

The Significance of Glutathione Peroxidase on Myocardial Protection in the rat Hearts:

The Key of Clarify the Cause of Vulnerability to Reperfusion Injury in Infantile Cardiac Operations

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Abstract

Selenium (Se) is an integral component of glutathione poeroxidase (GSHPx), and the serum selenium concentration is age-depend. We speculated that myocardial GSHPx had relation to reperfusion injury in open heart operations, especially in infants in whom GSHPx activity is low. This study correlated GSHPx activity with the serum and myocardial selenium concentrations in Wistar rats, which were divided into three groups, infants, Se-deficient rats, and control rats. Serum GSHPx activity in infant and Se-deficient rats (22.7 ± 3.5 U/g protein, 24.6 ± 22.2 U/g protein) was lower than that in controls (179 ± 12.0 U/g protein). The serum selenium concentration in infant and Se-deficient rats (3.81 ± 0.81 μ g/g protein, 2.06 ± 1.69 μ g/g protein) was also lower than that in controls (7.32 ± 2.96 μ g/g protein). The myocardial GSHPx activity was significantly lower in infants and Se-deficient rats ($4.76 \pm 1.05 \times 10^{-1}$ U/mg protein, $3.38 \pm 0.32 \times 10^{-1}$ U/mg protein) than that in controls ($8.03 \pm 0.57 \times 10^{-1}$ U/mg protein). However, the myocardial selenium concentration in infants ($1.42 \pm 0.24 \times 10^{-1}$ μ g/mg protein) was significantly higher than that in the other groups ($0.31 \pm 0.06 \times 10^{-1}$ μ g/mg protein, $0.28 \pm 0.04 \times 10^{-1}$ μ g/mg protein). Next, in Se-deficient and control rats, isolated hearts were perfused for aerobically with Krebs-Henseleit solution in the Langendorff mode for 15 minutes, followed by 60 minutes of global ischemia at 4°C and then reperfused for 30 minutes in a working mode. The hemodynamic parameters were measured. The aortic pressure, LV max dp/dt, aortic flow, cardiac output and stroke volume were significantly lower in the Se-deficient rats than those of the control rats. Immediately following these measurements, the hearts were frozend in liquid nitrogen, and the myocardial lipid peroxide (TBARS) concentration was assayed and found to be significantly higher in the Se-deficient rats. The lower myocardial GSHPx activity may play a important role in vulnerability to reperfusion injury in infants as in Se-deficient rats.

Key words: Selenium, Glutathione Peroxidase, Reperfusion Injury in Infancy, Myocardial Lipid Peroxide

索引用語: セレン, グルタチオンペルオキシダーゼ, 再灌流障害, 心筋内過酸化脂質.

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Introduction

The mammals, whose existence is based on oxygen, have defense mechanisms against active oxygen radicals. These defense mechanisms include superoxide dismutase (SOD), catalase, glutathione peroxidase (GSHPx), and other low molecular weight antioxidants. GSHPx catalyzes the breakdown of hydrogen peroxide, with glutathione serving as the hydrogen donor. GSHPx also catalyzes the reduction of hydroperoxides formed from fatty acids or from other substances^{1,2}.

Selenium was found to be a beneficial trace element for animals in the same year that GSHPx was discovered, by an interesting coincidence^{3,4}. ROTRUCK et al⁵ discovered that the formation of GSHPx in animals was dependent on selenium. The GSHPx is composed of four identical subunits, and it has been assumed that there is one selenium molecule per subunit⁶. Although the selenium atoms have not yet been shown to participate in the catalysis reaction, the unique chemical properties of selenium and its presence in stoichiometric amounts with the number of subunits suggest that it may function at the active site⁷. A combined deficiency of selenium and vitamin E induces degenerative heart disease in several species of domestic animals. A cardiomyopathy has been described in the inhabitants of Keshan country in the People's Republic of China. This disease, "Keshan disease", is characterized by multiple areas of focal myocardial necrosis and is characteristically seen in growing animals and in children aged 1 to 9 years. Affected individuals have very low serum selenium concentrations, and improve with selenium therapy⁸.

