IMPACT OF THE H3S10 PHOSHORYLATION IN RESPONSE TO INTENSE PHYSICAL ACTIVITY-INDUCED OXIDATIVE STRESS IN ELITE SOCCER PLAYERS – PILOT STUDY

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- H3S10 phosphorylation,
- oxidative stress,
- soccer,
- exercise-induced chromatin modifications.

Abstract:

Although the role of oxidative stress in mediating various physiological processes in humans is well-established, it is still little known about the impact of epigenetic mechanisms on modulating such signaling pathways. Epigenetic mechanisms, via regulating gene expression depending on wide range of the internal/environmental stimuli, serve as a very sensitive sensor and regulator of multiple organism-environment interactions. Consequently, they confer fast and adaptive response to different factors, including intense physical activity-induced oxidative stress. The latter, in turn, plays an essential role in athletes under long-term, high-intensity effort. Therefore, this pilot study, was aimed to examine one of the basic epigenetic regulation, manifested by the changes in global level of phoshorylation on serine 10 of histone H3 (H3S10ph), in response to strenuous physical activity in elite soccer players. Although, the slight changes in global H3S10ph levels were noted after soccer match, when compared to its pre-match amount, those changes were not statistically significant. The role of H3S10ph in regulation gene expression as well as methodological problems related to examination of this modification are also discussed

INTRODUCTION

It is widely accepted that strenuous physical activity is associated with oxidative stress that arises from overproduction of reactive oxygen species (ROS) and/or reduced rates of their scavenging orchestrated by a wide range of cellular antioxidant defense systems. Consequently, ROS abundance lead to oxidative damage to macromolecules, such as lipids, proteins and DNA. Although, many oxidative stress response mechanisms as well as oxidative stress response markers have been identified so far, novel key players in such defense pathways are still emerging [Colak 2008].

Oxidative stress mediates wide range of cellular processes, including changes in gene expression as well as proteins activity, cell death/survival, and mutagenesis. All of these alternations are modulated by epigenetic mechanisms [Jenuwein, Allis 200; Kreuz, Fishle 2016].

The term epigenetics refers to the mitotically and/or meiotically heritable changes in gene function, results from modifications of chromatin structure and cannot be explained by alternations in DNA sequence. These changes in chromatin structure are caused mainly by

chemical modifications of DNA and/or and histones (DNA-bound proteins), two main components of chromatin [Russo et al. 1996].

Histones are the most common chromatin proteins, therefore, any changes in their structure, abundance, posttranslational modifications or occurrence of non-canonical variants affect chromatin structure modulating gene expression as well as genome stability and replication [Kreuz, Fishle 2018].

One of the epigenetic mechanism that has an impact on chromatin structure and chromatin structure-mediated gene expression are posttranslational modifications (PTMs) of histones, among which histone phosphorylation plays an important role in stress signaling pathway [Zippo et al. 2009; Sawicka, Seiser 2014; Kreuz, Fishle 2016]. For example, phoshorylation of histone H2AX, a non-canonical variant of histone H2A, on serine 139 is engaged in signaling and double-strand DNA breaks (DSBs) repair pathways. Phoshorylation on serine 10 of histone H3 (H3S10ph) in interphase, in turn, is directly linked to transcriptional activation of many genes [Prigent, Dimitrov 2003].

THE AIM OF THE WORK

Although, role in the H3S10ph in mediating adaptive response to a wide range of extracellular stimuli that initiaties specific transcription programs [Sawicka, Seiser, 2012] is well-established, the effect of ROS on H3S10 phosphorylation is controversial [Kreuz, Fishle 2016]. Therefore, the aim of this pilot study was to examine the oxidative stress-driven phosphorylation on H3S10 in elite adolescent soccer players under competitive match conditions.

THE SUBJECTS AND THE METHODOLOGY

Subjects

Eight healthy adolescent soccer players from the Resovia SMS School participated in this study. On the day of the study, the subjects played a competitive soccer match. All participants as well as their parents were completely informed about the experimental procedures prior to signing the informed consent. The study was approved by the Ethics Committee of the Faculty of Medicine, the University of Rzeszów, Poland (no. 3/11/2017).

