**SHORT COMMUNICATION**

Age and gender dependent levels of glutathione and glutathione S-transferases in human lymphocytes

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Glutathione S-transferases (GSTs) are a family of enzymes involved in the detoxification of a wide range of chemicals including chemical carcinogens. Human cytosolic GSTs are divided into four major classes; α, µ, π and θ. This study was performed to evaluate the influence of age and gender on the GST isoenzyme expression and glutathione (GSH) content in lymphocytes. Blood was collected from 124 healthy controls, which were divided into age groups of 20–40 years (21 females, 20 males), 40–60 years (20 females, 21 males) and 60–80 years (20 females, 22 males). Lymphocytes were isolated by density centrifugation on Histopaque-1077. After homogenization, cytosolic fractions were isolated. Herein, GST isoenzyme levels were determined by densitometrical analysis of western blots after immuno-detection with monoclonal antibodies. Total GSH content was determined by high performance liquid chromatography after conjugation with monobromobimane. Spearman rank correlation and Wilcoxon rank sum tests were used for statistical evaluation. Lymphocytic GSTα and π levels were not correlated with age or gender. GSTζ was not detectable in lymphocytes. GSH contents were not different in males and females, but decreased with age in both males and females. In age group 60–80, GSH content was significantly lower as compared with age groups 20–40 years (21 females, 20 males), 40–60 years (20 females, 21 males) and 60–80 years (20 females, 22 males). Lymphocytes were isolated by density centrifugation on Histopaque-1077, according to the manufacturers instructions (Sigma diagnostic, St Louis, MO). Lymphocytes were pelleted and stored at −20°C until use. For preparation of cytosolic fractions, lymphocytes were thawed slowly, homogenized in 100 µl of 20 mM Tris–HCl buffer pH 7.4, containing 1 mM dithiothreitol and 1 mM sodium borohydride. Specific GST isoenzymes were determined as described previously (9), using bovine serum albumin as a standard. Specific GST isoenzyme levels were determined as described previously (10). In short, cytosolic fractions were subjected to SDS–PAGE [11% acrylamide (w/v)], and subsequently to western blotting, using a semi-dry blotting system (Novablot II, Pharmacia, Uppsala, Sweden). Western blots were incubated with monoclonal antibodies against human GST classes α, µ and π. Class α antibodies react with human GST A1-1, GST A1-2 and GST A2-2 (10). Class µ antibodies recognize human GST M1a-1a, GST M1a-1b and GST M1b-1b (11,12). Class π antibodies react with human GST P1-1 (13). The specific binding of the monoclonal antibodies to the isoenzymes was demonstrated by incubation with peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dakopatts, Glostrup, Denmark) and subsequent staining with 4-chloro-1-naphthol and hydrogen peroxide. Staining intensity on the immunoblots was quantified using a laser densitometer (Ultroscan XL, LKB, Bromma, Sweden). Known amounts of purified GSTs were run in parallel with the experimental samples and served as standards for the calculation of the isoenzyme levels in the cytosolic fractions. The detection limit of this assay is ~50 ng/mg protein. Total GSH was quantified by high performance liquid chromatography after reaction with monobromobimane, as described previously (14). In this assay, oxidized GSH present is reduced with sodium borohydride to the reaction mixture. A Spearman rank correlation test was used to correlate lymphocytic GSH content and GST isoenzyme expression with age and gender. A Wilcoxon rank sum test was used to assess statistical significance of differences between age

Little is known about the differences in GST and GSH expression between men and women. Also data on GST and GSH levels with respect to aging in humans are scarce. Loguercio et al. (8) showed that GSH content in body and antrum of the stomach decreased with age. They did not find a sex dependency in GSH content. To obtain information of the lymphocytic GSH/GST system during aging, we investigated GST isoenzyme levels and GSH contents in human lymphocytes from 124 healthy subjects, aged 20–80 years.

Blood was collected by venapuncture into sterile siliconized EDTA K3 10 ml vacutainer tubes (Beckton Dickinson, San Jose, CA). Controls were divided into three age groups; 20–40 years (21 females, 20 males), 40–60 years (20 females, 21 males) and 60–80 years (20 females, 22 males). Lymphocytes were isolated by density centrifugation on Histopaque-1077, according to the manufacturers instructions (Sigma diagnostic, St Louis, MO). Lymphocytes were pelleted and stored at −20°C until use. For preparation of cytosolic fractions, lymphocytes were thawed slowly, homogenized in 100 µl of 20 mM Tris–HCl buffer pH 7.4, containing 1 mM dithiothreitol using a glass/glass potter. Homogenates were centrifuged at 12 000 g (4°C) for 20 min. Aliquots of the supernatant were stored at −20°C until use. The investigations were approved by the local ethical committee on human experimentation.

Protein was assayed by the method of Lowry et al. (9), using bovine serum albumin as a standard. Specific GST isoenzyme levels were determined as described previously (10). In short, cytosolic fractions were subjected to SDS–PAGE [11% acrylamide (w/v)], and subsequently to western blotting, using a semi-dry blotting system (Novablot II, Pharmacia, Uppsala, Sweden). Western blots were incubated with monoclonal antibodies against human GST classes α, µ and π. Class α antibodies react with human GST A1-1, GST A1-2 and GST A2-2 (10). Class µ antibodies recognize human GST M1a-1a, GST M1a-1b and GST M1b-1b (11,12). Class π antibodies react with human GST P1-1 (13). The specific binding of the monoclonal antibodies to the isoenzymes was demonstrated by incubation with peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dakopatts, Glostrup, Denmark) and subsequent staining with 4-chloro-1-naphthol and hydrogen peroxide. Staining intensity on the immunoblots was quantified using a laser densitometer (Ultroscan XL, LKB, Bromma, Sweden). Known amounts of purified GSTs were run in parallel with the experimental samples and served as standards for the calculation of the isoenzyme levels in the cytosolic fractions. The detection limit of this assay is ~50 ng/mg protein. Total GSH was quantified by high performance liquid chromatography after reaction with monobromobimane, as described previously (14). In this assay, oxidized GSH present is reduced by adding sodium borohydride to the reaction mixture. A Spearman rank correlation test was used to correlate lymphocytic GSH content and GST isoenzyme expression with age and gender. A Wilcoxon rank sum test was used to assess statistical significance of differences between age

**Abbreviations:** GSH, glutathione; GST, glutathione S-transferase.


GSH and GSTs in human lymphocytes


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