Recent work has shown oxygen free radicals may be the principal pathogenic factor in the development of reperfusion injury in the hearts⁹⁻¹¹. We speculated that myocardial GSHPx may play an important role in reperfusion injury in open heart operations, especially in infants whose GSHPx activity is lower as a result of the low serum selenium concentration, and this is the key to clarify why myocardial reperfusion injury occurs more easily in infants than in adults. The purpose of this study is to test this hypothesis by measuring serum and myocardial GSHPx activity with their selenium concentrations and by measuring hemodynamic parameters using the isolated heart model in Wistar rats.

Method

Wistar rats were divided into three groups. One, infant rats 8 to 12 days after birth (infant rats $n=16$); two, adult rats fed a selenium-deficient diet for 3 months (Se-deficient rats $n=5$); and three, adult rats fed a normal diet for 3 months (control rats $n=5$). The rats were heparinized and anesthetized with 50 mg/Kg body weight intraperitoneal sodium pentobarbital. The abdomen was opened and the blood was collected from the abdominal aorta. The blood was prepared for assaying the serum selenium concentration and the serum GSHPx activity. Following the introduction of anesthesia in the same manner, the chest was quickly opened, and the beating heart was rapidly excised and homogenized in Hanks solution. After centrifugation at 105,000 g for 1 hour, the supernatant was prepared for assaying the myocardial selenium concentration and the myocardial GSHPx activity. The other Se-deficient rats ($n=6$) and the control rats ($n=6$) were heparinized and anesthetized with 50 mg/Kg body weight intraperitoneal sodium pentobarbital. The chest was then quickly opened, and the beating heart was rapidly excised, and briefly immersed in cooled (4°C) lactate Ringer's solution. The heart was then secured to a Langendorff column and was perfused with Krebs-Henseleit solution equilibrated with 95% O₂ and 5% CO₂ at 37°C. Following a 15 minutes

period of stabilization, the heart rate and coronary flow were measured. Then St. Thomas solution (4°C) was injected into the aortic root at a pressure of 50 cm H₂O for 2 minutes. During the 60 minutes arrest period, the myocardial temperature was maintained at 4°C by topical cooling. After that the heart was perfused with oxygenated Krebs-Henseleit solution by Langendorff's circulation for 15 minutes at 37°C, followed that an oxygenated Krebs-Henseleit solution was infused through the left atrium at a pressure of 13 cm H₂O at 37°C. The perfusate then passed to the left ventricle, from which it was ejected against a hydrostatic pressure equivalent to 70 cm H₂O. The coronary flow, aortic flow, aortic pressure, and left ventricular (LV) pressure and its first derivative (max dp/dt) were measured 30 minutes after the start of reperfusion. All hearts were maintained in a thermostatically controlled chamber at 37°C. After the measurement of these hemodynamic parameters, the hearts were quickly frozen with a clamp cooled in liquid nitrogen. Lipid peroxide (thiobarbituric acid reactive substance, TBARS) was analyzed in n-butanol extracts of the frozen tissue by Yagi's method¹². All animals received human care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 80-23, revised 1978).

Assay Methods

The serum and myocardial GSPHx activities were assayed according to the "coupled test" with a minor modification for applying the sample to a Cobas Bio centrifugal analyzer with program 8326. A unit of GSHPx activity was defined as the activity required to decrease the NADPH (nicotinamide adenine dinucleotide phosphate reduced form) concentration by 0.5 μ M per minute¹³. The serum and myocardial selenium concentrations were determined by electrothermal atomic absorption spectrometry according to a standard addition method with rhodium as a matrix modifier^{14,15}.

The protein concentration of each material was measured. All data were recorded per unit of protein.