Methods

Blood sampling and PMBCs isolation

The blood samples were collected before match, immediately after match and 24 hours after match. The blood samples were collected in vacutainer-EDTA tubes and immediately used for peripheral mononuclear blood cells (PMBCs) isolation. PMBCs were obtained with use of histopaque-1077 (Merck, Poland), according to the manufacturer's instructions. Immediately after isolation, PMBCs were snap frozen in liquid nitrogen and stored at −80 °C for later core histones extraction.

Core histone isolation and Western blot analysis

Core histones were extracted from PMBCs as described elsewhere [Arita et al. 2012]. The amount of proteins were determined by a standard Bradford protein assay (Merck, Poland) method, according to the manufacturer's manual. The global levels of unmodified histone H3 and its phosphorylated at serine 10 isofom were determined using commercial antibodies (cat. no. ab213257 and ab5176, respectively, Abcam, UK). The intensities of the Western blot signals were estimated using the ImageJ program. The levels of H3S10ph were normalized relative to the unmodified H3 and expressed in arbitrary units.

RESULTS

The analysis of the baseline values (determined before match) of hematological parameters and anthropometric characteristics of the soccer players showed that subjects were a relatively homogenous group (Tab.1).

The oxidative stress, which was induced under competitive soccer match conditions, resulted in an increase in global level of H3S10 phosphorylation immediately after match, followed by its decrease 24 hours later to the level comparable to the baseline (Fig.1). Nevertheless, the observed changes in H3S10ph levels were not statistically significant, which was verified by a nonparametric Friedman test.

Table 1: The baseline values of hematological and anthropometric characteristics parameters of the soccer players

Parameter	mean± SD (n=8)	unit
Red Blood Cell (RBC)	5.11 (± 0.38)	10^6/µl
White Red Cell (WBC)	5.9 (±1.68)	10^3/µl
Platelet (PLT)	225 (± 41.23)	10^3 /µl
Hemoglobin (HGB)	15.02 (± 0.82)	g/dl
Hematocrit (HCT)	43.64 (± 1.89)	%
Mean corpuscular volume (MCV)	87.36 (± 1.94)	fl
Mean corpuscular hemoglobin (MCH)	29.81 (± 0.95)	pg
Mean corpuscular hemoglobin concentration (MCHC)	33.72 (± 0.89)	g/dl
Age	17.38 (± 0.52)	years
Height	1.78 (± 0.06)	m

Weight	70.98 (± 4.76)	kg
BMI	22.39 (± 1.24)	$(kg \cdot m^{-2})$

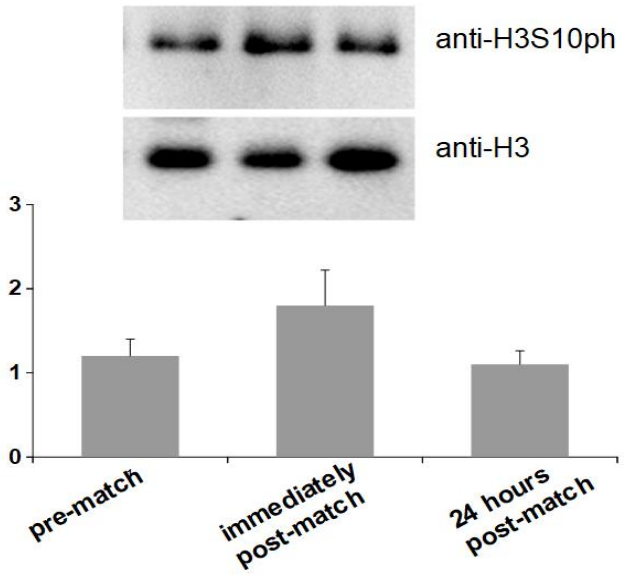

Fig. 1. Changes in global phosphorylation of H3S10 (representative Western blots) before and after the soccer match. Changes in modified histone levels are expressed in arbitrary units (diagrams). The global levels of unmodified histone H3 and its phosphorylated isofom were determined using commercial antibodies (cat. no. ab213257 and ab5176, respectively, Abcam, UK). The intensities of the Western blot signals were estimated using the ImageJ program. The levels of H3S10ph were normalized relative to the unmodified H3 and expressed in arbitrary units. Data details are presented as the means ± SD. The changes in global levels of H3S10ph are not statistically significant.

