The effect of selenium on redox state and thiols changes in lung tissue after Selol, a new organoselenium (IV) compound, administration

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Abstract

Selol is a product containing organoselenium compounds, where selenium (Se) exists at a +4 oxidation state. Bonding of Se (IV) into organic compounds is responsible for high bioavailability and low toxicity, while the presence of Se in its +4 oxidative state influences biological activity. Studies on sodium selenite (IV) show that it undergoes redox reactions in the presence of glutathione, which, in turn, produce reactive oxygen species. At the same time Se is widely known to be an anti-oxidative agent. Due to intracellular reactions Se affects an oxidation-reduction potential of cells. Changes of the potential correlate with the cell biological state: proliferation undergoes at a potential about –240 mV and apoptosis is induced at potentials higher than –170 mV.

The aim of the study was to test the effect of Selol 2% on the oxidation-reduction potential in lung tissue of healthy rats and to determine changes in concentration of thiol compounds involved in glutathione metabolism in relation to the amount of Se adsorbed by the lungs. A new HPLC method with sensitive fluorescence detection was developed to separate the relevant thiols: glutathione, cysteine, homocysteine, γ-glutamylcysteine, and cysteinylglycine. The Se content in lung tissue was assayed using ICP-MS.

A single administration of Selol 2% to healthy rats caused a ten-fold increase in Se content in lung cells, with a maximum value occurring at 2 h. During the first four hours, Selol showed a pro-oxidative effect characterised by decrease in GSH concentration and increase in redox potential level and CysGly concentration. After four hours an anti-oxidative effect related to decrease in the potential and an induction of GSH synthesis from its precursors was observed. The oxidative stress induced initially by Selol was finally overcame. The cells reached a better redox state than prior to the administration of Selol, which leads to the conclusion that the compound does not have a negative effect on healthy cells.

Key words: *Selol, organoselenium compound, redox state, glutathione, thiols.*

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Introduction

Selol is a product containing organoselenium compounds, where selenium (Se) exists at a +4 oxidation state, synthesised at the Department of Drug Analysis, Medical University of Warsaw (Polish patent no. 176530 granted in 1999) [1]. It is a mixture of selenitetriglycerides obtained by the chemical modification of sunflower oil. Compounds of different structure and degree of activity are obtained depending on the concentration of selenium used for the synthesis. Selol concentrations between 2-20% have been produced, which means that 1 ml of Selol contains between 20-200 mg Se (IV). Selol's structure has been determined by ^{1}H and ^{13}C NMR spectroscopy [2] (Fig. 1).

Pharmacokinetics and toxicity of Selol have been established on the basis of research carried out to date. Selol under-

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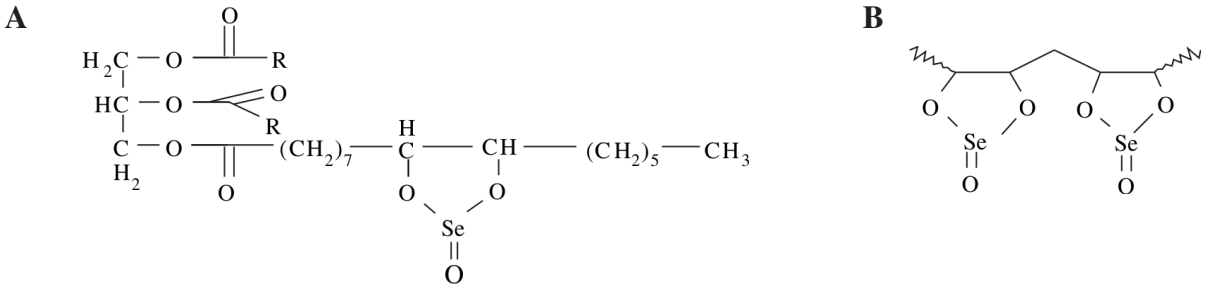

