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The role of metallothioneins in the maintance of zinc homeostasis and redox state in erythrocytes of cardiologic patients with the metabolic disorders

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ABSTRACT

The comparative analysis of the intracellular labile zinc pool, level of the reduced glutathione and cystein-rich proteins metallothioneins considering the viability of erythrocytes was conducted in patients suffered from coronary heart disease with diagnosed arterial hypertension and type 2 diabetes mellitus. Fluorescent probes (FluoZin-3-AM and Calcein-AM), Ellman's reagent and monoclonal antibody (anti-metallothionein UC1MT) have been used. The obtained results demonstrated an important role of zinc homeostasis (decrease in cytosolic Zn^{2+} level) in the etiopathogenesis of type 2 diabetes mellitus and in the development of metabolic syndrome in general. Increasing metallothioneins expression in erythrocytes of evaluated patients implied its functioning as an additional antioxidant in human erythrocytes defense system in this pathology. The present data show that these proteins could be selected as a target for some antioxidant treatment strategies for CHD patients with metabolic disorders.

Аннотация

Проведен сравнительный анализ внутриклеточного пула ионов цинка, содержания восстановленного глутатиона и цистеин-обогащенных белков металлотионеинов с учетом жизнеспособности эритроцитов у пациентов с диагностированной артериальной гипертензией и сахарным диабетом 2 типа на фоне ишемической болезни сердца. В работе использованы флуоресцентные зонды (FluoZin-3-AM и Calcein-AM), реактив Эллмана и моноклональные антитела (анти-металлотионеин UC1MT). Полученные результаты демонстрируют важную роль цинкового гомеостаза (снижение цитозольного уровня Zn^{2+}) в этиопатогенезе сахарного диабета 2 типа и развитии метаболических нарушений в целом. Увеличение уровня металлотионеинов в эритроцитах исследуемых пациентов свидетельствует о функционировании данных белков в качестве дополнительной антиоксидантной защитной системы эритроцитов человека при данной патологии. Полученные результаты демонстрируют, что эти белки могут быть выбраны в качестве мишени при назначении терапии кардиологическим пациентам с метаболическими нарушениями

Keywords: Metallothioneins, Labile zinc pool, Reduced glutathione, Erythrocytes, Type 2 diabetes mellitus, Coronary heart disease.

1. Introduction

Zinc (Zn^{2+}) is an essential trace element that controls the processes of proliferation, differentiation and cell death. It can mimic the action of hormones, growth factors, cytokines, thereby acting as a "signal molecule" [1]. At the end of 2010, the Protein Data Bank (PDB) contained 6170 structures of zinc-binding proteins but only 4882 are to be considered as true zinc proteins (they bind at least one Zn^{2+} with a physiological role). Over half of the true zinc proteins in the PDB (i.e., 2759 proteins) are enzymes, with most of the others being zinc finger proteins [2]. Currently there are already 14410 entries in PDB that contain Zn2+ cations. In metalloenzymes, zinc has three main functions: the involvement in catalysis, the maintenance of structural stability and the regulation of the cellular processes [3]. Knowledge about the proteins that control cellular zinc provides a basis for understanding how Zn^{2+} can regulate cellular processes.

Physiological concentration of zinc in human serum is

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about 2-15 μM, while its intracellular content is extremely low (~ 1 nM) [4]. The intracellular Zn^{2+} pool is controlled via the expression and the functioning of 14 zinc importers (ZIPs / SLC39s), 10 zinc exporters (ZnTs / SLC30s) and Zn-binding proteins such as metallothioneins (MTs) [1, 4, 5]. Zrt- and Irt-like (zinc and iron regulated transporter) proteins (ZIPs) are also named solute carrier family 39 (SLC39) A1–A14, while members of the zinc transporter (ZnT) family are denoted to SLC30A1–A10. A few of these transporters have an additional role in Mn^{2+} and Fe^{2+} transport, while MTs also have a function in Cu^{2+} metabolism [4]. Most of the ZIP and ZnT transporters are located in the plasma membrane, but others – in mitochondrial, Golgi network, lysosomal and vesicular or endoplasmic reticulum membranes [1, 4].

