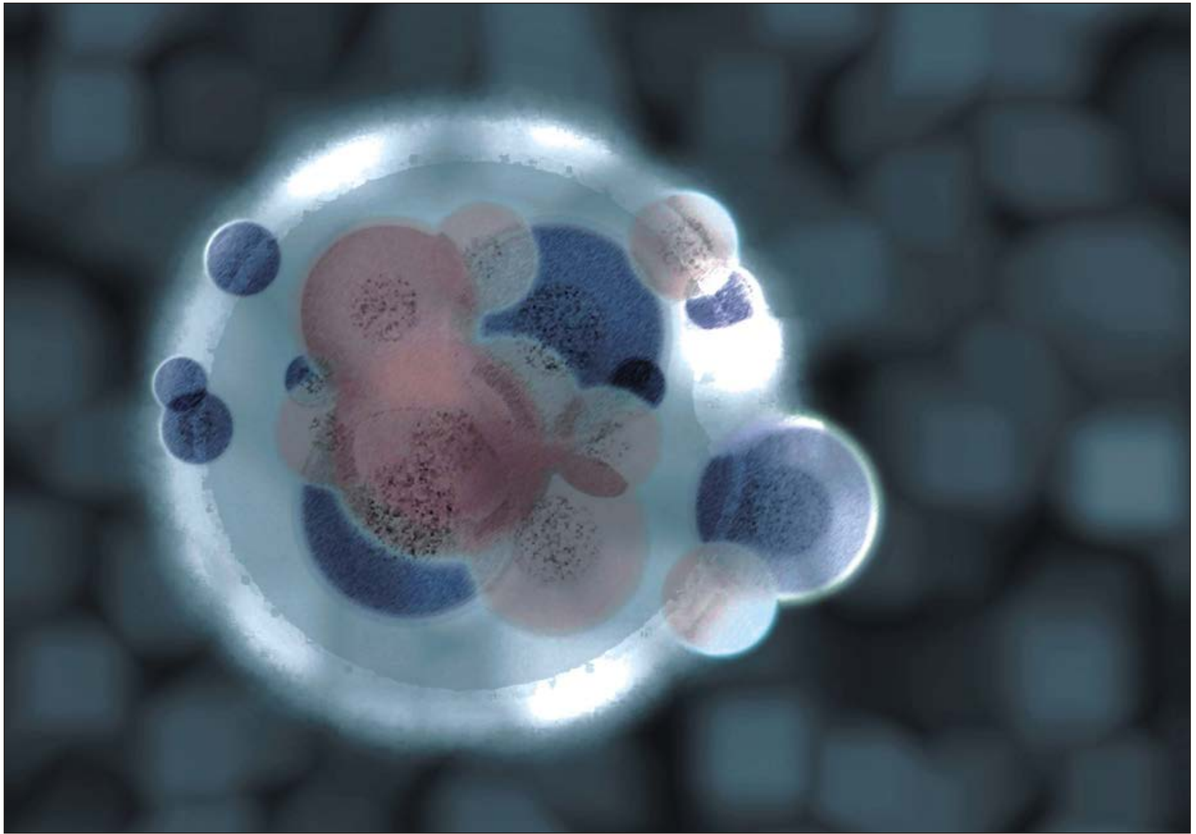

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Effect of Methylglyoxal on Antioxidant Enzymes of the Liver and Spleen of the Mice

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Abstract: In this study the effect of different doses of methylglyoxal (25-400 mg kg^{-1} body wt.) were examined using enzymes involved in the antioxidant function, superoxide dismutase (SOD), catalase (CAT), glutathion-S-transferase (GST), glyoxalase I (Glyo I) and glyoxalase II (Glyo II) in the liver and spleen of the mice after 6, 12 and 24 h. Significant changes were observed predominantly in the liver. The specific activities of (SOD), (GST), (CAT), (Glyo I), and (Glyo II) were found to decrease in the liver. The mode and magnitude of change in the specific activities were seen to depend on the dose of methylglyoxal and the time of the administration. The present findings suggested of the adverse effect of methylglyoxal on the antioxidant defense system. It is likely that methylglyoxal undergoes a redox cycle and generates the free radicals which in turn lower the antioxidant status in animals.

