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Abstracts
Poster Abstracts

36

Another challenge for personalized therapy: Efficacy of therapeutic influence on Peroxisome Proliferator Activated Receptor-gamma is determined by PPARG Pro12Ala gene polymorphism

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Introduction: Multiple studies showed that Peroxisome Proliferator Activated Receptor-gamma – NR1C3 (PPARG) plays an important role in various biological processes including lipid and glucose metabolism. PPARG agonists have been used in treatment of different metabolic disorders and non-alcoholic steatohepatitis (NASH) decreasing steatosis, inflammation, and fibrosis.
The aim of the study was to clarify the perspectives for individualized therapy with thiazolidinediones.

Methods: 249 patients with hypertension, dyslipidemia, metabolic syndrome participated in the study. Among them 50 patients with NASH were selected to form study group. PPARG agonist Pioglitazone administered 30 mg daily during 50–51 weeks. Genetic polymorphism (Pro12, Pro12Ala, Ala12Ala) of PPARG gene determined by PCR. Genotypes were: Pro12 (n = 32, 64.0%); Pro12Ala (n = 14, 28.0%); Ala12 (n = 4, 8.0%) Liver biopsies performed prior and after study.

Results: Pioglitazone improved glycemic control and glucose tolerance ($p < 0.001$), normalized liver aminotransferase levels as it decreased AST by 42.1 ± 1.17% $p = 0.014$; ALT by 57.5 ± 1.37%, $p < 0.001$; decreased hepatic fat by 54.6 ± 2.09%, $p < 0.001$; and increased hepatic insulin sensitivity by 48.5 ± 1.63% $p = 0.006$. Administration of pioglitazone caused improvement in histologic findings with regard to steatosis, ballooning necrosis, and inflammation. In 4 (8%) Ala12 patients no reliable changes were observed, except glycemic control and glucose tolerance.
Reduction in fibrosis did not change significantly. Statistically insignificant weight gain and mild lower-extremity edema developed in 2 subjects with Pro12Ala genotype, no other side effects were observed.

Discussion/Conclusion: Administration of thiazolidinediones leads to metabolic and histologic improvement in most patients with NASH. However, individual response may be affected by Pro12Ala polymorphism of PPARG gene. This study shows that carriers of Ala genotype whilst comparatively rare among NASH patients are much less sensitive to PPARG agonists' therapy.

Lipopolysaccharide induces neovascularization and immunosuppression, and must be considered as therapeutic target

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Introduction: Previous studies showed that endotoxin – lipopolysaccharide (LPS) is both angiogenic and immunosuppressing, thus promoting metastatic growth (MG). However, the role of LPS as a therapeutic target is unclear. We hypothesized that anti-LPS therapy may decrease MG.

Methods: Murine model including 3 groups (25 each) of adolescent mice was used. Metastatic process was modeled by i/v injection of 200 µl spontaneously metastasizing mammary adenocarcinoma cell culture suspension. Control group (CG) animals received 200 µl sterile saline intraperitoneal (i.p.), experimental group 1 (EG1) – 200 µl suspension of 10 µg LPS per mouse, experimental group 2 (EG2) – same plus 20 µg at 0.5 ml anti-LPS monoclonal antibodies. MG evaluated hystochemically within lung metastases.

Results: EG1 showed significantly higher ($p < 0.001$) MG compared with the control. MG was characterized by 61.2% higher mitotic index (MI) in the EG1 and 42.3% lower apoptotic index (AI). MI/AI ratio in the EG1 was 3.2 times higher ($p < 0.001$) than control. LPS injection resulted in reliably ($p = 0.002$) higher levels of serum VEGF than in control with strong positive correlation ($r = 0.971$) between circulating VEGF and LPS levels. Addition of anti-LPS monoclonal antibodies significantly decreased MG, MI and increased AI with respective change of MI/AI ratio. VEGF becomes insignificantly higher than in control whilst LPS concentration decreased reliably ($p = 0.014$).

