

O-GLYCOSYLATION IN THE CELLULAR STRESS RESPONSE

Doctoral (PhD) thesis

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SUMMARY

O-glycosylation is a dynamic, reversible protein post-translational modification which modulates the metabolic state of cells. It interacts with several other post-translational modifications including acetylation, methylation, ubiquitination and nitrosylation, and most importantly with phosphorylation. Recently, the role of O-glycosylation was revealed in stress adaptation and preconditioning.

In the first part of the work presented in my thesis we investigated the effect of acute oxidative stress on the dynamic change of O-glycosylation, its relationship with tau phosphorylation at various recovery times on the neuronal cell line SH-SY5Y. We found that intracellular O-glycosylation rapidly increased after acute stress, then returned to near the baseline at the end of the recovery period. Similar kinetic was observed at the expression level of the major enzymes involved in the regulation of O-glycosylation. In the case of tau phosphorylation, we found that tau phosphorylation at specific sites [Ps¹⁹⁹] and [Ps^{396/404}] changed in a reciprocal way compared to O-glycosylation. Our results show that O-glycosylation plays an important role in stress adaptation and understanding the relationship with tau phosphorylation may contribute to a more accurate understanding of the pathophysiology of Alzheimer's disease.

In the second part of the thesis, I present a study in which we investigated O-glycosylation regulation in white blood cells isolated from volunteers' blood samples after a single bout of physical activity. Our results showed that due to physical activity, significant O-glycosylation elevation occurred in the white blood cells in comparison to the samples collected during resting. In white blood cell subpopulations, O-glycosylation change was most prominent in the group of lymphocytes and monocytes. Although understanding this complex function requires further testing, measurement of O-glycosylation in human samples could already be a useful tool for laboratory diagnostics; for example, it may be suitable for assessing the physical condition of athletes or for the screening of metabolic syndrome or diabetes.

INTRODUCTION

Cells try to maintain their intracellular conditions amidst continuously changing external environment. Various microenvironment stressors such as heat shock, oxidative stress, toxic substances, osmotic shock may induce different cellular stress responses that either increase cell tolerance contributing to cell survival or induce cell death.

During preconditioning, stressors can activate stress proteins which, due to its general cellular property, make the cells more resistant to stressors. The most frequently studied mechanisms of preconditioning are ischemic preconditioning and exercise induced preconditioning. Both mechanisms are critical in mitigating harmful effects caused by inflammatory processes or oxidative stress. Cellular stress responses induced by these mechanisms are activated and regulated by various genomic, transcriptional, translational and post-translational processes. While transcriptional gene induction is a response to a severe stressor, post-translational modifications are quick responders to moderate stress. To maintain the integrity of the cell, post-translational modifications can induce reversible or irreversible structural, stability and functional changes on proteins.

O-glycosylation is a dynamic and reversible post-translational modification affecting serine (Ser) and threonine (Thr) amino acids of cytoplasmic, nuclear and mitochondrial proteins by addition of a single sugar molecule, O- β -N-acetylglucosamine (O-GlcNAc) to their hydroxyl groups. The number of known O-glycosylated proteins is rapidly increasing thanks to advances in mass spectrometry techniques – over 3000 O-glycosylation proteins has been identified so far, including regulators of gene expression, translation, signal transduction, cell cycle regulation and cellular stress response.

It is known that O-glycosylation can participate in the regulation of proteins by various mechanisms, including the interactions with other post-translational modifications. The interaction of O-glycosylation has been studied with acetylation, methylation, ubiquitination and nitrosylation. Probably the most investigated interaction of O-glycosylation is the competition with phosphorylation over the same Ser and Thr sites, but proximity site competition or cooperation have also been demonstrated. This interaction between O-glycosylation and phosphorylation can be observed on many proteins (for example proto-oncogenes, neurofilaments, cytoskeletal proteins) and play an important role in the development of chronic diseases such as diabetes, neurodegenerative disorders and tumors. Recent results have shown a significant degree of O-glycosylation of many proteins in neuronal tissues including microtubule associated tau protein. It is known that the disrupted

equilibrium between the tau protein phosphorylation and O-glycosylation can lead to hyperphosphorylated tau proteins, which may aggregate and form neurofibrillary bundles, one of the major elements of the pathophysiology of Alzheimer's disease. A number of research groups have investigated the relationship between O-glycosylation and tau phosphorylation, establishing that acute or long-term inhibition of O- β -N-acetylglucosaminidase (OGA) - the enzyme responsible for the removal of O-glycosylation - can cause increased tau O-glycosylation and decrease tau phosphorylation.

