Antioxidant Effects of Polyphenols in Experimental Hypobaric Hypoxia

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Abstract. Hypoxia/ischemia and reperfusion is associated to increased production of reactive oxygen species, hence the attempts to use antioxidants compounds in order to combat their deleterious effects. The aim of this study was to evaluate the antioxidant effects of a red grape seed extract in experimental hypobaric hypoxia. Rats were divided in 4 groups: 1 control group and 3 groups exposed for 24 hours to hypobaric hypoxia 5500 m simulated altitude. One of these groups was given grape seed extract before exposure and one group was given immediately after exposure. Oxidative stress parameters and antioxidant enzymes were measured in both plasma and brain homogenate. We found that hypobaric hypoxia exposure inhibits the activity of superoxide dismutase and catalase in brain and increases oxidative stress parameters. Polyphenols do not seem to influence the activity of antioxidant enzymes in the brain.

Keywords: oxidative stress; polyphenols; grape seed extract; hypobaric hypoxia.

INTRODUCTION

Oxygen is indispensable for aerobe organisms including humans. On the other hand, it is the source of several destructive and noxious forms, known as reactive oxygen species (ROS). Whenever ROS production is enhanced or the antioxidant systems of the body are over passed oxidative stress occurs. Over the years, it has been shown that oxidative stress is involved in a large number of both physiologic and pathologic conditions, many of them with clinical involvement (Benz and Yan, 2008; Lubos et al., 2008; Ceriello, 2008). Therefore, there seem justified the numerous attempts to use some antioxidant compounds in processes in which oxidative stress is either a cause or aggravates the disease (Seifried, 2007).

Experimental or cardiovascular, renal, respiratory diseases associated acute or long term hypoxia produces vascular, hemodynamic, metabolic and cellular changes associated to increased levels of ROS (Millar et al., 2007; Legrand et al., 2008).

The aim of this study was to estimate the effects of a red grape seed extract on oxidative stress parameters and on some antioxidant enzymes in rats exposed to hypobaric hypoxia.

MATERIALS AND METHODS

Experimental model: 40 male Wistar rats, weighing 180± 10 g, were used in the experiment. They were housed at a room temperature of 23±2°C, with a 12/12 hours light/dark cycle, with food and water ad libitum. They were allowed to acclimate for 1 week prior to experimental procedures. The animals were divided in 4 groups: group I controls.
(n=10), group II (n=10): animals exposed to hypobaric hypoxia, group III (n=10): animals exposed to hypobaric hypoxia and which were given 1 dose red grape seed extract before exposure, group IV (n=10): animals exposed to hypobaric hypoxia for 24 hrs and which were given 1 dose red grape seed extract right after exposure. 1 hour after bringing the animals to sea level, blood samples were collected and the plasma quickly separated and frozen at -80°C until used. The animals were sacrificed; brains were collected and kept at -80°C until used.

Hypobaric hypoxia exposure was achieved by keeping the animals for 24 hrs in a baric chamber, 5500 m simulated altitude.

The red grape seed extract (GSE), prepared as described elsewhere (Postescu et al., 2007), was administered by gavage, in a dose of 50 mg gallic acid equivalents/kg body weight.

This investigation conforms to local guidelines for animal research and was approved by the Ethics Committee of “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj.

Biochemical analyses

As oxidative stress parameters in plasma and brain homogenate we determined the levels of malondialdehyde (MDA), which is a product of decomposition of oxidized lipids, and of protein carbonyls (PC), which result from interaction of free radicals with proteins. MDA was measured by mean of thiobarbituric spectrophotometric method (Esterbauer and Cheeseman, 1994) and was expressed as nmol/ml. PC assay was performed by dinitrophenylhydrazine method (Reznick and Packer, 1994) and was expressed as nmol/mg protein.

The activity of the following antioxidant enzymes was also measured in both erythrocyte lysate and brain homogenate: superoxide dismutase (SOD) (McCord and Fridovich, 1969), catalase (CAT) (Pippenger et al., 1998) and glutathione peroxidase (GPx) (Flohe and Gunzler, 1984).

Statistical analysis: results are expressed as mean values. After assessing the normality of data the Student “t” test was used for comparing the groups. The statistical software package SPSS 13.0 was used for all data analysis.

RESULTS

Oxidation parameters

Table I presents the values of oxidative stress parameters. Hypobaric hypoxia increases the level of MDA and tends to increase the level of PC in brain homogenate. GSE administered before exposure decreases both MDA and PC levels in brain homogenate.