MTs are the family of intracellular cysteine-rich, low molecular weight (6–7 kDa) metal-binding proteins. They have a single peptide chain containing 61–68 amino acids, 20 of them are cysteines distributed in two domains α and β-clusters, and they bind in total 7 ions of divalent metals [6]. The single polypeptide chain of MT has the structure Cys-X-Cys, Cys-XY-Cys, and Cys-Cys where X and Y represent noncysteine amino acids; the stoichiometric form of the protein shows 7 ions for each 20 cysteines forming metal-thiolate complexes [6, 7, 8]. MT binds to metals through a thiol group (SH) found in cysteine residues; the metal-free protein named as apo-metallothionein (Apo-MT) or thionein. Four isoforms of MTs – I, II, III and IV were discovered. MT-I and MT-II which are the major isoforms found in the most tissues, whereas MT-III and MT-IV have cell type-specific expression [7]. Traditionally, MTs have been considered as an intracellular proteins which are localized in the cytoplasm and can also be found in the nucleus upon translocation; however, more recent reports suggest that MT can be localized in a variety of extracellular spaces [9]. MTs are involved in many physiological and pathophysiological processes such as intracellular storage, transport and metabolism of metal ions, whereas in vivo they regulate essential trace metal homeostasis such as zinc and copper and play a protective role in heavy metal detoxification reactions such as cadmium and mercury [6]. However, under physiological conditions MTs have high binding affinity exactly to Zn^{2+} and play a significant role in maintaining its stable intracellular availability through sequestration or release of zinc ions. Intracellular Zn^{2+} is strictly regulated by binding to MTs via compartmentalization through the activities of ZnT [1]. When intracellular Zn^{2+} level is insufficient to stabilize the protein, the MTs are rapidly proteolyzed, so the Zn^{2+} is released by the degradation of MTs causing the intracellular Zn^{2+} to remain at a balanced concentration [10].

In addition, MTs are vital proteins in the cellular defense antioxidant system, and their protective role against reactive oxygen species (ROS) damage in biological systems has been widely reported. Different studies have shown that the thiolate ligands in cysteine residues confer the redox activity of MTs; these residues can be oxidized by cellular oxidants, and during this process zinc is released causing a decrease in lipid peroxidation levels [11]. It is known that, when oxidative stress level increases, MTs are able to scavenge a variety of ROS including hydroxyl radicals and superoxide anion, hydrogen peroxide, radicals of reactive nitrogen species, and nitric oxide radicals [12]. Compared to other antioxidants such as super oxide dismutase, catalase, glutathione peroxidase, MT could be considered as a more effective antioxidant [13]. It is known that the low-molecular weight thiol glutathione (L-g-glutamyl-L-cysteinylglycine) (GSH) is the major attribute of a redox buffer in cells because of its high cellular abundance (2–10 mM) and low redox potential of -240 mV [14]. However, it was shown that all 20 cysteine sulfur atoms of MTs are involved in the radical quenching process, and the rate constant for the reaction of hydroxyl radical with MT is about 340-fold higher than that with GSH [15].

So, MTs and two zinc transporter families – zinc importers (ZIPs / SLC39s) and zinc exporters (ZnTs / SLC30s) – control the intracellular Zn^{2+} pool and dysfunction of such complex regulatory system may lead to Zn dyshomeostasis that is associated with many pathological processes in the human organism [1, 4, 5].

