PHARMACOLOGY AND TOXICOLOGY

Metabolic Preparation MP-33 as Inductor of Cell Glutathione and Glutathione-Dependent Enzymes

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Metabolic preparation MP-33 induces cell glutathione and activities of glutathione S-transferase and glutathione peroxidase. This effect depends on the preparation composition. Single injection of MP-33 to rats considerably activates glutathione-dependent systems in the liver, heart, and erythrocytes. MP-33 is a more potent inductor than reduced glutathione.

Key Words: *intracellular glutathione; glutathione S-transferase; glutathione peroxidase; metabolic preparation*

Reduced glutathione (GSH, γ-glutamyl-cysteinyl-glycine), the most abundant form of nonprotein thiol, is involved in many intracellular processes including detoxification of xenobiotics, protein synthesis, regulation and expression of cell cycle genes, and protection of proteins from autooxidation [1,3,5]. GSH is a cosubstrate for glutathione S-transferase (GST) catalyzing reactions of detoxification [8,9]. GSH is also involved in reduction of lipid peroxides and H_2O_2 catalyzed by glutathione peroxidase (GPX). Multifunctional role of GSH in tissues and its involvement in protein synthesis and regeneration determine the relationship between the level of cell glutathione and aging and various diseases (ischemic heart disease, Parkinson's disease, diabetes, arthritis, and cataract) [7]. Therefore, studies of molecular mechanisms of GSH regulation are of considerable importance. Here we studied the effects of original metabolic preparation MP-33 on cell level of GSH.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 180-200 g and kept under standard conditions. Water solution of MP-33, a mixture of equivalent volumes of L-glutamic acid, sulfur-containing amino acid (cysteine, cystine, or methionine), and glycine, was administered perorally in a dose of 6 mg/kg body weight (0.5 ml) to rats [2]. Control animals received equivalent volume of distilled water. Rats of other groups were perorally treated with GSH (100 mg/kg body weight), cysteine (2 mg/kg body weight), cystine (2 mg/kg body weight), and methionine (2 mg/kg body weight). Control and experimental animals were killed 30-40 min after treatment. Microsomal fraction and postmicrosomal supernatant of liver, kidney, and heart homogenates were isolated by two-step centrifugation at 12,000*g* for 20 min and 105,000*g* for 1 h. Erythrocytes from rat venous blood were obtained as described previously [4]. The content of GSH [14] and total glutathione (GSH+oxidized glutathione, GSSG) [15] were measured. GST activity was determined using 1-chloro-2,4-dinitrobenzene (Sigma) as the cosubstrate [6]. GPX activity was estimated by the decrease in NADPH (Serva) [13]. Protein concentration was determined by the method of Lowry [11]. The results were analyzed by Student's *t* test (probability 95%).

RESULTS

MP-33 considerably increased GSH level in rat liver, while GSH and individual amino acids had no effects on this parameter (Table 1).

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TABLE 1. Effects of GSH, Cysteine, Cystine, Methionine, and MP-33 (with Different Sulfur-Containing Amino Acid Components) on Level of GSH and GSH+GSSG in the Liver of Wistar Rats (*M±m*)

Content, μmol/g	Control (*n*=15)	GSH, 6 mg/kg (*n*=12)	Cysteine, 2 mg/kg (*n*=10)	Cystine, 2 mg/kg (*n*=10)	Methionine, 2 mg/kg (*n*=10)	MP-33 (6 mg/kg) with various sulfur-containing amino acids		
						cysteine (*n*=10)	cystine (*n*=10)	methionine (*n*=10)
GSH	3.51±0.32	3.54±0.31	3.45±0.36	3.43±0.38	3.48±0.32	4.80±0.39*	4.67±0.35*	4.16±0.34
GSH+GSSG	3.53±0.31	3.55±0.33	3.48±0.34	3.47±0.31	3.51±0.32	5.01±0.40*	4.69±0.36*	4.18±0.34

Note. *$p<0.05$ compared with control.

The increase in the content of thiols was mainly due to the increase in the content of GSH (but not GSSG). The effect of MP-33 depended on its sulfur-containing component. The preparations including cysteine, cystine, and methionine elevated the content of GSH by 2.7, 1.9, and 1.8 times, respectively, in comparison with the theoretical value, i. e. the content of GSH in the liver assuming that MP-33 contained equivalent volumes of ingredients and was utilized by the liver only (mean weight 4 g) [2]. Taking into account that ASC, X_C^-, and L are the main transport systems providing the passage of cysteine, cystine, and methionine, respectively, into the cell, and γ-glutamyltransferase is a selective transporter of cysteine and cystine [12], it can be assumed that activation of these transport systems leads to an increase in cell content of GSH. This effect probably results from reduction of -S-S- groups in active sites of transport proteins under the influence of thioredoxin and glutaredoxin, whose activities significantly increase due to the rise in the content of GSH after the initial passive transport of precursor amino acids into the cell. Glycine transport into the cell is less specific (through L, ASC, Gly, C, and Band-3 transport systems) [12]. L-glutamic acid transport is hindered although a Na^+-independent transmembrane Glu transporter was found. Nevertheless, MP-33 can promote glycine and L-glutamic acid transport, because thioredoxin and glutaredoxin increase conformational mobility of membrane-bound transport proteins. The effect was the most pronounced if the preparation included cysteine and cystine as sulfur-containing amino acids, because γ-glutamyltransferase is a specific transporter of these amino acids. The rise in the cell content of GSH increases its efflux from liver cells followed by rapid activation of transport systems of precursor amino acids in extrahepatic tissues due to direct effects of GSH on -S-S- groups in transporter active sites and their reduction by membrane-bound glutaredoxin using GSH as a cosubstrate. Besides, GSH can inhibit some protein kinases (primarily protein kinase C) activated by reactive oxygen intermediates. The inhibition of protein kinase C abolished (due to phosphorylation) the inhibition of γ-glutamylcystein synthase, the key enzyme of GSH biosynthesis [12]. This mechanism is responsible for a considerable rise of the level of GSH under the effect of MP-33.

