Abstract

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Title of Diploma Thesis: Immunohistochemical analysis of endoglin expression and biomarkers of inflammation during liver fibrosis after Carotuximab administration

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Background: The aim of this diploma thesis was to analyze changes in the expression of endoglin and markers of inflammation VCAM-1 and ICAM-1 in liver in an experimental mouse model of liver fibrosis, which was induced by administration of a diet containing 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-collidine (DDC). Another aim was to elucidate the effect of carotuximab administration on the expression of these markers after fibrosis induction, and to determine whether carotuximab administration effects the process of liver fibrosis by endoglin inhibition.

Methods: The experimental mice were divided into three groups – the control group, which was fed a standard (chow) diet, the DDC group, which was fed a DDC-supplemented diet, and the DDC/TRC105 group, which was administrated by carotuximab along with the DDC diet. Liver tissue samples from all three groups were subjected to histochemical staining to detect steatosis (light microscopy) and indirect immunohistochemical analysis to detect endoglin, VCAM-1 (light microscopy) and ICAM-1 (fluorescence microscopy) expression. In addition, immunofluorescence detection of endoglin and VCAM-1 colocalization was performed.

Results: The results of this diploma thesis show that the DDC diet induced the development of liver fibrosis and inflammation without the participation of endoglin. Endoglin expression was reduced in the DDC and DDC/TRC105 groups compared to the control group. The development of inflammation was confirmed in the DDC and DDC/TRC105 groups by an increased expression of ICAM-1 in endothelial cells and VCAM-1 in cholangiocytes. Endoglin and VCAM-1 colocalization was not proved. Carotuximab administration did not affect the changes in endoglin, ICAM-1 nor VCAM-1 expression.

Conclusion: The development of inflammation has been demonstrated in the DDC and DDC/TRC105 groups by detecting the expression of the inflammatory markers ICAM-1 and

VCAM-1. The decrease of endoglin expression in the liver tissue of animals treated with DDC is probably due to the direct action of DDC, regardless the development of fibrosis. Thus, carotuximab administration did not affect the development of fibrosis and the expression of the observed markers, as endoglin, the target of carotuximab, was already reduced by DDC administration alone. Therefore, the use of an experimental DDC model is not suitable for investigating the effect of carotuximab in the development of liver fibrosis.

Key words: carotuximab (TRC105), endoglin, ICAM-1, fibrosis, immunohistochemistry, inflammation, non-alcoholic fatty liver disease (NAFLD), VCAM-1