Dietary selenium intake affects cardiac susceptibility to ischaemia/reperfusion in male senescent rats

Stéphane Tanguy1, Marie-Claire Toufektsian1, Sophie Besse1, Véronique Ducros2, Joël de Leiris1, François Boucher1

1Laboratoire Stress Cardiovasculaires et Pathologies Associées, Université Joseph Fourier, Grenoble, France
2Département de Biologie Intégrée, Centre Hospitalier Régional de Grenoble, Grenoble, France

Address correspondence to: F. Boucher, Laboratoire Stress Cardiovasculaires et Pathologies Associées, Bâtiment Jean Roget – Domaine de la Merci, Faculté de médecine et de Pharmacie, Université Joseph Fourier, F-38700 La Tronche, France. Fax (+33) 4 76 63 71 17. Email: francois.boucher@ujf-grenoble.fr

Abstract

Background: cardiovascular ageing is associated with an increase in cardiac susceptibility to ischaemia and reperfusion. This has been suggested to be partly related to an increased sensitivity of the myocardium to the reactive oxygen species that are produced during post-ischaemic reperfusion. The aim of the present study was therefore to determine whether increasing cardiac glutathione peroxidase activity by a selenium-enriched diet could afford some protection against ischaemia and reperfusion to senescent rat hearts.

Methods: 22 months old male Wistar rats received either a high-selenium (1.5 mg Se/kg diet) or a low-selenium (0.05 mg Se/kg diet) diet for 10 weeks. At the end of the diet, hearts were submitted to ischaemia and reperfusion ex vivo and either fixed for semi-quantitative analysis of ultrastructural damage by electron microscopy or used for glutathione peroxidase activity assessment.

Results: high-selenium supply increased cardiac total, mitochondrial and cytosolic glutathione peroxidase activities. Moreover, this diet induced a significant improvement of cardiac post-ischaemic functional recovery. Finally, this preservation of cardiac function was associated with a significant limitation of ultrastructural alterations of sarcomeres and mitochondria.

Conclusion: our high-selenium diet considerably limits the sensitivity of senescent rat hearts to ischaemia and reperfusion. This finding suggests that peroxides might play a key role in the increase in cardiac sensitivity to ischaemia and reperfusion during ageing. Together with the observation that selenium status decreases with age in humans, our results indicate that reinforcing selenium supply could improve the prognosis of cardiovascular diseases in old patients.

Keywords: ageing, glutathione peroxidase activity, isolated rat heart, reactive oxygen species, reperfusion-injury, selenium

Introduction

Ample experimental evidence shows that reactive oxygen species (ROS) are produced in ischaemic tissues during ischaemia, or particularly during post-ischaemic reperfusion [1, 2].

Besides, it is now well established that ageing increases the sensitivity of the myocardium to ischaemia and reperfusion [3]. Although numerous studies have attempted to determine the origin of this phenomenon, the exact mechanisms responsible for this deterioration have not yet been fully elucidated.

In a previous work, we have evidenced profound modifications of the cellular enzymes that are involved in ROS detoxification during myocardial ageing [3]. Indeed, we have shown a decrease in catalase activity in old animals compared to adults and the disappearance of the correlation between the activity of cardiac glutathione peroxidase (GPx) and post-ischaemic functional recovery. On the basis of these findings, we suggest that any appropriate reinforcement of myocardial antioxidant defence systems might prevent the increased sensitivity to cardiac ischaemia in the elderly.

Because of the key role that peroxides play in the oxidative component of the pathophysiological situation of myocardial ischaemia/reperfusion, we attempted, in the present study, to improve the natural defence systems against these ROS in aged rats.
At the cellular level, the elimination of hydrogen peroxide and of organic hydroperoxides is catalysed by catalase and GPx. In cardiomyocytes, a seleno-dependent form of GPx (Se-GPx) is the main enzyme responsible for the elimination of these ROS [4].

It is well established that in most industrialised countries, selenium intake is so low [5] that it might limit GPx synthesis. The present study was therefore designed in order to assess whether an increase in endogenous myocardial GPx activity in the elderly might be able to improve the tolerance of the myocardium to ischaemia/ reperfusion.