Metabolic syndrome (MS) is a group of conditions that put people at risk for heart disease and diabetes. These conditions or so-called metabolic risk factors include high blood pressure, high blood glucose levels, high levels of triglycerides, low levels of high-density lipoprotein in the blood, excessive fat deposits around the waist. Diabetes mellitus (DM) is the major cause of morbidity and mortality world-wide, and also the major risk factor for early onset of coronary heart disease (CHD). Type 1 diabetes is characterized by the lack of insulin production and type 2 diabetes is characterized primarily by the resistance to insulin effects. It is known that pancreatic β cells contain large amounts of zinc which participates in the binding of insulin in hexamers [16]. Decade ago, it has been established that SLC30A8 gene, encoding zinc transporter ZnT8, is expressed exclusively in the secretory granules of the pancreatic β-cells [17], thereby playing an important role in the pathogenesis of type 2 DM, where single nucleotide polymorphism in SLC30A8, rs13266634 (Arg325Trp) has been reported [18]. Later, Rutter and Sladek et al. [19, 20] demonstrated that normal cellular zinc homeostasis is a crucial factor of the insulin release maintaining physiological glucose levels, and decreasing the behavioral risk for type 2 DM. The reduced Zn^{2+} level in atherosclerotic plaques of patients with type 2 diabetes mellitus, as well as in the blood plasma of patients with pathology under evaluation was shown [21, 22]. Zinc deficiency has also been observed in patients with metabolic syndrome [23] and arterial hypertension [24]. These results indicate that zinc is strongly associated with lipid and glucose status. It can be assumed that cellular zinc concentration could be a predictive for metabolic disorders. Singh et al. [25] concluded that lower

consumption of dietary zinc and low serum zinc levels were associated with an increased prevalence of CHD and several associated risk factors including hypertension and hypertriglyceridemia.

There are many studies [19, 26, 27] which have confirmed that type 2 DM caused by oxidative stress plays an important role in the development of cardiological disorders. At the same time, Zn dyshomeostasis (due to a change in the expression of zinc transporter ZnT8) also induces the development of oxidative stress in type 2 DM cells [1, 4, 5, 17], but MTs expression in this cell type was not investigated. Recently, it was revealed that the high urinary excretion of zinc caused the reduction in its blood plasma levels being indicative of disturbance in Zn-based antioxidant mechanism [28]. Thus, a simultaneous investigation of the parameters that characterize both cellular zinc metabolism and redox state of cardiologic patients with metabolic disorders may be useful for selecting a treatment strategy (e.g.: zinc or antioxidant supplementation) that could regulate the cellular metabolic processes.

The aim of this study is to investigate the level of expression of cystein-rich proteins metallothioneins in erythrocytes of patients suffered from coronary heart disease with the metabolic syndrome (representing arterial hypertension and type 2 diabetes mellitus) and to find out their role in the maintenance of zinc homeostasis (estimating the intracellular labile Zn^{2+} pool) and redox state (estimating the reduced glutathione concentration) under these pathological states.

It is known that when zinc compounds enter the human blood, more than 90% of Zn^{2+} accumulates in red blood cells [1]. So, human erythrocytes were used as a model system and as a potential indicator of zinc status in humans. Moreover, the absence of nuclei in mature mammalian red blood cells provides an opportunity to exclude a genotoxic effect of Zn^{2+}.

2. Material and Methods

All the chemicals in the experiment were used without further purification. FluoZin-3-AM was obtained from Molecular Probes (USA); anti-metallothionein UC1MT, anti -mouse IgG1-FITC, mouse IgG1 were obtained from Abcam (Great Britain); Calcein-AM, twin 20, paraformaldehyde, pluronic F-127, 5,5'-dithio-bis-[2-nitrobenzoic acid], bovine serum albumin (BSA) were obtained from Sigma-Aldrich (Germany); trichloroacetic acid (TCA) and all salts were obtained from “Belleschimcomplect” (Belarus).

Peripheral blood from patients with coronary heart disease (n = 18, age 59.7±2.7 years) was supplied by Republic scientific and practical Centrum "Cardiology" (Minsk, Belarus) and from healthy donors (n = 10) was supplied by Republic scientific and practical Centrum “Transfusiology and medical biotechnologies” of Ministry of Health of Belarus (Minsk, Belarus). All blood samples were in the conservative agent "citrate-Na". Erythrocytes were separated from serum by centrifugation of blood at 1500g, 15 min.

Diagnostic evaluation of CHD patients for formation of three tested groups were conducted by cardiologists of Republic scientific and practical Centrum "Cardiology" (Minsk, Belarus). The first group is CHD patients who had arterial hypertension and diabetes mellitus type 2 (Ah+DM+), the second group is CHD patients who had only arterial hypertension (Ah+DM–) and the third group is CHD patients without any diagnosed component of metabolic disorder (Ah–DM–).

