УДК: 61:615.1(043.2)
ББК: 5:52.8
ISBN: 978-985-21-0532-3

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THE INFLUENCE OF COUMESTROL ON SPHINGOLIPID SIGNALING PATHWAY AND INSULIN RESISTANCE DEVELOPMENT IN PRIMARY RAT HEPATOCYTES – A POSSIBLE TREATMENT METHOD?

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Background. Coumestrol (COM) is a phytoestrogen belonging to the coumestans family, large group of polyphenolic substances widely found in some plants including legumes, Brussels sprouts, spinach, clover and soybeans. Coumestrol seems to be an interesting compound, in aspect of potential usage in treatment of metabolic pathologies such as type 2 diabetes mellitus (T2DM) and obesity, due to its estrogen-like activity resulting from high affinity to the estrogen receptors ERα and ERβ.

Aim: the aim of the study was to evaluate whether coumestrol through influence on sphingolipid metabolism pathway affects insulin signaling and changes insulin resistance development in rat hepatocytes during lipid overload state.

Materials and methods. The study was performed on primary rat hepatocytes isolated during rat liver collagenase perfusion. The cells were incubated with COM and/or palmitic acid (PA) for 18 h. Additional groups were also incubated with insulin. Ceramide (CER) concentration was measured by high performance liquid chromatography. The expression of enzymes responsible for the *de novo* synthesis of ceramide in sphingolipid pathway (SPTLC1/LASS4) and proteins of insulin signaling pathway (AKT/GSK) were evaluated using Western Blot.

Results and discussion. CER concentration was significantly increased in hepatocytes incubated with COM or with PA. However, the accumulation of CER in rat hepatocytes incubated with both COM and PA was notably decreased compared to PA alone group. The expression of SPTLC 1 and LASS 4 were significantly higher in COM or PA group but hepatocytes incubated with PA + COM showed lower expression of these enzymes in comparison to the PA alone group. Rat hepatocytes in presence of PA + COM revealed significantly higher expression of pGSK and GSK. These results showed that PA as well as COM potentiates synthesis of CER, whereas incubation of the rat hepatocytes both with COM and PA resulted in decreased accumulation of CER by diminution of CER *de novo* synthesis pathway. Consequently, COM and PA together reduced insulin resistance development in rat hepatocytes.

Conclusions. The results of our study confirmed that coumestrol significantly changed sphingolipid pathway and insulin sensitivity. Coumestrol and other phytoestrogens should be studied more intensively as a potential drug in supportive treatment of metabolic pathologies especially T2DM and obesity.