Key words: Methylglyoxal, liver, spleen, antioxidant enzymes

INTRODUCTION

Methylglyoxal (2-oxopropanal, pyruvaldehyde)(MG) is a metabolit found widespread through biological life[1,2]. It was initially thought to be involved in mainstream glycolysis but the discovery of phosphorylated glycolytic intermediates led to its demise as a metabolite of major importance. This compound could influence the cell proliferation, differentiation, vesicle mobilisation and growth regulation[3]. Methylglyoxal is present in increased concentrations in diabetics and implicated in formation of advanced glycosylation end-products, and secondary diabetic complications[4]. It was also shown to be mutagenic[5,6]. Its mutagenicity was markedly enhanced in the presence of hydrogen peroxide[7].

Methylglyoxal was suggested to play an important role in the causation and complications of experimental diabetes mellitus[2,8,9]. It was shown that methylglyoxal influence the hepatic microsomal mono-oxygenase system in mice[10] and albino mice[7]. Also, it diminished the mitochondrial respiration[11,12].

It is important to note that methylglyoxal could interact with nucleic acids and proteins adversely affecting the expression of genetic information as well as various cell functions in wide variety of cells and species[5,13]. In the present study, therefore, the effect of methylglyoxal on some of antioxidant enzymes, superoxide dismutase, catalase, glutathion-S-transferase, glyoxalase I and glyoxalase II were examined and found to be adversely affected in the liver and spleen of the mice.

MATERIALS AND METHODS

Chemicals: All chemicals used in this study were purchased from Sigma chemical Co.. All other chemicals used were of analytical grade.

Animals: Male mice (6-7 weeks old) were used for the present study.

Preparation of homogenate: Mice were treated with different doses of methylglyoxal (25, 50, 100, 200 and 400 mg kg^{-1} wt.) for 6, 12 and 24 h, then sacrificed. Livers (perfused with 0.9% NaCl) and spleen of each case were taken out and homogenized in 0.1 M phosphate buffer (pH 7.0) using. Each homogenate was centrifuged at 20.000Xg for 30 min. The pellet was discarded and the supernatant recentrifuged at 100.000Xg for 60 min. The resulting supernatant used for the assay of enzymes.

Determination of enzyme activity: SOD was assayed according to Choudhary *et al.*[7] method which involves the inhibition of pyrogallol autoxidation at pH 8.0 (a single unit of enzyme was defined as the quantity of enzyme required to produce 50% inhibition of autoxidation). Catalase was estimated also according to

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Choudhary *et al.*[7]. GST was assayed using the method of Sady *et al.*[14], glyoxalase I was assayed according to the method of Thornalley[2] and glyoxalase II was assayed according to the method of Thornalley[15].

Protein determination: Protein was determined using Bradford[16] method using bovine serum albumin (BSA) as standard.

Statistical analysis: The significance of difference between the data pairs was evaluated by analysis of variance (ANOVA) followed by U test.

RESULTS

To test the toxicity, various doses of MG (25, 50, 100, 200 and 400 mg kg^{-1} body wt.) were given to mice intraperitonially (single dose) since it was found by Kalapos[18] that 800 mg kg^{-1} body wt. is lethal to animals.