For this purpose, two groups of 22 months old Wistar rats were fed for 10 weeks diets containing either a low- or a high-selenium level. The low-selenium diet contained 0.05 mg/kg selenium, which corresponds, in humans, to the most common mild deficiency. The high-selenium diet (1.5 mg/kg diet), which would correspond to a high selenium intake in humans, still remains within the range of physiological values.

Materials and methods

Animals and diets

Male Wistar rats of 22 months of age were randomly assigned to one of two experimental groups:

- a low-selenium group ('Low-Se', n=10), receiving a standard diet containing 0.05 mg selenium/kg food for 10 weeks, and
- a high-selenium group ('High-Se', n=9), receiving the same diet containing 1.5 mg selenium/kg food for 10 weeks.

Selenium content in both diets was adjusted by sodium selenite addition.

Rats were housed under conditions of constant temperature, humidity and standard light – dark cycle (12 h/12 h). They had free access to tap water and food. They received humane care in compliance with the guidelines formulated by the European Community for use of experimental animals (L358–86/609/EEC).

Isolated heart preparations

Ten weeks after the beginning of the diets, animals were anaesthetised with sodium pentobarbital (60 mg/kg body weight, i.p.) and heparin (100 IU) was administered via a femoral vein.

The heart was removed and immediately perfused through the aorta at a constant pressure of 9.81 kPa (100 cm H2O) using a physiological buffer containing (in mmol/l): NaCl 118.50; NaHCO3 25.00; KCl 4.75; MgSO4.7H2O 1.19; KH2PO4 1.18; CaCl2.2H2O 1.36; Glucose 11.10 at 37.0 ± 0.2°C and saturated with a gas mixture of O2–CO2 (95%–5%) (pH 7.4 ± 0.1).

A water-filled balloon [6], connected to a pressure transducer (Statham, P23ID) was inserted into the left ventricle and inflated to impose an end-diastolic pressure of 4.0 ± 0.5 mmHg. The volume of the balloon was kept constant throughout experiment. The sinus node was removed by cutting the right atrium, and the heart was electrically paced at 300 beats/min via a monopolar electrode.

After a 20-min stabilisation period, hearts were subjected to global-zero flow ischaemia for 20 min at 37°C. They were then reperfused for 30 min under control normoxic conditions.

Left ventricular developed pressure (LVEDP) and the maximal positive and negative first derivatives of left ventricular pressure (+dP/dt and –dP/dt) were recorded throughout the perfusion. Coronary flow (CF) was measured by timed-collection.

Electron microscopy and semi-quantitative assessment of ultrastructural alterations

At the end of the 30-minute reperfusion period, two hearts from each group were fixed with a solution of glutaraldehyde (2.5%; pH=7.4) [7]. Small samples (1 mm3) of the subendocardial part of the left ventricle were post-fixed, dehydrated in ethanol and embedded in Epon. Two blocks from each heart were selected for electron microscope examination. From each of these fragments, 16 ultra-thin (0.5 μm) sections were prepared and observed. Micrographs were taken following a random sampling procedure to obtain a representative overview of the whole section.

Structural alterations of mitochondria and sarcomeres were semi-quantitatively assessed by the method previously described by Pucheu et al. [8]. Briefly, mitochondria were counted and classified into two categories:

- type A: mitochondria with normal appearance,
- type B: swollen mitochondria, with a thinner matrix and/or presenting membrane ruptures.

Similarly, sarcomeres were classified into two categories:

- type A: sarcomeres with normal configuration,
- type B: sarcomeres with contraction bands and/or contraction band necrosis.

Biochemical assays

Blood plasma and red blood cells (RBC) were separated by centrifugation and stored at −80°C.

At the end of the perfusion, hearts were quickly frozen at liquid nitrogen temperature and kept at −80°C until use.

Heart samples were homogenised in 50 mM Tris–HCl buffer, pH=7.4.

