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Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

The effect of exogenous GSH, GSSG and GST-E on glutathione concentration and activity of selected glutathione enzymes in the liver, kidney and muscle of mice

Grażyna Świderska-Kołacz[1], Jolanta Klusek[1], Adam Kołątaj[2]

[1] Department of Animal Physiology, Institute of Biology, Świętokrzyska Academy, Świętokrzyska 15, 25-406 Kielce, Poland

[2] Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec, 05-552 Wólka Kosowska, Poland

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The experiment was conducted on randomly chosen 6 weeks-old mice, divided into four groups of 10 animals. The animals of control group were intraperitoneally injected with 250 μl of 0.9% NaCl whereas those of three experimental groups with reduced glutathione (GSH), oxidized glutathione (GSSG) and glutathione ethyl ester (GST-E) at doses 250 μg/kg body weight. Glutathione concentration as well as activity of glutathione enzymes was determined in liver, kidney and muscle tissues of control and experimental mice.
Injections of GSH led to significant increase in the concentration of reduced glutathione in the liver only, increased activity of glutathione transferase and glutathione peroxidase in the liver, kidney and muscle, and of glutathione reductase in liver and kidney.
Injections of GSSG caused the significant decrease in GSH concentration of the kidney while the activity of glutathione transferase rised in all three organs. The glutathione peroxidase rised only in the muscle.
Injections of GST-E increased the glutathione concentration in the liver and muscle, and glutathione transferase activity in all three tissues. Simultaneously, the activity of glutathione peroxidase in both liver and kidney as well as of glutathione reductase in the muscle dropped.

KEY WORDS: enzyme / glutathione / liver / kidney / mice / muscle

Glutathione is synthesized mainly in the thiol form (GSH – 98%), but is also present in the oxidized form (GSSG – 1.5%), thioether, mercaptide or other thioester forms [DeLeve and Kaplowitz 1990, Dringer *et al*. 2000, Lenartowicz *et al*. 1996].

GSH is the major intracellular non-protein thiol, connected with the cellular reducing environment that is essential for the optimum activity of some enzymes and other cellular macromolecules [DeLeve and Kaplowitz 1991, Lenartowicz *et al*. 1996]. Endogenously produced hydrogen peroxide is reduced by GSH in the presence of GSH peroxidase [Comhair *et al.* 2001, Czuczejko *et al.* 2003]. Intracellular GSH is maintained in the reduced form by the NADPH-requiring enzyme glutathione reductase [Nagaoka *et al.* 2004]. Glutathione also plays a major role in detoxication of many reactive metabolites either by spontaneous conjugation or by glutathione S-transferases [Armstrong 1997, Eaton and Bammler 1999, Hayes *et al*. 2005, Nobili *et al.* 2005].

Because decreased intracellular concentration of GSH might be undesirable in several clinical conditions, it is interesting from pharmacological point of view to correct it. In this report the effect of GSH, GSSG and GSH-E administration is described on the level of reduced glutathione and activity of glutathione enzymes in liver, kidney and muscle of mice.

Material and methods

We used 40 Swiss male mice, 6 weeks old, and weighing 20-25 g. The animals came from the mouse farm of the Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec. Animals were housed in standard cages in a controlled temperature (21°C) with a 12:12h dark:light cycle and fed the standard pelleted feed Murigran (Animal Food Company, Łomna near Warsaw, Poland) containing 16% protein, with free access to water. All mice received good veterinary care. The experimental protocol was approved by the respective Local Ethics Commission.

Mice were randomly divided into four groups of 10: 1 – control; 2 – injected with reduced form glutathione (GSH); 3 – injected with oxidized form glutathione (GSSG); 4 – injected with glutathione ethyl ester form (GSH-E).

The animals from experimental groups received for consecutive four days, at 8.00, 12.00 a.m. and 4.00 p.m. intraperitoneally 250 µg/kg body weight GSH (group 2), 250 µg/kg body weight GSSG (group 3) and 250 µg/kg body weight GSH-E (group 4). At those times and with the same intervals the control animals (group 1) were injected with 250 µl of 0.9% NaCl.

