BIOCHEMICAL AND MORPHOFUNCTIONAL ASPECTS OF ARONIA MELANOCARPA EXTRACT INTERVENTION IN EXPERIMENTAL ARTERIAL HYPERTENSION

Manuela Ciocoiu¹, Anca Miron², Codruta Badescu³, Anca Morosanu¹, Oana Badulescu¹, Magda Badescu¹

UNIVERSITY OF MEDICINE AND PHARMACY “GR. T. POPA” IASI, ROMANIA
1 - DEPARTMENT OF PATHOPHYSIOLOGY, FACULTY OF MEDICINE
2 – DEPARTMENT OF PHARMACOGNOSY, FACULTY OF PHARMACY
3- II- nd MEDICAL CLINIC, FACULTY OF MEDICINE
4- PhD STUDENT, DEPARTMENT OF PATHOPHYSIOLOGY, FACULTY OF MEDICINE

Summary

Various epidemiological studies have shown an inverse association between the consumption of polyphenols or polyphenol-rich foods and the risk of cardiovascular diseases. The purpose of the study was to emphasize the effects of the polyphenolic extract from the isolated and purified vegetable material represented by the mature fruit of the Aronia melanocarpa on biochemical parameters and histopathological modifications. The experiment was performed on the arterial hypertension model. The significant lipid peroxide diminution in the serum contained by the HTA+P group compared to the P group is a result of a considerable reduction in the MDA serum concentration. The antioxidant capacity of the serum is significantly improved (p<0.001) in the HTA+P rats, as well as the GSH concentration being normalized. In the arterial hypertensive model the cardio-protective effects of the polyphenolic extract from Aronia melanocarpa are represented by the antioxidant, hypocolesterolemiant intervention. The extract also ensures the integrity of the vascular endothelium. Any clinical applications using polyphenolic extract should rely on an accurate understanding of the action mechanisms that are relevant.

Key words: Arterial hypertension, Aronia melanocarpa, oxidative stress.

Introduction

Polyphenols are organic compounds synthesized by plants, including tannins, lignans and flavonoids. Isoflavones are flavonoid compounds with both antioxidant and estrogenic properties, such as the soybean isoflavones genistein and daidzein which can behave as estrogen mimics (Setchell, 1998).

Polyphenols vary strongly in their absorption and distribution. They show high affinity for different structures and may therefore be able to decrease oxidative damage mainly at such particular sites (Yao et al., 2004). On the other hand, since polyphenols are redox active compounds they may also cause increased radical formation if they uncouple electron pathways in the body or if they chelate transition metals in such a way that they become more reactive like in the experimental Fenton oxidation systems.

More evidence for a protective role of polyphenols against cardiovascular diseases arose from a number of clinical trials (Pascual-Teresa and Sanchez-Ballesta, 2008), experiments on animal models and mechanistic studies (Mennen et al., 2004). Various epidemiological studies have shown an inverse association between the consumption of polyphenols or polyphenol-rich foods and the risk of cardiovascular diseases (Bell and Gochenaur, 2006).

The purpose of the study was to emphasize the effects of the polyphenolic
extract from the isolated and purified vegetable material represented by the mature fruit of the *Aronia melanocarpa* on biochemical parameters and histopathological modifications. The experiment was performed on the arterial hypertension model.

**Material and methods**

Ripe berries of *Aronia melanocarpa* Michx. (Rosaceae, chokeberry) were shade-dried at room temperature for one week. Dried chokeberries (100 g) were chopped into small pieces and extracted with 3 x 700 ml ethanol using a magnetic stirrer (FALC F30ST), each time for 3 h. The combined extracts were taken to dryness by evaporation under reduced pressure (BÜCHI R-210 rotavapor, BÜCHI V-850 vacuum controller, BÜCHI V-700 vacuum pump). Total phenolics quantification was performed by Folin-Ciocalteu method. The absorbance was measured at 765 nm after 2 h of incubation at room temperature. A calibration curve was plotted using gallic acid as standard. The total phenolic content was expressed as mg gallic acid equivalents/g extract. Sample was assayed in triplicate and the results were given as the mean ± standard deviation.