Fig. 1. A tentative structure of Selol 2-5%. A – The structure of selenitetriglycerides containing 2% of Se, which forms mainly dioxaselenolane rings. B – The structure of selenitetriglycerides containing 5% of Se, which forms also double dioxaselenolane rings

goes fast resorption from the digestive system. Thanks to its lipophilic properties, Selol is highly distributed throughout the body. It easily crosses the blood-brain barrier [3]. Research indicates that it is extensively metabolised in the liver. Selol is mainly excreted through the kidneys, and is completely eliminated from the organism within 24 h after administration [4]. Acute toxicity studies were carried out in rats following a single dosage. After oral administration of Selol 2%, the LD_{50} was found at 100 mg Se/kg of body mass, while for 10% Selol this value is 68 mg Se/kg of body mass [5]. Selol 2% is over 30 times less toxic than sodium selenite (IV), the only compound containing inorganic Se (IV) in medical use. The LD_{50} value for sodium selenite (IV) is 3 mg/kg of body mass, p.o. [2]. As a result Selol may be administered in much higher doses than sodium selenite (IV). It was also established that it does not exhibit cumulative or chronic toxicity [2, 5]. The Ames test carried out on *Salmonella typhimurium* demonstrated a lack of mutagenic activity [2]. Tests carried out on leukaemia HL-60 and multidrug-resistant HL-60/Dox (resistant to doxorubicin) and HL-60/Vinc (resistant to vincristine) cell lines have shown that the administration of Selol reduces cellular proliferation, and even at higher concentrations induces apoptosis by the mechanism of changes in mitochondrial potential and caspase activity. It was observed that cells resistant to doxorubicin (HL-60/Dox) were more sensitive to Selol than HL-60 cells, which demonstrates that Selol may be able to overcome drug resistance [6]. Literature search shows that selenium compounds may reduce the risk of cancers of the prostate, lungs, colon [7], thyroid [8], and melanomas [9]. This brings hope that future research on Selol will lead to it coming into use in the prevention or even treatment of neoplasm.

Detailed mechanisms of Selol activity have not yet been fully understood. The compound differs in structure to selenium compounds described so far. Studies on inorganic sodium selenite (IV) showed that it undergoes redox reactions in the presence of glutathione (GSH), which, in turn, produce selenodiglutathione (GSSeSG), selenoglutathione (GSSeH), hydrogen selenide (H_2Se) and Se^0. The reactions also lead to formation of glutathione disulphide (GSSG) and reactive oxygen species (ROS) such as superoxide anions ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). Oxidative stress induced this way, is one of the key mechanisms responsible for cytotoxic effect of Se (IV) compounds, as well as their anti-cancer activity [10]. Additionally, GGSeSG was found to be a pro-apoptotic agent. At the same time Se is widely known to induce an anti-oxidative effect on cells. It is present in an active centre of selenoenzymes, including glutathion peroxidise and thioredoxin reductase, responsible for intracellular redox homeostasis [11]. Among many redox systems in cells the GSH/GSSG system is the most important in maintaining the appropriate oxidation-reduction potential (ORP). The ORP can be expressed as half-cell reduction potential (E_{hc}) based on GSH/GSSG ratio. Other redox systems such as the thioredoxin system TrxSS/Trx(SH_2) and the nicotinamide adenine dinucleotide phosphate system $NADP^+$/NADPH play a lesser role in maintaining cell ORP due to their significantly lower concentration than GSH. The presence of ROS in cells affects the relation between the concentration of reduced and oxidised forms of non-protein thiols. It was found that ORP changes correlate with the cell biological state: proliferation undergoes at a potential about –240 mV, differentiation at about –200 mV and apoptosis is induced at potentials higher than –170 mV [12].

The aim of the study was to test the effect of Selol 2% on ORP in lung tissue of healthy rats and changes in concentration of GSH–related thiol compounds in relation to the amount of Se adsorbed by the lungs. Non-protein thiols: GSH, cysteine (Cys), homocysteine (Hcy), γ-glutamylcysteine (γGluCys), and cysteinylglycine (CysGly) were assayed with a new HPLC method with sensitive fluorescence detection. The Se content in lung tissue was determined using ICP-MS.