Statistical Analysis

All values are expressed as mean $\pm$ standard deviation (SD). To assess the significance of the difference, the Student's unpaired test was used. The differences were considered significant if they reached the $p < 0.05$ levels.

Results

The serum GSHPx activity is shown in Fig. 1; it was 22.7 ± 3.5 U/g protein in the infant rats, 24.6 ± 22.2 U/g protein in the Se-deficient rats, and 179.6 ± 12.0 U/g protein in the control rats. The serum GSHPx activity in both infant and Se-deficient rats was significantly lower than in the control rats.

The myocardial GSHPx activity is shown in Fig. 2; $4.76 \pm 1.05 \times 10^{-1}$ U/mg protein in the infant rats, $3.38 \pm 0.32 \times 10^{-1}$ U/mg protein, and $8.03 \pm 0.57 \times 10^{-1}$ U/mg protein in the control rats. The myocardial GSHPx activity in the infant and Se-deficient rats was significantly lower than that in the control rats.

The serum selenium concentration is shown in Fig. 3; it was 3.81 ± 0.81 μ g/g protein in the infant rats, 2.06 ± 1.69 μ g/g protein in the Se-deficient rats, and 7.32 ± 2.96 μ g/g protein in the control rats. The serum selenium concentrations in both infant and Se-deficient rats were significantly lower than that in the control rats.

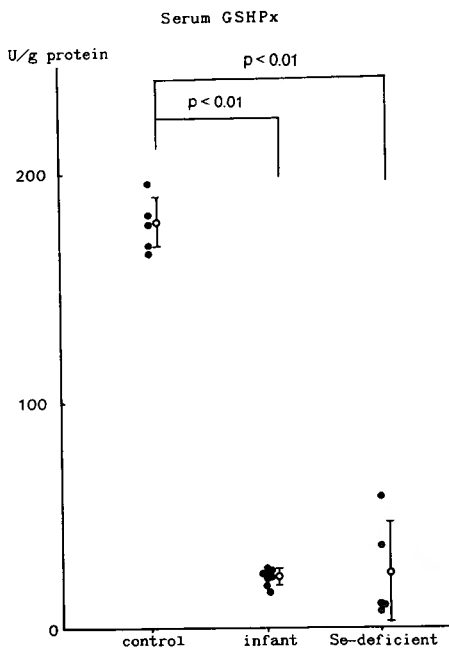


Fig. 1 Serum selenium concentration; The serum selenium concentrations in both infant and Se-deficient rats were significantly lower than that in the control rats.

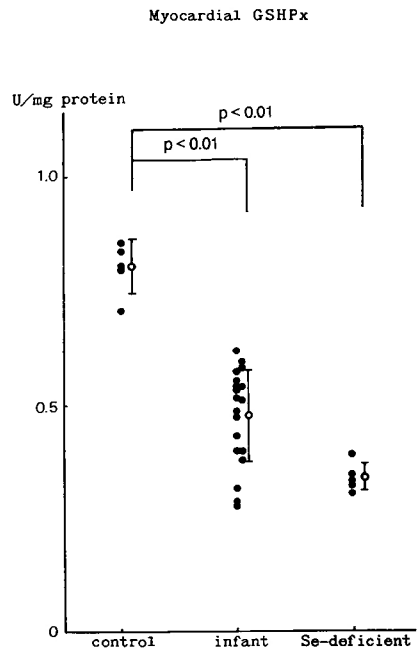


Fig. 2 Myocardial selenium concentration; The myocardial selenium concentration in the infant rats was significantly higher than that in both the Se-deficient and the control rats.

