ОРИГИНАЛЬНАЯ СТАТЬЯ

SELENIUM – GLUTATHIONE PEROXIDASE RELATION IN ERYTHROCYTES WITH G-6-PDH DEFICIENCY AND THE GP ACTIVITY DEVIATION DURING OXIDATION

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ABSTRACT. Selenium status and glutathione peroxidase (GP) activity were investigated in patients with normal and glucose-6-phosphate dehydrogenase (G-6-PDH) deficient erythrocytes among Azerbaijani population. It has been shown that the content of Se in G-6-PDH-deficient erythrocytes is a little (≈16%) different from the norm, while GP activity of in them is substantially lower (≈ 50%). The low activity of GP probably is due to decreased production of GSH, the basic substrate for GP, in G-6-PDH-deficient erythrocytes. Addition of GSH precursor (N-acetylcysteine) to the incubation medium increases the GP activity, suggesting that the low level of GP activity is possibly connected with low level of GSH. G-6-PDH-deficient erythrocytes demonstrate significantly greater susceptibility to oxidative stress than normal ones when exposed to ultraviolet radiation of the small (7 kJ/m^2) and moderate (15 kJ/m^2) doses, and to high tension electric field (HTEF ≈ 60 kV/m × 5 hours). In particular, the accumulation of lipid peroxidation products (malonic dialdehyde) under visible UV irradiation in G-6-PDH-deficient erythrocytes is ≈50-80% higher, than in the control. Nevertheless, in G-6-PDH-deficient erythrocytes HTEF have no significant effect on the accumulation of malonic dialdehyde, though the GP and catalase activity are falling faster than normal, and have initially lower level.

KEYWORDS: glucose-6-phosphate dehydrogenase deficiency, selenium, glutathione peroxidase, high tension electric field, ultraviolet radiation.

INTRODUCTION

It is known that the G-6-PDH deficiency is an erythrocyte enzymopathy widely spread in the world (WHO Working Qroup, 1989; Beutler, 1994; Cappellini, Fiorelli, 2008). The occurrence of this gene defect varies in different areas of the Globe. In Azerbaijan areas with its high prevalence also exist (Javadov et al., 1977; Rustamov, Kulieva, 1983; Krasnopolskaya et al., 1985).

The G-6-PDH enzyme has an important role in erythrocytes and it is a main enzyme of the pentose-phosphate pathway (Luzatto, Lippincott, 1995). Its deficiency limits the synthesis of NADPH, which is a product of the energy cycle, and it also cause lack of the GSH form of glutathione, which is the main substrate of antioxidant glutathione peroxidase (GP) enzyme. The defective erythrocytes show sensitivity to infections, toxins, some medicines, mechanical injuries, being especially vulnerable to oxidation (Luzatto, 1967; Luzatto, Lippincott, 1995; Vasilyeva, 2005; Yoshihito, 2012).

The ecological situation is aggravating due to urbanization last years, reducing resistance of human organism to the oxidation factors (Yoshihito, 2012; Erzurum, Kalayci, 2012). It is interesting to study the effect of endogenous and exogenous factors in organisms with low antioxidant status. Therefore, we have investigated the oxidative influence of physical factors on erythrocytes. The influence of UV radiation (high dose) (Guliyeva, Mamedova, 2010) and high tension electric field (HTEF, 50 Hz) in different dose on erythrocytes with G-6-PDH has been investigated and it has shown that such influence accelerates oxidation significantly (Huseynov et al., 2012b; Huseynov, Guliyeva, 2015). The dependence of oxidative process on HTEF dose is apparent. It is important to find out the regularities (stress phases) of oxidation stress under little and medium doses of UV radiation (Huseynov et al., 2012a). Also participation of selenium (Se) which is the main component of antioxidant defense system has been examined (Huseynov et al., 2012b;

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Huseynov, Yakhyayeva, 2014; Huseynov, Guliyeva, 2015). The investigation which have been done on Azerbaijan population shows that the amount of selenium in the blood is close to deficiency level (~ 90 µg/ml) (Huseynov et al., 2012c; Huseynov, Yakhyayeva, 2014). The subnormal amount of selenium has been found out in experiments on erythrocytes with G-6-PDH deficiency (Guliyeva, 2014). Therefore, it is important to investigate the relation between selenium and GP in erythrocytes with G-6-PDH deficiency, the state of oxidation processes, and some endogenous antioxidant components under the influence of the above mentioned induction factors.

