Activity of Glucose-6-Phosphate Dehydrogenase and Glutathione Levels in Liver Lobes of Mus Musculus

MUS MUSCULUS KARACİĞERİNİN FARKLI LOBLARINDAKİ GLUKOZ-6-FOSFAT DEHİDROGENAZ AKTİVİTESİ VE GLUTATYON DÜZEYİ

Ergül BELGE KURUTAŞ, MD,[a] Metin KILINÇ, MD,[a] Yıldız ÇAĞLAR, MD[b]

[a]Department of Biochemistry, Kahramanmaraş Sütçü İmam University Medical Faculty, KAHRAMANMARAŞ
[b]Department of Histology, Çukurova University Medical Faculty, ADANA

Abstract

Objective: Glucose-6-phosphate dehydrogenase (G6PDH) and glutathione (GSH) are two antioxidant systems that play important roles in the cellular response to oxidative stress. G6PDH activity and GSH levels in different liver lobes of mice lend themselves particularly to comparative investigation due to the similar homology of human and mouse G6PDH and GSH enzymes.

Material and Methods: Twenty males of Mus musculus albino mice were included in the experiments. The activity of G6PDH and GSH levels in liver were measured with the aid of the Beutler method.

Results: Mean and standard deviation per g liver for lobular G6PDH activity and GSH levels were found to be as follows: 3.30 ± 1.44 U/g and 1.84 ± 0.030 µmol/g for the median lobe; 1.56 ± 0.93 U/g and 0.57 ± 0.25 µmol/g for the right lobe; 2.43 ± 1.42 U/g, 1.06 ± 0.21 µmol/g for the left lobe; and 0.41 ± 0.17 U/g, 0.05 ± 0.02 µmol/g for the caudate lobe. The highest G6PDH activity and GSHs level were found in the median and left lobes. Moreover, significant differences in G6PDH activity and GSH levels were recorded among lobes ($p< 0.05$).

Conclusion: G6PDH activity and GSH levels were seen to have a heterogeneous distribution in the liver lobes of male mice.

Key Words: Mice, liver, glucose phosphate dehydrogenase, glutathione

Özet

Amaç: Oksidatif strese karşı hücresel yanıtta önemli rol oynayan antioksidan sistemler Glukoz-6-fosfat dehidrogenaz (G6PDH) ve glutatyon (GSH)'dur. İnsan ve fare G6PDH enziminin homoloji göstermesinden dolayı, farelerin farklı karaciğer loblarında G6PDH aktivitesi ve GSH düzeyleri araştırıldı.

Gereç ve Yöntemler: Yirmi erkek Mus musculus albino fare çalışmada kullanıldı. Karaciğer loblarında G6PDH aktivitesi ve GSH düzeyi Beutler yöntemi ile ölçüldü.

Bulgular: Farklı karaciğer loblarında G6PDH aktivitesi ve GSH düzeyinin ortalama ve standart sapması sırasıyla: Orta lob; 3.30 ± 1.44 Ü/g karaciğer, 1.84 ± 0.030 µmol/g karaciğer; sağ lob; 1.56 ± 0.93 Ü/g karaciğer, 0.57 ± 0.25 µmol/g karaciğer, sol lob; 2.43 ± 1.42 Ü/g karaciğer, 1.06 ± 0.21 µmol/g karaciğer, kaudat lob; 0.41 ± 0.17 Ü/g karaciğer, 0.05 ± 0.02 µmol/g karaciğer. Maksimum G6PDH aktivitesi ve GSH düzeyi medyan ve sol lobda bulunmuştur. Bunun yanı sıra, G6PDH aktivitesi ve GSH düzeyinin loblar arasında anlamlı farklılıklar gösterdiği saptanmıştır ($p< 0.05$).

Sonuç: G6PDH aktivitesi ve GSH düzeyi erkek fare karaciğer loblarında heterojen dağılım göstermektedir.