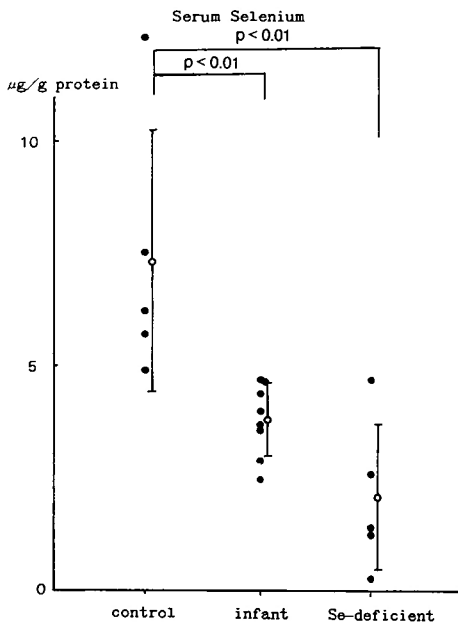


Fig. 3 Serum glutathion peroxidase (GSHPx) activity; The serum GSHPx activity in both infant and Se-deficient rats was significantly lower than in the control rats.

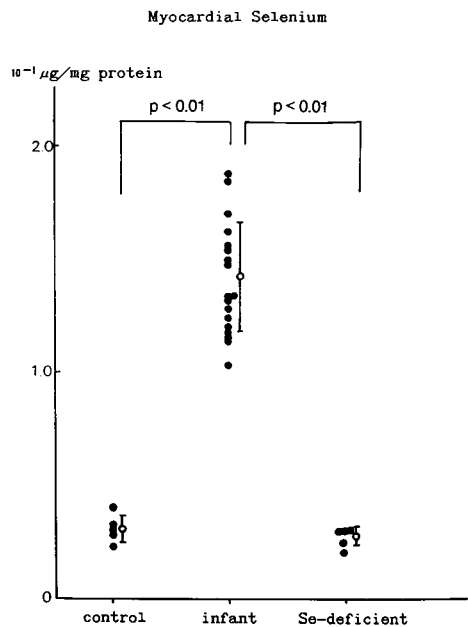


Fig. 4 The myocardial glutathione peroxidase (GSHPx) activity; The myocardial GSHPx activity in the infant and Se-deficient rats was significantly lower than that in the control rats

The myocardial selenium concentration is shown in Fig. 4; it was $1.42 \pm 0.24 \times 10^{-1} \mu\text{g}/\text{mg}$ protein in the infant rats, $0.31 \pm 0.06 \times 10^{-1} \mu\text{g}/\text{mg}$ protein in the Se-deficient rats, and $0.28 \pm 0.06 \times 10^{-1} \mu\text{g}/\text{mg}$ protein in the control rats. The myocardial selenium concentration in the infant rats was significantly higher than that in both the Se-deficient and the control rats.

The heart rate during Langendorff's circulation before the cardiac arrest was 232 ± 47 beats/minutes in the Se-deficient rats, and 267 ± 35 beats/minutes in the control rats, which was not significantly different from the Se-deficient rats. The coronary flow during Langendorff's circulation before the cardiac arrest was $6.3 \pm 1.9 \text{ ml}/\text{g}$ cardiac wet weight in the Se-deficient rats, and $7.4 \pm 1.6 \text{ ml}/\text{g}$ cardiac wet weight in the control rats, which also not significantly different from the Se-deficient rats. The hemodynamics at 30 minutes after reperfusion, which followed 60 minutes of ischemia were as follows (Fig. 5):

Heart rate: 248 ± 31 beats/minutes in the Se-deficient rats, and 258 ± 24 beats/minute in the control rats, which was not significantly different from the Se-deficient rats.

LV systolic pressure: $86.3 \pm 18.9 \text{ mmHg}$ in the Se-deficient rats, and $99.0 \pm 8.1 \text{ mmHg}$ in the control rats. There was not significant $99.0 \pm 8.1 \text{ mmHg}$ in the control rats. There was not significant difference.

LV diastolic pressure: $7.5 \pm 4.0 \text{ mmHg}$ in the Se-deficient rats, and $6.5 \pm 3.0 \text{ mmHg}$ in the control rats, which was not significantly different.