MATERIALS AND METHODS

For the experiment blood samples of 24 healthy donors and 31 patients with G-6-PDH deficiency (age 18–40 years) were used. The samples were obtained from the Scientific Research Institute of Hematology and Transfusion of the Ministry of Health of Azerbaijan. Blood was taken from the cubital vein; erythrocytes were separated from heparinized blood, centrifuged (1000g × 10 min) and washed 3 times by isotonic 0.15 M NaCl solution (Dodge, 1963). Washed erythrocytes were exposed to HTEF (60 kV/m; 5, 8, 24, 96 hour exposition) and relatively small (7 kJ/m^2) and medium (15 kJ/m^2) dose of the UV radiation. The amount of hemoglobin was determined using cyanmethemoglobin method. The accumulation of methemoglobin (MetHb) has been estimated according to the equation offered by Winterbourn (Winterbourn, 1990). The amount of GSH (Luzatto, Lippincott, 1995) and catalase activity (Aebi, 1984) were determined spectrophotometrically. GP activity in erythrocyte lysate was determined by Moin method (Moin, 1986). The amount of selenium in investigated samples was determined by the fluorimetrical method with 2,3-diaminonaphtalin (Nazarenko et al., 1975). The intensity of oxidation process was estimated by accumulation of thiobarbituric acid reactive products (Mengel, Kann, 1966). Statistical analysis was carried out using Students t-test (Khudson, 1990).

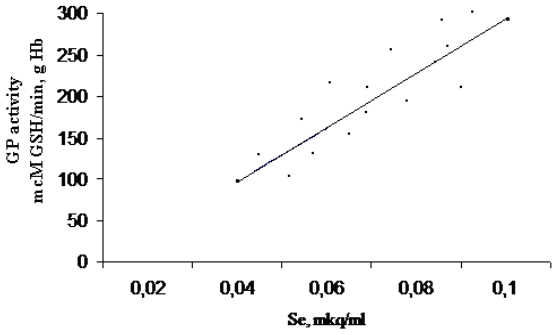
RESULTS AND DISCUSSION

The results of the measurements of Se amount and GP activity in erythrocytes with G-6-PDH deficiency and control erythrocytes are shown in Table 1.

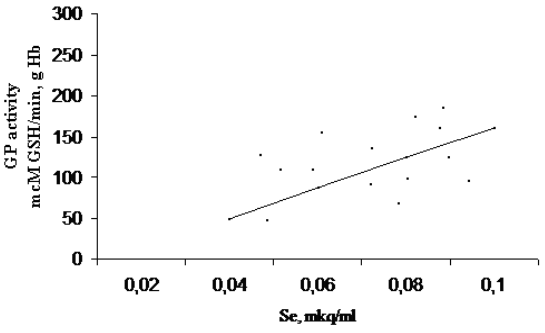
Despite a small difference in Se amount, a significant difference between GP activities was found out. This situation can be explained with small amount of GSH, the main substrate for GP, in erythrocytes with G-6-PDH deficiency (Luzatto, Lippincott, 1995; Pace et al., 2003). For clarifying this, amount of GSH in the samples have been evaluated (Table 2).

In order to ensure normal substrate supply for GP activity in erythrocytes with G-6-PDH deficiency, N-Acetyl-Cysteine, a proximate precursor of GSH, has been used. It became clear that it is possible to bring GP activity to the normal level (≈ 5 µmol/g Hb and more) by increasing GSH amount (Table 3).

The results gave us opportunity to put forward an idea that the low activity of GP in erythrocytes with G-6-PDH deficiency is connected with small amount of reduced glutathione that occurs on the background of this enzymopathy. It is clear that GP is an active indicator of Se metabolism. In G-6-PDH-deficient erythrocytes the correlative relationships between Se amount and GP activity is breaking down: (r = 0.69 at "norm" and r = 0.35)[1] at G-6-PDH deficiency (Fig. 1.)



a) r = 0.69; n = 22; p < 0.01



b) r = 0.35; n = 22; $p \leq 0.05$

Fig. 1. *The correlation between Se and GP activity in erythrocytes with at G-6-PDH deficiency: a) control erythrocytes, b) erythrocytes with G-6-PDH deficiency*

[1] r – is a correlative coefficient of selenium amount and GP activity.