The research was performed on Wistar white rats, with an average weight of 250-280 g, which were divided into 4 groups of 12, namely: - Group W - control, normal animals, that didn’t receive natural polyphenols; - Group HTA - animals which were administered L-NAME 40 mg/kg body/day, i.p., at every 2 days, for 8 weeks; - Group P – animals that were administered polyphenols under the form of solution, from the extract obtained from the *Aronia melanocarpa* fruit, with a dosage of 0.040 g/Kg body, p.o. (by tube feeding), at every 2 days, for 8 weeks; - Group HTA+P – animals which were administered polyphenols in the dosage mentioned p.o. at every 2 days, concomitantly with L-NAME, for 8 weeks.

The experimental study fulfils all the requirements of the guide regarding the use of laboratory animals and biological preparations issued by the International Society of Pain Study (IASP) and the European Council Committee (86/609/EEC).

**Reduced glutathione (GSH)** was also determined by the Beutler method (Beutler *et al.*, 1990), through the use of 5,5' ditio-bisnitro-benzoic acid (DTNB) and was expressed in μg GSH/mg protein or g Hb in erythrocyte. The **malondialdehyde** (**MDA**) **concentration** – the index of lipid peroxidation – was determined by the Ohkawa method using the tiobarbituric acid (TBARS) (Ohkawa *et al.*, 1979).

The exploration of the **lipid profile** included the measurement by photocolorimetry, in the serum obtained after separation, of the concentration of total cholesterol (Ch-T), of triglycerides (TG) (Allain, *et al.*, 1974), of total lipids (LT), [the method with sulfovaniline], of high-density lipoproteins (HDL) (Lopes-Virella, 1977), of low-density lipoproteins (LDL) [according to the Friedewald formula] for all the animals included in the experiment.

**Statistical data interpretation.** All the data are shown as mean value ± standard error of the mean (SEM). Statistical analysis was performed with the paired or unpaired t-test. Statistical data interpretation considered the corresponding differences for a given significance threshold: p>0.05 statistically insignificant; p<0.05 statistically significant; p<0.01 strong statistical significance; p<0.001 very strong statistical significance.

**Results and discussion**

Oxidative stress generates free radicals and oxidants that play a role in increasing lipid peroxidation, as confirmed by the high levels of MDA, of serum lipids and fractions of membrane lipids. MDA has been proposed as an indicator of lipid peroxidation because this molecule is one of the end products of this oxidative process (Nielsen *et al.*, 1997).
There are highly significant values (p<0.01) for group P when compared with group W and for group HTA +P when compared with group HTA and extremely significant values (p<0.001) for group HTA when compared with group W, as shown by the statistical analysis of the MDA values (Table I).

Malondialdehyde, the most abundant among the reactive aldehydes derived from lipid peroxidation, was significantly increased in blood as well as in peripheral mononuclear cells. These aldehydes have been implicated as causative agents in cytotoxic processes, and it is reasonable to suppose that releases from cell membranes may diffuse, interact, and induce oxidative modifications in other cells and in LDL molecules, thereby increasing the risk of cardiovascular damage (Steinberg et al., 1989).

The significant lipid peroxide diminution in the serum contained by the HTA+P group compared to the P group is a result of a considerable reduction in the MDA serum concentration.

Reduced levels of GSH have been related to an extensive number of metabolic and gene expression disturbances, since the tripeptide is not only an efficient antioxidant but also an important regulatory substance in biological systems. Whether the low GSH levels and activity of the antioxidant enzymes is the cause or the consequence of the increased oxidative status needs further evaluation, but the fact that the low activity included several systems points to the reduction being more a consequence than a cause.