Multitude of studies also demonstrated the importance of O-glycosylation in cellular stress response. Elevated O-glycosylation levels were found during cellular stress response induced by heat stress, oxidative stress, DNA damage stress (doxorubicin, UV radiation), hypoxia, ER stress (tunicamycin) ischemia / reperfusion injury or traumatic bleeding. It was proposed that increased O-glycosylation may enhance the tolerance to stress and thus may improve cell survival. In experiments with rat heart muscle cells, it has been shown that O-glycosylation has a mediator role in ischemic preconditioning by its cardioprotective effect. The relationship between exercise induced preconditioning and O-glycosylation have been studied in a limited number of animal models with controversial results. Peternejl et al. showed elevated O-glycosylation levels in the rat skeletal muscle after acute exercise (Peternejl et al. 2014). In contrast, Medford and his team studied the effects of acute exercise in the myocardial tissue of mice, concluding that after 15 minutes of treadmill training the O-glycosylation level decreased, while after a half-hour training they could not find any significant change (Medford et al. 2013).

Increasing number of evidence suggest that O-glycosylation levels significantly change in inflammatory processes, congenital and acquired immune responses and tumor diseases. The role of O-glycosylation in the immune system was mainly studied in lymphocyte cells. Kears and Hart demonstrated the role of O-glycosylation in the early stage of T lymphocyte activation (Kears and Hart 1991). Several transcription factors translocated to the nucleus during T cell activation or during B-cell reprogramming have been found to contain O-glycosylation modification sites.

The effect of physical activity on the immune system is a widely studied area. It is generally accepted that moderate- intensity exercise can enhance the immune system, while strenuous exercise for long period can cause temporary depression of the immune system. The cellular response to physical activity can be observed through natural killer cell (NK-cell) activity, neutrophil function and lymphocyte response.

A number of studies revealed the influence of O-glycosylation on the immune system. Likewise, the impact of physical activity seems to be evident on the immune system, however there are no data available yet about the relationship between immune system, O-glycosylation and physical activity.

AIMS

The aim of this work was to investigate the role of O-glycosylation in acute stress in two models.

1. First, using hydrogen peroxide treatment on human neuroblastoma cell line as acute stress, we investigated:

- *the dynamic changes of O-glycosylation at various recovery times on human neuroblastoma cells exposed to oxidative stress for a short time.*
- *the interaction between O-glycosylation and tau phosphorylation after acute stress.*
- *the mRNA expression level of the major enzymes involved in the O-glycosylation process.*

2. In the second model, we investigated the relationship of short-term physical activity and O-glycosylation on human blood cells collected from volunteers. For this purpose, the following steps were performed:

- *Detection of biochemical factors that exhibit significant changes after physical activity.*
- *Analysis of O-glycosylation levels of white blood cells isolated from human blood after acute exercise.*
- *Analysis of O-glycosylation modification of white blood cell subpopulations.*

MATERIALS AND METHODS

1. Cell culturing, treatments methods

Experiments were performed on SH-SY5Y (ATCC CRL-2266 human neuroblastoma) and Jurkat (ATCC TIB 152 human acute T-cell leukemia) cell lines (kind gift from the Dept. of Immunology and Biotechnology of the University of Pécs).

To stimulate acute oxidative stress, we used 0.5 mM H₂O₂ for 30 min. After the treatment, the medium was replaced by complete medium and the SH-SY5Y cells were incubated for the following recovery times: 0, 30, 60 min. and 2, 4, 24, 48 and 72 hrs.