The homogenates were centrifuged at 2000 g for 10 min to remove all nuclear debris and then centrifuged at 20,000 g for 20 min to separate the cytosol (supernatant) and mitochondria (pellet). The mitochondrial pellets were resuspended in phosphate buffered saline (KH2PO4 50 mM, pH=7.0).
Glutathione peroxidase activity was determined in mitochondrial and cytosolic extracts by the modified method of Flohe and Günzler [9].

Selenium assays

Plasma and RBC selenium contents were evaluated by gas chromatography coupled to mass spectrometry technique [10, 11].

Table 1. Effect of the 10 week selenium diets on body weight, heart weight and selenium status

<table>
<thead>
<tr>
<th></th>
<th>Low-Se (n=10)</th>
<th>High-Se (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>533 ± 20</td>
<td>498 ± 20</td>
</tr>
<tr>
<td>Heart weight (mg)</td>
<td>1614 ± 58</td>
<td>1661 ± 107</td>
</tr>
<tr>
<td>Heart to body weight ratio (mg/g)</td>
<td>3.1 ± 0.2</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Plasma selenium (µmol/l)</td>
<td>4.23 ± 0.28</td>
<td>4.97 ± 0.10*</td>
</tr>
<tr>
<td>RBC selenium (mmol/g Hg)</td>
<td>15.80 ± 0.60</td>
<td>27.30 ± 2.80**</td>
</tr>
</tbody>
</table>

Groups are defined in the text. RBC=red blood cells. Values are mean±SEM. * P<0.05; ** P<0.01 versus the corresponding Low-Se value. Mann-Whitney test.

Figure 1. Effect of the 10-week selenium-diets on myocardial glutathione peroxidase activity. * P<0.05; ** P<0.01 versus Low-Se. Means ±SEM. Mann-Whitney test.

Table 2. Effect of the 10 week selenium diets on cardiac function under pre-ischaemic conditions of perfusion and after the 30 min post-ischaemic reperfusion period

<table>
<thead>
<tr>
<th>Condition of perfusion</th>
<th>Experimental group</th>
<th>LVDevP (mmHg/g w wt)</th>
<th>+ dP/dt (mmHg/s/g w wt)</th>
<th>−dP/dt (mmHg/s/g w wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ischaemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-Se (n=10)</td>
<td>9.84 ± 0.80</td>
<td>53.6 ± 1.8</td>
<td>2594 ± 80</td>
<td>2032 ± 76</td>
</tr>
<tr>
<td>High-Se (n=9)</td>
<td>9.59 ± 0.50</td>
<td>52.9 ± 1.8</td>
<td>2498 ± 125</td>
<td>1989 ± 160</td>
</tr>
<tr>
<td>Post-ischaemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-Se (n=10)</td>
<td>5.12 ± 0.51</td>
<td>23.6 ± 3.1</td>
<td>1064 ± 128</td>
<td>752 ± 90</td>
</tr>
<tr>
<td>High-Se (n=9)</td>
<td>7.48 ± 0.75</td>
<td>45.0 ± 5.4*</td>
<td>1809 ± 209*</td>
<td>1691 ± 287*</td>
</tr>
</tbody>
</table>

Groups are defined in the text. CF=coronary flow; LVDevP=left ventricular developed pressure; ± dP/dt=positive and negative first derivatives of left ventricular developed pressure. Values are mean±SEM. * P<0.05 versus the corresponding Low-Se value. Mann-Whitney test.

Selenium and myocardial ageing

Statistical analysis

All results are expressed as mean ± SEM and compared with a non-parametric Mann-Whitney test. The condition of mitochondria (or sarcomeres), expressed as the ratio between the number of mitochondria (or sarcomeres) in one category divided by the total number of mitochondria (or sarcomeres) studied, was analysed using a proportion comparison test (χ²) corrected for continuity using the Yates’ method. In all cases, the significance threshold was fixed at P=0.05.

Results

General aspects

Selenium intake did not affect growth rate of the animals. Moreover, heart weight/body weight ratio, expressed as mg wet tissue per g body weight, remained similar in both groups (Table 1).

As expected, high selenium supply induced a significant increase in plasma (P<0.05) and RBC (P<0.01) selenium levels when compared to the Low-Se group (Table 1). Moreover, total GPx activity was significantly increased in the myocardium of High-Se compared to Low-Se (Figure 1). This phenomenon resulted from an increase in both cytosolic and mitochondrial GPx activities (Figure 1).