Intracellular labile zinc pool ($[Zn^{2+}]i$) was assessed using fluorescent dye FluoZin-3-AM. Cells were loaded with 2 μM FluoZin-3-AM (λex/em=494/516 nm) in the presence of 0.02% Pluronic F-127 for 30 min at 37°C. Cells were washed (2000g, 10 min) and incubated for an additional 30 min at room temperature in PBS buffer (KH2PO4 – 2 mM, Na2HPO4*12H2O – 10 mM, NaCl – 137 mM, KCl – 2.7 mM, pH 7.4) with 0.3% BSA. After loading, erythrocytes were washed twice with the same solution. Then cells were analyzed by the flow cytometry at the FL-1H channel.

The concentration of reduced glutathione (GSH) in erythrocytes was estimated by Ellman’s method. For this purpose 10% TCA was added to cell suspension for 15 min at room temperature. After that cells were centrifugated (2000g, 10 min) and then 0.1 M Na-phosphate buffer pH 8.0 supplemented with 1 mM Ellman’s reagent (5.5'-dithio-bis-[2-nitrobenzoic acid]) were added to supernatant for 15 min at room temperature. Then optical density of solution was measured and GSH concentration (mM) was calculated (ε=13.6 $mM^{-1}cm^{-1}$, L=1 cm).

Metallothioneins expression level (MT-I/MT-II) was evaluated using monoclonal antibody – anti-metallothionein UC1MT. Erythrocytes suspension was fixed by 2% paraformaldehyde (60 min, t=4°C), washed (2000g, 10 min), permeabilized using 0.5% twin 20 in PBS pH 7.4 (10 min, t=4°C) and incubated with UC1MT overnight at 4°C. Then cells were rinsed in PBS (2000g, 10 min) and further incubated with secondary antibodies labeled with fluorophore – anti-mouse IgG1-FITC (dilution 1:500) for 120 min at 4°C. Mouse IgG1 was used as an isotype control that was incubated in parallel with cells as describe above. After washing in PBS a cytofluorimetric analysis was performed. Fluorescence intensity of complex: metallothioneins-UC1MT-FITC was used as a marker of these proteins expression in erythrocytes.

The viability of erythrocytes was controlled by cell esterase activity using high lipophilic dye calcein-AM – a highly lipophilic dye that rapidly enters to viable cells, where it is converted by intracellular esterases to its deesterified form Calcein with an intense green emission (CAL, λex/ em=496/516 nm). It should be noted that Calcein is retained by cells with intact plasma membrane [29]. Erythrocytes were loaded with 5 μM Calcein-AM in PBS for 40 min at 37° C. After cells were washed (2000g, 10 min) and analyzed by flow cytometry at the FL-1H channel.

Cytofluorimetric analysis was performed on flow cytometer FACSCanto II (Beckton Dickenson) and spectrophotometric analysis – on the Specord M-40 spectrophotometer.

Statistical analysis of experimental results was carried out using non-parametric Mann–Whitney U test and Spearman test. Data were expressed as mean ± SEM. The results were considered statistically significant at $p<0.05$. Statistical analysis program (STATISTICA, version 8.0) was used for evaluations.

3. Results and discussion

At the first stage, the cytofluorimetric study of the intracellular level of labile zinc pool in erythrocytes of patient's blood samples was carried out. We have used a recently developed fluorimetric probe (FluoZin-3) with high affinity to Zn^{2+} (Kd=15 nM) and low affinity to Ca^{2+} or Mg^{2+} [30]. FluoZin-3 is particularly sensitive to changes in zinc concentration because of the high ratio between the fluorescence of the probe saturated with Zn^{2+} and that in its absences [31]. A significant decrease (on 15–20%) of the intracellular zinc level in red blood cells of CHD patients with presenting arterial hypertension and type 2 diabetes mellitus (Ah^+DM^+) in comparison to the last one in healthy donors was established (Figure 1A). But it was revealed that there is only a tendency to reduction of the cytoplasmic zinc pool in groups of CHD patients without diagnosed type 2 diabetes mellitus (Ah^+DM^-) and without both signs of metabolic disorders (Ah^-DM^-) (Figure 1A).