The tripeptide γ-glutamylcysteinylglycine or GSH is the major nonenzymatic regulator of intracellular redox homeostasis, ubiquitously present in all cell types at millimolar concentration. This cysteine-containing tripeptide exists either in reduced (GSH) or oxidized (GSSG) form, better referred to as glutathione disulfide, and participates in redox reactions by the reversible oxidation of its active thiol. Reactive oxygen species oxidized GSH to GSSG, leading to a decrease in GSH and an increase in GSSG concentrations. Moreover, even though the increment in ROS may upregulate the antioxidant enzymes under higher amounts of pure oxygen or related species, consumption by ROS can overcome the increased production, leading to the low activity observed.

Since polyphenols may modulate eNOS via O$_2^-$ mediated activation of src kinase (Anselm et al., 2007), it seems relevant to further investigate the source(s) and role(s) of O$_2^-$ and other ROS in soy isoflavone mediated activation of eNOS and antioxidant genes. Under conditions of oxidative stress, upregulation of Hsp90 expression (Whittier et al., 2004) and increased intracellular Ca$^{2+}$ will promote turnover and proteosomal degradation of proteins such as calmodulin and eNOS (Squier, 2006) and thereby affect NO bioavailability. The ability of dietary polyphenols to generate both NO and ROS in endothelial cells and activate ARE/EpRE (Antioxidant response element/Electrophile response element) mediated gene expression underlies their cardioprotective properties (Lee-Hilz et al., 2006).

Dietary polyphenols may counteract oxidative stress in vascular and inflammatory diseases (Rahman et al., 2006) by modulating key redox sensitive gene transcription via NF-κB and Nrf2/ARE (Hernandez-Montes et al., 2006) signaling pathways.

The balance between antioxidant and pro-oxidant characteristics of polyphenols have been attributed not only to their structural features, but also to the concentration, suggesting induction of antioxidant defence metabolism by low concentrations and ROS production at high concentrations (Masella et al., 2005).

Dietary polyphenols may offer an indirect protection by activating endogenous defense systems and by modulating cellular signalling processes.
such as NF-κB activation, glutathione biosynthesis, MAPK proteins, and PI3-kinase/Akt pathway.

The higher GSH levels in the heart of animals subjected to experimental arterial hypertension is an adaptive reaction triggered by the activation of the non-enzymatic antioxidant systems. GSH may be covalently bound to proteins through a process called glutathionylation and acts as a coenzyme of numerous enzymes involved in cell defense. The antioxidant capacity of the serum is significantly improved (p<0.001) in the HTA+P rats, as well as the GSH concentration being normalized (Table I).

Table I. GSH and MDA modifications in the studied groups.

<table>
<thead>
<tr>
<th></th>
<th>W</th>
<th>P</th>
<th>HTA</th>
<th>HTA+P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (μmol/l/mL)</td>
<td>7.42±0.19</td>
<td>7.93±0.28*</td>
<td>4.91±0.58***</td>
<td>6.88±0.28##</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>0</td>
<td>0</td>
<td>8.73 x 10⁻²***</td>
<td>6.92 x 10⁻²##</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Statistical analyses *- p<0.05; **- p<0.01; ***- p<0.001, vs. W group. #- p<0.05; ## - p<0.01; ###- p<0.001 vs. HTA group.

It should also be noted that the total cholesterol and triglycerides-lowering activity of Aronia melanocarpa extracts was found in the case of rats fed with standard, non-hypercholesterolemic diet supplemented with high doses of chokeberry anthocyanins for 4 weeks (Wro´blewska et al., 2008).

When comparing total cholesterol and LDL-col levels, the results show that these are significantly higher in the HTA group than in the W group. There are significant improvements taking place against the dislipidemia occurring in arterial hypertension as a result of the administration of polyphenols extracted from Aronia melanocarpa fruit. The serum LDL levels in the HTA+P group were kept within normal limits by the polyphenolic protection (Table II). From the viewpoint of the variability coefficient (%), the mean values obtained are typical of the series considered. Research comparing the HTA+P and HTA groups shows that the HDL cholesterol is significantly higher in the first group.