Anahtar Kelimeler: Mus musculus (fare), karaciğer lobları, glukoz-6-fosfat dehidrogenez, glutatyon

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Production of free radicals continuously occurs in all cells as part of normal cellular function. They usually derive from oxygen and their various reactive intermediators, but also derive from metabolic reactions involving outer-shell electrons of transition metals. Many disorders are associated with an excessive generation of reactive oxygen species (ROS) and oxidative stress. ROS are very reactive molecules produced when one is exposed to pollution, cigarette smoke, stress, ionizing radiation, inflammation, excessive sunlight and toxic compounds.[1] If the oxidative stress increases in cells, it may cause oxidative DNA damage which in turn modulates the mutation rate and ultimately the cancer incidence.[2] It has been estimated that metabolism of normal endogenous compounds alone might cause approximately 10^4 to

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Yazışma Adresi/*Correspondence:* Ergül BELGE KURUTAŞ, MD
Kahramanmaraş Sütçü İmam University,
Medical Faculty, Department of Biochemistry,
46050, KAHRAMANMARAŞ

10^5 oxidative hits per cell per day.[3] Antioxidants prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. Some of the endogen antioxidants are Glucose-6-phosphate dehydrogenase (G6PDH) and Glutathione (GSH).[3]

G6PDH enzyme is the key enzyme of pentose phosphate pathway. The reducing equivalents (NADPH) generated by G6PDH is for ultimate electron acceptor in oxidative stress due to its biochemical role found in all cell types and organisms.[4,5]

GSH (γ-glutamyl-cysteinyl-glycine) is the most important low-molecular-weight non-enzymatic antioxidant in mammalian tissues.[6] The ability of GSH to react with peroxides and several electrophiles, such as carcinogenic-epoxide metabolites, has prometed interest in it as a factor carcinogenesis.[7]

According to our knowledge, none of the previous researches investigated G6PDH activity and GSH levels in liver lobes of mouse. They both have very important role to protect cells from the free radicals. Therefore, it was aimed to investigate variations of G6PDH activity and GSH level in different liver lobes of mice.

Material and Methods

Chemicals: Nicotinamide adenine dinucleotid phosphate (NADP), glucose-6-phosphate (G6P) were obtained from Sigma Chemical Co. All other chemicals were analytical grade products of Merck (Darmstald, Germany).

Animals: Twenty non-inbred male Mus musculus albino mice (30-45 g) were obtained from the Medical Experimental Research Center of the University of Çukurova (Adana, Turkey). Ten week-old mice were fed with a standard laboratory diet and tap water. Ambient temperature had a range of 22-24˚C. We followed the Guide for the care and use of laboratory animals. Besides, we obtained approval from ethic commitee

Preparation of Homogenate: As soon as the experimental mice were sacrified, their livers were quickly removed, and blotted on filter paper. Tissue samples (200-300 mg wet weight/per mouse) were obtained from various sites (Figure 1). Liver lobes were homogenized with 10 volumes of ice-cold 0.25 M sucrose, centrifuged in 14.000 g to measure the activity of G6PDH and level of GSH in resulting supernatant.[8]

Determination of enzyme activity: G6PDH activity was determined from supernatant at 37˚C according to Beutler.[9] The reaction mixture contained 1 M Tris-HCI pH 8.0, 6 mM G6P Na, 2 mM NADP, 0.1 M $MgCI_2$ and supernatant of liver lobes in total volume of 3 mL. One unit of enzyme activity is the amount catalyzed the reduction of 1 mM of NADP per minute.

Determination of GSH level: GSH level was determined according to Beutler.[9] The rection mixture contained filtrate, phosphate buffer and DTNB (5,5'-dithiobis 2-nitrobenzoic acid) in final volume of 10 mL. A blank was also prepared by using precipatiating reagent and distilled water instead of filtrate. The absorbances were immediately read in a spectrophotometer at 412 nm before and after addition of DTNB. The values were determined from the standard curve.