As mentioned above, type 2 diabetes mellitus mediated the changes in redox state behavior in blood cells [19, 26, 27]. Therefore, in our previous study, the alterations in intracellular zinc ions pool of erythrocytes and activation of the programmed cell death process under H_2O_2-induced oxidative stress in vitro were found [32]. So, in order to avoid the experimental artifacts associated with cell death, it is necessary to take into account their viability when evaluating the intracellular Zn^{2+} pool.

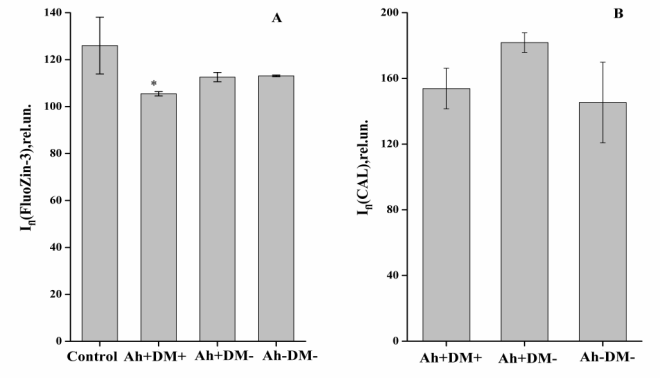

Figure 1 | Fluorescence intensity of FluoZin-3 (A) and Calcein (B) incorporated into erythrocytes of donors (Control) and patients with coronary heart disease (Ah^+DM^+, Ah^+DM^-, Ah^-DM^-); * – $p<0.05$ compared to control. Интенсивность флуоресценции FluoZin-3 (А) и Кальцеина (Б), встроенного в эритроциты доноров (контроль) и пациентов с ишемической болезнью сердца ($АГ^+СД^+$, $АГ^+СД^-$, $АГ^-СД^-$). * – $p<0.05$ по сравнению с контролем

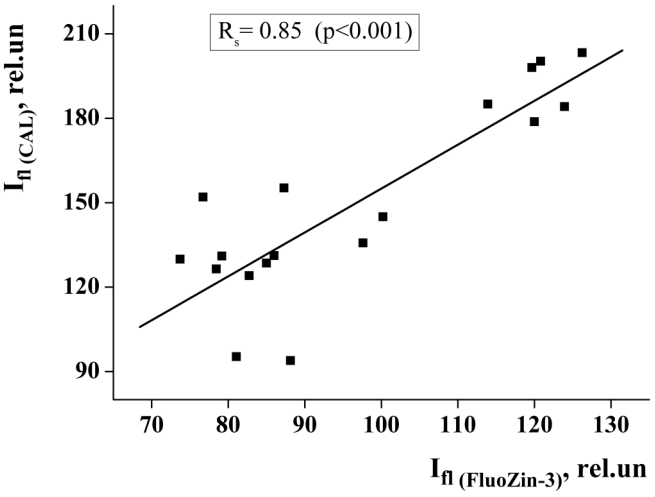

Figure 2 | Correlation between values of FluoZin-3 fluorescence intensity and Calcein fluorescence intensity in erythrocytes of patients with coronary heart disease where Ifl – fluorescence intensity; CAL – desterified form of calcein. Корреляционная связь между значениями интенсивности флуоресценции FluoZin-3 и кальцеина в эритроцитах пациентов с ишемической болезнью сердца, где, Iфл – интенсивность флуоресценции; CAL – деэстерифицированная форма кальцеина

Additionally a comparative evaluation of the erythrocytes esterase activity (a marker of their viability) using Calcein-AM in the tested groups of patients with coronary heart disease was conducted. This methodology is based on the ability of living cells to hydrolyze calcein-acetoxymethyl ester by intracellular esterases and on the association of Calcein-AM hydrolysis rates with a specific cell status (e.g. pre-apoptotic stage), both in physiological and pathological conditions [29]. However, we did not reveal the significant difference in the fluorescence of Calcein for tested cells (Figure 1B). But, the viability of erythrocytes in CHD patients was slightly reduced in comparison to donors' cells. Based on the correlation analysis (positive correlation parameters were approximately Rs=0.85, p=0.001), we found that values of fluorescence intensity for FluoZin-3 (characterizing the cytosolic labile zinc ions level) and Calcein (characterizing cell viability) correspond to CHD patients of the third tested groups (Figure 2). These results may help to predict the risk of involvement of Zn dyshomeostasis in activation of apoptotic process in cells of CHD patients.

