

## CHANGES IN OXIDATIVE STATUS OF THE HEART IN RATS RECEIVING VITAMIN C SUPPLEMENTS

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**Abstract** — We studied the oxidative status of the heart in rats receiving by two doses of vitamin C over a period of four weeks. The activities of copper zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), and catalase, hydrogen peroxide concentration, the level of lipid peroxidation, and total vitamin C content were determined in the heart of the experimental animals, as well as the concentration of vitamin C in their serum. Our results indicate that, apart from the ability of rats to synthesize vitamin C, supplementation leads to additional antioxidative protection.

**Key words:** Vitamin C, heart, antioxidative protection, rats

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### INTRODUCTION

During respiration, a small portion of oxygen molecules is transformed into superoxide radicals ( $O_2^{\bullet-}$ ) through mitochondrial complexes I and III (Turrens, 1997). Hydrogen peroxide, produced by  $O_2^{\bullet-}$  dismutation, can be further converted to more reactive oxygen species (ROS), such as the hydroxyl radical. Thus, the level of respiration is an important factor for the susceptibility of tissues to oxidative stress.

The heart is an organ with high oxygen consumption, and increasing evidence suggests that ROS play a major role in the pathophysiology of heart diseases (Keith et al., 1998). As a consequence, antioxidative protection may be of great importance for its function. Vitamin C (L-ascorbic acid, ascorbate) is a hydrosoluble vitamin with confirmed antioxidative properties. Unlike humans, rats have the ability to synthesize it endogenously, and one can expect that in this way they satisfy normal daily needs for this nutrient. However, it is possible that additional vitamin C intake may improve antioxidative defense in these animals. Thus, our aim was to study the influence of vitamin C on the antioxidative status of rats receiving supplements of it for four weeks. We determined the activities of copper zinc superoxide

dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), and catalase, hydrogen peroxide concentration, the level of lipid peroxidation and total vitamin C content in the heart of experimental animals, as well as the concentration of vitamin C in their serum.

### MATERIALS AND METHODS

#### 2.1. Design of experiment

Male rats of the Wistar strain (*Rattus norvegicus*) weighing  $200 \pm 45$  g were used for the experiments. The animals were acclimated to  $22 \pm 1^\circ\text{C}$  and maintained under conditions of 12-h periods of light and dark, with free access to tap water and commercial rat food.

The rats were divided into three groups, each consisting of six animals. The first group was formed of control rats, which drank tap water. The second and the third groups consisted of animals receiving low (0.75 mg of ascorbic acid/kg body weight daily) or high (25 mg of ascorbic acid/kg body weight daily) doses of vitamin C dissolved in tap water.

#### 2.2. Vitamin C supplementation

According to results of a previous experiment

performed on a group of six animals over four weeks, the average daily consumption of water in rats was  $240 \pm 5$  ml per kilogram of body weight. Both doses of vitamin C were therefore dissolved in an adequate volume of tap water every day and administered to the appropriate group of animals. This supplementation was constant throughout four weeks.

### 2.3. Sample preparation

Animals were killed by decapitation with a Harvard guillotine without anesthesia, as recommended by the local ethical committee. After decapitation, the heart was removed and blood was collected.

The left half of the heart was excised and divided into two equal portions. One portion was homogenized in 25 mM phosphate buffered saline (PBS) (pH 7.0) and centrifuged at 9000g in a semi-preparative Sorvall Super T21 centrifuge for 20 min at 4°C. The supernatants were used for determination of catalase, CuZnSOD, and MnSOD activities, as well as for measurement of the  $H_2O_2$  concentration and total content of lipid hydroperoxides (the level of lipid peroxidation). The other portion of the heart and blood serum were used for determination of vitamin C content by using a similar procedure, except that 6% trichloroacetic acid (TCA) was used instead of PBS.

### 2.4. Methods

Total superoxide dismutase activity was determined in PBS samples by the adrenaline method of Misra and Fridovich (1972) using potassium cyanide as a selective CuZnSOD inhibitor for differential calculation of MnSOD activity (Weisiger and Fridovich, 1973).

Catalase activity was measured in PBS samples by the method of Beutler (1982).

Both lipid hydroperoxides and  $H_2O_2$  were determined in PBS samples by the ferrous ion oxidation (FOX) assay (Wolf, 1994). There are two versions of FOX assay, FOX-1 and FOX-2 (Banerjee et al., 2002). The concentration of hydrogen peroxide was measured by FOX-1 (Gay and Gebicki, 2000)

and calculated against hydrogen peroxide standard curve absorption values. The concentration of lipid hydroperoxides was measured by FOX-2 (Jiang et al., 1991), with the level of lipid peroxidation in samples expressed as a percent of the lipid peroxidation level in control group of animals.

