Summary

Lensoside Aβ (quercetin 3-O-[6-OE-caffeyl)-β-D-glucopyranosyl(1→2)]-β-D-galactopyranoside-7-O-β-D-glucuropiranoside) is a derivative of quercetin and belongs to flavonoids, compounds commonly found in plants. Flavonoids have recently become the subject of numerous studies due to their different biological and pharmacological properties. *In vitro* and *in vivo* studies confirm their positive effect, both at the cell and the whole organism level. They exhibit antioxidant, anti-inflammatory, anti-atherosclerotic, anti-allergic and other activities. Their anticancer activity is particularly important. One of the targets for flavonoids are cell membranes. At the same time, lensoside Aβ is a new compound isolated from the aerial parts of edible lentil (*Lens culinaris*) cultivar *Tina*. So far, there is very little data about secondary metabolites isolated from the leaves and stems of this plant.

The main aim of the doctoral thesis was to determine the ability of lensoside Aβ to interact with membranes (both with membrane lipids and proteins), to determine how this compound interacts with membranes, to investigate its localization and orientation in membranes, and to evaluate its effects on the cells. In order to explain how this flavonoid interacts with membranes and to confirm the influence of these interactions on normal and cancer cells, various research methods were applied such as: Fourier transform infrared absorption spectroscopy (FTIR), nuclear magnetic resonance (^{1}H NMR), differential scanning calorimetry (DSC), confocal fluorescence lifetime imaging microscopy (FLIM), patch clamp, transmission and scanning electron microscopy (TEM, SEM), light microscopy, fluorescence microscopy and flow cytometry. In the experiments multilayer vesicles (MLV), giant unilamellar vesicles (GUV) made of dipalmitoylphosphatidylocholine (DPPC) and *in vitro* cell cultures: cervical cancer (HeLa), laryngeal cancer (Hep-2) lines and human skin fibroblast line (HSF) were used.

The studies have shown that lensoside Aβ significantly affects on model membranes formed with DPPC. The tested compound incorporates into membranes and binds to the lipid polar head groups by hydrogen bonds between the C – O – P – O – C segment and hydroxyl groups of flavonoid.

Moreover, ^{1}H NMR and DSC analysis showed that the tested flavonoid has ordering effect on DPPC membranes and changes their physical properties. A slight decrease of the

main phase transition temperature, the disappearance of the pretransition and a decrease in the calorimetric enthalpy and the co-operativity of the transition were found. FLIM investigation has revealed roughly parallel orientation of the examined compound with respect to the membrane. To determine the modulating effect of tested flavonoid on membrane transport proteins, patch-clamp technique was used. Lensoside Aβ has changed the activity of potassium channels in human skin fibroblasts, which resulted in their increased opening.

FTIR analysis confirmed that the tested compound, interacts with both proteins and lipids of the cells at the molecular level. The studied flavonoid interacts with the cell lipids in the region of the choline heads of phospholipids in HeLa and Hep-2 cells via the formation of hydrogen bonds with the $-PO_2^-$ groups and the C-O-P-O-C segment. The lensoside Aβ clearly affects the spectral profile of proteins from the amide I spectral zone, which was manifested by changes in their secondary structure and reduction in their amount. On the other hand, it acts differently on normal cells of the HSF line by inducing the production of additional proteins and a slight ordering effect of the lipid fraction of the polar head groups region.

Results obtained by the neutral red and the LIVE/DEAD tests have revealed a cell line - dependent reduction in cell viability. Lensoside Aβ has been shown to have little effect on cell survival. It has the greatest effect on human cervical cancer cells. Its cytotoxic effect is correlated with the induction of apoptosis in HeLa and Hep-2 cancer cells whereas human skin fibroblasts are not sensitive to the cytotoxic effect of the tested flavonoid.

Cell analyses with application of transmission and scanning electron microscopy as well as light and fluorescence microscopy have shown the effects of lensoside Aβ on cancer cells. Changes in their shape, surface, morphometric parameters and ultrastructure were observed. Cells had features characteristic of apoptotic cells, such as: shrinkage of cells and nuclei, reduction or loss of microvilli, fragmentation of chromatin, strong vacuolation of cytoplasm, presence of autolysosomes, presence of membrane connections and the appearance of apoptotic bodies. At the same time, lensoside Aβ did not change the morphology and ultrastructure of normal human skin fibroblast cells.

The final stage of the research involved determining the effect of LAβ on the induction of oxidative stress in the cells. Studies have shown a dualistic mode of action of the tested compound. The flavonoid has induced oxidative stress in HeLa and Hep-2 cells and at the same time showed an antioxidant effect on normal human skin cells.

In conclusion, the results included in this dissertation, confirmed the influence of lensoside Aβ on cell membranes. By interacting with proteins and lipids of the cells, including the membrane lipids, the flavonoid changes their dynamic and structural properties. As a result, of this interaction, changes in a morphology and ultrastructure of cancer cells, which is mostly consistent with the apoptotic cell phenotype, are being observed. Simultaneously, the examined compound does not induce apoptosis in normal cells, does not also exhibit cytotoxic activity against them and enhances their protein synthesis. Such properties of lensoside Aβ make it a potential therapeutic agent, used alone or in combined therapy.

Key words:

flavonoids, liposomes, cancer cells, electron microscopy, FTIR

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