

REVIEW

Biological Effects of Selenium Compounds With a Particular Attention to the Ontogenetic Development

I. OŠŤÁDALOVÁ^{1,2}

¹Centre for Cardiovascular Research, Prague, Czech Republic, ²Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

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Summary

Selenium is a trace element that is essential for living organism. Its beneficial effect is, however, expressed in a very narrow dosage range: the high and low doses of selenium are connected with pathological manifestations. The toxicity depends on the chemical form of selenium, state of organism, interactions with heavy metals and on the stage of ontogenetic development. Whereas one dose of sodium selenite (20 $\mu\text{mol/kg}$ b.w.) is lethal in adult rats, suckling rats are entirely resistant. However, within one week after administration of the same dose, cataract of eye lens developed. The highest incidence of cataract was observed in 10-day-old animals and it decreased until day 20. From postnatal day 20 to day 40 the rats were resistant to both the lethal and cataractogenic effects of selenium. The incidence of cataract may be suppressed by premature weaning, lower hydration of suckling, change of water soluble/water insoluble lens protein ratio, thyroxine treatment, and by interaction with mercury. By means of its oxidative and reduction properties, selenium is involved in the maintenance of the cell redox homeostasis. Typical example is its possible cardioprotective effect: selenium decreased number of arrhythmias, reduced infarct size and improved the contractile recovery after ischemia/reperfusion injury. Selenium supplementation may thus increase cardiac tolerance to ischemic damage.

Key words

Development • Selenium toxicity • Cataractogenic effect • Lethal effect • Cardioprotective effect

Corresponding author

I. Ošťádalová, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic. E-mail: iostadal@biomed.cas.cz

Introduction

Selenium is a trace element that is essential for living organisms. The only source of selenium for mammalian food chain is the soil. The amount of selenium in the soil is not equal; it depends on the nature of soil and in particular on its pH. There are geographic areas with high content of selenium e.g. in China, USA and Canada. On the other hand, there are areas with very low content of selenium as other areas in China, Finland and New Zealand (Robinson and Thompson 1981, Diplock 1993). Water washes the selenium out of soil so that plants take in selenium not only from soil but also from water. The concentration in plants is not identical, some plants are known as selenium accumulators since they are able to accumulate selenium against a concentration gradient. At the end of the food chain are people taking up selenium from a plant food directly, through animal food, and from water. In the organism, selenium is transported to the organs and cells; it passes well over placental barrier as well as through mother milk to sucklings (Allen and Miller 1981, Enjalbert *et al.* 1999). After ingestion by humans and animals, selenium is partly expired and mostly returned to the soil through urine and feces. Some relatively inert chemical forms of selenium are converted to selenite and selenate by soil bacteria.

Selenium compounds

Inorganic selenium forms, selenite and selenate, predominate in soil and water, whereas organic selenium

compounds, selenomethionine and selenocysteine, predominate in plants and carnal food and milk. In contrast to thiols, selenols are largely ionized at neutral pH and they are anionic in the enzymes. The lower redox potential of selenol as compared with corresponding thiol may explain the presence of selenols in redox catalysts (Stadtman 1980). The discovery of selenium as an essential trace element of considerable physiological significance in warm-blooded animals comes from Schwarz and Foltz (1957). Rotruck and coworkers (1972) discovered that selenium is located in the active site of glutathione peroxidase, the enzyme which protects liver cells against mitochondrial swelling, and they described the exact structure of this enzyme.

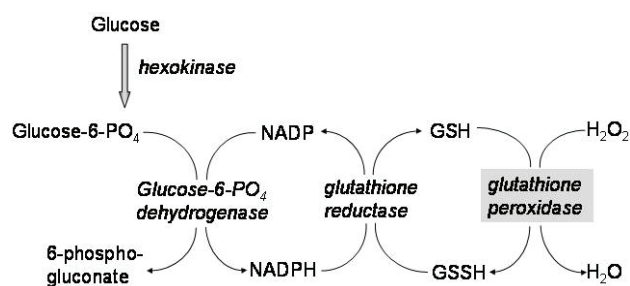


Fig. 1. Decomposition of ROS.

Selenium is a component of several enzymes. Cellular glutathione peroxidase (GPx) – the first identified selenium-dependent enzyme – converts reduced glutathione (GSH) to oxidized glutathione (GSSG) and simultaneously decomposes hydrogen peroxide. The reduction of hydrogen peroxide is coupled with the reduction of NADPH and conversion of glucose-6-phosphate to 6-phosphogluconate. Glucose is necessary for preservation of GPx activity (Fig. 1). Tripeptide GSH, synthesized in most if not all mammalian cells, is a critical source of reducing power and is involved in a number of different functions (detoxication, disulfide bond formation, maintenance of thiol status, antioxidant defense, and modulation of proliferation, differentiation and apoptosis). The central cysteine group is essential in the regulation of sulfide bonds and in the disposal of oxidants. The antioxidant function is mediated by the redox active thiol group, which becomes oxidized when GSH simultaneously reduces target molecules. GSH (at millimolar concentrations in cells), can influence the redox potential, and can interfere with redox signalling. Later, other glutathione peroxidases were discovered: phospholipid hydroperoxide glutathione peroxidase

(GPX4, Ursini *et al.* 1985), extracellular glutathione peroxidase (GPX3, Takahashi and Cohen 1986), and gastrointestinal glutathione peroxidase (GPX2, Chu *et al.* 1993). At present, eight isozymes are known; they are encoded by different genes and vary in substrate specificity and cellular localization.

Iodothyronine deiodinases (Berry *et al.* 1991, Bianco *et al.* 2002) represent a new family of eukaryotic selenoproteins. They are localized in strategically important locations in the cells enabling them to act as gate-keepers to the nuclear receptors (Köhrle 1996). Three iodothyronine deiodinases were described: type I and II activate the thyroid hormone by converting T₄ (thyroxine) to T₃ (triiodothyronine), type III inactivates T₃; the thyroid gland is thus rich in selenium. The experiments showed that the low levels of serum selenium or selenoprotein P do not necessarily interfere with regular functioning of the thyroid hormones axis. It seems that the 5'-deiodinase isoenzymes are preferentially supplied with selenium and that they are even less dependent on serum or brain selenium levels. Thyroid gland has a top priority in respect to the hierarchy of selenium supply in the organism (Schomburg *et al.* 2005). Expression of these selenoenzymes plays a role during embryonic development and in the regulation of metabolic rate.