It worth to notice that decrease of erythrocytes viability under modification of zinc homeostasis enables us to assume the crucial role of these processes in the etiology and pathogenesis of metabolic disorders in general and of type 2 diabetes mellitus in particular.

Previously, it was established that among the pathogenetic mechanisms underlying some metabolic and endocrine diseases, the relationship between the alteration of Zn^{2+} level

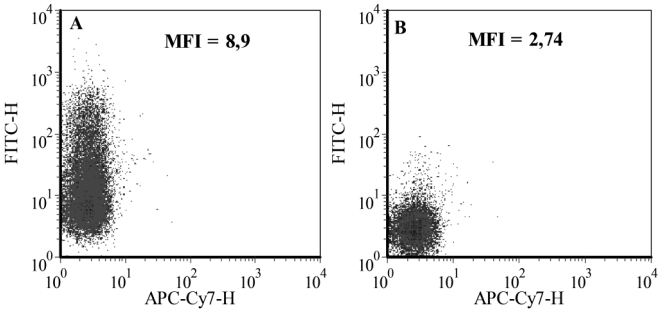

Figure 3 | Representative diagrams of distribution of UC1MT-IgG1-FITC fluorescence intensity (A) and IgG1-FITC fluorescence intensity (B) in the total population of donor's erythrocytes where MFI – mean fluorescence intensity. Репрезентативные диаграммы распределения интенсивности флуоресценции комплексов UC1MT–IgG1–FITC (А) и IgG1–FITC (Б) в суммарной популяции эритроцитов донора где, MFI – значение интенсивности флуоресценции комплексов.

and activation of free radical processes is of particular importance due to disturbance of glutathione homeostasis and cellular cascade of reactions dependent on NFκβ [26]. Moreover, Zn^{2+} is an important regulator of GSH synthesis. The importance of zinc in the metabolism of glutathione underscores the finding that in many studies a decrease of reduced glutathione under zinc deficiency conditions was revealed [33, 34]. Therefore, in the next step, the content of GSH, as the main cellular antioxidant, in erythrocytes was evaluated. Its significant reduction (2–2.8-fold) was found in all tested groups of CHD patients: 0.36±0.02 mM – in Ah^+DM^+ group; 0.40±0.02 mM – in Ah^+DM^- group; 0.47±0.03 mM – in Ah^-DM^- group. At the same time, GSH concentration in erythrocytes of healthy donors was approximately 0.91±0.07 mM.

It is known that cysteine-rich low-molecular proteins metallothioneins are markers of oxidative stress of both mRNA and protein levels [11, 12]. Nordberg and Arnér in their study [35] have demonstrated that MTs "co-operate" with also low-molecular weight thiol – GSH, in maintaining the cellular redox state and act as an additional antioxidant in the cellular defense system [35]. These results testify about antioxidant features of MTs in the extreme conditions under oxidative stress. Moreover, the enhanced MTs expression in the cell line with blocked glutathione synthesis was detected [36]. In our previous studies, it was found an increased MTs level in human erythrocytes, both under H_2O_2-induced oxidative stress when zinc releases from its intracellular depots [32], and Zn-deficient state [37]. Kumar et al. [38] showed that the introduction of Zn^{2+} into the diet of diabetic mice led to a significant inhibition of lipid peroxidation and to a decrease in the level of superoxide anions and oxidized glutathione. Besides, they also found that the introduction of Zn^{2+} causes the decrease in GSH level and MTs mRNA expression, and also superoxide dismutase activation.