Table 1: Effect of MG on the specific activity of SOD , CAT, GST, Glyo I and II in the liver of mice

Dose mg kg^{-1} body wt.	Specific activities time (h)		
	6	12	24
SOD			
None	8.15±0.522	8.10±0.66	8.12±0.700
25	8.02±0.466	7.77±0.499	8.09±0.549
50	7.44±0.310	7.36±0.522	7.68±0.422
100	7.30±0.282	7.18±0.510	7.47±0.287
200	7.12±0.222	7.07±0.420	7.20±0.202
400	7.08±0.240	7.02±0.400	7.32±0.200
CAT			
None	29.88±0.888	27.25±0.741	27.01±0.681
25	27.67±0.920	25.38±0.701	25.00±0.542
50	26.77±0.812	23.10±0.660	21.80±0.522
100	24.20±0.676	21.77±0.605	20.06±0.522
200	22.85±0.514	20.53±0.422	18.77±0.508
400	20.00±0.818	18.66±0.420	16.02±0.440
GST			
None	1.442±0.068	1.428±0.088	1.398±0.182
25	1.368±0.046	1.335±0.094	1.322±0.023
50	1.356±0.043	1.330±0.080	1.321±0.034
100	1.266±0.031	1.218±0.040	1.301±0.034
200	0.776±0.036	0.680±0.038	0.228±0.042
400	0.512±0.044	0.444±0.038	1.298±0.117
Glyo I			
None	0.626±0.078	0.633±0.049	0.630±0.055
25	0.512±0.031	0.532±0.044	0.606±0.022
50	0.487±0.047	0.489±0.077	0.499±0.018
100	0.411±0.038	0.421±0.061	0.455±0.017
200	0.401±0.030	0.398±0.077	0.297±0.013
400	0.380±0.022	0.379±0.061	0.291±0.070
Glyo II			
None	0.122±0.014	0.118±0.010	0.120±0.009
25	0.114±0.009	0.111±0.007	0.110±0.004
50	0.102±0.007	0.096±0.005	0.102±0.007
100	0.098±0.008	0.091±0.006	0.094±0.003
200	0.038±0.006	0.029±0.003	0.025±0.005
400	0.016±0.005	0.009±0.002	0.002± 0.006

Table 2: Effect of MG on the specific activities of Catalase, GST, Glyo I and II in the spleen of mice

Dose (mg kg^{-1} body wt.)	Specific activities time (h)		
	6	12	24
Catalase			
None	6.240±0.006	6.311±0.011	6.330±0.043
25	4.336±0.028	4.210±0.077	4.467±0.081
50	4.108±0.064	4.031±0.063	4.450±0.026
100	3.407±0.092	3.850±0.085	3.936±0.010
200	3.100±0.117	3.659±0.099	3.900±0.088
400	2.255±0.057	3.052±0.103	3.440±0.076
GST			
None	1.450±0.050	1.522±0.074	1.771±0.099
25	1.420±0.044	1.421±0.072	1.400±0.080
50	1.440±0.046	1.456±0.068	1.380±0.055
100	1.402±0.068	1.400±0.034	1.338±0.056
200	1.386±0.060	1.366±0.056	1.312±0.033
400	1.337±0.022	1.305±0.077	1.288±0.043
Glyo I			
None	0.446±0.044	0.444±0.042	0.428±0.050
25	0.440±0.041	0.440±0.039	0.468±0.018
50	0.438±0.040	0.436±0.026	0.402±0.019
100	0.433±0.022	0.420±0.021	0.361±0.033
200	0.410±0.066	0.381±0.020	0.312±0.022
400	0.382±0.020	0.344±0.016	0.282±0.030
Glyo II			
None	0.100±0.007	0.080±0.005	0.112±0.012
25	0.088±0.003	0.077±0.002	0.096±0.007
50	0.066±0.003	0.068±0.001	0.080±0.008
100	0.042±0.002	0.0.33±0.007	0.037±0.003
200	0.022±0.001	0.024±0.004	0.026±0.002
400	0.020±0.001	0.021±0.001	0.018±0.001

The specific activity of SOD, GST, CAT, Glyo I and II were determined 6, 12 and 24 h after administration of MG. The specific activities of the first three enzymes in the liver were found to decrease with the increase in the concentration of MG at these three intervals of time (Table 1). The mode and magnitude of the change in the specific activities of these three enzymes were somewhat irregular and depended on the dose and time after the treatment with MG. It was clearly visible that the effect of MG diminished with time since the specific activities of these enzymes were found to be higher at 24 h compared to those at 6 h and 12 h after treatment of MG. However, the significant effect on catalase persisted up to 24 h.

