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Fund “Nauka” Project № 17015 Resume – Competition-Based Session 2017:

“Studying the effects of sulfur-containing mineral waters on the sulphur metabolism of humans”

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The object of the investigation was the contribution of sulfur-containing mineral water from the Varna Basin for metabolic import and utilization of sulfur at the level of intestinal absorption.

Sulfur is a macronutrient with important metabolic functions. In addition to maintaining redox status at the cellular and extracellular levels, it is necessary for the posttranslational modification of proteins and for maintaining their native conformation. Finally yet importantly, sulfur is a substrate in the sulfation reactions in the metabolism of xenobiotics, including many drugs.

According scientific data, sulfur and inorganic sulphates from various foods, beverages and medicines are almost completely absorbed in the gastrointestinal tract. On the other hand, the role of sulfurous mineral waters in metabolic import of sulfur remains underestimated and scientific data on the subject are scarce. At the same time, thousands of people use the mineral waters from the Varna basin every day for drinking needs.

The aim of the project was to investigate the effect of sulfur-containing mineral water on the activity of enzymes involved in sulfation processes in human intestinal cells.

Sulfatation is an important reaction in Phase II of the metabolism of many xenobiotics, drugs and endogenous compounds. This reaction is catalyzed by a super family enzymes named sulfotransferases (SULTs). A universal donor of sulfate and a mandatory co-substrate for sulfatation reactions is 3'-phosphoadenosyl-5’-phosphosulfate (PAPS). The low availability of PAPS is shown to be a limiting factor for sulfotransferase activity. Therefore, the rate of PAPS synthesis might be an important factor in determining the sulfatation activity in tissues during metabolism of drugs and xenobiotics, and hence to affect their biological activity.

Biochemical and molecular biology **methods** were applied to perform the experimental tasks envisaged in the work program:

- Cultivation of pure line of human intestinal epithelial cells (HIEC6)
- MTT assay was applied to determine cytotoxicity of sulfur-containing mineral water from a selected spring with preliminary measured physicochemical parameters and the content of hydrogen sulfide and dissolved sulfides.

- Incubation of cells with different volume concentrations of sulfur-containing mineral water in the culture medium
- Messenger RNA extraction and Real-Time Polymerase Chain Reaction (RT-PCR) was performed to determine the gene expression of two isoforms of human sulfotransferases (SULT1A1 and SULT1A3) and the two isoforms of human PAPS synthases (PAPSS1 and PAPSS2).

Analysis of the results showed that the treatment of human intestinal epithelial cells with sulfur-containing mineral water resulted in a significant increase in SULT1A1 gene expression, regardless of the volume concentration of the mineral water in the culture medium. A similar stimulating effect of the water was observed on PAPSS1 expression but only at the lowest concentration applied. It was interesting to note that higher volume concentrations of water content significantly inhibited the gene expression of both PAPS synthases, despite the stimulated expression of sulfotransferases. It could be suggested that this effect on the expression of both types of enzymes is due to some of the sulfur-containing active components dissolved in the water. Scientific data indicate that organosulfides contained in some foods, such as sulforaphane in cabbage and broccoli could induce the gene expression of Phase 2 enzymes of xenobiotics metabolism, including sulfotransferases. No data about similar effect of organosulfides from foods on PAPS synthases were found.

In the available scientific literature there is currently no information about studies focused on the metabolic effects of sulfur-containing mineral waters. It is logical to suggest that sulfur-containing substances imported with the water would have an effect comparable to that of organosulfides from foods.

In conclusion, sulfurous mineral water should be considered as an important dietary factor contributing to the sulfur metabolism at intestinal levels.

Contributions from the implementation of the project:

Data obtained are the first scientific evidence about direct link between sulfurous mineral water and metabolic challenge of the enzymes involved in sulfur utilization and its metabolism.

From a scientific point of view, revealing such a correlation could be a starting point for new research focused on the contribution of mineral waters for the metabolic import of active components. On the other hand, we believe that the results obtained would be of interest to health professionals and pharmacists regarding the consumption of sulfur-containing mineral waters by people on drug and hormone replacement therapy, bearing in mind the involvement of the studied enzymes in xenobiotic metabolism, including medicines.

In addition to scientific and public contribution, the project has an undoubted contribution in improving the skills and career development of team members.

The project implementation supported a PhD student, a member of the research team, who successfully defended the dissertation in the beginning of 2020. On the other hand, the work on the project gave the opportunity for knowledge transfer from senior scientists to their younger colleagues and to the students. Traditionally, the Department of Biochemistry, Molecular Medicine and Nutrigenomics works actively with students, involving them in the implementation of scientific tasks within elective courses and scientific projects. We believe that the transfer of experience and knowledge to students with an interest in science will support their motivation and will be our contribution to attracting young people to science.

Dissemination of results:

The project overview was presented in Scientific and Practical Conference within the Fifth Pharmaceutical Business Forum in October 2018. In addition, the major outcomes will be reported in the International Congress of Obesity, in the topic Basic and Experimental Science "Gut signaling and function", scheduled for September 2020. A full-text scientific publication in a journal with IF is forthcoming.

Achieved results:

The sulfur-containing mineral water was poured from two public fountains in the city of Varna, "Aquarium" and "Dom Mladost". It has a previously known physicochemical composition and content of hydrogen sulfide and soluble sulfides. In a previous study, the physicochemical composition and hydrogen sulfide content was estimated in samples from these sources as well as the changes in hydrogen sulfide concentration in water at different time points of residence after pouring: 24 hours, 3 days and 7 days. Based on these data, the treatment scheme of the cells was determined. Based on the cytotoxicity test, three volume concentrations of mineral water in the nutritional medium were determined: 2%, 4% and 8%.

The results analysis showed that the treatment of human intestinal epithelial cells with sulfur-containing mineral water leads to a significant increase in the expression of the two sulfotransferase genes – SULT1A1 and SULT1A3 in cells treated 24 hours after water sampling from both sources. This effect is strongly emphasized depending on the percentage content of mineral water in the nutritional medium.

On the other hand, the effects on the two PAPS synthases are divergent, but with marked inhibition of expression, and these effects are more pronounced in cells treated with water from the "Dom Mladost" fountain. According to preliminary data, the hydrogen sulfide concentration in the water of this source is in higher concentration compared to the "Aquarium".

It may be assumed that the sulfur-containing active ingredients dissolved in the water have an inhibitory effect on the expression of PAPS, while inducing sulfotransferases. Data about similar effects have been reported for sulfur-containing active substances in plant-derived foods, such as cruciferous organosulfides. Similar data are available for plant

polyphenols, which have been shown to affect the expression of metabolic and antioxidant enzymes as well as of sulfotransferases.

In conclusion, it could be assumed that the sulfur-containing mineral water from the Varna basin could influence the detoxification processes by affecting the expression of the second phase enzymes. Furthermore, this effect markedly depends on the residence time of water after pouring. The results obtained in this project should be considered when sulfur containing mineral water is consumed with certain drugs and additives that are substrates of sulfation processes.