Thioredoxin reductase is a ubiquitous protein with redox active cysteine residues. The reduced thioredoxin acts as an electron donor and also scavenges intracellular H₂O₂ while it is catalyzed by a family of thioredoxin-dependent peroxidases (it reduces thioredoxin and thereby regulates cellular protein thiol redox status) (Park and Suzuki 2007). Other biologically active selenium compounds are mitochondrial capsulae selenoprotein in the mouse spermia (Karimpour *et al.* 1992), selenoprotein W in skeletal muscles (Vendeland *et al.* 1995), and selenoprotein P (Yang *et al.* 1987) used as a biomarker of selenium status (Burk *et al.* 2003).

Considering the importance of selenium for different essential functions, a marker of selenium status would be very useful. However, there are different opinions. Blood platelets were considered in this respect because they have a relatively short biological life span and they seem to be useful for determination of actual selenium status (Levander *et al.* 1983). The other possibility is represented by erythrocytes, since they are at greater risk from peroxides than most other cells, due to their much higher concentration of oxygen (Chambers *et al.* 1986); they contain glutathione peroxidase which is

a good indicator of long-term selenium intake. However, Xia *et al.* (2005) suggested that selenoprotein P requires a greater selenium intake than full expression of plasma glutathione peroxidase; selenoprotein P was thus recommended as a better indicator of selenium nutritional status than glutathione peroxidase.

Biological effect of selenium

Selenium is an essential trace element and its beneficial effect is expressed in a very narrow dosage range. The high and low doses of selenium are connected with pathological manifestations (Fig. 2). However the availability and biological effects of selenium are dependent on a number of circumstances.

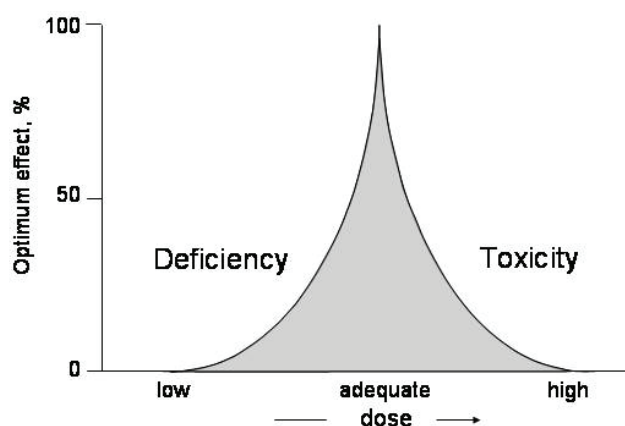


Fig. 2. Dose-dependent biological effects of selenium.

Dose

The effects of selenium are dose-dependent (Fig. 2). Recommended dietary allowance of selenium for healthy adults is 55 µg/day (Schelenz 1987, Burk 2002); the dose for children, pregnant and lactating women has, however, not yet been determined.

Excessive dose of selenium induces toxicity both in humans and in animals. Acute human intoxication is rare and it is almost invariably fatal, manifested by stupor, hypotension and respiratory depression. Chronic selenium poisoning, reported in some areas in China, produces selenosis in humans and induces changes in the liver, skin, hairs and nails. Garlic odor of the breath is the evidence for expiration of dimethyl selenide and is an indicator of selenium intoxication (Barceloux 1999). Selenite biotransformation to dimethyl selenide involves a six valence reduction to H₂Se and subsequent methylation. Both steps are exhausting and induce

depletion of methyl groups and antioxidative reserves.

Toxic syndrome in different farm species of animals is more frequent. The first reasonable data came from Nebraska (Moxon 1938): the disorder was characterized by loss of hair and soreness of the feet and was named „alkali disease“, since the farmers believed that it was caused by the alkali (high salt) waters and seepages of the area. Conclusive evidence that a high dose of selenium was responsible for this disease arose from the experiments of Franke and Potter (1936). Alkali disease was considered as a chronic stage of selenium intoxication, a state associated with the dizziness and designated as „blind stagger“; it represents an acute form of intoxication.

Toxicity of selenium in rats is dependent on their selenium state. It was demonstrated that pretreatment with a low dose of selenium protects the rats against a lethal effect of other selenium compounds (Pařízek 1976, Kalousková and Pavlík 1982).

However, even selenium deficiency is connected with pathological states in animals: liver necrosis in vitamin E-deficient rats, exudative diathesis in chicken and „white muscle disease“ in lambs and calves (Schwarz 1976). Moreover, selenium deficiency markedly enhances lipid peroxidation in the heart cells *via* depressed glutathione peroxidase activity that might be responsible for the decrease of Ca²⁺-ATPase and Ca²⁺ uptake activities in the sarcoplasmic reticulum in selenium-deficient animals (Schwarz 1976, Wang *et al.* 1993).

In humans, selenium deficiency has been implicated as a pathogenic factor in the development of Keshan disease. It is a potentially fatal form of dilated cardiomyopathy restricted to the endemic areas in China (northeastern Chinese county Keshan) and seen in residents having an extremely low selenium status, with a prevalence in children (Yang 1985). Selenium deficiency is probably a necessary but not sufficient factor for the development of this cardiomyopathy. To trigger the disease, the infection by coxsackie B virus is essential. The administration of selenite inhibited the replication of coxsackie B virus and demonstrated the prophylaxis of Keshan disease (Cermelli *et al.* 2002). Pathological, biochemical as well as clinical studies of Keshan disease demonstrated an increase of enlarged and swollen cardiac mitochondria with distended crista membranes, together with a significant reduction in the activity of oxidative phosphorylation. It was, therefore, suggested that mitochondria are the predominant target of the pathogenic factors of the disease. On the basis of these

results it was considered that Keshan disease might be classified as „mitochondrial cardiomyopathy“ endemic in China (Fuyu 2006). Selenium deficiency and antioxidant status in general increase the sensitivity to viral infections. Severe pathology of hearts was demonstrated in mice infected with a myocarditic coxsackie virus B3 (CVB3/20) (Beck *et al.* 1994). Another syndrome of selenium deficiency in humans is the Kashin-Beck illness. This disease is induced by the selenium deficiency, together with the iodine deficiency, and results in atrophy, degeneration and necrosis of cartilage tissue (Moreno-Reyes *et al.* 1998).