Histological analysis: Tissues for histological analysis were fixed in neutral buffered 10% (v/v) formalin, dehydrated in alcohol and embedded in

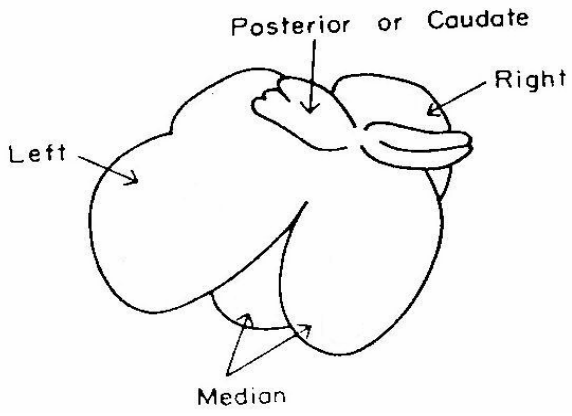

Figure 1. Nomenclature used for mouse liver lobes. The liver is viewed from the ventral-posterior position.
The sampling sites of tissue samples are indicated in the figure by 1 to 6.

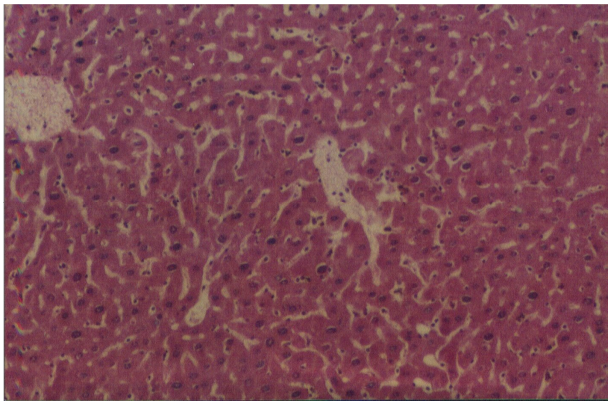

Figure 2. Normal histological observations of Mus musculus liver tissue.
Tissue samples obtained from site 1 and 6 were fixed and then stained with hematoxylene-eosin x 200

paraffin. Paraffin sections (about 5 µ thick) were stained by a routine method with hematoxylin and eosin.[10] The present study, the normal liver were used according to histopatologic results (Figure 2).

Statistical analysis: Data were expressed as mean values ± SD and median-range. Statistical analyses of differences in G6PDH and GSH levels between liver lobes were performed by using Kruskal-Wallis variance analysis followed by post-hoc Mann-Whitney U test. The correlations between G6PDH and GSH levels were determined using Spearman correlation coefficients. A p value less than 0.05 was accepted as significant. Statistical analyses were performed using SPSS 9.05 for Windows (SPSS Inc., Chicago, IL).

Results

G6PDH activity and GSH levels were found as heterogeneously distributed in liver lobes of male mice as statistically (Chi-Square for G6PDH: 17.14, p: 0.001; Chi-Square for GSH: 43.23, p: 0.000). The maximum activity of G6PDH and the maximum level of GSH were found higher in median and left lobes than in the other lobes as shown in Table 1 and 2.

There was a good corelation in all of lobes between G6PDH activities and GSH levels (rho: 0.575, p: 0.000). After then, The corelation analyses for each lobe were done between G6PDH activities and GSH levels. There was a negative corelation between Caudate G6PDH activities-left GSH levels and median GSH levels-caudate GSH levels (rho: -0.744, p: 0.006; rho: -0.61, p: 0.035 respectively).

Discussion

Tissues are exposed to many exogeneous and endogeneous oxidative agents. Liver is the most important tissue that eliminates the oxidative stress products. Increased formation of ROS and/or decreased antioxidant defence can be defined as oxidative stress, which is widely recognized as an important feature of many disease including hepatocellular carcinoma.[11] Free radicals are known to increase membrane permeability and inactivate the receptors in membrane and enzymes by lipid peroxidation as being an important membrane component that causes membrane disflow. They occur both in cytoplasm by cytochrome P450 2E1 system and in mitochondria.[3] The highly reactive free radicals generated during the course of the reaction are detoxified by GSH

Table 1. Activity of G6PDH in liver lobes of mice.