Effect of selenium supply on cardiac function

As shown in Table 2, cardiac haemodynamic parameters assessed ex vivo at the end of the control normoxic perfusion period, were equivalent in both groups.

Besides, post-ischaemic recovery of LVdevP was significantly improved from the 15th minute of reperfusion in the High-Se group compared to Low-Se (Figure 2, Table 2). A similar cardioprotective effect was observed in terms of +dP/dt and −dP/dt (Table 2).

This preservation of cardiac function was associated with a non significant improvement of coronary flow recovery.

Effect of selenium supply on post ischaemic cardiomyocyte ultrastructure

Selenium intake by itself did not significantly affect cardiomyocyte ultrastructure on our model since no
obvious difference has been evidenced between the two experimental groups under basal conditions.

After 30 min of reperfusion, morphological alterations, characteristic of the ischaemic-reperfused myocardium, were present: clearing of the mitochondrial matrix, destruction of mitochondrial cristae, oedema, absence of mitochondrial glycogen, membrane ruptures, disruption of tubular systems and disorganisation of sarcomeres. High selenium intake significantly reduced the adverse effects of reperfusion on both the sarcomeres and mitochondria (Table 3).

Discussion
The present study clearly shows that selenium intake conditions blood selenium content and cardiac GPx activity in senescent rats. Moreover, high selenium intake was associated with a significant reduction of cardiac sensitivity to post-ischaemic reperfusion, assessed by the better functional recovery and the limitation of ultrastructural alterations.

Selenium and cardiovascular diseases
It is well established that selenium plays a major functional role in numerous intracellular enzymes [for a review see 12].

One of the main biological functions of selenium relates to its role in the enzyme GPx which is one of the major systems involved in ROS elimination in cardiomyocytes [4]. It has been shown for a long time that GPx functions as an antioxidant enzyme, reducing hydrogen peroxide and organic hydroperoxides [13]. The assessment of its activity has been extensively used as an index of body selenium status and nutritional requirements [5, 14].

In humans, the most commonly described cardiovascular pathology associated with low selenium intake is Keshan’s disease. This syndrome is associated with a high mortality that can be limited by selenium supply [15]. Besides Keshan’s disease, some of the cardiac complications that are observed in patients undergoing total parenteral nutrition might also be linked to a selenium deficiency [for review see 16]. Indeed, in such situations, selenium supplementation has also been shown to reduce the specific cardiac disorders. While these two situations remain exceptional and are

Table 3. Effect of the 10-week selenium diets on cardiac sarcomeric and mitochondrial alterations after 20 min of ischaemia and 30 min of reperfusion

<table>
<thead>
<tr>
<th></th>
<th>% Mitochondria</th>
<th>% Sarcomeres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type A</td>
<td>Type B</td>
</tr>
<tr>
<td>1 – Low-Se</td>
<td>75.8</td>
<td>24.2</td>
</tr>
<tr>
<td>2 – High-Se</td>
<td>85.2</td>
<td>14.8</td>
</tr>
</tbody>
</table>

Mitochondria: Type A: mitochondria with normal appearance; Type B: swollen mitochondria, with a thinner matrix and/or presenting membrane ruptures. Low-Se: n=747 and High-Se: n=881 mitochondria observed.
Sarcomeres: Type A: sarcomeres with normal configuration; Type B: sarcomeres with contraction band and/or necrosis. Low-Se: n=770 and High-Se: n=668 sarcomeres observed.

*P<0.01 versus the corresponding Low-Se value. \( \chi^2 \) test corrected for continuity using the Yates’ method.
associated with severe selenium deficiencies, mild deficiencies have also been proposed to be related to cardiovascular disease [5, 17]. Salonen et al. [18] have reported a negative relationship between plasma selenium level and the incidence of heart diseases. Selenium status in humans, therefore appears to be a major factor determining both the incidence and the prognosis [19] of cardiovascular disease. This observation is crucial since nutritional selenium supply is considered to be very low in most industrialised countries [5].

