Abstract

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Title of master thesis: Immunohistochemical analysis of TRC105 effects on the liver fibrosis

The subject of this diploma thesis was the analysis of the expression of liver fibrosis biomarkers in mice liver. The aim of our work was to test the effect of the drug TRC105 (carotuximab) on a fibrotic liver model after 4 weeks of DDC (3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-colidine) administration. Collagen deposition and expression of α-SMA, GFAP and endoglin were examined and compared. Animals were divided into 3 groups: control group (n=6), DDC group (n=6) and TRC105 group (n=6). The control group was fed a standard diet, while the DDC and the TRC105 groups were fed a diet with 0.1% DDC. Physiological solution was administered to the control and DDC groups while carotuximab was administered to the TRC105 group. The analysis of fibrosis biomarker expression was then performed on fixed liver sections. Collagen expression was evaluated by Picro-Sirius Red staining and α-SMA, GFAP and endoglin expression by immunohistochemistry with fluorescence imaging.

Histological staining of collagen fibers with Picro-Sirius Red showed that the DDC diet induced the development of liver fibrosis. Greater staining intensity compared to the control group was observed in the DDC and TRC105 groups. There was no significant difference between the staining of collagen fibers in the DDC and TRC105 groups. In the case of immunohistochemical detection of markers α-SMA and GFAP, the intensity of their expression was similar in both DDC and TRC105 groups. Endoglin expression was reduced in the DDC and TRC105 groups.

The comparable intensity of expression of collagen fibers in histological staining and the similar expression of α-SMA and GFAP markers in DDC and TRC105 groups in immunohistochemical staining, point to the ineffectiveness of the drug TRC105 for preventing liver fibrosis. The reduced expression of endoglin in the DDC and TRC105 groups may suggest that endoglin is not a key factor involved in the development of liver fibrosis**.**

The results of our work show the induction of liver fibrosis by DDC, but the expected decrease in area of fibrotic tissue did not occur after the administration of carotuximab. Surprisingly, we did not observe changes in the expression of markers that characterize cells involved in the development of liver fibrosis between the groups. The reduced expression of endoglin in DDC and TRC105 groups may indicate that endoglin does not play a key role in the process of liver fibrosis. Our results need to be verified by other methods such as Western Blot analysis.

Key words

Liver fibrosis, Collagen, TRC105, α-SMA, GFAP, Endogline, Imunohistochemistry