	**G6PDH (U/g liver)			
	*n	X ± SD	median	min.-max.
Median lobe	20	3.30 ± 1.44	3.43	1.00-5.17
Right lobe	20	1.56 ± 0.93	1.93	0.01-2.76
Left lobe	20	2.43 ± 1.42	2.72	0.10-4.67
Caudate lobe	20	0.41 ± 0.17	0.37	0.10-0.74

* Number of liver lobes.
** Significant differences ($p < 0.05$) in G6PDH activities between median-right lobes, between median-caudate lobes, between left-caudate lobes were observed.

Table 2. The levels of GSH in liver lobes of mice.

	**GSH (µmol/g liver)			
	*n	X ± SD	median	min.-max.
Median lobe	20	1.84 ± 0.30	1.83	1.35-2.38
Right lobe	20	0.57 ± 0.25	0.53	0.23-0.97
Left lobe	20	1.06 ± 0.21	1.07	0.50-1.35
Caudate lobe	20	0.05 ± 0.02	0.05	0.02-0.08

* Number of liver lobes.
** Significant differences ($p < 0.05$) in GSH levels between median-right lobes, between median-left lobes, between median-caudate lobes, between right-left lobe, between right-caudate lobes, and between left-caudate lobes were observed.

and/or by the end electron acceptor NADPH produced by the G6PDH enzyme.[12,13]

Mouse is a good animal model for various study of experimental disease and cancer research and mouse liver is similar to human liver as anatomic and histologic structure.[14,15] Liver of mice is an organ on where high metabolic activities take place and hepatocytes contain various numbers of mitochondrias. Those hepatocytes also take nutritions absorbed from intestines in sinuosides with the aid of fenestres and metabolize them through oxygen supplied by arterial blood. Oxygen has very important role for the change of nutrition to metabolic energy. Lipids become oxidized in mitochondria during this metabolic activity in order to supply them for organism. If there is no enough oxygenation, lipid metabolism occurs under suboptimal conditions and toxic radicals become available after oxygenation comes back.[16] Moreover, liver may be exposed to toxic substance via various causes. Matsubara et al. reported the liver toxicity in rats after CCl_4 application. They also found out severe damages in median and right lobes although mitochondrial enzymes were more in left lobes. It was suggested that toxic substances were successfully inactivated after being metabolized due to perfusion in left liver.[17]

It has been suggested that liver lobes perfusion is an important factor to eliminate the toxic substances. Liver is fully filled with strong antioxidant system against free radicals that are oxidative stress factors. The liver G6PDH enzyme of mouse showed induction with exposure to halothane anesthesia in the previous study.[12] Maiti et al. suggested that acute arsenic exposure in rats were significantly depleted GSH levels. In liver arsenic metabolism appears dependent upon the GSH. Arsenic induced GSH depletion and increasd in lipid peroxidation.[18] Shan et al reported that chronic hypoxia effects of amounts and/or levels of detoxification systems (G6PDH and GSH ets.) and chronic hipoxia may be increased susuceptibility of cells to chemical injury.[19] Hypoxia and ischemia, harmfull substances in the liver, give rise to the decrease of antioxidant enzymes in liver causing severe damages due to unthrown oxidative radicals in the organ. They have different effects on lobes. Once blood flow turns back to normal process, left lobe has been remarkably in good condition respect to other lobe becasue it had more blood flow.[19]

Intensive blood flow may cause fast metabolic activity which causes lipid peroxidation products in high rates; however, antioxidant systems may successfully companse this process. That can be the reason why liver lobes might be less affected from those toxic products. High blood flow in left lobe increases microsomes and mitochondrial metabolism. Antioxidants have been found more than the others although the occurrence of free radicals are higher in this lobe.[20] In the present study we demostrated that the maximum activity of G6PDH and the maximum level of GSH were found higher in median and left lobes than in the other lobes. Consequently, the distinct blood flow according to liver lobes might cause different antioxidant enzyme level and detoxification in these areas. We believe that the results of the present study should be investigated further.

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