In this connection, it is necessary to mention that selenium pathology is a subject of a new scientific discipline – geomedicine – dealing with the influence of natural factors on the geographical distribution of problems in human and veterinary medicine (Steinnes 2009).

Chemical form

For the biological effects of selenium its chemical form is also very important. Selenium is usually determined as its total concentration. However, different chemical forms of selenium have different toxic potentials and effects. Organic compounds of selenium are more effective in comparison with inorganic compounds. Selenium as selenomethionine has nearly twice the bioavailability of selenium as selenite (Xia *et al.* 2005). The different bioavailability of selenium compounds contributes to the controversy of selenium action.

State of the organism

Another important factor for biological effect of selenium is the state of the organism, the inherited characteristics, previous history, physiological state, gender, nutritional status, the supply of other trace elements, and concomitant effects of other simultaneously acting factors of the environment (Pařízek 1975, Pařízek *et al.* 1980). Selenium enters into interactions with nutritional status of the animals and humans. For example, the compensated selenium deficiency can become decompensated by simultaneous vitamin E or C deficiency or by prooxidative nutrients (Mertz 1985, Duarte and Lunec 2005). It is interesting to mention that the prooxidant vs. antioxidant properties of vitamin C are in its concentration, since *in vitro* data suggest that at low levels of vitamin C it can act as a prooxidant, but as an antioxidant at high levels. However, *in vivo* evidence for this contention is lacking, and there are data showing that vitamin C predominantly reduces *in*

vivo oxidative damage (Buettner and Jurkiewicz 1996).

Interactions with other elements

Selenium enters into the interactions with a number of other elements. Selenite acts as a strong oxidative agent with a powerful lethal action (Ošťádalová *et al.* 1978). We have shown (Pařízek and Ošťádalová 1967) that administration of selenite prevents completely the toxic effect of sublimate, and on the contrary, a single dose of sublimate stopped lethal effect of selenite. The protective effect is, however, not connected with the increased excretion, but with increased retention of both mercury and selenium in the organism, and with a decrease of excretion of both elements (Pařízek *et al.* 1971a). Similar treatment of pregnant rats decreases the transfer of these elements into fetuses and analogous treatment of lactating rat females decreases the content of these elements in the milk and their transfer to sucklings (Pařízek *et al.* 1969a, 1969b, 1971a, 1971b). It might be supposed that selenium and heavy metals, e.g. mercury, form a complex or that they may compete for binding sites of proteins or other compounds. Mercury increases the content of selenium in the organism, however, in the nonavailable form, and in this way decreases both the disposal of biological effective selenium at low doses (Gasiewicz and Smith 1978, Skerfving 1978, Agarwal and Behari 2007, Su *et al.* 2008), and toxicity of high doses of selenium compounds. Similar interactions were also observed between selenium and cadmium (Pařízek 1978, Pařízek *et al.* 1980).

Developmental aspects of biological effect of selenium

While the beneficial effect of selenium during ontogeny is not known, the toxic actions are markedly dependent on the stage of the ontogenetic development. Prenatal administration of selenium has a teratogenic effect (Puzanová *et al.* 1976). In adult rats, one dose of sodium selenite (20 $\mu\text{mol/kg}$ body weight) induces acute lethal syndrome. The animals die usually within the first two hours after the subcutaneous injection of selenite (Ošťádalová *et al.* 1978, 1979) with signs of cardiopulmonary failure (Fishbein 1977).

Cataract of eye lens

Suckling rats are entirely resistant to the lethal dose of selenite in comparison with adults. However, within one week after the same dose, the cataract of eye

lens develops (Ošťádalová *et al.* 1978); this original observation was later confirmed (Shearer *et al.* 1980, Bunce and Hess 1981, Palmquist *et al.* 1986). A single dose of sodium selenite administered on postnatal day 5 induced 98 % of cataracts, 76 % of cataracts when administered on day 10, 48 % was found when injected on day 15 and the incidence of cataracts decreased almost to zero in animals that received selenite on day 20. In older rats, cataracts of eye lens were never observed (Fig. 3A). It should be mentioned that high incidence of selenite-induced mortality occurred in 50-day-old rats and that the rats in the period between days 20 and 40 were markedly resistant to both cataractogenic and lethal effect of selenite. The increase of the selenite dose extended the lethal effect during the early stages of postnatal development, the period of sensitivity to cataractogenic action was, however, not changed (Fig. 3B) (Ošťádalová *et al.* 1979).

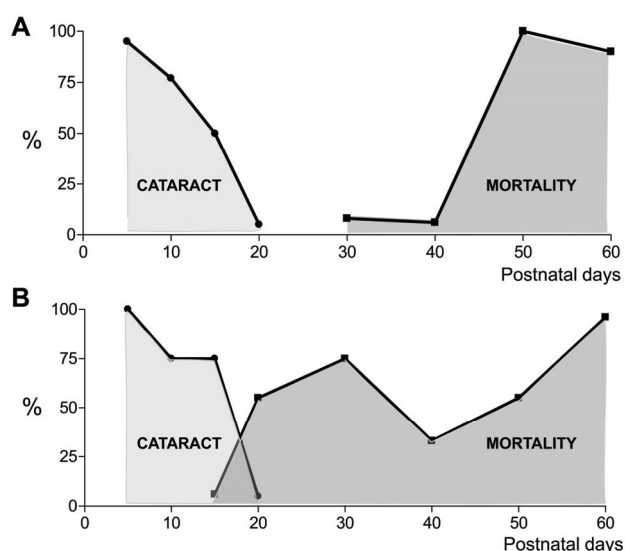


Fig. 3. Age dependence of cataractogenic and lethal effect induced by selenium. A single dose of sodium selenite: **A)** 20 µmol/ kg b.w. s.c., **B)** 40 µmol/ kg b.w. s.c. was administered on postnatal day 5, 10, 15, 20, 30, 40, 50, 60. Number of rats in the groups was more than 20. Values are expressed as percentage of injected animals at the age of 70 days. (Data from Ošťádalová *et al.* 1979.)

