Original Article

DETERMINATION OF GLUTATHIONE PEROXIDASE EXPRESSION IN AORTIC WALL OF SPONTANEOUSLY HYPERTENSIVE RATS UNDER DIET OF DIFFERENT SELENIUM CONTENT

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Summary

The aim of the study was to investigate the influence of diets with different selenium (Se) content on the expression of glutathione peroxidase (GPx-1) in the aortic wall of spontaneously hypertensive rats (SHR). Twenty-four male, 8 months old SHR, divided into 3 groups, were used: for 8 weeks G1 received a low-Se diet, G2 a diet with adequate Se content, and G3 received a diet with Se supplementation. Histological investigations of aortic walls showed atherosclerotic changes at different stage of development between groups. Fluorescence microscopy image analysis showed different degrees of expression of GPx-1 in aortic walls, determined by immuno-fluorescence. Se supplementation increases GPx-1 expression in aortic wall of SHR and decreases pathological changes.

Key words: selenium, SHR, aortic wall, GPx expression, immunofluorescence

Introduction

Selenium (Se) is an essential trace element, incorporated in selenoproteins. Tissue expression of these enzymes depends on daily Se intake. Selenium enters the organism mainly with food. It has been established that a diet containing 0.1мg Se/g of food is enough for normal growth and reproduction. Se-dependant cellular glutathione peroxidase (GPx-1) is the most abundant intracellular isoform of the GPx antioxidant enzyme family. GPx-1 is a cytoplasmic homotetramer that is involved in the control of cellular oxidative state [1]. There is evidence that under normal physiological conditions, a low GPx activity may be compensated by other antioxidants such as vitamins E and C. However, prospective effects of GPx are of particular importance when the organism is exposed to additional stress factors. Low GPx activity is the main predictor of cardiovascular events in patients with coronary artery disease [2, 3].

The aim of the study was to investigate the influence of diets with varying selenium (Se) content on the expression of GPx-1 in the aortic wall of spontaneously hypertensive rats (SHR).

Methods

The experiment was performed in accordance with the Animal Welfare Act Regulations and was approved by University Ethic's Committee.

Twenty-four male 8- months- old SHR (Okamoto-Aoki), divided into 3 groups were used: G1 - on a low Se diet (0.05мg Se/g of food; n=8), G2 - on an adequate Se diet (0.11мg Se/g; n=8), and G3 that received Se supplementation (0.25мg Se/g; n=8). Their systolic blood pressure, equaled to 184±6 mm Hg, was measured indirectly using a tail cuff. Six months old rats were put on diets, differing in Se content for 8 weeks. The rats were placed in single chambers and had water and food *ad libitum*. Daily intake of food was checked for each rat. At the age of 8 months, aortas of the rats were extirpated completely under light ether anesthesia. Aortas were separated into 2 parts - thoracic and abdominal, and then fixed in a 10% solution of formaldehyde and embedded into paraffin blocks. Longitudinal slices were prepared and stained by hemalaun-eosin (HE). Histomorphological changes were described. Slides were also prepared for immuno-fluorescence staining at the laboratory of pathomorphology at the Department of Clinical Pathology of Medical University - Pleven. Tissue sections for slides were 5 m thick. Imunofluorescence cell staining was performed at the immunohistological laboratory of Institute of Biology and Immunology of Reproduction of Bulgarian Academy of Sciences - Sofia, using kits and protocols of Santa Cruz Biotechnology, Inc. [4]. Deparaffinization and hydratation were performed. The slides were washed in phosphate buffer saline (PBS). The specimens were incubated with 10% normal blocking serum (normal donkey sera: sc-2044) for 20 minutes to suppress non-specific binding of IgG. The next step was incubation with primary antibody (GPx-1 /H-19/ goat polyclonal antibody: sc-22146) at dilution range 1:100 for 60 minutes. For negative control, sections were prepared without primary antibody (with PBS only). After that slides were incubated in fluorochrome conjugated secondary antibody (donkey anti-goat IgG-FITC: sc-2024) at dilution range 1:200 for 45 minutes. Then coverslips were mounted with Ultra Cruz mounting medium (sc-24941) and examined under a fluorescence microscope ("Light"-Austria) with appropriate filters. Photos were taken and the results compared, using quolitative analysis of changes.