Jurkat cells cultured in EMEM and Ham's F12 medium were treated as follows for 1 hour: a) with serum before and after a single-bout of exercise, b) with 5,10,20 mM lactic acid (PTE

Pharmacy); c) with 1 μ M and 100 μ M epinephrine. Jurkat cells were incubated with 3, 5, 50, 100, 1000 pg / ml IL-6 for 1 and 6 hours. After the ending of the incubation periods, the cells were stored at -76 ° C as pellets.

2. Experimental design, blood sampling

The experiment included healthy male volunteers who completed the 2 mile run according to US Army physical fitness test. The experimental setup was designed to contain two phases; 1) *resting* and 2) *exercise*. The two phases were separated by 3 weeks period. In each phases, the participants were asked to report at the laboratory in the morning after 12 hours of fasting and 48 hours of restrain from any strenuous physical exercise. In the resting phase, after standard breakfast (~600kcal, 80% carbohydrate) the volunteers were asked to restrain from any physical activity for 3,5 hours before collecting the second set of blood samples. In the exercise phase the volunteers were asked 3 hours after breakfast to complete a two-mile running exercise which was followed by the final blood sample collection.

Venous blood samples were collected in tubes without additives and in tubes containing potassium ethylenediaminetetra-acetic acid (K-EDTA) and sodium-fluoride (NaF). Tubes with K-EDTA anticoagulant were used for testing cellular blood parameters (Cell-Dyn 3700, Abbott Diagnostics) and to isolate WBCs. Tubes containing NaF as glycolysis inhibitor were used for plasma glucose and lactate analysis, while tubes without additives were used to obtain ion homeostasis, kidney and liver parameters, metabolism and tissue damage processes. After blood collection plasma and serum were separated by centrifugation (10 min, room temperature, 1500 rcf). Plasma and serum parameters were measured by Cobas 8000 Modular Analyzer (Roche Diagnostics).

Approximately 2.5 ml of K-EDTA anticoagulated whole blood was used to isolate mononuclear cells, which was layered on Histopaque-1077 (Sigma-Aldrich) solution and prepared by isopycnic centrifugation (20 min, RT, 500 rcf) immediately after collection. Mononuclear cells were collected from the plasma/Histopaque-1077 interface and washed 2x in ice-cold PBS and then cells were stored at -76 ° C as pellets.

3. Western blot analysis

The pretreated SH-SY5Y and Jurkat cell as well as isolated human mononuclear cells were harvested in RIPA buffer kept on ice for 30 min. and centrifuged for 10 min at 4 °C at 3000 rpm. From the supernatant the total protein concentration was determined using Bio-Rad Dc Assay Kit. Proteins were separated on 8% SDS-PAGE and transferred onto polyvinylidene

difluoride membranes. Blots were probed with anti-O-glycosylation antibody CTD110.6 and RL2. Blots were also probed with rabbit anti-tau [Ps¹⁹⁹] phosphospecific antibody, rabbit anti-tau [Ps²⁶²] antibody, PHF1 (anti-tau Ps^{396/404}) and anti-actin IgG antibody according to the manufacturer's protocol followed by their respective HRP conjugated secondary antibodies. Blots were developed using Femto chemiluminescent substrate.

4. Cell viability test

Cells were lifted by trypsin and quickly washed 2x in ice-cold PBS. Approximately 10⁶ cells/sample were stained with Propidium Iodide and Annexin V-FITC. The fluorescence signal was detected with a Cytomic FC 500 flow cytometer (Beckman Coulter). Defining the gating of dead cells (positive for propidium iodide and negative/positive for Annexin V-FITC) and live cells (negative both for propidium iodide and FITC Annexin V) was performed on control samples and identical boundaries were utilized for all samples.

5. Immunfluorescence microscopy

SH-SY5Y cells were grown on coverlips and after oxidative stress/recovery treatments they were washed twice in ice-cold PBS, then the cells were fixed in 10% PBS-buffered formaldehyde for 30 min at room temperature. After permeabilization and blocking with bovine serum albumin (BSA, Sigma-Aldrich), the coverslips was incubated at room temperature with CTD110.6 monoclonal antibody. After rinsing three times with PBS, the samples were incubated with Alexa Fluor 594 goat antimouse IgM secondary antibody. Nuclei were counterstained with Hoechst dye. Image acquisition was performed with a Zeiss Axiovert 35 inverted fluorescent microscope with CellD software.