Aortic systolic pressure: $59 \pm 2.0 \text{ mmHg}$ in the Se-deficient rats, and $77 \pm 9.0 \text{ mmHg}$ in the control rats. The aortic systolic pressure in the Se-deficient rats was significantly lower than that in the control rats ($p < 0.01$).

LV max dp/dt: $2033 \pm 153 \text{ mmHg}/\text{second}$ in the Se-deficient rats, and $2711 \pm 13 \text{ mmHg}/\text{second}$ in the control rats. LV max dp/dt was significantly lower in the Se-deficient rats than in the control rats ($p < 0.05$).

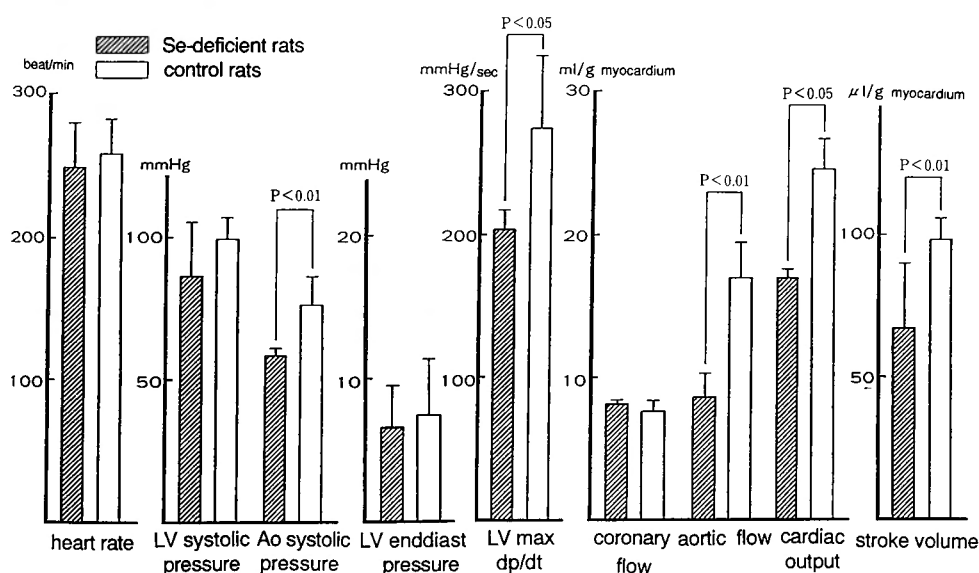


Fig. 5 The comparison of hemodynamic parameters; LV: left ventricle, Ao: aorta, LV max dp/dt: maximal rate of the first derivative of left ventricular pressure

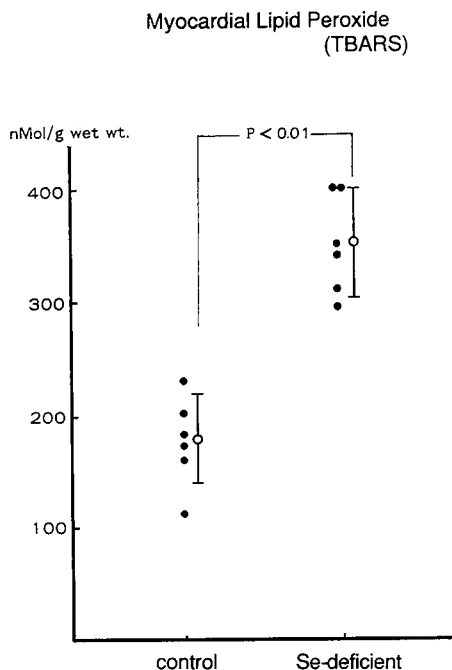


Fig. 6 The myocardial lipid peroxide (thiobarbituric acid reactive substance TBARS) level; The myocardial TBARS in Se-deficient rats was significantly higher than that in the control rats.

179 $\pm$ 41 nMol/g cardiac wet weight in the control rats. The myocardial lipid peroxide level in Se-deficient rats was significantly higher than in the control rats (Fig. 6).

