Determination of enzymes of glycolysis and oxidative phosphorylation in lymphocytes in patients with immuno-dependent diseases

https://doi.org/10.53529/2500-1175-2023-2-54-56

T. V. Radygina 1, S. V. Petrichuk 1, O. V. Kurbatova 1, D. G. Kuptsova 1, A. S. Potapov 1,2

1 National Medical Research Center for Children’s Health, 2, str. 1, Lomonosovskiy Pr-t, Moscow, 119296, Russia
2 Sechenov First Moscow State Medical University, Moscow, Russia

Keywords: oxidative phosphorylation, glycolysis, lymphocytes, immune-dependent diseases.


Relevance

One of the modern directions in immunological research is studying metabolic profile of immuno-competent cells, depending on the stage of their differentiation and proliferative activity. Glycolysis and oxidative phosphorylation (OXPHOS) are the basic metabolic processes, providing ATP formation in cells. Glycolysis generates ATP as well as provides intermediate metabolites, necessary for building cell structures. There is a metabolism switching of resting lymphocytes from OXPHOS to aerobic glycolysis [1] with antigen stimulation of immune naive cells and their transition to effector cells with proliferative process activation. Specialized T-lymphocyte populations with unique metabolic phenotype are formed during differentiation. All subpopulations of activated CD4+ cells are characterized by increased glycolysis. Besides, the balance between fat synthesis and fatty acid oxidation is important for differentiating Th17-lymphocytes and regulatory T-lymphocytes (Treg). In rheumatoid arthritis, it was shown that glycolysis inhibition in T-lymphocytes with the help of 2-deoxyglucose led to a decrease in the inflammatory process [2]. Patients with inflammatory bowel disease (IBD) experienced increased expression of HIF-2α that activates glycolysis processes in intestinal mucosa cells and results in tissue damage [3].

Objective — to evaluate glycolysis processes and OXPHOS in lymphocytes of patients with immuno-dependent diseases as compared with conditionally healthy children.

Materials and methods

The study involved 32 conditionally healthy children and 25 patients with immune-dependent diseases (IDD) (IBD — 15, vulgar psoriasis — 10) in an exacerbation of the disease. The age of the examined children is 8-18 years. The study was conducted by multiplexed method on the flow fluorimeter Bio-Plex™-200 Protein Assay System (Bio-Rad, USA) in lysates of mononuclear cells, using the commercial kit Human Oxidative Phosphorylation (OXPHOS) Magnetic Bead Panel (Merck, Germany). T- and B-lymphocyte
populations were received after sorting the general lymphocyte population on the microfluidic cell sorter SH800 (Sony Biotechnology, Japan). Lysates of mononuclear cells were obtained by standard method, using protease and phosphatase inhibitors. Detection of protein complexes OXPHOS, NNT and glycolysis enzymes in cell lysates were determined by the fluorescence intensity with protein rationing per sample (IF). Quantitative determination of protein content was conducted, using bicinchoninic acid (BCA protein reaction kit; Sigma, USA) by Paul Smith method. The amount of apoenzyme in complexes OXPHOS was defined: NADPH-ubiquinone oxidoreductase (complex I), succinate ubiquinone oxidoreductase (complex II), ubiquinone cytochrome C oxidoreductase (complex III), cytochrome C oxidase (complex IV), ATP synthase (complex V) and integral protein of the inner mitochondrial membrane — nicotinamide nucleotide hydrogenases (NNT). Glycolysis was measured by the amount of 7 proteins: ENO1 (enolase 1), G6PI (glucose-6-phosphate isomerase), HIF-1α (hypoxia-inducible factor 1-α), LDHA (α-chain L-lactate dehydrogenase), LDHB (β-chain L-lactate dehydrogenase), PKM2 (M2-isoform pyruvate kinase), TKT (transketolase). Informed voluntary consent of parents and children over 14 years old was obtained for all patients according to Helsinki Declaration. Statistical processing of the results was performed using the package Statistica 10.0 (StatSoft, USA). Descriptive statistics of quantitative traits are presented in the format: median (lower and upper quartile) — Me (Q0,25–Q0,75). The reliability of the difference between the groups was evaluated using nonparametric Mann-Whitney U-test. The correlation analysis was conducted using Pearson’s correlation coefficient (r). Differences were considered as statistically significant at p < 0.05.