Results

Histological investigations showed athero-sclerotic changes at different stage of development in the three groups and difference in aortic wall engagements - from endothelial hyperplasia, proliferation of miocytes and macrophages to fibrosis and hialinosis. The rats of G1 showed severe atherosclerotic changes and many atherosclerotic spots, spread throughout the thoracic to abdominal aorta. In the thoracic aorta, plaques with cellular proliferation predominated. Plaques with fibrosis and hialinosis predominated in the abdominal aorta, and were located near the branches of arteries. They confluenced, deforming the lumen of the

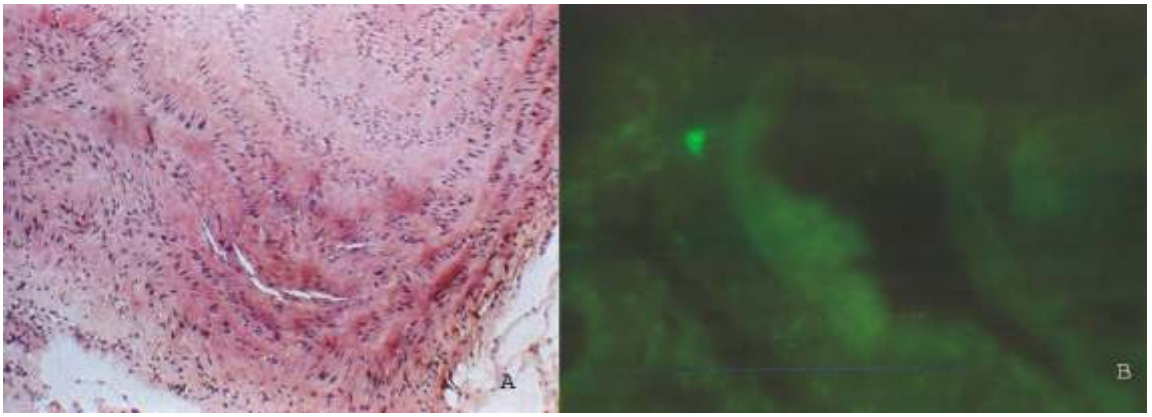

Figure1. Representative photomicrograph of aortic wall of rat from group G1.
Aorta - fibro sclerotic plaque with multiple necroses and lipid drops in media. Whole wall of the vessel, from the endothelium to adventitia, is engaged. HE - (magnification x16) A; Immunofluorescence - expression of GPx (magnification x40) B.

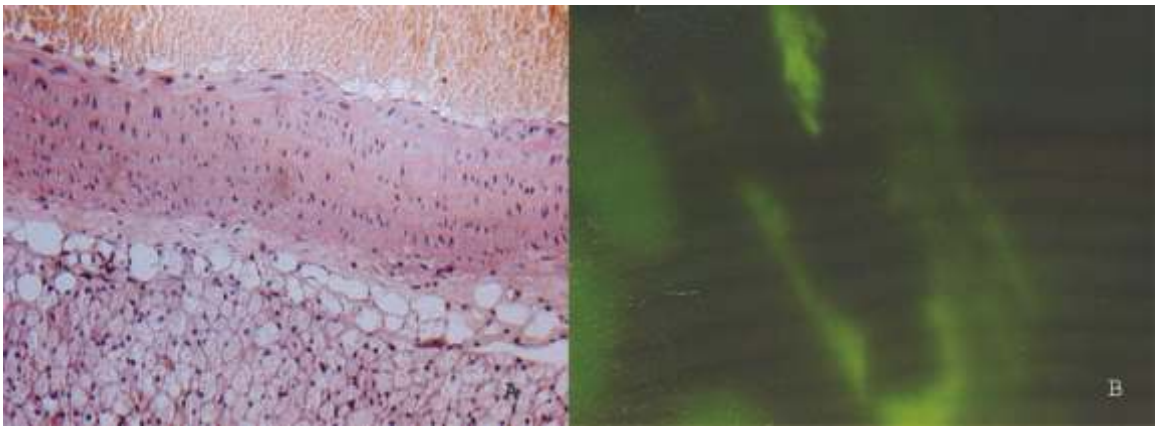

Figure 2. Representative photomicrograph of aortic wall of rat from group G2.
Aorta - proliferation of endothelial cells, prominated to the lumen, single necrobiotic changes and small lipid drops, situated at media. HE - (magnification x16) A; Immunofluorescence - expression of GPx (magnification x40) B.

aorta and causing stenosis (Fig.1.A). All rats of G2 had endothelial hyperplasia, erosions and plaques, which engaged the media. There were foci of medionecrosis, cell proliferation of myocytes, and lipid drops (Fig.2.A). The histology of aortic walls showed small number and slow evolution of the atherosclerotic changes in G3 (Fig.3.A). Immunofluorescence examination of GPx revealed predominant expression in endothelium and weak localization in the medial smooth muscle cells. Fluorescence microscopy image analysis showed the different degree of fluorescent expression in SHR rats on a diet of different Se content: it was low in G1 (Fig.1.B); moderate in G2 (Fig.2.B) and high in G3 (Fig.3.B).