Several experimental studies on animal models have been designed to elucidate the repercussions of selenium supply on the cardiovascular system [11, 20–23]. These studies have unanimously shown that low selenium intakes are associated with an increase in myocardial sensitivity to reperfusion injury in young as well as in adult rats.

Finally, the serum selenium levels achieved in our two experimental groups of senescent rats are consistent with previous findings reported in the literature in adult male rats [11, 20]. However, in a study on young (60–80 g) female Wistar rats [23] subjected to a mildly low selenium diet (0.16 mg Se/kg) for 10 weeks we found quite low plasma selenium levels (3.9 ± 0.1 μmol/l versus 4.23 ± 0.28 μmol/l). Such differences might be due to the combination of both gender and age differences. Further studies will be required to test this last hypothesis.

Myocardial ageing and cardiac ischaemia/reperfusion

In humans, myocardial ageing appears to be associated with both an increase in the incidence and a worsening of the consequences of cardiovascular diseases. Indeed, numerous studies have reported an increased mortality associated with myocardial infarction in aged patients [24, 25].

Most of the experimental approaches to cardiovascular ageing have been performed in the rat on the basis that the modifications to its cardiovascular system during the 30–36 months of its normal life span are comparable to the effects of cardiovascular ageing in humans. Generally, 4-month-old male Wistar rats are considered as adults or mature rats, 16-month-old rats as older adults or old rats, and 24-month-old rats as senescent rats [26].

Consistent with the observations reported in humans, several studies have shown that biological and morphological changes that occur in old rats are associated with an increased myocardial sensitivity to ischaemia and reperfusion [3, 26–29]. An increasing body of evidence shows that the alteration of cardiac function occurring during post-ischaemic reperfusion is due, at least in part, to an overproduction of ROS, leading to an overwhelming of the natural defences against these species [for a review see 30]. Moreover, this phenomenon has been shown to be increased in aged rat hearts [3], therefore reinforcing the hypothesis of an increased sensitivity to oxidative stress of the aged myocardium.

In a previous study we have shown that myocardial catalase activity is significantly reduced in the myocardium of old rats compared to young adults [3] suggesting that the higher sensibility of the aged myocardium to the reperfusion syndrome might be related to a reduced ability of the heart to eliminate hydrogen peroxide, and more generally organic hydroperoxides. In addition, cellular ageing has been shown to be characterised by an increased cytosolic density that could represent an overall basis for a further down-regulation of the cellular enzymes involved in ROS elimination [31, 32].

The present study clearly demonstrates, for the first time to our knowledge, that high selenium intake reduces the susceptibility of senescent rat hearts to ischaemia and reperfusion. This cardioprotective effect is directed to cardiomyocytes and does not involve any major vascular effect since no significant difference in coronary flow was evidenced between groups at any time of our protocol. We therefore propose that the increased activity of myocardial GPx induced by the high-selenium diet could compensate for the impairment of hydrogen peroxide eliminating systems in aged cardiomyocytes. This hypothesis is supported by both a functional and an ultrastructural preservation of cardiac cells during ischaemia and reperfusion in high-Se rats.

Together with the observation that selenium status decreases with age in humans [33], our results suggest that reinforcing selenium supply might improve the prognosis of cardiovascular diseases in old patients.

Additional experiments are now required to further investigate the intracellular mechanisms that are responsible for the cardioprotective action of high selenium intake in senescent rats. For instance, high-energy phosphate myocardial content and lactate release assessments on isolated heart preparations are now in progress in our group in order to verify whether selenium allows a metabolic preservation in the ischaemic senescent myocardium.

Key points

- Peroxides play a key role in the increased cardiac sensitivity to ischaemia and reperfusion during ageing.
- Reinforcing selenium supply might improve the prognosis of cardiovascular diseases in old patients.

Acknowledgements

The authors would like to thank Dr Claude Sebban and Brigitte Decros for kindly providing senescent rats. Stéphane Tanguy was supported by ‘Centre Evian pour l’Eau’.

References


Received 5 April 2002; accepted in revised form 29 October 2002