Material and methods

Experimental animals

All procedures used in the present study were in compliance with Animal Care Committee guidelines approved

by Council of Science (the organ of the Ministry of Science and Higher Education). The studies were performed on 57 healthy 10-week-old male Wistar rats of an average body mass 200-220 g, fed with a standard laboratory diet and given water *ad libitum.* The rats were kept under conventional standardized conditions (room temperature 22.5-23.0˚C; relative humidity 50-70%; 12 h day/night cycle). Animals were divided randomly into 14 groups: control group (No 1) and groups differing in time after Selol 2% administration (No 2-14) – 0.5; 0.75; 1; 1.25; 1.5; 2; 2.5; 3; 4; 6; 8; 12 and 24 h. Groups No 2-14, consisted of 3 animals each, were given orally a single dose of Selol 2% (20 mg Se(IV)/kg of body mass) diluted with sunflower oil, in a volume about of 10 ml/kg. Group No 1 (control animals) was divided into 6 subgroup from 1a to 1f, consisting of 3 rats each. In subgroup 1a animals received no sunflower oil and in subgroups from 1b to 1f animals received only the oil in a volume equivalent to that applied to animals treated with Selol 2% (volume of about 10 ml/kg^{-1}). At specified time after Selol administration the rats were anaesthetized with halothane and decapitated. The lungs were collected and stored at –80˚C until analysed. In case of control animals tissue samples were collected at 0; 0.5; 2; 6; 24 h.

Reagents and chemicals

All reference standards including L-cysteine (Cys), DL-homocysteine (Hcy), L-glutathione (GSH), L-cysteinylglycine (CysGly), γ-L-glutamyl-L-cysteine (γGluCys), selenium (1 mg/ml Se in nitric acid) were obtained from Sigma (Sigma-Aldrich, St. Louis, MO, USA). For derivatization procedure 7-fluorobenzofurazan-4-sulfonic acid amonium salt (SBD-F) obtained from Fluka (St. Louis, MO, USA) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) obtained from Sigma (Sigma-Aldrich, St. Louis, MO, USA) were used.

Sodium hydroxide 0.1 M (NaOH), hydrochloric acid 0.1 M (HCl) and disodium ethylenediaminetetraacetate dihydrate (EDTA) were purchased from POCH (Gliwice, Poland). Phosphate buffered saline (PBS) were obtained from Institute of Immunology and Experimental Therapy (Wrocław, Poland), disodium tetraborate decahydrate (borax) from AppliChem (Dramstadt, Germany) and nitric acid 65% (HNO_3) from Merck (Dramstadt, Germany). Triethylamine (TEA) from Fluka (St. Louis, MO, USA), acetic acid 99.9% from POCH (Gliwice, Poland) and methanol (MeOH, gradient grade) form LabScan (Dublin, Ireland) were used as mobile phase constituents.

Highly-purified deionised water was prepared using EASY Pure RF deionizer (Barnstead-Thermolyne, Dubuque, IA, USA). All buffers and solutions applied to a chromatography were filtered through hydrophilic polypropylene 0.45 μm membrane filters (Pall Corporation, Ann Arbour, MI, USA).

PBS/EDTA, a diluent for tissue homogenization was prepared as follows: EDTA was dissolved in PBS solution to obtain the final concentration of 2 mM of EDTA. Borate buffer for derivatization purpose was prepared as follows: borax and EDTA were dissolved in deionized water to reach the final concentration of 125 mM borate and 4 mM EDTA.

Single stock solutions of all the thiols were prepared at concentrations of GSH (6.5 mM), Cys (10.0 mM), Hcy (8.9 mM), CysGly (5.6 mM), γGluCys (4.0 mM) in 0.1 M HCl with 0.1 mM EDTA. For thiols determination by HPLC method, single thiols solutions were mixed in a way to obtain final concentration at a level corresponding to averages of rat tissues.

Determination of selenium

ICP-MS technique was applied for total Se assay in rat lungs with use of VG PlasmaQuad 3 inductively-coupled plasma mass spectrometer (Thermo, Elemental). The excitation power of plasma was evaluated: 1380 V. The flow rate was as follows: plasma gas: 12.6-12.7 l/min; nebulization gas: 0.74-0.80 l/min and auxiliary gas: 0.77-0.85 l/min. The baseline of the background was below 10 cps. Samples were aspirated by 180 s and the measurement took 15 s in a triplicate [13].