6. Real-time PCR analysis

Total RNA was isolated from SH-SY5Y cells previously treated with hydrogen peroxide, using RNeasy Mini Kit. Reverse transcription into cDNA was performed with iScript cDNA Synthesis Kit. Pre-designed TaqMan assay (Roche Applied Science) was used to determine mRNA expression levels of human O-linked N-acetylglucosamine transferase (OGT) and glucosamine-fructose-6-phosphate aminotransferase (GFAT). As a reference gene, human porphobilinogen deaminase (PBGD) was used. Real-time PCR was performed in a LightCycler® thermal cycler.

7. Flow cytometry

For isolation of white blood cells whole blood samples anticoagulated with K-EDTA, were lysed and fixed using Lyse/Fix Buffer (BD Biosciences). Fixed WBCs were washed once in PBS and permeabilized with 0.5% Triton X-100 and blocked with 5% BSA-PBS. Next, the cells were incubated with anti-O-glycosylation antibody RL2 for 30 min at 37°C. After being rinsed in PBS, the cells were incubated with the secondary antibody fluorescein-conjugated goat anti-mouse IgG. Forward, side scatter (FS and SS) values and fluorescence intensities (detected at 525 nm on the FL1 channel) per cells were measured with a Cytomics FC 500 flow cytometer.

8. Data analysis

Data are presented as means \pm standard deviations and \pm standard error of mean throughout. Statistics were performed by Student's t-test and one-way ANOVA plus Bonferroni's post-hoc test in case of multiple comparisons. Statistically significant differences between groups were defined as p values <0.05 and are indicated in the legends of figures.

RESULTS AND DISCUSSION

Effect of acute stress on O-glycosylation on neuroblastoma cell line

Results

1. Effect of moderate oxidative stress on SH-SY5Y cell survival

To simulate acute oxidative stress and subsequent recovery, neuroblastoma cells exposed to 0.5 mM H₂O₂ for 30 min followed by washing steps and refreshing growth media to allow for recovery. The damage caused by oxidative stress was most prominent 24 hours post-stress (the proportion of dead cells increased from 6.3% to 22.6% compared to the ratio of dead cells in the control sample). This ratio did not increase further significantly after 48 and 72 hours post-stress.

2. Changing the O-glycosylation dynamics detected with immunofluorescence after acute oxidative stress

To assess the dynamics of O-glycosylation, SH-SY5Y cells grown on coverlips and treated with 0.5 mM H₂O₂ for 30 min and were fixed in formalin after different recovery times and then labeled with CTD110.6 (anti-O-glycosylation, monoclonal) antibody. We found that the cytoplasm of neuroblastoma cells showed a diffuse, abundant granular O-glycosylation.

Although oxidative stress resulted in increased O-glycosylation level in each cell, significant changes were found between the 0 minute and 2 hours recovery times.

3. Analysis of the relationship between O-glycosylation and phosphorylation on tau proteins in SH-SY5Y neuroblastoma cells

Next, we investigated whether the dynamic O-glycosylation change can be verified by western blot. We found that proteins labeled with CTD110.6 anti-O-glycosylation antibody significantly increased O-glycosylation intensity 2-4 hours following oxidative stress, which returned to near baseline levels after 48 hours.

To investigate the relationship between O-glycosylation and phosphorylation, the proteins obtained from pretreated neuroblastoma cells were labeled with phosphospecific [Ps¹⁹⁹] and [Ps²⁶²] and PHF1 antibodies. Phosphospecific [Ps¹⁹⁹] and PHF1 tau protein levels were decreased significantly after oxidative stress, indicating an opposite dynamic with O-glycosylation. On the other hand, phosphospecific [Ps²⁶²] tau proteins levels followed a parallel dynamic with O-glycosylation.

4. Oxidative stress induced changes of the mRNA expression level of GFAT and OGT

In our experiments, mRNA expression level of GFAT and OGT were also investigated after H₂O₂ induced oxidative stress. In response to oxidative stress, the mRNA level of both GFAT and OGT was increased by up to ~3x the normal expressional rate peaking at 4 hrs post-stress, then the expressional levels gradually decreased close to the normal levels in the next 3 days.

