indicated that moderate hypothermia induces the generation of ROS, as confirmed by increased levels of PC in plasma and tissues. (Klichkhanov, 2001).

An in vitro study has showed favorable effects of L-carnitine added to plasma, in terms of oxidative and nitrosative denaturation of plasma proteins induced by peroxynitrite (ONOO-) (Kolodziejczyk, 2011). Exposure to hypothermia, for longer periods of time, induces OS in the liver and small intestine, heart, kidney, thymus, adrenals and lungs of the animals. Some reports indicate that OS occurs in rats even in other tissues, such as: brain, brown adipose tissue and skeletal muscle, although some researchers have not shown such results.

A possible explanation for these discrepancies may depend on the accuracy of the methods used to determine lipid peroxidation. However, the information available in the literature, shows that low temperatures induce OS in various animal tissues (Kaushik and Kaur, 2003, Kolosova, 1995, Tnimov, 1984, Venditti, 2004, Venditti, 2006, Venditti, 2010,).

As it concerns the AO systems of liver tissue, exposure to cold causes a decrease in AO defense by total SOD, CAT and GSH, and an increase in AO defense by GSH-Px and vitamin E (Venditti, 2010, Zlatković and Filipović, 2011). It seems that cold-induced changes of hepatic AO system does not follow a clear, consistent pattern. In vitro studies, sustain the lack of the total antioxidant capacity modification in the liver after 2 days of exposure, but after 10 days of exposure there was a decrease of it (Venditti, 2004). These results emphasize the idea that individual changes of AO system components can reduce the overall efficiency of AO in the
liver, in stress conditions induced by cold. The decrease of the AO capacity underlines the increased activity of free radicals, that induce oxidative destruction of proteins and lipids. Exposure to cold seems to increase the unsaturated PUFA and Fe\(^{2+}\), which promotes oxidative processes and it may be possible for the liver subjected to cold to be more sensitive to oxidants and to be more easily affected by oxidative stress (Venditti, 2010).

Vitamin E has had protective effect on skeletal muscle of rats exposed to low temperatures, demonstrating the AO effect of the compound, evidenced by reduced levels of hydroperoxides and PC in the muscle, and increased values of the GSH in the tissues (Venditti, 2009). Acute intraperitoneally administration of carnitine improved total AO activity in the brain (Tsakiris, 2008).

**CONCLUSIONS**

Chronic hypothermic intermittent stress determines alterations of oxidant/antioxidant balance in serum and tissues by increasing the OS and by reducing the AO defense indicators.

Carnitine supplementation, in groups exposed to chronic hypothermic stress, has protective effects, described by decreased levels of the OS and increased levels of AO indicators, in serum and tissues.

**REFERENCES**