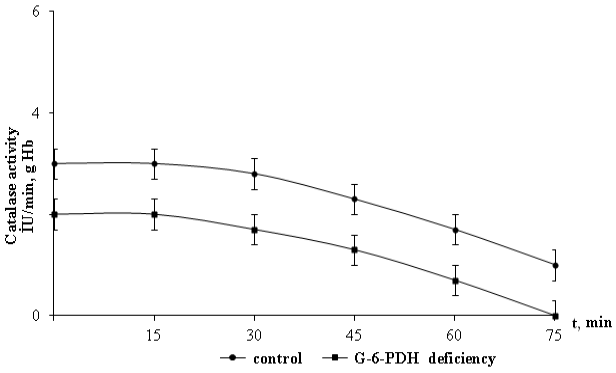


Fig. 2. *Influence of UV radiation (10 W/m^2) to the catalase activity in erythrocyte lysate at G-6-PDH deficiency (0.1 M phosphate buffered saline, pH=7.2)*

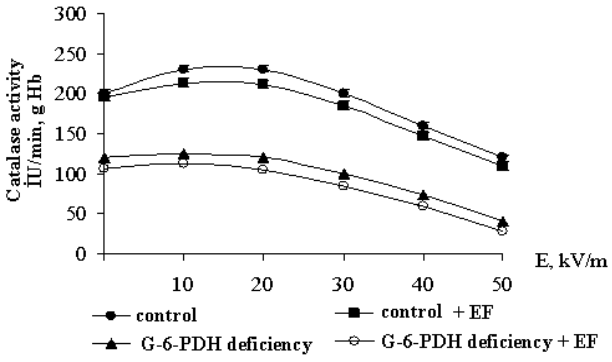


Fig. 3*. The changing of catalase activity in erythrocytes at G-6-PDH deficiency under HTEF influence (≤ 60 kV/m × 5 hours)*

Table 1. ***Se amount and GP activity in erythrocytes with G-6-PDH deficiency***

Group	Se (μg/l)	GP (mmol/g Hb)
Control (n = 24)	0.116 ± 0.034	460 ± 40
G-6-PDH deficiency (n = 31)	0.098 ± 0.028 $p \leq 0.05$	280 ± 30 $p < 0.01$

Table 2. ***Amount of GSH in the lysates of control erythrocytes and erythrocytes with G-6-PDH deficiency***

Group	GSH μmol/g Hb
Control (n = 15)	4.7 ± 0.7
G-6-PDH deficiency (n = 15)	3.1 ± 0.6

Table 3. ***GP activity after adding N-Acetyl-Cysteine to the erythrocyte lysate (hematocrit ≈ 5)***

+ N-Acetyl-Cysteine NAC (n = 5)	GSH μmol/g Hb (n = 5)	GP activity μmol/g Hb (n = 5)
0	3.1±0.5	280
1×10^{-3}M	4.5±0.7	290
5×10^{-3}M	5.3±0.8	340
10×10^{-3}M	6.5±1.0	345

Table 4. ***Influence of «small» (7 kJ/m2) and «medium» (15 kJ/m2) doses of UV radiation on oxidation process in erythrocytes at G-6-PDH deficiency***

UV	Samples			
	Control		G-6-PDH deficiency	
	GP activity μmol GSH/min×g Hb	MDA μmol/ml	GP activity μmol GSH/ min×g Hb	MDA μmol/ml
0	357±21	0.24±0.03	270±18	0.34±0.05
7 kJ/m^2	438±25	0.18±0.04	210±20	0.48±0.04
15 kJ/m^2	415±22	0.31±0.04	186±14	0.57±0.08

Table 5. GP ***activity and MDA amount in erythrocyte suspension at G-6-PDH deficiency under exposure to HTEF***

Groups		Exposure time					
		8 hour		24 hour		96 hour	
		MDA nmol/ml	GP μmol/min×g Hb	MDA nmol/ml	GP μmol/min×g Hb	MDA nmol/ml	GP μmol/min×g Hb
Donor	control	5.82±0.60	370±53	5.40±0.85	280±18	12.34±1.82	102±33
	experiment	6.93±0.73	430±69	5.83±0.91	210±23	9.36±1.12	62±12
G-6-PDH deficiency	control	9.44±0.77	210±48	8.02±0.98	160±25	10.30±1.10	47±11
	experiment	11.08±0.96	260±47	10.31±0.87	123±19	14.60±1.25	23±8

Oxidation in erythrocytes with G-6-PDH deficiency was determined by MDA amount and change in GP activity under UV-irradiation (Table 4).