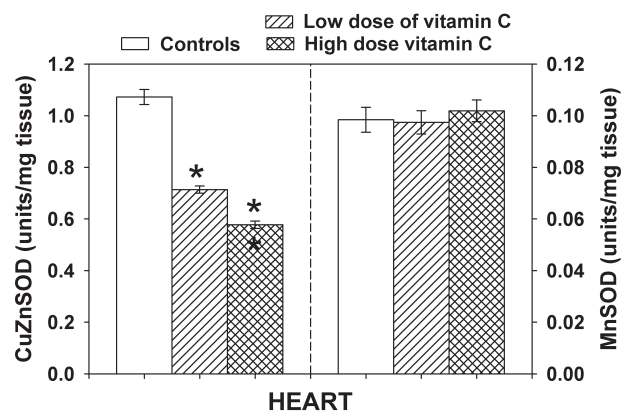
Total vitamin C content was determined in TCA samples by the method of Roe and Kuether (1943) against vitamin C standard curve absorption values.

### 2.5. Statistical analysis

The data are expressed as means  $\pm$  SEM. One-way ANOVA was used for multiple range comparison, with posterior testing for determination of significant differences among groups. The minimal level of probability of significance was set at  $P < 0.05$ .

## RESULTS

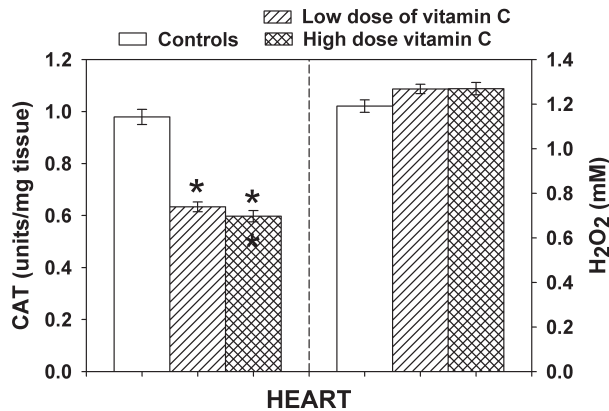
As presented on Figs. 1 and 2, both doses of vitamin C decreased the activities of heart CuZnSOD and catalase, while the activity of MnSOD and  $H_2O_2$  concentration remained unchanged.



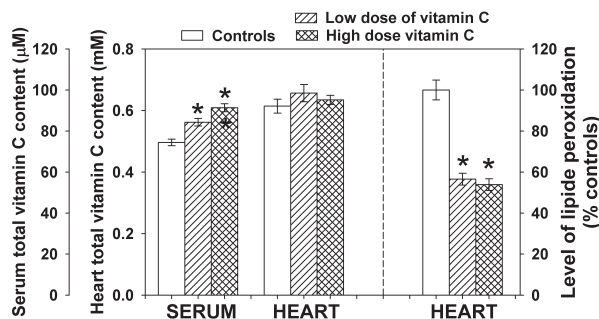
**Fig. 1.** Influence of low (0.75 mg/kg b.w. per day) and high (25 mg/kg b.w. per day) doses of vitamin C on activity of CuZnSOD (left) and MnSOD (right) in the rat heart. Statistically significant differences ( $P < 0.05$ ) in relation to the control are marked with an asterisk above the columns, while statistically significant differences between the two doses of the vitamin C are marked with an asterisk inside the columns.

Vitamin C supplementation increased the total content of ascorbate in the serum, but did not affect

its concentration in the heart (Fig. 3). As presented on the same figure, the level of the heart lipid peroxidation was decreased.



**Fig. 2.** Influence of low (0.75 mg/kg b.w. per day) and high (25 mg/kg b.w. per day) doses of vitamin C on activity of catalase (left) and concentration of H<sub>2</sub>O<sub>2</sub> (right) in the rat heart. Statistically significant differences ( $P < 0.05$ ) in relation to the control are marked with an asterisk above the columns, while statistically significant differences between the two doses of the vitamin C are marked with an asterisk inside the columns.



**Fig. 3.** Influence of low (0.75 mg/kg b.w. per day) and high (25 mg/kg b.w. per day) doses of vitamin C on total vitamin C content (left) and the level of lipid peroxidation (right) in the rat serum and heart. Statistically significant differences ( $P < 0.05$ ) in relation to the control are marked with an asterisk above the columns, while statistically significant differences between the two doses of the vitamin C are marked with an asterisk inside the columns.