Indeed, MP-33 increased the content of GSH and GSH-dependent enzymes in the liver, heart, and kidneys (Fig. 1). GSH content in the liver increased by 1.6 times (5.01±0.40 *vs.* 3.51±0.32 μmol/g liver in the control). Maximum activation of cytosolic GST (1.6-fold) was observed in erythrocytes (2.04±0.07 *vs.* 1.29±0.06 μmol/min/mg hemoglobin in the control), while the most pronounced increase (1.5-fold) in microsomal GST activity was found in the liver (121±13 *vs.* 82±7 nmol/min/mg microsomal protein in the control). GPX activity was the highest in the heart (2.05±0.26 μmol/min/mg cytosolic protein) and exceeded the control value (1.37±0.21 μmol/min/mg cytosolic protein) by 1.5 times. It can be assumed that MP-33 increased GST and GPX activities because the rise of intracellular GSH altered the cell redox status and, therefore, changed the content of transcriptional factors NF-κB and AP-1. We propose the following sequence of processes. The inhibition of protein kinase activities induced by a GSH increase suppresses activation of

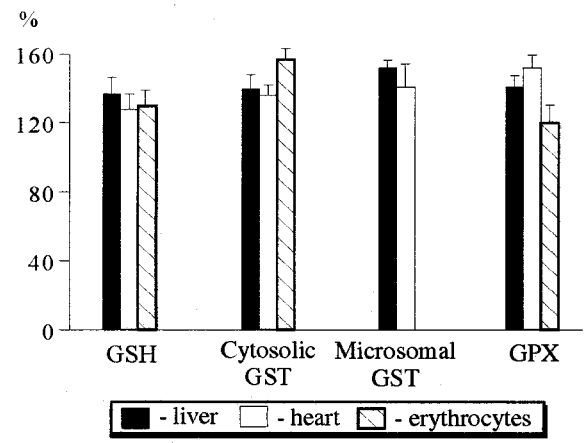

Fig. 1. Level of reduced glutathione (GSH) and activities of glutathione S-transferase (GST) and glutathione peroxidase (GPX) in the liver, heart, and erythrocytes of Wistar rats after administration of 6 mg/kg MP-33 containing cysteine. Here and in Fig. 2: results are expressed in % of the control (*n*=12).

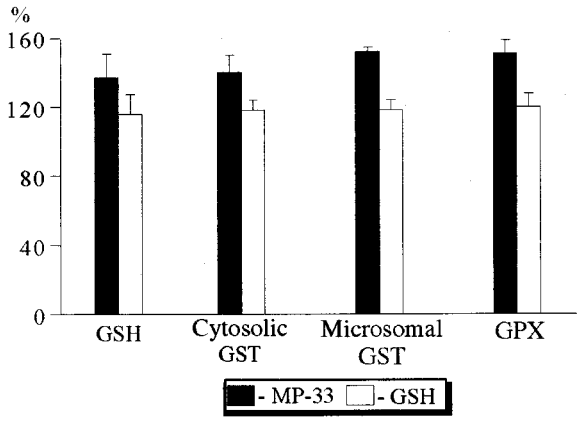

Fig. 2. Effect of MP-33 (6 mg/kg) containing cysteine and reduced glutathione (GSH, 100 mg/kg) on activity of glutathione S-transferase (GST) and glutathione peroxidase (GPX) in Wistar rats.

NF-κB and expression and activation of c-Fos and c-Jun proteins, components of AP-1 [10]. Thus, cytosolic levels of active NF-κB and AP-1 decrease. However, the activation of thioredoxin and glutaredoxin induced by a GSH rise promotes transcription of NF-κB and AP-1 due to reduction of -SH- groups in critical sites of these proteins. This mechanism maintains the nuclear content of NF-kB and AP-1 that can cause induction of some proteins including GST and GPX. Long-term action of the preparation can amplify this effect.

MP-33 was superior to GSH (applied in a higher dose) in activating GSH and GSH-dependent enzymes (Fig. 2). In comparison with the tripeptide, MP-33 increased the level of GSH 1.26-fold (3.80±0.30 *vs.* 3.52±0.31 μmol/g liver in the control), cytosolic GST activity 1.24-fold (1.18±0.03 *vs.* 1.20±0.03 μmol/min/mg cytosolic protein in the control), microsomal GST activity 1.4-fold (86±9 *vs.* 79±7 nmol/min/mg microsomal protein in the control), and GPX activity 1.26-fold (13.7±2.8 *vs.* 12.3±2.4 μmol/min/mg cytosolic protein in the control). Thus, MP-33 is a more efficient regulator of intracellular GSH than exogenous tripeptide, whose passage through the cell membrane is difficult.

In conclusion, MP-33 is a potent modulator of the GSH-dependent system and can be used for the therapy and prevention of some diseases.

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