ABSTRACT

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Title of the diploma thesis: Study of the variations in the expression of different adhesion and cytoskeletal proteins of podocytes (E-Cadherin, Podocin, Vimentin) due to Bisphenol A

Bisphenol A (BPA) is one of the most widespread compounds in the world, producing over 6 billion metric tons per year. It is widely used as part of polycarbonate plastics and epoxy resins, from which reusable plastic bottles, food boxes and some medical equipment are made. It is also used to coat the inner layer of the cans.

Previous studies have shown that BPA contributes to many chronic diseases in the human body, such as kidney disease - diabetic nephropathy. Podocytes - terminally differentiated cells of the Bowman's capsule in glomerulus - are an integral part of the filtration barrier, where they play an important role in preventing the plasmatic proteins from penetrating to the urine. Therefore, in this study, we looked at the effect of BPA on these cells and their particular proteins, using both *in vivo* and *in vitro* methods.

First, we demonstrated the harmful effect of BPA 100 nM on mice kidneys by immunohistochemistry (IHC) method. This was manifested mainly by the enlargement of the distal and proximal convoluted tubules of the glomerulus and Bowman's capsule. In the samples with induced diabetes mellitus, besides the above-mentioned manifestations, a significant mesangial expansion occurred. Using IHC, we also observed a decreased density of podocytes in the glomerulus, and decrease in their size and function, resulting in hypertrophic stress of the podocytes and glomerular collapse.

A previous study by R. Bosch’s laboratory dealing with BPA toxicity to the mice podocytes has shown a clear association between BPA exposure and proteinuria in

mice. Therefore, we investigated the effect of BPA on human podocytes by examining the toxicity of BPA on individual proteins of these cells. In particular, we dealt with E-Cadherin, an adhesive type of protein whose function is primarily to ensure the integrity and polarity of renal epithelial cells. The decrease in protein expression was induced by BPA exposure at all used concentrations (1 nM, 10 nM, 100 nM) in the Western Blot (WB) method. The decrease of E-Cadherin was also manifested in immunocytochemical (ICC) and IHC methods. E-cadherin deficiency is a signal of epithelial and mesenchymal transition, a process in which cells lose cell-cell contact and delaminate from the epithelium. As a result, cell adhesion is impaired. Another protein of interest was Podocin, a cytoskeletal type protein specific for mature podocytes, located in the slit diaphragms. Its function is to regulate the glomerular permeability. In this case, the trend of decreasing protein expression was demonstrated at all of the above-mentioned BPA concentrations used in WB. The ICC method showed a significant decrease of Podocin due to BPA 100 nM treatment on the podocytes. The lack of this protein results in decreased function of podocytes, leading to increased proteinuria. The last studied protein was Vimentin, representing the group of cytoskeletal proteins found in all eukaryotic cells with the mesenchymal origin. The function of this protein is mainly to maintain the stability and shape of the podocyte. Here again, BPA toxicity was manifested by a decrease in Vimentin levels in the cells. The greatest decrease occurred with BPA exposure of 100 nM. The lack of this protein disrupts the already mentioned cell stability, including their shape.

All the measured results confirm the original assumption of a harmful effect of BPA onto human podocytes. Not all of these results were statically significant, but the trend of decreasing values for all proteins was visible.