Discussion

The biological role of selenoproteins in mammals includes optimal endocrine and immune function, prevention of cardiovascular and neoplastic diseases. Nowdays, there are 30 selenoproteins defined, grouped into 3 families of enzymes: glutathione peroxidases, thioredoxin reductases, and iodothyronine 5[1] deiodinases. Their expression is coded by 25

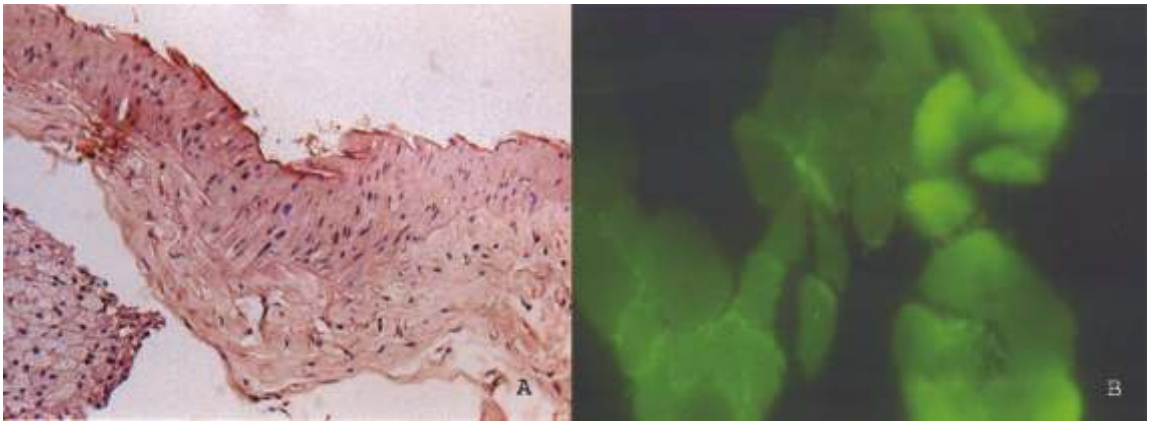

Figure 3. Representative photomicrograph of aortic wall of rat from group G3.
Aorta - uniform thickness of aortic wall, moderate endothelial proliferation, erosions, light hyperplasia of miocytes and small lipid drops. HE - (magnification x16)A**;** Immunofluorescence - expression of GPx (magnification x40) B.

selenoprotein genes. Six isoenzymes of glutathione peroxidase that participate in antioxidative defense of mammalian organism have been evaluated. Thioreductases control cellular redox systems. Researchers have identified 3 iodothyronine deiodinases, which catalyze release of iodine from 5 or 5' position of iodothyronin substrates and play an important role for activation and inactivation of thyroid hormones in all tissues. Selenoprotein expression depends on daily selenium intake.

Cellular antioxidant enzymes such as GPx-1 and superoxide dismutase (SOD) play a major role in the control of reactive oxygen species (ROS), which participate in atherogenesis and in pathogenesis and development of hypertension. GPx-1 deficit, which is due to low Se intake, directly induces increase of oxidative stress, leading to endothelial dysfunction. Low GPx-1 activity decreases bioactive nitric oxide (NO), which is synthesized by the endothelium. NO contributes to vascular tone, preserves endothelial integrity, inhibits smooth muscle cell migration and proliferation. Endothelial cells are continuously exposed to blood flow and are the primary target of oxidant-induced injury. Hypertension-induced target organ damage (TOD) is one of the leading causes of morbidity and mortality. Low Se intake worsens the antioxidant status and is a risk factor for the development of cardiovascular diseases (CVD), whereas Se supplementation has a positive effect in reducing TOD. Our results have revealed the important evidence for this hypothesis, because the therapeutic benefit of Se administration in prevention and treatment of CVD still remains insufficiently documented [1, 2, 5, 6, 7].

Conclusions

Selenium supplementation increases GPx-1 expression in aortic wall of spontaneously hypertensive rats and slows down the development of pathological changes.

Acknowledgements

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