Two hours after the last injection the mice were killed by breaking the spinal cord and decapitation. Liver, left kidney and left thigh muscle were immediately isolated. The liver was perfused in 4°C physiological salt solution, and similarly (as remaining tissues), homogenized in 0.1 M phosphate buffer with addition 10 mM EDTA, pH 7.4, in a teflon POTTER-ELVEJHEM homogenizer. Homogenates were centrifuged for

15 min with 12,000 rev./min using JANETZKI K-24 centrifuge.

The GSH level (in mmol/g of tissue) was determined after Ellman [1959] using a SPECOL 20 spectrophotometer at the wavelength 412 nm. Activity of glutathione transferase was measured with the method of Habig *et al.* [1974], activity of glutathione peroxidase by the method of Chiu *et al.* [1976], the activity of glutathione reductase by method of Szczeklik [1974] and total protein according to Lowry *et al.* [1951] as modified by Kirschke and Wiederanders [1984]. The enzymatic activity was expressed in nmol/mg protein per min. All substrates were obtained from SIGMA.

The results were evaluated by analysis of variance (ANOVA).

Results and discussion

Table 1 shows that in relation to control mice the **reduced glutathione** level increased significantly ($P<0.05$) in liver after GSH and GSH-E administration (up to 120.6 and 130 %, respectively) and in the muscle after injections of GSH-E (up to 120.2%). After GSSG injections observed was also a decreased level of reduced glutathione in the kidney (77.5% of control).

In all examined tissues significant increase of **glutathione transferase** activity was found after GSH, GSSG and GSH-E administration (Tab. 1). The particularly high increase was observed in the muscle and in the kidney after GSSG injection (up to 290.2% and 217% of activity found in control mice).

Table 1 shows that the GSH administration increased the **glutathione peroxidase** activity in the liver, kidney and muscle, while administration of GSSG in the muscle only. GSH-E administration led to decreased glutathione peroxidase activity in the liver and kidney (down to 74.0% and 56% of controls).

The GSH injections increased **glutathione reductase** activity (Tab. 1) in the liver and in the kidney (up to 128.6 and 135.9% of activity found in control mice, respectively). After GSSG administration an increase in the enzyme activity occurred in the liver, in the kidney and in the muscle (up to 132.5, 155.3 and 123% of control mice). GSH-E injections decreased significantly the enzyme activity in the liver and in the muscle (down to 84.4 and 54.5% of controls).

Our results presented here indicate that reduced glutathione concentration in control animals was higher in liver (6.54 mmol/g tissue) than in kidney (4.27 mmol/g tissue) and in muscle (2.41 mmol/g tissue). The liver serves as a glutathione-generating organ and the principal mechanism of hepatocyte glutathione metabolism appears to be the cellular efflux system. The kidney also plays an important role in GSH organism homeostasis.

The results presented revealed a significant increase in concentration of glutathione in the liver only, but not in the kidney and muscle, after administration of reduced glutathione.

Exogenous GSH also significantly increased the activity of glutathione transferase, glutathione peroxidase and glutathione reductase in the tissues studied.

Table 1. Means and their standard deviations (SD) for reduced glutathione concentration and activity of glutathione enzymes in the liver, kidney and muscle of mice as related (%) to control (NaCl) group

Item	Injection		Liver	Kidney	Muscle
Reduced glutathione (mmol/g tissue)	NaCl	mean	6.54	4.27	2.41
		SD	0.53	0.29	0.50
		% of control	100	100	100
	GSH	mean	7.88*	4.11	2.65
		SD	0.33	0.90	0.46
		% of control	120.6	96.4	109.8
	GSSG	mean	6.45	3.31*	2.52
		SD	0.34	0.64	0.49
		% of control	98.6	77.5	105.0
	GSH-E	mean	8.50**	4.43	2.90*
		SD	0.71	0.63	0.28
		% of control	130.0	103.9	120.2
Glutathione transferase (nmol/mg protein/min)	NaCl	mean	7.00	3.51	3.00
		SD	0.28	0.59	0.38
		% of control	100	100	100
	GSH	mean	11.91***	8.13***	5.91***
		SD	0.61	0.71	0.31
		% of control	170.1	231.7	196.8
	GSSG	mean	12.29***	7.61***	8.72***
		SD	0.90	0.85	0.70
		% of control	175.4	217	290.2
	GSH-E	mean	8.76	5.18***	4.06**
		SD	0.84	0.55	0.57
		% of control	125.0	147.7	135
Glutathione peroxidase (nmol/mg protein/min)	Control	mean	0.0536	0.0361	0.0152
		SD	0.027	0056	0.005
		% of control	100	100	100
	GSH	mean	0.0635*	0.0436*	0.0213**
		SD	0.0038	0.0137	0.003
		% of control	118.4	120.9	140.3
	GSSG	mean	0.0613	0.0407	0.0194**
		SD	0.0036	0.0094	0.004
		% of control	114.4	113	127.7
	GSH-E	mean	0.0397*	0.0202***	0.0138
		SD	0.0046	0.003	0.002
		% of control	74.03	55.9	90.8
Glutathione reductase (nmol/mg protein/min)	Control	mean	0.154	0.103	0.057
		SD	0.016	0.024	0.014
		% of control	100	100	100
	GSH	mean	0.198**	0.140**	0.059
		SD	0.045	0.036	0.01
		% of control	128.6	135.9	103.1
	GSSG	mean	0.204**	0.160***	0.070*
		SD	0.035	0.021	0.013
		% of control	132.5	155.3	123.0
	GSH-E	mean	0.130*	0.098	0.031***
		SD	0.027	0.014	0.009
		% of control	84.4	95.1	54.5