The cataractogenic effect is dependent on the chemical form of selenium (Ošťádalová and Babický 1980). While selenium in a higher oxidative form as selenate, D,L-selenomethionine, and D,L-selenocystine induces the cataract of the eye lens, other compounds, as dimethylselenide and trimethylselenonium ion, fail to induce it (Ošťádalová and Babický 1980). Critical period for cataractogenic action of selenite in rats correlates with the suckling period (Babický *et al.* 1970, Ošťádalová *et al.*

1978, 1979).

Our results indicate a strong dependence of selenium toxic effect on the ontogenetic development and suggest a different, developmental mechanism of detoxication or a different sensitivity to the adverse impact of selenium as an oxidant. There is a marked developmental difference in the retention and excretion of toxic doses of selenite. In suckling rats, almost 10 times higher concentration of selenium was observed in the blood, liver, kidney and heart up to day 7 after selenite administration. The highest concentration in the immature rats was in the liver while in the adults it was in the kidney (Table 1) (Ošťádalová *et al.* 1988). Surprisingly, during 2 hours after selenite administration, the suckling rats excrete selenium in the urine only; the adults excreted 5 times higher amount equally in urine and breath (Table 2) (Ošťádalová *et al.* 1982). The methylated metabolites were present in a several times higher proportion in adults as compared with sucklings (Table 3, Fig. 4) (Ošťádalová *et al.* 1982).

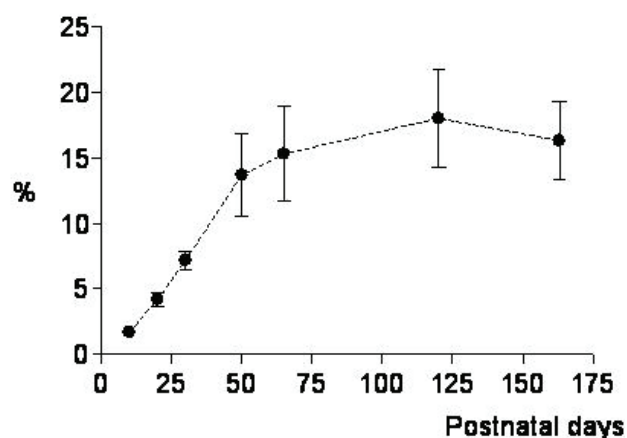


Fig. 4. Age dependence of $(\text{CH}_3)_3^{75}\text{Se}^+$ concentration in rat urine after administration of $\text{Na}_2^{75}\text{SeO}_3$. Content of trimethylselenonium ion during 2 h after injection of 20 µmol/kg b. w. $\text{Na}_2^{75}\text{SeO}_3$ s.c. expressed as a percentage of whole activity in urine. 6-8 rats per group, values are expressed as mean \pm S.E.M. (Data from Ošťádalová *et al.* 1982.)

Possible mechanisms of the selenium-induced cataract

There are numerous ontogenetic differences that might be responsible for the different response to selenium in sucklings and adults. The main difference is in the nutrition. Mother milk is the source of both nutrients and water for the pups. It is known that one of the initial phases of cataract formation is the permeation of water into the lens due to the disturbance of the permeability of lens epithelium membrane, resulting in lens opacity (Obenberger 1977, Martinez and de Jongh 2010).

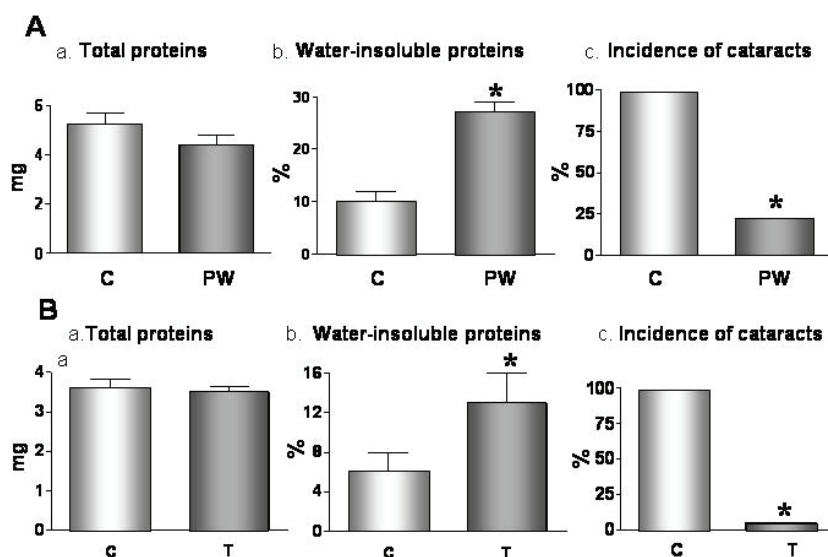


Fig. 5. A. Effect of premature weaning on the eye lens proteins and selenium-induced cataract. On postnatal day 14 the groups of youngs were weaned from the mothers (PW), the control groups (C) remained at the mothers. On day 20 the total lens proteins in mg (a) and water-insoluble proteins as % of total proteins (b) were estimated (8 rats per groups). The incidence of cataracts induced on day 14 (simultaneously with premature weaning) by a single dose of 30 $\mu\text{mol/kg}$ b. w. Na_2SeO_3 was evaluated as a percentage on postnatal day 60 (c) (10 rats per groups). Means \pm S.E.M., * $p < 0.05$. (Data from Babický *et al.* 1982.). **B.** Effect of thyroxine (T) on the eye lens proteins and selenium-induced cataract. Thyroxine was administered in s.c. doses daily (the first week of postnatal life 1 $\mu\text{g}/\text{rat}$, the second week of postnatal life 2 $\mu\text{g}/\text{rat}$), the control groups (C) remained without a treatment. The total lens proteins in mg (a) and water-

insoluble proteins as % of total proteins (b) were estimated on postnatal day 14 (8 rats per groups). The incidence of cataracts induced on day 14 by a single dose of 30 $\mu\text{mol/kg}$ b. w. Na_2SeO_3 in thyroxine pretreated youngs (c) was evaluated as a percentage on postnatal day 60 (10 rats per group). Means \pm S.E.M., * $p < 0.05$.