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Plasma MDA nmol/ml</th>
<th>Brain MDA nmol/mg protein</th>
<th>Plasma PC nmol/mg protein</th>
<th>Brain PC nmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.25±0.3</td>
<td>0.04±0.01</td>
<td>0.9±0.1</td>
<td>1.75±0.25</td>
</tr>
<tr>
<td>II</td>
<td>1.7±0.2</td>
<td>0.12±0.1*</td>
<td>1.0±0.05</td>
<td>2.3±0.15*</td>
</tr>
<tr>
<td>III</td>
<td>1.1±0.1</td>
<td>0.06±0.03*</td>
<td>1.3±0.2</td>
<td>1.24±0.05**</td>
</tr>
<tr>
<td>IV</td>
<td>1.55±0.3</td>
<td>0.11±0.01</td>
<td>1.15±0.1</td>
<td>1.83±0.1</td>
</tr>
</tbody>
</table>

Antioxidant enzymes

As shown in Fig. 1, hypobaric hypoxia exposure decreases SOD activity in erythrocyte lysate. GSE, administered both before and after exposure, increased the SOD activity.
As shown in Fig. 2, erythrocyte CAT activity is increased when the GSE is administered before hypoxia. After exposure administration has no effect on CAT activity.

As for GPx in erythrocyte lysate, its activity increases significantly when the GSE is administered after exposure (Fig. 3), while in brain homogenate GSE depresses it in both timings (Fig. 4).

Fig. 1. Superoxide dismutase in erythrocyte lysate
Fig. 2. Catalase in erythrocyte lysate

Fig. 3. Glutathione peroxidase erythrocyte lysate
Fig. 4. Glutathione peroxidase in brain lysate

Fig. 5. Superoxide dismutase in brain lysate
Fig. 6. Catalase in brain lysate
Both SOD and CAT activity in the brain are decreased by hypobaric hypoxia (Fig. 5, Fig. 6). GSE administered before exposure decreases even more the SOD activity in the brain (Fig. 5). GSE after exposure increases the CAT activity in the brain (Fig. 6).

DISCUSSIONS

The main types of hypoxia are: hypoxic, anemic, circulatory and hystotoxic. Ischemia and/or hypoxia lead to tissue necrosis unless the blood flow is rapidly reestablished. Therefore, reperfusion represents the chance for survival, but it is accompanied by suplementary injuries due to increased ROS production (McMichael and Moore, 2004).

Increased values of brain MDA and PC in animals exposed to hypobaric hypoxia in our study are a consequence of ROS action on lipids and proteins. We also found a decreased activity of SOD and CAT in erythrocyte lysate and brain homogenate of animals exposed to hypoxia. Other studies have shown that high altitude exposure results in increased formation of reactive oxygen and nitrogen species (RONS), which causes oxidative damage to lipids, proteins and DNA (Maiti et al., 2006). Another finding of our study was that the activities of SOD and CAT decreased in brain homogenates after 24 hours exposure to hypobaric hypoxia, while GPx activity did not change. Imaizumi and coworkers also reported low levels of antioxidant enzymes in rat brain at 3,6 and 24 hours after hypoxia, concluding that SOD and CAT depletion indicate a certain vulnerability toward reperfusion (Imaizumi et al., 1994).

Polyphenols are secondary plant metabolites found in foods and beverages that act directly as antioxidants by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. They also inhibit of the redox-sensitive transcription factors, the "pro-oxidant" enzymes and induce phase II and antioxidant enzymes (Frei and Hingdon, 2003; Scalbert et al., 2005).

In our study, GSE administration prior to hypobaric hypoxia exposure significantly improved both SOD and CAT activities in the erythrocyte lysate, but did not change their activities in the brain. By contrary, GSE administration immediately after exposure prevented the decrease of both enzymes activity. On the other hand, only when GSE is administered before exposure, it effectively manages to decrease oxidative stress parameters in the brain. Other studies also have shown that black or green tea polyphenols prevented decreased activity of SOD during carcinogen exposure (Das et al., 2002) or infections (Guleria et al., 2002).

CONCLUSIONS

Hypobaric hypoxia exposure inhibits the activity of SOD and CAT in brain and increases oxidative stress parameters. GSE administration improves SOD, CAT and GPx activity in erythrocytes lysate. GSE administered before exposure decreases both MDA and PC levels in brain homogenate.

Polyphenols do not seem to influence the activity of antioxidant enzymes in the brain.
REFERENCES