In case of SOD, it was found to be significant at 6 h intervals only. Higher doses (200 and 400 mg kg^{-1} body wt.) altered the activity of GST significantly at 6 and 12 h. Intraperitoneal administration of 25 mg kg^{-1} body wt. did not affect the specific activities of both Glyo I and II, whereas, the administration of 50, 100, 200 and 400 mg kg^{-1} body wt of MG diminished the specific activities of these two enzymes (Table 2).

It was shown that the effect of low doses of MG (25, 50 and 100 mg kg^{-1} body wt.) on the specific activity of Glyo I decreased with time whereas with high doses of MG (200 and 400 mg kg^{-1} body wt.) its effect increased at

12 and 24 h. In the case of Glyo II, the specific activity was significantly inhibited by higher doses (200 and 400 mg kg^{-1} body wt.)

In the spleen, MG also affected the specific activity of CAT. The pattern of change was quite similar to that found in the liver. The most interesting observation that no significant changes were observed in the specific activities of SOD and GST in the spleen. The specific activity of Glyo I in the spleen was significantly affected 6 h after the administration of MG above 50 mg kg^{-1} body wt. (Table 2). However, the changes affected by MG on the specific activity of Glyo II in the spleen were not significant.

Present results showed that higher concentration of MG (10 mM) was mainly effective in inhibiting the specific activity of antioxidant enzymes to the extent of around 20-25%. The lower concentration (0.1 mM) had very little or no effect. Also, it was shown that Glyo I was affected the most by MG among all the enzymes studied. The specific activity was reduced by around 60% within 15 min of incubation with 10 mM of MG.

DISCUSSION

In mammals, MG is synthesised by bacteria of the gut flora and by the host itself can be taken up from foodstuffs[17-19]. The glycolysis bypass, acetone metabolism and amino acid breakdown are important pathways of production of MG[20]. Levels of MG could increased under certain physiological and pathological conditions[13]. MG is a toxic and it is also a substrate for the glyoxalase system which play a vital role in cellular function.

The results of the present study suggested that MG affected the specific activities of SOD and catalase which are important members of the defense system against the oxidative damage. Superoxide and H_2O_2 generated in the electrons reduction of molecular oxygen, or redox reactions, are known to be catalytically metabolised by SOD and catalase, respectively to less toxic or non-toxic products[21]. Even though, the SOD was not affected very much, the decrease in the activity of catalase would lead to the accumulation of H_2O_2. The importance of GST in the protective mechanism is now well established[13]. Thiols act as protective agents against electrophile radical damage and oxidative stress. GST is one of the enzymes which catalyse antioxidant processes of thiols. The adverse effects on GST might reduce the protective mechanism in animals[22] thus, MG weakens the antioxidant enzymes and their modulation may contribute to an oxidative stress[10].

The inhibition of antioxidant enzymes was suggestive of the involvement of hepatotoxic effect of MG and its direct interaction in modulation of specific activities of these antioxidant enzymes[15,22,23]. Therefore, alterations in the specific activities of antioxidant enzymes in the present study might have resulted from the liver damage.

An active glyoxalase system is present through embryogenesis, tissue maturation and persists until cell death[24]. The coupled reaction catalysed by Glyo I and II converts electrophile and cytotoxic 2-oxoaldehydes to less reactive chemical species[7]. Thus, glyoxalases are considered as a part of reduced glutathion (GSH) dependent detoxifying enzymes[14]. As shown in the present results, MG decreased the specific activities of both Glyo I and II in the liver (Table 1). It is likely that the inhibition of Glyo I was related to its direct interaction with MG, whereas inhibition of Glyo II was linked to the indirect effect of MG[25,26]. Interestingly, MG is a substrate for Glyo I , therefore, the decrease in the specific activities of glyoxalase system particularly Glyo I which has GSH as a cofactor would lead to the accumulation of MG and significant generation of free radicals[10] which might further potentiate the damage and affect the vital biological functions.

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