Table 1. ^{75}Se -concentration in tissues and urine of young and adult rats.

Day	% of administered dose					
	Blood %/g	Liver %/g	Kidney %/g	Heart %/g	Urine %/ml	
Young	1	3.96 \pm 0.15	11.85 \pm 0.96	3.87 \pm 0.16	1.27 \pm 0.06	2.62 \pm 0.19
	7	1.54 \pm 0.09	6.46 \pm 1.11	2.00 \pm 0.10	0.55 \pm 0.03	0.31 \pm 0.06
Adults	7	0.43 \pm 0.03	0.51 \pm 0.04	0.80 \pm 0.10	0.09 \pm 0.02	1.92 \pm 0.20
	7	0.11 \pm 0.003	0.16 \pm 0.01	0.34 \pm 0.03	0.05 \pm 0.003	0.03 \pm 0.01

Values found after s.c. injection of 30 $\mu\text{mol/kg}$ b.w. $\text{Na}_2^{75}\text{SeO}_3$ to young rats (14 days old) or 15 $\mu\text{mol/kg}$ b.w. $\text{Na}_2^{75}\text{SeO}_3$ to adult rats (90 days old), 6-8 rats per group, values are expressed as means \pm S.E.M. (Adapted from Ošťádalová *et al.* 1988.)

Table 2. Ontogenic changes in ^{75}Se excretion.

Age, day	% of administered dose		% of total excretion	
	10	65	10	65
Expired air	0.06 \pm 0.01	5.52 \pm 0.77	2.5	50.3
Urine	2.33 \pm 0.27	5.46 \pm 0.54	97.5	49.7
Total	2.39 \pm 0.27	10.98 \pm 0.94	100.0	100.0

^{75}Se excretion during 2 h after the injection of 20 $\mu\text{mol/kg}$ b.w. $\text{Na}_2^{75}\text{SeO}_3$ s.c., 6-8 rats per group. Values are expressed as means \pm S.E.M. (Adapted from Ošťádalová *et al.* 1982.)

The hydration of the pups after birth markedly differs in comparison with the older age. The newborns face the severe sudden stop of transplacental nutrition. It means that during the first several hours after birth the neonates overcome the hunger and the thirst. Their hydration is depressed and no selenium-induced cataract

in these days was observed. During the following days, lactation as well as the hydration of sucklings is increasing. The next period of lower hydration starts at the end of the suckling period, i.e. on day 16. The amount of maternal milk is not adequate; the youngs start to consume solid food. Presumably the balance between

Table 3. Selenium metabolites in urine in young and adult rats.

Age (days)	10	65
Metabolites in urine (%)		
1. $^{75}\text{SeO}_3^{2-}$	54.5±5.8	66.1±5.0
2. $(\text{GS})_2^{75}\text{Se}$	15.1±1.6	7.2±1.0
3. Start line	27.7±6.4	8.5±1.5
4. $(\text{CH}_3)_3^{75}\text{Se}^+$	1.8±0.3	14.6±3.6
Total	99.1±8.8	96.4±6.4

Selenium metabolites excretion during 2 h after injection of 20 $\mu\text{mol/kg}$ b. w. $\text{Na}_2^{75}\text{SeO}_3$ s.c., 6-8 rats per group. Values are expressed as means \pm S.E.M. (Adapted from Ošťádalová *et al.* 1982.)

supplied calories and water in the mother milk is disturbed by the intake of other calories from the solid food. It seems that this is the reason why water intake follows the intake of solid food, since the immature rats have to keep the osmotic balance (Babický *et al.* 1970, 1972, 1973). It seems that by the end of the suckling period, the hydration decreases and sensitivity to cataractogenic effect of selenite disappears.

Premature weaning on day 14 after birth decreases significantly the hydration of youngs and coincides with a decreased incidence of selenium cataract (Fig. 5A) (Babický and Ošťádalová 1982, Ošťádalová and Babický 1984). It is possible to speculate that higher hydration, typical for neonates, at the time of the advanced milk intake, is probably the essential prerequisite for the cataract development.

To analyze this problem the rats with a disturbance of hydration were investigated. For this purpose the neonates of homozygotes of Brattleboro rats were used. These rats have a congenital defect in water metabolism, resulting from a complete lack of vasopressin synthesis in the hypothalamus, subsequently inducing diabetes insipidus. Their hydration is significantly lower (increased hematocrit, plasma osmolarity, Valtin and Schrier 1997). It has been observed that these rats do not develop selenium cataract at all (Table 4) (Babický *et al.* 1982).

It thus appears that water content is one of the factors important for the maintenance of eye lens transparency and it is regulated by an active transport of Na^+ and K^+ in the lens epithelium. The uptake of rubidium $^{86}\text{Rb}^+$ – analogous to K^+ uptake – into rat eye lens in the experiments *in vitro*, differs substantially with age. A rapid drop in the uptake of ^{86}Rb was found in the lens during neonatal period, critical for the development of selenium cataract (Sládková *et al.* 1984). In the neonatal period, ^{75}Se uptake by lenses *in vivo* is almost

500 times higher and *in vitro* 5 times higher in comparison with the adult rats. It may reflect the age-dependent differences in the structure and function of cellular and subcellular membranes (Babický *et al.* 1985).