Lung tissue of weight of 0.4 g was taken from each animal in the group. Samples were mineralized with addition of 3 ml of 65% HNO_3 in teflon crucibles with a microwave mineralizer MDS-2100 (CEM Corporation, USA): first step: 10% max energy (1 min); second step: 20% max. energy (2 min); third step: 25% max. energy (2 min). Mineralizates were transferred to 100 ml volumetric flasks and diluted with deionized water.

Selenium reference standard were diluted with 1% HNO_3 in way to reach a concentration range 0.5-10 ppb. 1% HNO_3 was used as a blank sample. Se content in lung tissue was calculated with use of external calibration curve.

Tissue sample preparation

All the samples and reagents were kept in ice during sample preparation. Lung tissue of weight of 0.7-1.0 g was homogenized in electrical glass grinder with addition of PBS/EDTA in a volume equivalent to fourfold mass of the sample (final dilution 5× m/v). The homogenate was centrifuged at 14000 rpm for 10 min with cooling down to 15˚C (Sigma 3K15, Osterode am Harz, Germany). A supernatant was collected and subsequently deproteinized by ultrafiltration. Before use, the ultrafiltration membrane 10 kDa cut-off (Microcon Ultracell YM-10 cartridge, Milipore, Bedford, MA, USA) was pretreated by centrifugation of deionised water (200 μl) at 14000 rpm for 10 min at 15˚C. 200 μl of the supernatant was placed in the cartridge and centrifuged at 8000 rpm for 60 min at 15˚C. Ultrafiltrates were directly taken for derivatization.

Derivatization procedure

For reduced thiols determination 200 μl of ultrafiltrate was adjusted to alkaline pH with 80 μl of borate buffer. Next

20 µl of 1 mM SBD-F solution in water was added, vortexed thoroughly and heated at 60°C for 40 min.

Oxidized thiols were calculated as a difference between total and reduced species. Total thiol content was performed as follows: 100 µl of ultrafiltrate was mixed with 50 µl of 20 mM TCEP water solution, mixed and incubated at room temperature for 10 min. Next the pH of the sample was adjusted with 110 µl of borate buffer. Derivatization was accomplished by addition of 40 µl of 1 mM SBD-F, vortexing and heating at 60°C for 40 min.

Each tissue sample was tested in duplicate. The content of reduced and oxidized thiols was expressed as mean ± SEM in nmol per 1 g of tissue taking into account all the dilution steps applied.

Chromatography

The chromatographic separation was performed using Dionex Ultimate 3000 HPLC system (Dionex, Sunnyvale, CA, USA) consisted of a dual low-pressure gradient pump allowing a ternary gradient formation, a degaser, an autosampler and a column oven. Separation of the analytes was achieved with a reverse phase Supelcosil LC-18-T column, 150 × 4.6 mm, 3 µm (Supelco, Bellefonte, PA, USA) protected with Supelguard LC-18-T 2 cm security guard column (Supelco, Bellefonte, PA, USA). Chromeleon software v. 6.8 was used for data acquisition and analysis. During chromatographic runs samples were kept at 10°C and injection volume was 20 µl. Peaks were measured at fluorescence detector. (RF2000, Dionex, USA) with ex/em wavelength 385/515 nm. Elutions were performed at 40°C with 1 ml/min mobile phase flow in a ternary gradient mode (Tab. 1). Buffer for mobile phase contained 100 mM TEA adjusted with acetic acid to pH 5.15.

Results

Method development

Deproteinization is an important step of preparation of the biological material for thiol determination. A commonly used method is based on precipitation of proteins by strong acids such as trichloroacetic, perchloric or sulphosalicylic [14, 15]. In general acidic media have preserving effect on thiols however sulphosalicylic and perchloric acids are able to oxidise thiols [16]. On the other hand acids disturb the following derivatization step which requires an alkaline environment. High acid content coming from deproteinization has to be neutralized that introduces a lot of additional ions changing the ionic strength of the sample, which in turn may negatively affect chromatography results. Additionally, sulphosalicylic acid reacts with the labelling reagent SBD-F making the quantification of thiols inappropriate. As a result we selected an ultrafiltration using membranes with 10 kDa cut-off point for the deproteinization of the samples. An advantage of this method is the successful removal of compounds of high molecular mass without affecting the sample composition. It was also decided to use no internal standard which is usually a compound with a reactive –SH group. Internal standard affected changes of the thiol profile because of a series of redox reactions taking place among thiols in the sample.