In this regard, an assessment of the content of MTs, functioning as an auxiliary protective antioxidant system [12, 13, 15], in erythrocytes of tested groups of CHD patients was conducted. Figure 3 demonstrates the dot diagrams of the fluorescence of UC1MT-IgG1-FITC and IgG1-FITC complexes showing the order of fluorescence intensities values of these complexes (MFI parameter – mean fluorescence intensity) in donor's erythrocytes. It is known that UC1MT are the specific mouse monoclonal antibodies for two classes of MTs – MTI and MTII. We estimated the relative content of MTs in human erythrocytes by using the ratio of the fluorescence intensity of the UC1MT-IgG1-FITC complex (I1) to the fluorescence intensity of the isotype control IgG1-FITC (I2) which characterized the non-specific binding of antibodies in cells. It is clearly seen that the ratio of the fluorescence intensity of donor's erythrocytes antibodies to the isotype control was about 3.04±0.60, while in the cells of CHD patients with arterial hypertension (Ah^+DM^-) – 4.16±0.38 (Figure 4). At the same time, this value was in the range of 5.6±0.6 for the group of patients having arterial hypertension and type 2 diabetes mellitus (Ah^+DM^+).

A comparative analysis of the content of metallothioneins in the erythrocytes of tested groups of patients revealed a principal trend – an increase in the content of low-molecular weight polypeptides with antioxidant properties in red blood cells of CHD patients. Moreover, in the Ah^+DM^+ group these changes were more pronounced as 1.8-fold increase in the MTs content was observed compared to the healthy donors' erythrocytes.

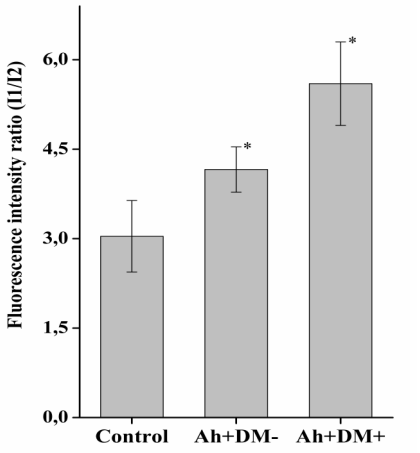

Figure 4 | Relative level of metallothioneins in the donor's erythrocytes and two examined groups of patients with coronary heart disease: Ah^+DM^- and Ah^+DM^+, where, control – healthy donors; I1 – the fluorescence intensity of the UC1MT-IgG1-FITC complex (relative units); I2 – intensity of fluorescence of isotype control IgG1-FITC (relative units). * – $p<0.05$ compare with control. Относительный уровень содержания металлотионеинов в эритроцитах доноров и двух обследованных подгрупп пациентов с ИБС: $АГ^+СД^-$ и $АГ^+СД^+$, где, контроль – здоровые доноры; I1 – интенсивность флуоресценции комплекса UC1MT–IgG1-FITC (отн. ед); I2 – интенсивность флуоресценции изотипического контроля IgG1 -FITC (отн. ед). * – $p<0.05$ по сравнению с контролем

5. Concluding Remarks

Thus, it was revealed that the observed decrease in the intracellular level of labile zinc in CHD cells appears to be an inductor of modification of cellular redox state (2–2.8-fold decrease in GSH concentration). The increase in metallothioneins expression and significant decrease in the reduced glutathione level in erythrocytes of CHD patients with the metabolic disorders imply functioning cysteine-rich proteins as an additional antioxidant in human erythrocytes defense system under these pathologies. It is clear that the potential of MTs as the scavengers of reactive species is not fully understood, but the present data show that these proteins could be selected as a target for some antioxidant treatment strategies for CHD patients with metabolic disorders in general and with type 2 diabetes mellitus in particular.

Заключение

Обнаруженное снижение внутриклеточного уровня лабильного цинка в эритроцитах ИБС-пациентов, по-видимому, является индуктором в нарушении редокс-статуса клеток, что проявляется в 2–2.8-кратном снижении концентрации восстановленного глутатиона. Увеличение уровня экспрессии металлотионеинов на фоне значительного снижения уровня восстановленного глутатиона в эритроцитах ИБС-пациентов с метаболическими нарушениями свидетельствует о функционировании данных цистеин-содержащих белков в качестве дополнительной антиоксидантной защитной системы эритроцитов человека при данной патологии. Разумеется, роль MTs, как захватчиков свободных радикалов до конца не выявлена, но представленные данные демонстрируют, что эти белки могут быть выбраны в качестве мишени при назначении терапии кардиологическим пациентам с метаболическими нарушениями в целом и при наличии сахарного диабета 2 типа в частности.

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