Table 4. Incidence of selenium-induced cataract in rats Brattleboro strain.

Age (days)	65	65	
Heterozygotes		Homozygotes	
Male (n=10)	90	Male (n=7)	0
Female (n=12)	92	Female (n=10)	0

Incidence in 2 months old rats after injection of 30 $\mu\text{mol/kg}$ b. w. Na_2SeO_3 s. c. on day 10 of postnatal life. (Data from Babický *et al.* 1982.)

The other important developmental change is the content of total proteins in the eye lens. This parameter increases during the entire suckling period. However, the fraction of water insoluble proteins markedly decreases after birth with a maximum on day 5 and increases again to postnatal day 20. The decay of this protein fraction corresponds with the period of sensitivity to the cataractogenic effect of selenite (Fig. 6A, B).

It is known that the treatment with exogenous thyroxine accelerates the maturation of young rats. The rats open their eyes earlier (10.5±0.5 postnatal day) than the controls (14.2±0.2 postnatal day), they terminate the intake of maternal milk 3-4 days earlier but they are not different from controls in the onset of solid food intake. The fraction of water-insoluble proteins in their eyes increases significantly in comparison with non-treated controls and the incidence of cataracts in thyroxine-treated youngs is significantly limited (Hrdličková *et al.* 1985). It seems that the protein composition in eye lenses plays a crucial role in the development of selenium cataract (Fig. 5B).

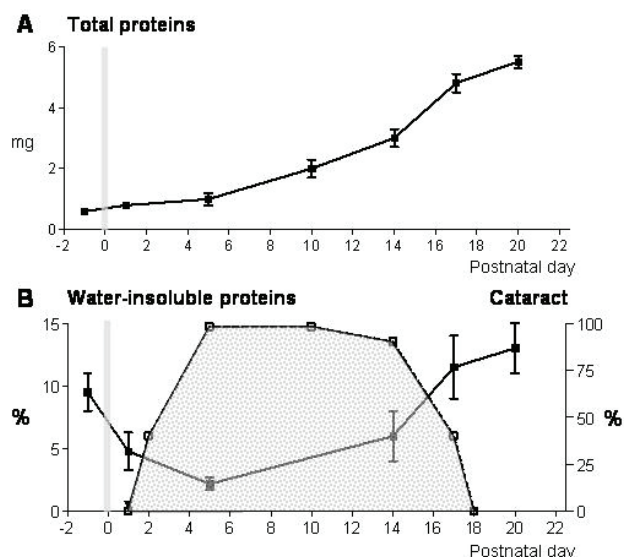


Fig. 6. Dependence of selenium-induced cataracts on the postnatal development of lenticular proteins. Estimation of total proteins in mg from day 1 to 20 (A), and fraction of water-insoluble proteins expressed as % of total proteins (8 rats per group, full square) in relation to incidence of cataracts (empty square) induced by a single dose of 30 $\mu\text{mol/kg}$ b. w. Na_2SeO_3 on day 1, 2, 5, 10, 14, 15, 17 and 18 expressed as a percentage (B), (7-9 rats per group).

It is interesting that the above mentioned mercury-selenium interaction in the relation to mortality may be demonstrated even in the relation to the development of eye lens cataract. In young rats, which are sensitive to the cataractogenic effect, mercury inhibits the development of selenium-induced cataract (Shearer *et al.* 1983). The protective effect of mercury is, however, not accompanied by the decrease of selenium compounds, because mercury increased ^{75}Se uptake into lenses in experiments *in vitro* (Kopoldová *et al.* 1985, Ošťádalová *et al.* 1989) as well as *in vivo* (Ošťádalová *et al.* 1985a,b). Similarly, selenium interacts in the eye lens with thallium: simultaneous administration of thallium together with cataractogenic dose of selenite protects the young rats against the development of selenium-induced cataract (Ošťádalová and Babický 1986, 1987). In this connection it is very interesting to mention that the rats in the developmental period between day 18 and 40, when no cataract was observed, was also resistant to the lethal effect of selenium. This question should be the topic of further experiments.

Selenium-induced cataract became an extremely rapid and convenient model of nuclear cataract, widely used for modelling of various mechanisms of cataract formation and for the screening potential of anti-cataract agents (Yilmaz *et al.* 2000, Zigler *et al.* 2007, Aydın *et al.* 2009, Doganay *et al.* 2009, Kyselová 2010).

The role of selenium in cell signalling

Selenium occurs in the periodic table between sulfur and tellurium; it is very similar to sulfur in a number of respects and forms the analogous compounds in a different oxidative level: elemental selenium (0), selenide (-2), selenite (+4) and selenate (+6). The possibility to convert selenium into a different oxidative level is a very important property for transfer of oxygen radicals.

There are two major pathways for cell signalling: i) phosphorylation of proteins or ii) changes in the thiol status of proteins due to changes in the redox environment of the cell. „Redox state of the cell“ is defined as a balance between reactive oxygen species (ROS) production and their removal by antioxidant systems. ROS are derived from many sources; however, their main source is NADPH oxidase. Both oxidative and reductive stress can trigger redox cascades that bring about changes in the thiol status of the cell. Oxidative stress develops as an imbalance between the production of ROS and the antioxidant defense. However, it is no longer acceptable that ROS are always detrimental to the organism and that high levels of antioxidants must be beneficial due to their scavenging properties (Upham and Trosko 2009). Actually ROS are considered not only a by-product of metabolism but a critical regulator of multiple intracellular signaling cascades. More reactive radicals, such as superoxide and the hydroxyl radical, are unstable and they are unlikely to travel significant distances from the site of their synthesis. They are removed by antioxidant enzyme such as superoxide dismutase that reduces superoxide and hydroxyl radicals to H_2O_2 . However, H_2O_2 has a very low molecular weight, not much different from that of water, and thus can readily traverse the channels and gap junctions and can consequently serve as ideal second messengers in a network of signalling pathways to maintain tissue homeostasis.