The determination of biologically important thiols in body fluids (plasma [17-20] and urine [21]) have been described by a few authors. They proposed precolumn derivatisation with SBD-F and HPLC methods generally based on 0.1-0.2 M acetate buffer of pH ranging between 4.5-5.5 with a methanol gradient. The reagent selectively forms stable derivatives without fluorescent by-products. One of the advantages of SBD-F is the increased solubility in a water-based medium. On the other hand this can result in high polarity of analytes and poor retention in reversed phase chromatographic separation [22-23]. As we were studying both the precursors and the product of GSH degradation and the material was lung tissue, it was necessary to develop a new HPLC method in order to separate the analytes.

Ion pair chromatography was selected, which ensures greater selectivity for thiol derivatives under investigation, because of a different separation mechanism. Thiol SBD-F derivatives are negatively charged mainly because of the sulphonate group ($-SO_3^-$), therefore triethylamine (TEA) was used as a counter ion. The ion pairs formed tends to adsorb on a stationary phase resulting in an increase in the retention time of the analytes. The HPLC method was optimised taking into consideration buffer concentration, pH, the temperature of the separation, and the methanol gradient. The best results were obtained using the 100 mM TEA acetate buffer at pH 5.15, and the temperature of 40°C.

Table 1. Gradient time program for thiol determination

Time [min]	MeOH [%]	H_2O [%]	Buffer [%]
0	25	0	75
9	22	7	71
13	20	20	60
17	20	60	20
27	20	60	20
35	25	0	75

Method validation

The developed method for thiol determination was validated for specificity, selectivity, linearity, limits of detection and quantitation, precision, intermediate precision, and recovery.

The specificity of the method resulted from the use of SBD-F, which reacts with the thiol group under the given conditions. The TEA-based mobile phase ensures selectivity, therefore matrix does not interfere with the analyte

peaks. The chromatogram in Figure 2 shows analyte peaks well separated and additional peaks do not interfere with the integration.

A linear relationship between the peak area and the concentration was observed in the range of thiol concentration found in the samples. The limit of detection (LOD) for individual analytes was defined as the concentration giving the signal-to-noise ratio equal 3, while the limit of quantitation (LOQ) as the concentration at which the signal-to-noise ratio is 10. A recovery was tested by the addition of the standards to the ultrafiltrate. The recovery ranged between 87.0% for γGluCys and 100.2% for GSH.

Method validation was completed by checking precision and intermediate precision. Relative standard deviation (RSD%) for precision was below 2.3%, while RSD% for intermediate precision was below 5.5%. Validation results are shown in Table 2.

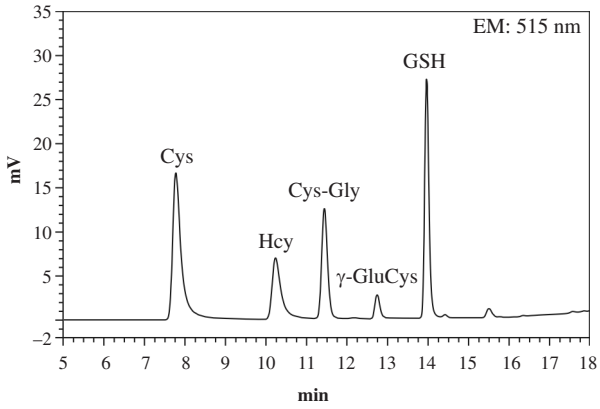

Fig. 2. Chromatogram of mixture of thiols in optimized conditions described in the section *Chromatography*

Selenium assay

ICP-MS results show that following the administration of a single dose of Selol Se is accumulated in lungs reaching the highest concentration after 2 h. Maximum concentration was about 52 nmol/g of tissue and it was 10 times higher than the control value. Se content in the tissue decreased over time, although its concentration at 24 h after Selol administration was still about 3 times higher than the control sample. Changes in Se levels in rat lung tissue are shown in Figure 3.