In a combined therapy, chokeberry extracts were given as supplements with the diet of patients after myocardial infarction, as an addition to the statin treatment. Compared to the control group, treated only with statins, patients receiving additional Aronia extract for 6 weeks had significantly lower LDL-chol oxidation status as well as reduced levels of serum 8-isoprostanes and increased adiponectin levels, which indicate diminished oxidative stress and reduced endothelial inflammation (Naruszewicz et al., 2007).

Table II. Lipid profile in the studied groups.

<table>
<thead>
<tr>
<th></th>
<th>W</th>
<th>P</th>
<th>HTA</th>
<th>HTA+P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch-T (mg/dL)</td>
<td>73.41±4.56</td>
<td>65±1.72</td>
<td>95.3±6.74***</td>
<td>70.4±3.77###</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>86.53±6.62</td>
<td>72.54±6.66*</td>
<td>144±15.38***</td>
<td>95.7±22.85###</td>
</tr>
<tr>
<td>HDL-col (mg/dL)</td>
<td>34.21±4.36</td>
<td>32.56±3.62</td>
<td>21.74±4.88***</td>
<td>28.27±3.42##</td>
</tr>
<tr>
<td>LDL-col (mg/dL)</td>
<td>23.41±5.32</td>
<td>20.2±2.79</td>
<td>42.83±5.41***</td>
<td>27.12±7.36###</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Statistical analyses *- p<0.05; **- p<0.01; ***- p<0.001 vs. W group. *- p<0.05; ## - p<0.01; ###- p<0.001 vs. HTA group.
Standard H-E (hematoxylin and eosin) stain and special stains for the various tissue components, such as van Gieson's stain for elastin, were used for classical histopathological diagnosis setting. The sections were examined at the optical microscope with a x10, 20 and 40 lens. In the HTA group, the injuries were located mainly in the heart (Fig. 1). Hypertrophic myocardial fibers and thin fibrosis trabecula in the interstice were noticed. The vessels were characterized by collagenized adventitia, which was surrounded by fibrosis strips.

Areas of hypertrophic myocardial fibers and small areas of atrophied myocardial fibers were revealed (Fig. 2).

In the HTA+P group, the myocardial modifications are significantly more diminished when compared with the group HTA. The myocardial fiber with nuclei suggesting the normalization of the histological aspect (Fig. 3). In the HTA+P group, no morphological alterations in the aorta were revealed (Fig. 4). No aorta alterations, such as atheromatous plaques, were noticed.

Our understanding of endogenous mechanisms of hypertension by oxidative processes has advanced greatly in the last decade, yet the description of the molecular action of predisposing factors must be further elucidated to prevent and properly treat cardiovascular diseases.
Conclusions

In the arterial hypertensive model the cardio-protective effects of the polyphenolic extract from *Aronia melanocarpa* are represented by the antioxidant, hypcholesterolemiant intervention. The extract also ensures the integrity of the vascular endothelium.

The explanation for the inconsistencies between the *in vitro* and *in vivo* data lies in the limited bioavailability of the dietary polyphenols and their extensive metabolism in humans. Due to the fact that most of them exert multifacet action, any clinical applications using these substances should rely on an accurate understanding of the action mechanisms that are relevant.

Acknowledgements. The work was supported by the Romanian Ministry of Education and Research, CNCSIS, plan PN2, program IDEI, section PCE, research grant ID-2519/2008.

References

Anselm, E; Chataigneau, M; Ndiaye, M; Chataigneau, T; Schini-Kerth, V.B.: Grape juice causes endothelium-dependent relaxation via a redox-sensitive Src- and Akt-dependent activation of eNOS. Cardiovasc. Res. 73, 404 – 413, 2007.