## DISCUSSION

Chemically, ascorbic acid is a simple glucose-related carbohydrate with rather unique properties. The presence of an enediol group in the molecule confers electron lability, which makes it a member of the oxidation-reduction system. Loss of the first

electron results in formation of the ascorbate free radical (Mehlhorn et al., 1989), which can be further oxidized to give dehydroascorbic acid. Both ascorbic acid and the ascorbate free radical have a reducing potential low enough to react with most of the physiologically important radicals and oxidants (Buettner, 1993), enabling vitamin C to act as a powerful hydrosoluble antioxidant in body fluids (Frei et al., 1989), scavenging reactive oxygen and nitrogen species (Halliwell, 1996).

Our experiments showed that vitamin C supplementation lowered the activity of heart CuZnSOD, but did not affect MnSOD activity (Fig. 1). According to these results, ascorbate exerts different effects in different cell compartments of the heart. It seems that in cytoplasm, where CuZnSOD is located, vitamin C plays an antioxidative role. However, this is not the case with mitochondria which are MnSOD containing parts of cell.

Mitochondria are considered to be the major source of superoxide in eukaryotic cells because they consume most of the oxygen used by the cell. From this point of view, the unchanged MnSOD activity may imply that mitochondrial antioxidative defense has an intrinsic capacity sufficient to cope with oxidative stress under physiological conditions. Moreover, the presence of CuZnSOD in the intermembranous space of mitochondria has been confirmed (Okado-Matsumoto and Fridovich, 2001). Thus, any O<sub>2</sub><sup>•-</sup> entering the mitochondria would be scavenged by mitochondrial CuZnSOD.

In the cytoplasm of cardiomyocytes, vitamin C exerts an antioxidant role. It has become evident that oxidative stress in the heart may involve ROS production by various highly specialized enzymes, not only respiratory ones (Finkelel, 1999). There are several potential sources of O<sub>2</sub><sup>•-</sup> in the cytoplasm of heart cells, for example xanthine oxidase (Berry and Hare, 2004) or NADPH oxidases (Cave et al., 2005). Probably acting through the removal of superoxide radicals, ascorbate reduces activity of CuZnSOD, thus preventing further ROS generation and lowering the level of heart lipid peroxidation (Fig. 3).

The function of SODs is catalytic dismutation of  $O_2^{\bullet-}$  with hydrogen peroxide as a final product. Thus, decreased CuZnSOD activity may imply reduced  $H_2O_2$  production, followed by consequent catalase activity decrease. Our data show that vitamin C supplementation decreased activity of catalase in the heart, while the concentration of hydrogen peroxide remained unchanged (Fig. 2). The purpose of this could be to keep of the  $H_2O_2$  content at the optimal, rather than the minimal level (Clement and Pervaiz, 2001), since it acts as one of the most important redox regulators in cell (Thannickal and Fanburg, 2000). Thus, it seems that maintaining the hydrogen peroxide concentration at the basal level in the heart is not linked with the activity of SODs, but with some other, probably cytoplasm-derived sources of  $H_2O_2$  (Shah and Channon, 2004).

It is interesting that vitamin C supplementation increases ascorbate concentration in the blood, but not in the heart (Fig. 3). This phenomenon has no adequate explanation, inasmuch as the concentration of vitamin C in the heart is among the lowest in the organism. It is possible that the heart's ascorbate content must be kept at a low level in order to maintain an appropriate ROS concentration. If that is the case, the extensive ascorbate turnover with blood could be responsible for vitamin C homeostasis in the heart.

In conclusion, the presented results demonstrate that vitamin C supplementation exerts additional antioxidative protection in the heart of rats, apart from their ability to synthesize vitamin C.

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## ПРОМЕНЕ ОКСИДАТИВНОГ СТАТУСА СРЦА ПАЦОВА ПРЕХРАЊИВАНИХ ВИТАМИНОМ С

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У овом раду испитиван је ефекат прехрањавања двама дозама витамина С на оксидативни статус срца пацова. Одређивани су активност бакар цинк супероксид дисмутазе, манган супероксид дисмутазе и каталазе, ниво липидне пероксидације и концентрација водоник пероксида и

укупног витамина С у срцу експерименталних животиња, као и концентрација витамина С у серуму. Добијени резултати указују да егзогено дат витамин С повећава антиоксидативну заштиту у срцу пацова, без обзира на способност ових животиња да га и ендогено синтетишу.