Discussion

Optimal myocardial protection during open heart operation in infants is still controversial. Although the use of cold cardioplegia with mild hypothermia is a well established method of myocardial protection in adults, this is not uniformly accepted in infants. Current cardioplegic technique, largely based on experience with adult patients, may be inadequate for the infant, particularly for the neonate. Many investigators have reported structural, metabolic and functional differences between the neonatal and adult hearts. These features in the neonatal heart include the underdevelopment of the sarcoplasmic reticulum^{16,17}, a lower sarcoplasmic reticular calcium ATPase activity with less active calcium sequestration¹⁸, and the greater dependence of the myocardial cells on extracellular Ca^{++} for excitation-contraction coupling¹⁹. Also isometric force development and the extent and velocity of shortening at any load are lower in the neonatal heart which is related to its lower intrinsic enzymatic activities of actomyosin or myosin²⁰. The water content of the neonatal heart is larger than that of the adult heart, therefore myocardial edema may be more likely to occur during reperfusion²¹. On the other hand, some investigator has reported that the newborn myocardium was more tolerant of hypoxia and ischemia than the adult heart because of increased glycogen stores for greater anaerobic glycolysis²²⁻²⁵. The tolerance of immature hearts to ischemia is related to amino

Coronary flow: 8.3 $\pm$ 0.2 ml/g cardiac wet weight in the Se-deficient rats, and 7.6 $\pm$ 0.8 ml/g cardiac wet weight in the control rats. There was not significant difference between these two groups in regard to coronary flow.

Aortic flow: 8.7 $\pm$ 2.7 ml/g cardiac wet weight in the Se-deficient rats, and 17.0 $\pm$ 2.5 ml/g cardiac wet weight in the control rats. Aortic flow was significantly lower in the Se-deficient rats than in the control rats ($p < 0.01$)

Cardiac output (the sum of coronary and aortic flow): 17.0 $\pm$ 4.6 ml/g cardiac wet weight in the Se-deficient rats, and 24.6 $\pm$ 2.0 ml/g cardiac wet weight in the control rats. Cardiac output was significantly lower in the Se-deficient rats than in the control rats ($p < 0.05$).

Stroke Volume: 67.5 $\pm$ 11.6 l/g cardiac wet weight in the Se-deficient rats, and 95.6 $\pm$ 9.8 l/g cardiac wet weight in the control rats. Stroke volume in the Se-deficient rats was significantly lower than that in the control rats.

The myocardial lipid peroxide (TBARS) level was 352 $\pm$ 49 nMol/g cardiac wet weight in the Se-deficient rats, and 179 $\pm$ 41 nMol/g cardiac wet weight in the Se-deficient rats, and

acid utilization by transamination and increased substrate level phosphorylation. This substantial difference between the neonatal and adult myocardium has not yet been confirmed.

In the present studies, the state of low myocardial GSHPx activity was induced by Se-deficient diet in Wistar adult rats. The hemodynamics at 30 minutes after the reperfusion of ischemic heart were significantly depressed in the Se-deficient rats. These results suggest the low myocardial GSHPx activity produced the more lipid peroxide at reperfusion of the ischemic heart, and related to vulnerable myocardial protection in the Se-deficient rats. And present studies confirmed that the state of the serum and myocardial GSHPx activity which was induced in Se-deficient rats was similar one found in the infant rats. LOMBECK et al⁽²⁶⁾ have reported that serum selenium concentrations exhibit a clear-cut age dependency, and are lower in early infancy (lowest in infant 1-4 months old) than in adults⁽²⁷⁾.