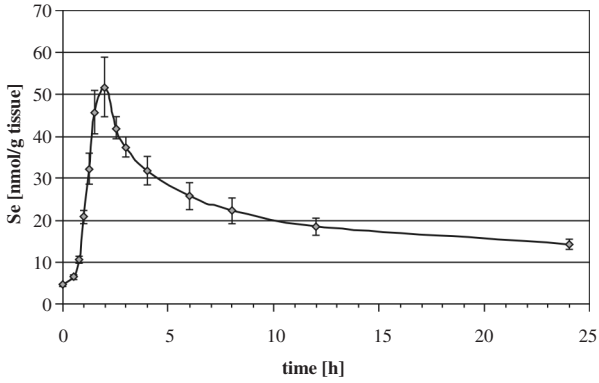

Fig. 3. The dynamics of Se changes in rat lungs within 24 h after Selol 2% administration that corresponds to 20 mg Se(IV)/kg body mass

Changes in thiol profiles

Content of thiols in lung tissue of rats from the control group remained unchanged at all time points, indicating that administration of sunflower oil has no influence on the cellular thiol concentration.

A decrease in GSH concentration to 45% of control level was observed in lung homogenates within 1 h of Selol

Table 2. Validation results: linearity range, detection and quantification limits, precision, intermediate precision and recovery

	Linearity range [nmol/ml]	LOD [nmol/ml]	LOQ [nmol/ml]	Precision	Intermediate precision	Recovery [%]
GSH	0.064-1.926 $r^2 = 1.0000$	0.001*	0.003*	1.2% ($n = 5$)	4.6% ($n = 7$)	100.2
Hcy	0.028-0.855 $r^2 = 0.9999$	0.002	0.006	2.3% ($n = 5$)	5.5% ($n = 7$)	90.5
Cys	0.654-19.620 $r^2 = 0.9999$	0.010*	0.034*	1.4% ($n = 5$)	3.9% ($n = 7$)	98.0
γGluCys	0.068-1.028 $r^2 = 0.9999$	0.007	0.023	2.1% ($n = 5$)	4.2% ($n = 9$)	87.0
CysGly	0.033-0.499 $r^2 = 0.9999$	0.001*	0.002*	1.5% ($n = 5$)	5.3% ($n = 6$)	97.5

** based on extrapolation of calibration curve and evaluation of peak S/N value*

2% administration (Fig. 4). A rapid increase was observed next, bringing the concentration to above its initial level, reaching a maximum value 2 h after administration of Selol 2%. After that GSH concentration dropped again, falling to 30% of the initial level 4 h after administration. From that point on, slow constant increase of GSH concentration was observed, reaching 23.7 nmol/g of tissue within 24 h, a value 1.6 times greater than control.

For the oxidised form of GSH a small drop of 25% in concentration was observed within 2.5 h. An increase in GSSH content was observed from 3 h onwards, reaching maximum value after 12 h, when the GSSG concentration was 2.3 times greater than control. A decrease of GSSG content was observed next, although its level was still 1.5 times higher than control after 24 h.

The E_{hc} value was calculated using the Nernst equation with parameters adjusted to 37˚C (310 K) and pH 7.4 [12]:

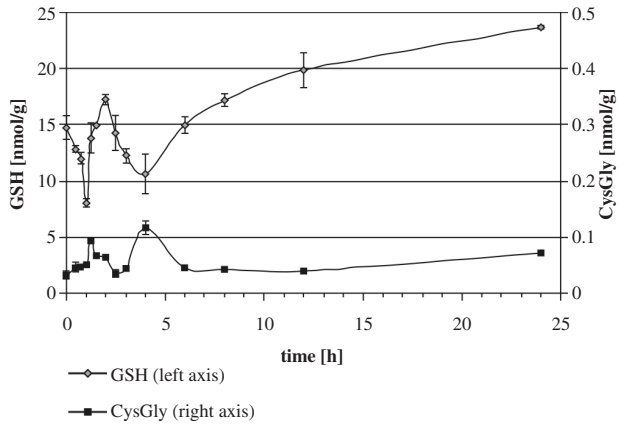

Fig. 4. The dynamics of GSH and its degradation product CysGly changes in rat lungs within 24 h after Selol 2% administration