**Results and discussion**

When comparing fluorescence intensity of proteins of the successive stages OXPHOS the highest values were obtained for I complex — 1,11 [0,9–1,31]. This indicator was adopted as 100% for further comparative evaluation of the rest respiratory chain enzyme complexes. IF II complex was 0,79 [0,57–1,04] c.u. (66% of IF complex I; p = 0,0002), IF III complex — 0,71 [0,37–1,09] c.u. (63% of IF complex I; p = 0,037), IV complex — 0,54 [0,26–1,33] c.u. (52% of IF complex I; p = 0,404). It was also shown that for ATP synthase (complex V) IF level was the lowest — 0,23 [0,14–0,30] c.u. (22% of IF complex I; p = 4*10 −5). Direct correlation was found between IF complexes I and II (R = 0,68; p = 0,005), and also between IF cytochrome C oxidase and NNT protein (R = 0,87; p = 1*10 −5). In assessing glycolysis proteins, the highest IF intensity in the group of conditionally healthy children is identified for TKT, G6PI, LDHB. The comparison of the patients with IDD in conditionally healthy children showed significant reduction in OXPHOS protein: I complex by 28% (p = 0,002), II complex by 56% (p = 5*10 −3), III complex by 49% (p = 0,009). Moreover, the children with IDD experienced significant increase in IF glycolysis proteins: G6PI of 18% (p = 0,024), HIF-1α of 51% (p = 0,012), TKT of 29% (p = 3*10 −5). PKM2 showed a reduction of IF by 69% (p = 0,004).

Thus, in the acute stage the patients with IDD experienced the reduction in proteins of I–III complexes OXPHOS with the increase in G6PI proteins (initial stage of glycolysis), transketolase (activation of pentose phosphate cycle), PKM2 decrease (final stage of glycolysis). There has been a significant increase of hypoxia-inducible factor 1-α in the patients with IDD.

Due to the fact that T- and B-lymphocytes have different metabolic profiles, in the next stage of work processes of OXPHOS and glycolysis were compared in the patients with IDD in an exacerbated stage and in the group of conditionally healthy children. In the comparison group it was found that OXPHOS processes prevail in T-lymphocytes as compared to B-lymphocytes: in I complex by 8% (p = 0,01), IV complex by 74% (p = 0,02), V complex by 11% (p = 0,01) and for NNT protein by 83% (p = 0,001). The average increase in IF was 2,97 times. While evaluating glycolysis of conditionally healthy children, IF indicator in T-lymphocytes turned out to be significantly higher, compared to B-lymphocytes, expect for ENO1 and HIF1α proteins. The highest ration of IFT/IFB was observed for G6PI (32 times) and TKT (9,4 times). Glycolysis prevalence in B-lymphocytes relative to T-lymphocytes was observed only for HIF1α protein by 2,4 times (p = 0,038). The ratio of OXPHOS enzymes in the patients with IDD between T- and B-lymphocytes was consistent with that in the conditionally healthy patients, except for increase in proteins of III complex (p = 0,03). As for glycolysis processes, the patients with IDD experienced a significant increase in the amount of ENO1 protein by 4,1 times (p = 0,03) and HIF1 protein by 4,3 times (p = 0,03). The comparison of metabolic processes in T-lymphocytes in the patients with IDD and healthy children showed a considerable increase in all glycoly-
sis proteins, except for ENO1 and HIF1 (on average by 3.2 times) with the growth in IV complex OXPHOS (cytochrome C oxidase, p = 0.004). The comparison of the patients with IDD and healthy children in B-lymphocytes also showed a considerable increase in all glycolysis proteins on average by 6.4 times and growth in all OXPHOS complexes (on average by 6.7 times).

**Conclusion**

Therefore, a comprehensive evaluation of oxidative phosphorylation and glycolysis processes in patients with immuno-dependent diseases in an exacerbation stage showed a significant increase in glycolysis processes both in T- and B-lymphocytes. Besides, there was a considerable increase of OXPHOS processes in B-lymphocytes. The patterns we have identified are consistent with changes of metabolic processes, described when activating T- and B-cells [1, 4]. The study of metabolic processes in lymphocytes with different immune-dependent diseases opens up attributability and implementation of targeted therapy for controlling pathological process.

**REFERENCES /ЛИТЕРАТУРА**