Discussion

In the first part of our work we investigated the effect of acute oxidative stress on the dynamic change of O-glycosylation, and its relationship with tau phosphorylation at various recovery times on the neuronal cell line SH-SY5Y. Our results showed that following oxidative stress, protein O-glycosylation modification and mRNA expression levels of GFAT and OGT, are all changed in a very similar manner over time. Significantly increased O-glycosylation and OGT and GFAT mRNA expression levels were found 2–4 hrs post-stress which after 24-78 hrs post-stress returned to baseline levels. These results suggest that O-glycosylation modification is a sensitive and dynamic marker of cellular stress response.

The regulatory role of O-glycosylation is known in many intracellular processes, such as cell cycle, epigenetics, stress adaptation, Ca²⁺ signaling and phosphorylation. Hypoxia and oxidative stress induced O-glycosylation modification was studied mostly in rodent

myocardial and skeletal cells, resulting in a global O-glycosylation increase shortly after stress. In myocardial cells, the elevated O-glycosylation may have a protective role, since it seems to improve myocardial contractility and decrease infarct size. This protective role of O-glycosylation is explained by a number of theories, but the exact survival mechanism(s) by O-glycosylation is still unclear.

In our experiments we have found that SH-SY5Y cell line shows similar O-glycosylation dynamics as was shown in the literature in cardiomyocytes. These results suggest that oxidative stress induced O-glycosylation elevation in neurons is also an actively regulated process. It is known that O-glycosylation modification is also an important regulatory mechanism in Alzheimer's disease. In Alzheimer's disease, the dynamic balance between phosphorylation and O-glycosylation modification on tau proteins is shifted in favour of the phosphorylation leading to hyperphosphorylated tau which can aggregate into neurofibrillary tangles. However, it has been shown that elevated O-glycosylation levels are able to prevent the hyperphosphorylation of tau proteins. In our experiments, we have found that although oxidative stress can induce a temporal increase in some of the phosphorylations, other tau phosphorylation sites showed reciprocal dynamic pattern as O-glycosylation levels. The explanation of the relationship between the two processes can be characterized by either same site competition or by proximal site effect.

It is known that diabetes and insulin resistance, as risk factors can play a critical role in the pathophysiology of Alzheimer disease, increasing the ROS production levels. The role of O-glycosylation in this process is still unclear, however it has to be noted that O-glycosylation and the carbohydrate metabolism of the cell is closely connected.

In this part of the work we demonstrated that short-term oxidative stress can cause a rapid and temporary increase in O-glycosylation in SH-SY5Y neuroblastoma cells. We have also shown that oxidative stress can induce an inverse modification in tau phosphorylation compared to the dynamic changes of protein O-glycosylation modification during recovery. Taken together, we conclude that studying O-glycosylation modification parallel with tau phosphorylation in oxidative stress exposed neuronal cells should contribute to a more accurate understanding of the development of Alzheimer's disease.

Changes of O-glycosylation in human white blood cells after a single bout of exercise

Results

1. The effect of a single bout of exercise on human blood parameters

All the routine laboratory parameters from the fasting blood samples were found to be within normal range. There was no significant difference between the fasting blood samples collected in resting phase or in the exercise phase.

However, after exercise, several parameters were found to be significantly elevated: phosphate, lactate, creatinine, lactate-dehydrogenase (LDH), albumin levels, white blood cell counts, lymphocyte and platelet count increased. In contrast, no such change was observed in samples collected in the post-resting phase. In order to exclude daily fluctuations and postprandial effects, intraday changes were compared to intraday fluctuations in the rest period and physical activity.

2. O-glycosylation pattern of isolated mononuclear cells after exercise

Mononuclear cells were isolated from volunteers' anticoagulant blood samples with Histopaque-1077. Western blot was used to measure the amount and pattern of O-glycosylated proteins using CTD110.6 and RL2 anti-O-glycosylation antibodies. As expected, no significant difference was observed in rest phase, however, we found a significant O-glycosylation increase after exercise, compared to the samples collected before exercise.