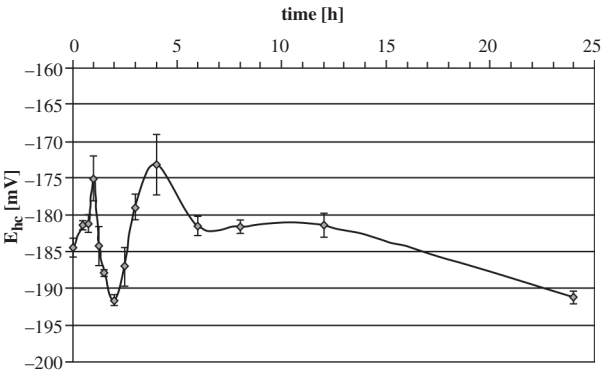

Fig. 5. The dynamics of half-cell reduction potential (E_{hc}) of GSH/GSSG changes in rat lungs after Selol 2% administration. The half-cell reduction potential (E_{hc}) were calculated using the Nernst equation see section *Changes in thiol profiles*

$$E_{hc} = -264 - \frac{61.5\ \text{mV}}{2} \log \frac{[GSH]^2}{[GSSG]}.$$

Changes in the E_{hc} of lung tissue over time (Fig. 5) was found reversed to the observed changes in GSH concentration. An increase of E_{hc} occurred at 1h and 4h after Selol administration, with the highest value of –173 mV observed at 4 h. Each peak of potential was followed by a fall, which was especially rapid after 1h, while after 4 h the potential value decreased systematically until 24 h, reaching a final value of – 191 mV.

Changes in the levels of CysGly, a direct product of GSH catabolism, were found different from GSH. CysGly concentration reached maximum values at 1.25 h and 4 h, and remained steady at the control level after 6 h (Fig. 4). In case of GSH precursors, γGluCys and Cys, changes in concentrations correlated with changes in GSH levels (Fig. 6). The level of both compounds decreased during the first hour, then increased until 2.5 h and decreased again after 4 h. A difference in the profile was significant at 8 h, when the precursor concentration dropped and GSH levels tended to increase. Changes in concentration of the oxidised precursors were analogous to the changes in concentration of their reduced forms.

Hcy content in lungs from the control group was assayed at very low level (0.004 nmol/g of tissue ± 0.000 SEM), between LOD and LOQ. 0.5 h after the administration of Selol 2%, Hcy concentration increased and remained almost unchanged at a level 18 times higher than control until 8 h. After that Hcy concentration decreased slowly until it reached 0.022 nmol/g of tissue, which was still almost 6 times higher than the initial value.

Discussion

Administration of 20 mg Se(IV)/kg body mass as seleniteriglycerides in Selol 2% in rats initiated changes in the concentrations of GSH and related thiols. Such a high dose of Se(IV) was likely to trigger strong oxidative stress, similarly to administration of sodium selenite [10]. A rapid fall in GSH levels was observed during the first hour after Selol 2% administration, corresponding with a four-fold increase in Se level in lung tissue. A pool of cellular GSH was activated as the first response to the oxidative stress, observed at 2 h as a rapid increase in GSH content above the control value. It is possible that the demand for GSH was covered due to glutathione reductase activity as a decrease in GSSG levels was observed during the same time period. As a result of the significant pro-oxidative effect of Se on lung tissue, GSH was still being used in redox reactions after 4 h. This trend was reversed during subsequent hours, and GSH content increased significantly above the control value, which may be explained by the dominance of GSH synthesis in cells. The process turned out to be so efficient that oxidative stress caused by the administration of Selol 2% was eliminated.