Oxidants at noncytotoxic doses can reversibly control the expression of genes. There are a number of genes and signal proteins reported to be sensitive to the redox state in the cell. A response of a cell to growth factors is a transient production of H_2O_2 , as a product of NADPH oxidase. Besides glutathione peroxidase, the cells possess the other antioxidant enzyme that reduces hydrogen peroxide such as catalase (it reduces H_2O_2 to H_2O). However, whereas glutathione peroxidase acts on relatively low levels of hydrogen peroxide, catalase acts

to remove high concentrations or bursts of hydrogen peroxide formation (Meister 1982), and is present mainly in the peroxisomes. To protect against the ROS-induced damage, the GSSH/GSH ratio has a fundamental importance in the cardiomyocytes, because it markedly modulates the cellular redox state. The GSSH/GSH ratio is thus predominantly maintained by two enzymes – glutathione reductase and selenoenzyme glutathione peroxidase (Fig. 2). Glutathione peroxidase is the first well-defined (but not the only) enzyme containing selenium, which is able as the other selenoproteins to take part in electron transfer reactions (Stadtman 1974).

Changes in the cellular redox environment can alter the status of the cell. Schafer and Buettner (2001) and Ng *et al.* (2007) opened a new field of quantitative biology, a rationale of cellular mechanisms associated with cell growth, development, signalling and reduction or oxidative stress. They presented that the changes of the half-cell reduction potential (E_{hc}) of the GSSH/GSH appear to correlate with the biological status of the cell: proliferation, differentiation and apoptosis corresponds to $E_{hc} \approx 240$ mV, ≈ 200 mV, and ≈ 170 mV, respectively. The rate of removal of H_2O_2 was a direct function of GPx activity \times GSH (effectivity GPx activity). The predicted cellular average GPx and H_2O_2 for their study are approximately $GPx \leq 1 \mu m$ and $H_2O_2 \approx 5 \mu m$ based on available rate constants and an estimation of GSH. Thus GPx is an adaptive enzyme increasing in response to oxidative stress, ageing, physical activity, iron deficiency anaemia (Kok *et al.* 1987) and level of bioavailable selenium which defines its activity.

The example of significant changes connected with ROS production is the newborn heart. At birth the mammalian heart meets suddenly with an extremely high ROS concentration as a consequence of the dramatic changes of the living conditions during the delivery: barometric pressure increases more than 3 times (from 226 to 760 mm Hg), PO_2 in the air increases more than 3 times (from 47 to 160 mm Hg) and arterial O_2 saturation increases even more than 5 times (from 18 to 97 %). ROS attack the proteins and other molecules and produce autophagocytized material – lipofuscin like pigments (LFP) (Heintz 2004, Kuma *et al.* 2004, Gustafsson and Gottlieb 2009). The analysis of cardiac LFP concentration during the early postnatal period in the rat heart revealed marked oscillations: a high value on day 1, a sharp decay on day 4 and an increase again on day 7 followed by a decrease to day 15 (Ošťádalová *et al.* 2010). These alterations are probably induced by the

adaptation to the squall of ROS after the birth. LFP concentration in the immature heart (10-day-old) can be significantly influenced by selenium supplementation of the pregnant and lactating females. The developmental oscillations of LFP were significantly reduced and ROS production was thus counterbalanced in order to maintain the redox potential (Fig. 7). The maximum value of heart weight/body weight ratio, that is at control rats on day 4 (Ošťádal *et al.* 1967, Ošťádalová *et al.* 1993), was in selenium-supplemented neonates shifted on day 7 (Ošťádalová *et al.* 2010).

Redox-sensitive signalling pathways have a key cellular importance in maintaining the balance between processes such as growth, proliferation, differentiation, migration, apoptosis and death (Sauer *et al.* 2001, Schafer and Buettner 2001, Ushio-Fukai and Alexander 2004, Ushio-Fukai 2007, Upham and Trosko 2009). By means of oxidative and reduction properties, selenium participates in the keeping of „redox homeostasis“, and it is able to reset the redox potential to the original state. Selenium has thus an essential role for the fundamental cellular functions. A typical example of the role of selenium in the redox-sensitive signalling is its possible role in the cardioprotective action.

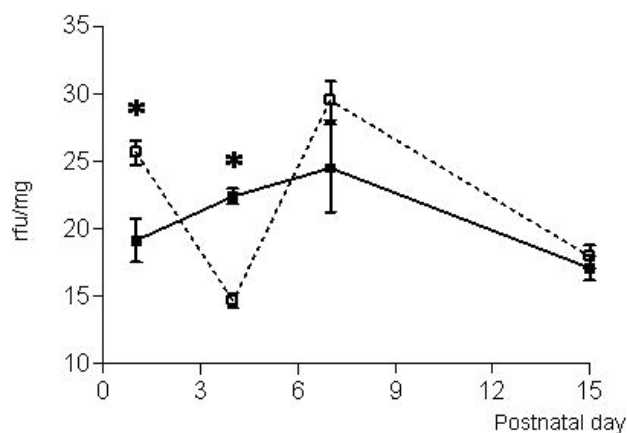


Fig. 7. Effect of long-time selenium supplementation on the development of myocardial LFP concentration (LFP fluorophore 350/450, expressed as rfu/mg, full line, $n=8$). The rats were fed by mothers that were drinking 2 ppm of Na_2SeO_3 in water – starting from the conception, during the whole pregnancy up to day 10 after delivery. Controls (dotted line, $n=8$) were fed by control mothers. Means \pm S.E.M., * $p<0.05$. (Data from Ošťádalová *et al.* 2010.)

Cardioprotective effect of selenium

At present, cardiovascular diseases represent the most important health risk because they are responsible

for more than 50 % of the total mortality. The leading cause of morbidity and mortality is the ischemic heart disease (for review see Ošťádal 2009). Ischemia/reperfusion injury is closely related to the development of ROS. It is, therefore, understandable that many investigators are interested in the analysis of the role of oxidative and antioxidative compounds. Selenium thus became the focus of clinical and experimental observations.

