Michał Grabowski “Analysis of selected molecular factors affecting the homocysteine level in the pathogenesis of ischemic stroke”

Stroke is the third leading cause of death and the first cause of disability in developed countries. Both social and economic costs of stroke places the disease as one of the most intensively studied diseases. Despite the intensity of research, we still do not fully understand the mechanism responsible for the development of stroke. Many studies have distinguished factors that may lead to the development of a stroke. Among the many risk factors, hyperhomocysteinemia - the accumulation of homocysteine in blood can be specified. Homocysteine is an endogenous amino acid that participates in the metabolism of the methyl group. Homocysteine accumulation induces a state of oxidative stress and contributes to a significant acceleration of atherosclerotic plaque formation. Hyperhomocysteinemia is caused mainly by disturbances in the enzymes of the homocysteine metabolic pathway - methionine synthase and methylenetetrahydrofolate reductase caused by mutations in the genes of both proteins. Changes in the sequences encoding the enzymes of the homocysteine metabolic pathway, they can lead to a significant reduction of their activity or to the appearance of a thermal-sensitive effect. Oxidative stress caused by accumulation of homocysteine leads for overproduction of heat shock proteins (Hsp). Overexpression of heat shock proteins in the region of atherosclerotic plaque suggests that they may play a role in reducing the effects of homocysteine accumulation. Verification of this hypothesis was carried out using bacterial methionine synthase – MetH. Under thermal stress conditions, heat shock proteins efficiently protected this enzyme from inactivation. The presence of vitamin B12 significantly increased the level of protection. In the case of earlier inactivation, heat shock proteins restored MetH activity only in the presence of cobalamin. In addition, studies on the impact of heat shock proteins on the thermal sensitive variant of methyltetrahydrofolate bacteric reductase - MetF117, corresponding to the human C677T polymorphism, were performed. The thermal-sensitive MetF177 protein was purified and activity tests were performed under stress conditions in the presence of heat shock proteins. The experiments showed the activity of heat shock proteins eliminating the effect of mutation and allowing maintaining activity of MetF177 protein, despite unfavorable conditions. Overexpression of heat shock proteins, despite positive effects on the enzymes of the homocysteine metabolic pathway, could lead to an increase in the inflammatory reaction in the region of the atherosclerotic plaque and thus increase the rate of its growth. Experiments , which aim was to clarify the possible correlation between the level of antibodies against heat shock proteins and risk factors for stroke have been made in collaboration with the Department of Adults' Neurology at GUMed. The tests were performed using the enzyme immunoenzymatic ELISA. The results of the statistical analysis confirmed the correlation between

the increased level of antibodies against heat shock proteins and the occurrence of stroke. There was also a correlation between the level of antibodies and risk factors for stroke. The action of heat shock proteins can lead to a decrease in the level of homocysteine in the blood by sustaining the action of enzymes. Additionally, published literature suggests a reduction in the level of homocysteine through the use of soybean products containing mainly genistein in the diet. Genistein as the main soy isoflavone is used as a substitute for estrogen in hormone replacement therapy. The use of genistein reduced the level of homocysteine in blood. The enzyme MetF and LDH (lactate dehydrogenase) were used to test the mechanism of action of genistein. The experiments have demonstrated the inhibitory effect of genistein on the activity of methylenetetrahydrofolate reductase (MetF), which should lead to the accumulation of homocysteine. Further experiments showed a mixed type of inhibition resulting from the interaction of genistein with the enzyme and one of the substrates - NADH. Additionally, the formation of the genistein – homocysteine complex, eliminating genistein activity was confirmed.