The above mentioned observations were also confirmed by the changes in OPR. At 1h and 4h, the reactive environment present in cells caused an increase in potential to a level close to pro-apoptotic (about –170 mV) [12], although this was temporary and from 4 h a gradual restoration of balance, previously disturbed by oxidative stress, was observed. The return of OPR to its initial level indicates that oxidative stress was entirely eliminated, and its further decrease (about –194 mV) demonstrates that the cell became in a better redox state than prior to the administration of Selol. It can be supposed that the reason why Se levels were 3 times higher 24 h after the administration of Selol than the initial value was a consequence of the element becoming incorporated into active centres of enzymes taking part in the cell anti-oxidative defence. This is confirmed by other studies [2], where plasma glutathione peroxidise levels in rats and mice increased following the administration of Selol.

The comparison of concentration levels of GSH related thiols in time (Fig. 4 and 6) provided valuable information regarding other processes occurring in lung tissue cells following the administration of Selol 2%. Changes in concentration of CysGly, the product of GSH degradation, which were reversed to the changes in GSH concentration and occurred at the same time points, indicate that the decrease of GSH concentration was correlated in time with its catabolism. As a result of strong oxidative stress, causing significant disturbance to the GSH-GSSG balance, the cell can remove excess GSSG as well as reducing it. It is an adverse process as it leads to a decrease of the GSH cellular pool, but it is also an important mechanism of cellular protection compensating for changes in the redox potential. The increase in extracellular GSH and GSSG catabolism as a result of γ-glutamyl transpepsidase activity leads to the accumulation of CysGly, observed at 1.25 and 4 h. The increased amount of CysGly may have a toxic effect on the cell because of the formation of a thiol radical, which may further intensify oxidative stress. According to literature data, oxidative stress is an important factor responsible for the induction of γ-glutamylcysteine synthetase activity, an enzyme that determines the rate of GSH biosynthesis [24]. An observed decrease in γGluCys levels at 0.75 h and 4 h leads to the conclusion that γGluCys was being used immediately for GSH synthesis. An analogous relation may be noticed in Cys, used for γGluCys synthesis all along. The availability of Cys limits the biosynthesis of GSH, and its cellular concentration is significantly lower than other substrates necessary for GSH production. The observed increase in Hcy concentration may be explained by natural cell response, aiming to increase the pool of Cys via transsulphuration process of Hcy [26]. The demand for Cys also influenced the escalation of CySS transport into the cell, observed as an increase of its content at 2 h, when GSH synthesis was also observed.

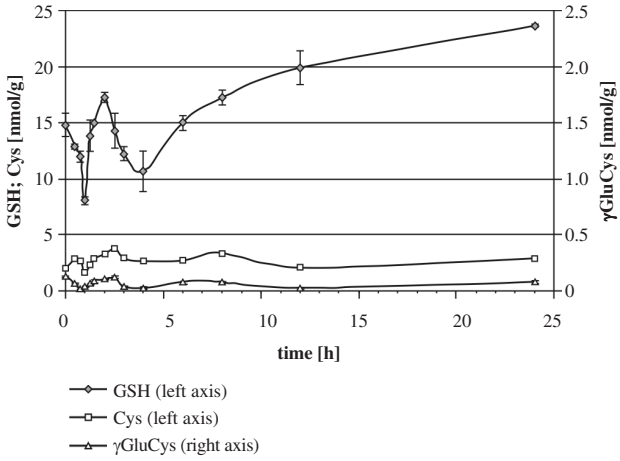

Fig. 6. The dynamics of GSH, its biosynthesis substrates γGluCys and Cys changes in rat lungs within 24 h after Selol 2% administration

In summary we can state that a single administration of selenium as Selol 2% to healthy rats caused a ten-fold increase of Se content in lung cells, with a maximum value occurring at 2 h. During the first four hours, Selol showed a pro-oxidative effect characterised by decrease in GSH concentration and increase in OPR level and CysGly concentration. After four hours an anti-oxidative effect related to decrease in OPR and an induction of GSH synthesis from its precursors was observed.

Conclusions

The administration of Se (IV) as selenitetriglycerides, and the further metabolism of these compounds, affects cellular OPR and initially causes oxidative stress, followed by the induction of glutathione synthesis. It also allows the elimination of consequences of stress, therefore indicate an anti-oxidative effect. Finally, the cell reaches a better redox state than prior to the administration of Selol, which leads to the conclusion that the compound does not have a negative effect on normal cells.

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