Glutathione and mercury conjugate with glutathione in ex-miners' erythrocytes

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All forms of mercury cause toxic effects in a number of tissues and organs, depending on the chemical form of mercury, the level of exposure, the duration of exposure, and the route of exposure. Although mercuric ions will bind to numerous nucleophilic groups on molecules, they have a greater predilection to bound to reduced sulphur atoms, especially those on endogenous thiol-containing molecules, such as glutathione, cysteine, homocysteine, N-acetylcysteine, metallothionine and albumin to participates in a variety of detoxification, transport, and metabolic processes. In order to determine if there are any residual effects of long-term past exposure to mercury vapour (Hg°), glutathione concentration (GSH) in the erythrocytes of 28 mercury miners, 21 ex-mercury miners, and 41 non-exposed people as control group were measured.

The miners were intermittently exposed to Hg° for periods of 7 to 18 and ex-miners of 13 to 31 years. The last occupational exposure to Hg° for both exposed groups was 1.2 to 28.2 years ago. The total number of exposure periods varied from 13 to 51 for the miners and from 51 to 119 for the ex-miners. Actual Hg concentration in urine of miners, ex-miners and control group ranged from 0.50 to 7.20, 0.10 to 2.70, and 0.10 to 5.00, respectively.

The ratio between reduced and oxidized glutathione (GSH/GSSG) was used to evaluate oxidative stress status in biological systems. Blood was collected into a 3 mL Vacutainer Tube (Becton Dickinson) containing K3-EDTA and 9,3 mmol/L N-ethylmaleimide (NEM). The hemoglobin concentration and erythrocyte count was determined with a Beckmann/Coulter Gen S hematology analzyer.

Reduced and oxidised glutathione were determined in hemolysed erythrocytes and deproteinised hemolysate with capillary electrophoresis system SpectraPhoresis 1000. Hemolysate was deproteinised with Sartorius Centrisart filter cut off values 10 000 D. Analyses were performed in an uncoated fused silica capillary size 70 cm × 50 µm I.D. with detection window located at the distance of 35 cm and measured with direct ultraviolet detection at 200 nm. The capillary electrophoresis operating conditions were optimised to allow simultaneous measurement of GSH and GSSG in samples. The buffer solution was sodium tetraborate pH 9.4, 40 mmol/L. Samples were injected into the separation capillary by the 3 s hydrodynamic injection load. Electrophoretic separation was performed by applying 20 kV in the constant voltage mode at ambient temperature. With N-ethylmaleimide (NEM) the oxidising of GSH to GSSG was prevented.

The reduced glutathione concentration in erythrocyte (mean ± SD), expressed as mmol/gHb, was determined for the three groups: control, miners, and ex-miners as 11.66 ± 2.66, 13.03 ± 3.705 and 9.64 ± 0.94 mmol/gHb, respectively. The corresponding value for the oxidised glutathione GSSG/Hb was 0.53 ± 0.23, 0.56 ± 0.22, and 0.39 ± 0.09 mmol/gHb respectively. Total glutathione concentrations for the correspondings groups were: 12,76 ± 2.90, 13,79 ± 3.17, and 10.59 ± 1.56 mmol/gHb, and for the calculated GSH/GSSG ratio were: 25.87 ± 13.39, for miners 25.69 ± 16.03 and for ex miners 23.29 ± 5.11.

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We found that the concentrations of reduced and total glutathione in erythrocytes of the ex miners were significant (p=0.01) lower than the concentrations for the control and miner's group. Mercury conjugate with glutathione was measured also with capillary electrophoresis simultaneously with oxidised (GSSG) and reduced (GSH) glutathione. The kinetic of reaction shows the formation of GS-Hg-SG conjugate. The linearity of the conjugate was up to 30 μ mol/L of $HgCl_2$. The sensitivity of the conjugate determination was 0.1 – 0.2 μmol/L $HgCl_2$ that is above the concentration of blood mercury.

Higher concentrations of mercury induced the glutathione synthesis in liver and excretion in blood. Lower GSH/GSSG ratio in blood from miners and control could indicate the exposure to the stress but not to the accumulated mercury after long-term occupational exposure.

The antioxidant activity of GSH is not reduced. The GSH and GSSG concentrations were about 2.8-3.6 times higer in hemolysate as in deproteinised hemolysate probably because mixed glutathione disulfides forms with proteins did not pass the filter membrane and only free glutathione was determined in deproteinised hemolysate.

The sensitivity of the GS-Hg-SG conjugate determination with capillary electrophoresis was too low and other analytical methods would be suitably.

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