We speculated that in infant rats the low myocardial GSHPx activity related to vulnerable myocardial protection as like in Se-deficient rats, and the low myocardial GSHPx activity was as the result of the low myocardial selenium concentration as like that in Se-deficient rats, too. The myocardial GSHPx activity in infant rats was significantly lower than that in the control rats. However, the myocardial selenium concentrations was significantly higher in the infant rats, which was the only difference between the infant rats and the Se-deficient rats. The reason for the higher myocardial selenium concentration despite the lower serum selenium concentration in the infant rats is unclear. It is also unclear why the myocardial GSHPx activity is lower despite the higher myocardial selenium concentration. There are several possible explanations for these findings. First, selenium must be incorporated into the polypeptide chains of GSHPx in the form of selenocystein residue in order for fully functional GSHPx to be produced. Selenocystein is synthesized from selenomethionine and inorganic selenium compounds, which are found in some foods, by the coupling reactions of cystathionine β -synthase and cystathionine γ -lyase⁽²⁸⁾. In infant rats both of those enzyme maybe low-functioning. Second, more than 80% of the selenium in selenoproteins exists in the form of a selenocysteine residue, and a half of this in mature rats is associated with GSHPx⁽²⁹⁾. However, in infant rats there may be large amounts of selenoprotein which is not associated with GSHPx, and the biologic role of these protein is unknown. Third, COHEN et al⁽³⁰⁾ reported there is about a 20 day time lag to synthesized GSHPx. Therefore, the myocardial selenium in infants may contribute to the future synthesis of GSHPx but is not strictly related to the current synthesis of GSHPx. In infant rats selenium does not manifest an effective function for GSHPx despite its high level by these suspected reasons.

Based upon our data, we are convinced that Se-deficient rats adequately reflected infant rats with regard to GSHPx. And we inferred that the low myocardial GSHPx activity in infant rats may play an important role in occurrence of reperfusion injury from the evidence confirmed in Se-deficient rats, although it was difficult to examine the hemodynamics in neonatal rat heart using the working heart model by technical reason.

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和文抄録

心筋保護におけるグルタチオンペルオキシダーゼの重要性 —ラット心を用いて—

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必須微量元素セレン (Se) は、フリーラジカルスカベンジャーの一つであるグルタチオンペルオキシダーゼ (GSHPx) の主要な構成成分である。血清 Se 濃度は年齢により変化し新生児期、乳児期は低い。このことが乳児期の開心術における再灌流障害に関与しているのではないかと推論した。ウィスター系ラットを乳児期ラット (乳児群), Se 欠乏食ラット (Se群), 対照成熟ラット (対照群) の3群に分けた。乳児群, Se 群の血清 GSHPx 活性は、対照群と比べ有意に低値を示した (順番に 22.7 ± 3.5 , 24.6 ± 22.2 , 179.0 ± 12.0 U/g protein)。乳児群, Se 群の血清 Se 濃度も同様に対照群と比べ有意に低値を示した (3.81 ± 0.81 , 2.06 ± 1.69 , 7.32 ± 2.96 $\mu\text{g/g}$ protein)。乳児群, Se 群の心筋 GSHPx 活性は対照群と比べ有意に低値を示した

($4.76 \pm 1.05 \times 10^{-1}$, $3.38 \pm 0.32 \times 10^{-1}$, $8.03 \pm 0.57 \times 10^{-1}$ U/mg protein)。

しかし乳児群の心筋 Se 濃度は Se 群, 対照群と比べ有意に高値を示した ($1.42 \pm 0.24 \times 10^{-1}$, $0.31 \pm 0.06 \times 10^{-1}$, $0.28 \pm 0.04 \times 10^{-1}$ $\mu\text{g/mg}$ protein)。これとは別に Se 欠乏食ラットと対照成熟ラットの摘出心を用いて Neely JR らの working heart model により, 4°C 60分間の心停止後の心機能パラメーターを測定した。大動脈圧, 左室 max dp/dt, 大動脈流量, 心拍出量, 一回拍出量は Se 群で有意に低値を示した。また心筋内過酸化脂質 (TBARS) 濃度は, Se 群で有意に高値を示した。このことから, 心筋内 GSHPx 活性の低下は心筋の再灌流障害と非常に関連が深いことが示唆された。