DISCUSSION

It has been reported that variety of stress signals stimulate induction of H3S10 phosphorylation, a part of the nucleosomal response, that triggers a cascade of events from histone modifications to a transcriptional activation of the immediate-early response genes [Mahadevan et al. 1991]. Such a mechanism of an induction of stress-inducible genes enables very fast and plastic response to wide range of different external/internal stimuli [Sawicka, Seiser 2014]. Additionally, H3S10ph seems to be a key player in nucleosomal response-dependent activation of stimuli-responsive genes, because it triggers a histone crosstalk between H3S10ph and acetylation of histone H4 at lysine 16. (H4K16ac) [Zippo et al. 2009] that generates the combination of histone modifications, a specific histone code (Jenuwein and Allis, 2001), responsible for activation and transcription elongation of stress-inducible genes [Zippo et al. 2009].

The abovementioned molecular mechanism have been reported for serum-inducible *FOSL1* gene, which serves as one of the extensively studied gene models. The authors [Zippo et al. 2009] showed that in serum-stimulated cells, H3S10ph enrichment, observed at the *FOSL1* enhancer, was responsible for recruitment of the adaptor protein 14-3-3. The latter, after its binding to serine 10-phosphorylated histone H3, triggered the histone acetyltransferase MOF, which action, in turn, led to H4K16 acetylation. The combination of H3S10ph and H4K16ac - recognized by BRD4 protein, a „reader" of such a histone crosstalk - promoted a recruitment of the positive transcription elongation factor b (P-TEFb) directly responsible for releasing a promoter-proximal paused RNAP [Zippo et. 2009] to start productive elongation [Liu et al. 2015].

The very recently published data showing that in human cells oxidative stress rapidly stabilizes promoter-proximal paused RNAP in a massive scale [Nilson et al. 2017], raises a question about a role of H3S10ph in oxidative stress-driven genome-wide regulation of RNAP switch between paused-to-elongating state.

The results presented here, however, do not answer this question clearly. Although, a slight increase in H3S10ph, followed by its decrease, were observed immediately after and 24 hours after physical activity-induced oxidative stress, respectively, when compared to the baseline, those rises were not significant. This effect may result from i.e. methodological problems related to analyzing so „sensitive" modification that can be very quickly and easily triggered by a wide range of internal and/or external stimuli. Moreover, in contrast to cell line models, that are commonly analyzed under fully controlled laboratory conditions, the whole organisms, epecially humans, are subjected to many different „uncontrollable" environmental factors that strongly influence their homeostasis. Therefore, examination of such easily inducible signaling pathways is a real challenge. Nevertheless, such studies provide data concerning the real interactions between organism and its environment, that – it should be highlighted – strongly depend on epigenetic control of gene expression, including genes induced by physical exercise [Stahopoulos-Ntanasis et al. 2013].

CONCLUSIONS

This pilot study focuses on global changes in H3S10ph level. Although the obtained data are not statistically significant, they show a trend in global H3S10 phosphorylation upon intense physical activity, manifested by an increase in H3S10ph level, followed by a decrease in its amount immediately after and 24 hours post-match, respectively. To clearly answer the

question concerning the changes of global H3S10ph induced by strenuous physical activity, the number of the subjects included to study, should be significantly increased.

Besides the examination of global changes in H3S10ph level, more detailed questions concerning H3S10 phosphorylation should be answered: Which genes are activated via phosphorylation of H3S10ph induced by physical activity-dependent oxidative stress? What is the role of the individual gene-specific enrichments of H3S10ph in modulating response to oxidative stress upon intense physical exercise in athletes? Finding the answers to these questions is kind of challenge, but it is also of high importance.

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