The results of clinical studies are very controversial. Some of them demonstrated a beneficial effect of selenium: lower plasma concentration of selenium was connected with higher concentrations of proinflammatory cytokines of TNF- α and IL-6 in patients, suffering from the acute myocardial infarction (Hassanzadeh *et al.* 2006). A relation of selenium and the degree of myocardial damage in ischemic heart disease was observed: dietary selenium supplementation may provide a method for increasing antioxidant protection, particularly in the individuals at risk for ischemic heart disease or in those, undergoing clinical procedures, involving transient periods of myocardial hypoxia (Venardos *et al.* 2007). C-reactive protein showed a significant positive correlation with the markers of cardiac damage (cTnT and cTnI) and a significant negative correlation with glutathione peroxidase. It suggests that the selenium level is related to the degree of myocardial damage and thus plays a role in the pathogenesis of ischemic heart disease (Altekin *et al.* 2005). The patients maintained for a long time on total parenteral nutrition, who are compromised from selenium deficiency, are recommended for a selenium supplementation (van Rij *et al.* 1981, Baker *et al.* 1983).

Other studies indicate no positive effect of selenium supplementation on the primary prevention of cardiovascular disease (Néve 1996, Stranges *et al.* 2005). Similarly, a randomized trial among the U.S. physicians (Salvini *et al.* 1995) showed no signs of protective effect of selenium and no reduced risk of myocardial infarction. The results of longitudinal studies are conflicting (Huttunen 1997) and the treatment of cardiovascular diseases with selenium still remains insufficiently documented. Rapid alterations in the serum level of selenium during the acute phase of myocardial infarction, as it was recently presented, indicates the relevance of time of selenium sampling and contributes to controversies of published results (Kutil *et al.* 2010). The inconsistency of the above results is not easy to explain. The dose and the chemical form of selenium, the

interactions, previous history of patients as well as the time of sampling have to be taken into consideration. Further epidemiological studies and randomized clinical trials across populations with different selenium status should be conducted to determine the causal effect of selenium on cardiovascular disease and risk factors (Stranges *et al.* 2010).

The experiments demonstrated that selenium reduces myocardial arrhythmia score and infarct size (Tanguy *et al.* 1998, 2004, 2010), improves recovery of the heart after ischemia/reperfusion injury (Poltronieri *et al.* 1992, Pucheu *et al.* 1995, Dhalla *et al.* 2000, Venardos *et al.* 2004, Lymbury *et al.* 2006), limits post-infarction cardiac remodelling and inhibits nuclear translocation of NF- κ B during myocardial infarction (Turan *et al.* 2005, Panicker *et al.* 2010).

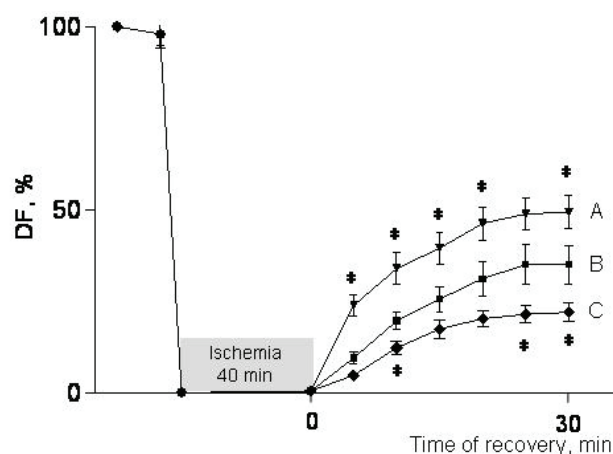


Fig. 8. Effect of addition of Na_2SeO_3 into Krebs-Henseleit solution (in a final concentration of 75 nmol.l^{-1}) on the recovery of isolated heart contractility (measured as a developed force) after global ischemia (expressed as a percentage of baseline value) in 10 day-old rats; controls (**B**), preischemic perfusion with selenium (**C**) and postischemic perfusion with selenium (**A**). Means \pm S.E.M., * $p < 0.05$. (Data from Ošťádalová *et al.* 2007.)

In all these experiments exclusively adult rats were used while the data concerning the immature heart were lacking. The aim of our study was, therefore, to find out whether selenium can protect also the immature heart against ischemia (Ošťádalová *et al.* 2007). We have observed that the addition of selenium to the perfusion solution during reperfusion significantly increased the recovery of the isolated neonatal rat heart. However, addition of selenium into perfusion solution before global ischemia had an adverse effect: the recovery after ischemia-reperfusion injury was markedly decreased (Fig. 8). These experiments suggest that the moderate

concentration of ROS developing during perfusion before ischemia is probably necessary, for their signalling role; the addition of selenium as antioxidant limits their function (Suzuki *et al.* 1997, Sauer *et al.* 2001). On the other hand, the production of ROS during reperfusion is high (Zweier *et al.* 1988) and the addition of antioxidative substance is thus beneficial.

A similar protective effect was also observed after long-lasting treatment of the immature hearts. Adult female rats were supplemented with selenite during pregnancy until day 10 *post partum*. Their fetuses were taking selenium through placenta till birth; after birth the sucklings were supplemented with selenium through mother milk. The isolated, perfused hearts of these selenium-supplemented 10-day-old sucklings were significantly more resistant against global ischemia-reperfusion injury in comparison with controls. In addition, serum concentration of NO in selenium-supplemented sucklings decreased, suggesting better antioxidative equipment (Ošťádalová *et al.* 2007).

Moreover, selenium supplementation increased significantly the sensitivity to the inotropic effect of isoproterenol. This is in a good agreement with recently published data demonstrating, that production of ROS in adult mouse cardiomyocytes contributes to the β -adrenergic stimulation (Sayar *et al.* 2000, Gomez *et al.* 2003, Andersson *et al.* 2011).

The results reviewed in this paper showed both beneficial and toxic effects of the trace element – selenium. However, the questions of optimal levels of selenium in the diet, possible toxicity and interactions should be the topic of further studies.

Conflict of Interest

There is no conflict of interest.

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