As it can be seen from Table 4, under the influence of 15 kJ/m^2 in both samples the accumulation of thiobarbituric acid reactive products increases. Relating to control, in erythrocytes with G-6-PDH deficiency it increases up to ≈ 100%. Despite adaptive increasing of GP activity (≈ 25% under 7 kJ/m^2 and ≈ 15% under 15 kJ/m^2) in control samples, in the samples with G-6-PDH this effect did not occur. Oxidation under the influence of UV radiation for erythrocytes with G-6-PDH deficiency is ≈ 1.8–2.5 times greater.

The changing of catalase activity is reflected in Fig. 2 and it decreases faster in comparison with control.

Also we examined oxidation in erythrocytes with G-6-PDH deficiency at different exposures to HTEF used as a stress factor. Since under the conditions of physiological temperature during long-term exposure auto-oxidation occurs rapidly, the effect of HTEF on erythrocytes was investigated at low temperature. The results are shown in the Table 5.

The obtained results from the experiments show that HTEF intensifies oxidative processes influencing the GP activity in erythrocytes. This reflects itself in accumulation of MDA and change of GP activity. At the same time, changing in catalase activity occurs in the samples (Fig. 3)

Thus we should note that there is a discrepancy between the Se amount and GP activity in erythrocytes with G-6-PDH deficiency. And this is caused by the overspending of antioxidant resources of erythrocytes with G-6-PDH deficiency during the oxidation processes. In other words, even though the amount of Se is close to normal, GP activity in the erythrocytes with G-6-PDH deficiency is low. This discrepancy is increasing more under influence of physical stress factors on the erythrocytes.

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СООТНОШЕНИЕ СЕЛЕНА И ГЛУТАТИОНПЕРОКСИДАЗНОЙ АКТИВНОСТИ В Г-6ФД ДЕФИЦИТНЫХ ЭРИТРОЦИТАХ И ИЗМЕНЕНИЕ ГП АКТИВНОСТИ В ПРОЦЕССЕ ИХ ОКИСЛЕНИЯ

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РЕЗЮМЕ. Рассмотрен статус селена и активность глутатионпероксидазы (ГП) в эритроцитах у пациентов с дефицитом глюкозо-6-фосфатдегидрогеназы (Г-6ФД) среди населения Азербайджана. Показано, что содержание Se в Г-6ФД-дефицитных эритроцитах мало (≈ 16%) отличается от нормы, в то время как активность ГП в них существенно ниже (≈ 50%). Низкий уровень активности ГП, вероятно, связан с пониженной выработкой GSH, основного субстрата для ГП, в Г-6ФД-дефицитных эритроцитах. Добавление в инкубационную среду предшественника GSH (N-ацетилцистеина) увеличивает активность ГП, свидетельствуя о том, что низкий уровень активности ГП, возможно, связан с низким уровнем GSH. Г-6ФД-дефицитные эритроциты показывают значительно более высокую чувствительность к окислению по сравнению с нормой при облучении малыми

(7 кДж/м^2) и умеренными (15 кДж/м^2) дозами ультрафиолета и воздействии электрического поля высокой напряженности (ЭПВН) (≈ 60 кВ/м × 5 ч). В частности, накопление продуктов перекисного окисления липидов (малонового диальдегида) при видимом УФ-облучении для Г-6ФД-дефицитных эритроцитов на ≈ 50–80% выше, чем в контроле. Однако в Г-6ФД-дефицитных эритроцитах ЭПВН не оказывает заметного влияния на накопление малонового диальдегида, хотя активность ГП и каталазы снижаются быстрее, чем в норме, и имеют изначально более низкий уровень.

КЛЮЧЕВЫЕ СЛОВА: дефицит глюкозо-6-фосфатдегидрогеназы, селен, глутатионпероксидаза, электрическое поле высокой напряженности, ультрафиолетовое излучение.