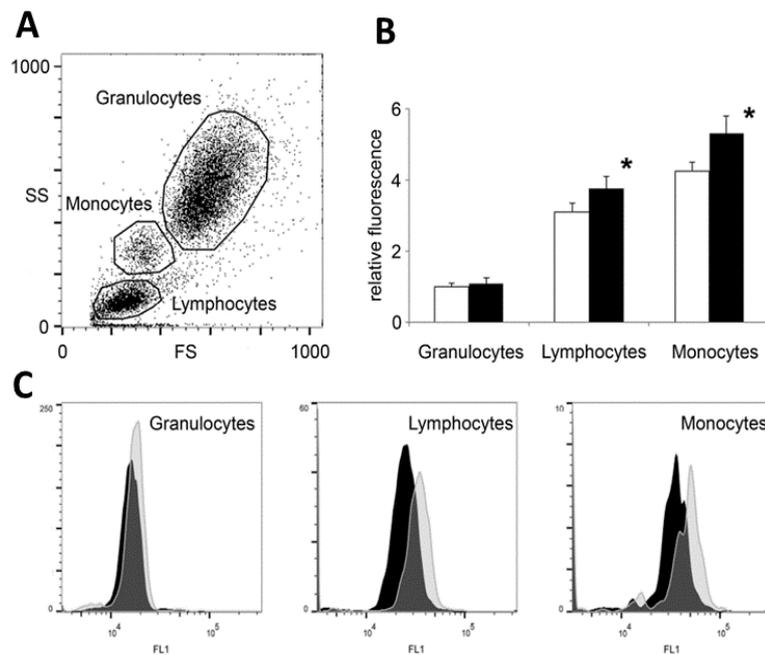
3. O-glycosylation in Jurkat cells following various treatments

Jurkat cells were treated with human serum collected from volunteers either before or after physical exercise. We have also treated Jurkat cells with various concentrations of lactic acid and epinephrine for 1 hour and with IL-6 for 1 and 6 hours. In the treated cells, the proteins O-glycosylation were detected by western blot with CTD110.6 antibody. Although a discrete O-glycosylation increase observed in the use of serum after exercise was not significant. Similarly, we could not induce significant changes in O-glycosylation by either lactic acid, epinephrine or IL-6 treatment in Jurkat cells.

4. Analysis of O-glycosylation modification in white blood cells subpopulation

To analyze O-glycosylation in white blood cell subpopulations, isolated and fixed white blood cells of volunteers were labeled with a RL2 anti-O-glycosylation antibody and detected

by flow cytometry. Using FSc and SSc values, the cells were divided into three groups according to their size and granularity, thus separating granulocytes, lymphocytes and monocytes. In the 3 cell populations, O-glycosylation levels could be separately analyzed by FL1 fluorescence. O-glycosylation levels were similar in the granulocyte cell groups before and after exercise, however lymphocytes and monocytes showed significantly increased O-glycosylation after exercise.



O-glycosylation changes in white blood cells subpopulations after exercise detected with flow cytometry. **A.** Classification of granulocytes, lymphocytes and monocytes based on FSc (x axis) and SSc (y axis). **B.** Relative FL1 fluorescence levels of O-glycosylation labeled blood cells, collected before (open bars) and after (black bars) exercise. Data are shown as mean \pm SD, * $p < 0.05$ vs before exercise. **C.** The O-glycosylation staining (FL1 channel) of the three regions of cells displayed individually by histograms. Distribution of cells collected before exercise are presented by black histograms, while the distribution of cells collected after exercise are presented by light gray histograms.

Discussion

In the second part of the thesis, we have studied the effect of exercise on the level of O-glycosylation modification in leukocytes collected from human volunteers. We have also analyzed the effect of exercise on various, routinely measured clinical laboratory biomarkers.

We have investigated the changes in O-glycosylation modification in isolated white blood cells after exercise compared to samples collected from resting volunteers. In our study we have found that physical activity can induce measurable changes in the O-glycosylation levels

mainly in lymphocytes and monocytes. O-glycosylation levels of granulocytes remained relatively unchanged.