The differences significant in relation to control: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

The GSH content of tissue depends on GSH neosynthesis and GSH regeneration from GSSG [Sen 1997, Włodek *et al.* 2002].

The GSH monoester led to increase in the SH glutathione level of liver and muscle. Moreover, it increased the glutathione transferase activity in all three tissues, decreased the glutathione peroxidase activity in the liver and kidney and lowered reductase activity in the liver and muscle. GSH ester is particularly important in protecting cells from radiation [Wellner *et al*. 1984], effective against some toxic compounds including heavy metals [Naganuma *et al*. 1990] and cancerogenic agents [Teicher *et al.* 1988].

An increase in glutathione transferase, glutathione reductase and glutathione peroxidase activities may result from the damage caused by oxidative stress, as the these enzymes protect cell membranes from damage induced. Particularly important are changes in glutathione reductase activity because the enzyme substitutes the reduced form of glutathione at the NADPH oxidation when a large amount of glutathione disulphide is formed in the cell.

We assume that the revealed changes in the activity of the investigated enzymes reflect the adaptation reactions to metabolic disorders caused by the excess GSH, GSSG or GSH-E. It may be suggested that reduced glutathione and GSH-E may be useful in the disease therapy and be indicators of metabolic reactivity.

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Grażyna Świderska-Kołacz, Joanna Klusek, Adam Kołątaj

Wpływ egzogennego GSH, GSSG i GST-E na stężenie glutationu i wybranych enzymów glutationowych w wątrobie, nerce i mięśniu myszy

S t r e s z c z e n i e

Badania przeprowadzono na 6-tygodniowych samcach myszy, pochodzących z kojarzeń losowych. Zwierzęta podzielono losowo na 4 grupy, po 10 osobników. W iniekcji dootrzewnowej osobniki kontrolne (grupa 1) otrzymywały przez 4 kolejne dni po 250 µg 0,9% NaCl. Osobnikom doświadczalnym (grupa 2, 3 i 4) podawano przez 4 kolejne dni tą samą drogą glutation zredukowany (GSH), glutation utleniony (GSSG) i ester etylowy glutationu (GST-E) w dawkach po 250 µl/kg masy ciała.

W grupie 2 stwierdzono istotny wzrost stężenia glutationu zredukowanego w wątrobie, wzrost aktywności transferazy glutationowej i peroksydazy glutationowej w wątrobie, nerce i mięśniu oraz wzrost aktywności reduktazy glutationowej w wątrobie i nerce. Podawanie glutationu utlenionego (grupa 3) prowadziło do istotnego zmniejszenia koncentracji GSH wyłącznie w nerce, wzrostu aktywności transferazy glutationowej w wątrobie, nerce i mięśniu oraz wzrostu aktywności peroksydazy glutationowej jedynie w mięśniu. Iniekcje GST-E (grupa 4) skutkowały wzrostem koncentracji glutationu zredukowanego w wątrobie i mięśniu, wzrostem aktywności transferazy glutationowej w wątrobie, nerce i mięśniu, spadkiem aktywności peroksydazy glutationowej w wątrobie i nerce oraz reduktazy glutationowej w mięśniu.