ABSTRACT

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Department of Biochemical Sciences

Candidate: Bc. Gabriela Kocurová

Supervisor: Prof. MUDr. Jaroslav Dršata, CSc.

Title of diploma thesis: Investigation of proteolytic enzymes expression in different tissues at the transgenic animal model of Huntington´s disease by means of biochemical and immunohistochemical methods

Background: Huntington's disease (HD) is a neurodegenerative disorder that is caused by an expansion of a polyglutamine (polyQ) domain in the huntingtin (Htt) protein. Because it is known that mutant Htt and especially its small proteolytic fragments are toxic to neurons (particularly those in the striatum and cortex), it has been suggested that proteolysis of mutant huntingtin (mHtt) might play an important role in HD pathogenesis. Therefore, the aim of the present study was to examine the expression of endogenous and mtHtt and possible participation of the proteolytic enzymes from the group of caspases, matrix metalloproteinases (MMPs), kallikreins (KLKs) and calpains in HD pathology of brain tissue.

Methods: In this study we used WT and TgHD minipigs for N-terminal part of the human mtHtt (548aaHTT-145Q, both F2 generation, age 36 months; F3 generation, age 48 months in additional experiment), R6/2 mice were used as positive controls. Htt and proteases were examined immunohistochemically (IHC) and by immunofluorescence (IF) on cryostat sections, or biochemically by Western blotting (WB) using the following primary antibodies: anti-BML-PW0595, anti-EPR5526, anti-MAB2166, anti- 1C2, anti-3B5H10, anti-MW8, anti-caspase-3, anti-caspase-8, anti-MMP-9, anti-MMP- 10, anti-KLK-10, anti-calpain-5.

Results: Endogenous and transgenic Htt was detected in the brain sections of TgHD minipig (F2 generation, 36m), as well as the presence of small proteolytic fragments was confirmed. IHC revealed formation of aggregates of mtHtt. Biochemical detection of proteases in the TgHD minipig brain showed higher levels of caspase-3, MMP-9 and its multiple proteolytic cleavage products (generated from mHtt). Using IHC and WB, we demonstrated significantly increased expression of caspase-3 in nucleus caudatus and cortex area of TgHD minipigs in comparison to WT animals. Immunohistochemically detected MMP-10 level was very weak in all animals studied (TgHD and WT) with small differences between them. Increased levels of MMP-9 were observed by IF in retinal pigment epithelial cells (RPE) of TgHD minipig (48m). In contrary to that the most proteolytic enzymes revealed the same or increased expressions in TgHD brains, the decreased expression of kallikrein-10 was detected in these brains in comparison to WT brains.

Conclusion: Due to different expression of proteolytic enzymes in TgHD brains further studies are necessary to clarify the exact role of these enzymes in etiology of HD.

Key words: proteolytic enzymes, transgenic animal model, Huntington's disease, biochemistry, immunohistochemistry