The protective role of O-glycosylation is known against various type of stressors, moreover it is also suspected to be involved in preconditioning processes. Most previous studies have been performed mainly on myocardial tissues of rodents, cells of the immune system have not been studied so far. However, physical activity and exercise also affects the function of the immune system, so it is important to know which molecular mechanisms are taking place in the background. The immunomodulatory effect of exercise can be influenced by the length, intensity, aerobic or anaerobic nature of exercise and training. It is well known that strenuous exercise can lead to immunosuppression, while moderate exercise is beneficial for the immune system and reduces the incidence of infections. O-Glycosylation may modulate many of the signaling element of the immune cell activation; e.g. the nuclear translocation of NF- κ B, which is a key transcriptional regulator in immune responses, is known to be influenced by O-glycosylation modification.

O-glycosylation is directly related to cellular metabolism by its substrate UDP-GlcNAc, therefore it seems to be a logical choice for the mediation of changes in metabolism (either stress or preconditioning). The direct activator of O-glycosylation elevation in our experimental setup is not known yet. The candidates for inducing O-glycosylation increase are many: hypoxia, elevated glucose levels, acidosis, myokines, IL-6, etc. Our preliminary experiments on Jurkat cell line suggest that a single molecule or activator (lactic, epinephrine, IL-6) alone is probably not sufficient to elicit a significant increase of O-glycosylation. Based on these, our conclusion is that both physical and biochemical factors are responsible for the significant O-glycosylation elevation.

Taken together, in second part of our study we demonstrated that an acute exercise can enhance protein O-glycosylation levels in WBCs, most prominently in lymphocytes and monocytes. Although understanding the complex function of O-glycosylation in leukocytes requires further testing, our results can help to clarify the role of O-glycosylation in stress adaptation mechanisms. We also believe that - regardless of the mechanism - the analysis of O-glycosylation in human samples can be of practical value in developing new diagnostical methods in sports medicine and in the laboratory screening of cardiovascular and metabolic disease.

SUMMARY OF NEW RESULTS

In our present work, we investigated the relationship between O-glycosylation and acute stress in cell cultures and isolated human leukocytes.

In human neuroblastoma cell line treated with hydrogen peroxide for 30 minutes we have found the followings:

- The level of O-glycosylation modified proteins were increased significantly reaching peak values 2-4 hours after treatment and returned to the baseline near the end of the recovery period.
- Oxidative stress induced a decrease of tau phosphorylation at site [Ps¹⁹⁹] and PHF1, but in contrast phosphorylation at site [Ps²⁶²] showed similar dynamic pattern to O-glycosylation levels.
- The mRNA expression levels of GFAT and OGT increased 2-4 hours after stress similar to the dynamic of O-glycosylation modified proteins.

Investigating the relationship between acute exercise and O-glycosylation on human blood cells collected from volunteers, the following results were obtained:

- Levels of glucose, lactate, phosphate, and creatinine have shown significant changes after exercise compared to resting.
- Significantly elevated O-glycosylation levels were detected in isolated white blood cells after exercise.
- Significantly elevated O-glycosylation levels were found in monocytes and lymphocytes after exercise.

PUBLICATIONS

Articles related to the thesis

- **Káta** E, Pál J, Poór VS, Purewal R, Miseta A, Nagy T. *Oxidative stress induces transient O-GlcNAc elevation and tau dephosphorylation in SH-SY5Y cells*. Journal of Cellular and Molecular Medicine. 2016 Dec; 20(12): 2269–2277. IF: 4,499
- Tamás Nagy, **Emese Káta**i, Viktória Fisi, Tamás Tibor Takács, Antal Stréda, István Wittmann, Attila Miseta. *Protein O-GlcNAc Modification Increases in White Blood Cells After a Single Bout of Physical Exercise*. Front Immunol. 2018 May 3;9:970. IF: 5,511 (2017)

Articles not related to the thesis

- Fisi V, **Káta**i E, Orbán J, Dossena S, Miseta A, Nagy T. *O-Linked N-Acetylglucosamine Transiently Elevates in HeLa Cells during Mitosis*. Molecules. 2018 May 26;23(6). IF: 2,861
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