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Tytuł pracy: *„Ocena apoptozy w procesie starzenia kory móżdżku u myszy niewytwarzających interleukiny 6”*

SUMMARY

Aging of multicellular organisms is the universal process that exists commonly. Humans are also affected by it. In the last century, the average life span increased significantly and number of aging people triplicates. It is projected that by the year 2025 20% of the population will be over the age of 65. Increasing with age and in neurodegenerative diseases IL-6 expression was recognized as an accelerator of senescence, and occurring with aging symptoms were connected with neuronal loss. Recently, it was indicated that 80% of CNS neurons are located in the cerebellum, and that Purkinje cells constitute the most vulnerable cell population susceptible to changes taking place in senescence.

Because degenerating cells are removed from tissues by apoptosis, the aim of this study was to evaluate whether inherited lack of IL-6 may have an influence on apoptotic cell death of cerebellar cortex and on mechanisms involved in regulation of this process.

Forty male 4- and 24-months old C57BL/6J $^{IL6-/-tm1Kopf}$ (IL-6 KO) and C57BL/6J (WT) mice were used in the study. Animals were obtained from the Center of Experimental Medicine of the Medical University of Bialystok and were originally purchased from Jackson Laboratories (USA).

Histological examination was performed on paraffin sections stained with hematoxyline and eosin (H+E). Apoptosis was evaluated by TUNEL method and by immunohistochemical assessment of active form of caspase-3 protein. Cellular localization of astrocytes’ GFAP protein was estimated by immunofluorescence. Moreover, expression of proteins involved in regulation of apoptosis: p53, MDM-2, Bax, and Bcl-2, as well as GFAP protein was evaluated by means of Western blotting.

Performed experiments indicated, that cerebellar mass was comparable in 4-month old animals of both genotypes. Since in the cerebellar mass of 24-months old WT mice an significant decrease, and in IL-6 KO animals similar increase was observed, the difference between these groups reached the level of statistical significance.

There were no changes in the morphological picture of cerebellum in young animals of both genotypes in H+E staining. However in 24-months old animals degeneration and loss of Purkinje cells was observed. In many of Purkinje cells shrinkage of perikarion and loss of characteristic

shape was present. The cytoplasm of these cells was intensively stained with eosin and in some of them the nucleus was pycnotic. These changes were more pronounced in 24-month old WT than in age related IL-6 KO animals.

Because aging is associated with hyperplasia and hypertrophy of glial cells, evaluation of glial activation was performed by use of immunofluorescence method with antibody directed against present in astrocytes' GFAP protein. While, in young animals of both genotypes the immunofluorescence of GFAP observed in cell bodies of astrocytes and their processes was very week, while in cerebellar cortex of old animals it was very strong, especially in WT animals. This result was supported by Western blotting that revealed significant increase of GFAP protein in cerebellum of 24-months old WT mice in comparison to genotype-related young ones and insignificant increase in 24-months old IL-6-deficient mice as compared to young WT animals. Moreover, in perykarions of Purkinje cells and accompanying them Bergmann glial cells in old animals of both genotypes autofluorescence of lipofuscin granules was observed.

For the evaluation of the significance of apoptosis in age-related loss of Purkinje cells TUNEL method was applied. Unexpectedly, there were no TUNEL-positive cells in cerebellar cortex of young animals and only single TUNEL-positive cells in molecular layer of 24-months old animals. Low apoptotic activity of cerebellar cortex was in accordance with lack of caspase-3 activity in neurons of all cerebellar layers evaluated by immunohistochemical method. This result indicate that for observed in aging Purkinje cell loss other than apoptosis mechanisms are responsible.

Moreover, evaluation of the expression of proteins involved in process of apoptosis by means of Western blotting method was performed. Among these proteins were tested: activated by cell injury p53 protein, MDM-2 protein - a negative regulator of p53, pro-apoptotic protein Bax and anti-apoptotic protein Bcl-2. Obtained results indicate significant increase of p53 protein in 24-months old animals of both genotypes, in comparison to genotype related young animals, without any change in MDM-2 protein expression. Although the significant increase of p53 protein expression in 24-months old mice of both genotypes as compared to genotype-related young mice was observed, in old IL-6 KO mice this expression was significantly lower than in old WT animals. Moreover, only slight increase in Bax and Bcl-2 protein expression in cerebellar cortex of old animals was observed.

Conclusions:

1. Apoptosis does not play a significant role in Purkinje cell loss present in physiological aging, because in cerebellar cortex of 24-months old animals of both genotypes only single TUNEL-

positive cells were observed, accompanying by slight increase of Bax protein expression and absence of active form of caspase-3, in spite of significant increase in p53 protein expression.

2. IL-6 deficiency protects against connected with aging neuronal accumulation of pathological changes, what was indicated by less prominent Purkinje cells neurodegeneration and significantly lower p53 protein expression in cerebellar cortex of 24-months old IL-6 KO mice in comparison to age related WT animals.
3. IL-6 deficiency decreases activation of glial tissue present in aging, what was indicated by less intensive immunofluorescenece of astrocytes GFAP protein in cerbellar cortex of 24-months old IL-6 KO mice, as compared to age related WT animals, supported by its significant lower expression.
4. Results of present study indicate that IL-6 deficiency inhibits development of age-related pathological changes in cerebellar cortex evoked by chronic neuroinflammation.