Discussion/Conclusion: Despite the well-established role of LPS as pro-inflammatory, pro-proliferator and pro-neovascularization factor, its role in carcino-genesis remains under evaluated.
Our findings show that targeted anti-LPS therapy may impact tumor growth due to prevention of neovascularization and inflammation as well as inducing apoptosis.

38

Selective inhibition of cyclooxygenase-2 in vitro induces apoptosis and decreases proliferation of the human liver tumor cell

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Introduction: Recent studies showed that selective cyclooxygenase-2 (COX-2) inhibitors suppress growth of colon cancer cells and have chemopreventive potential during colon cancerogenesis. However, it is still debatable whether COX-2 contributes to the malignant growth and whether inhibition of COX-2 modifies the malignant potential of liver tumors. The aim of the study was to clarify the pro-apoptotic and anti-proliferative effect of selective COX-2 inhibition.

Methods: HCC cells lines Hep G2/Hep 3B were cultivated in modified media seeded onto well plates. Celecoxib 50 μmol/l added in study group cultures. Apoptosis related cytokines were analyzed by Western blotting. Apoptotic nuclei were visualized with the TUNEL-staining protocol and cells viewed with a fluorescence microscope (magn. x400). The number of apoptotic cells calculated in percentage of total nuclei.

Results: COX-2 inhibition related changes become evident in Hep G2/Hep 3B cell lines after 48 hours of treatment leading to a significant time-dependent reduction of cell numbers of up to 80% ($p < 0.05$). Cells became sparse, rounded, and detached from the dishes representing morphologic signs of apoptosis. This correlated with activation of caspase-9, caspase-3, and caspase-6 cytokines. However, exposure of cell cultures to 3 g/ml PgE_2 eliminated the COX-2 inhibiting and pro-apoptotic effect on cells. This indicates that the antineoplastic properties of COX-2 inhibiting are dependent on reduces conversion of arachidonic acid to PGE_2 attributable to COX-2 inhibition.

Discussion/Conclusion: Selective inhibition of COX-2 causes marked growth inhibition of human liver tumor cells, based on the induction of apoptosis and inhibition of proliferation. The mechanism by which COX-2 inhibiting-related apoptosis is realized is still unclear as well as involvement of other factors into antiproliferative effect of COX-2 inhibitors.

40

Expression and function of Atrophin 2 in chronic liver disease

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Introduction: The atrophin gene family has been characterized after identification of atrophin as the cause of dentatorubral-pallidoluysian atrophy. Atrophin family proteins have been identified as nuclear receptor corepressors. They are implicated in the regulation of various biological processes including migration and orientation as well as apoptosis. Furthermore, altered atrophin expression has been shown in neurodegenerative disease and cancer. Atrophin 1 is not essential, whereas Atrophin 2 (ATN2) is crucial for normal mouse embryonic development.

The aim of this study was to analyze the expression and function of ATN2 in chronic liver disease.

Methods and results: Hepatic ATN2 expression was significantly increased in different murine models of chronic liver injury (chronic CCl_4 injury or dietary models of non-alcoholic steatohepatits [NASH]). Furthermore, we observed increased ATN2 expression in hepatic tissue of patients with NASH or liver cirrhosis of different origin. Moreover, ATN2 was significantly increased in primary murine and human hepatic stellate cells (HSC) during the course of *in vitro* activation. By transient transfection with siRNA we achieved more than 50% reduction of ATN2 in activated HSCs, and functional analysis revealed effects on attachment and proliferation. Because of the known association of ATN2 with cancer we analyzed ATN2 expression also in different human HCC cell lines and tissues and ascertained a marked upregulation compared to primary human hepatocytes and non-tumorous tissue.

Conclusion: Our data indicate ATN2 as a functionally relevant transcriptional regulator in activated HSCs. Furthermore, increased expression in HCC cells suggests that ATN2 also affects tumorigenicity. Herewith, ATN2 appears as a potential therapeutic target to inhibit the fibrosis and cancerogenesis in chronic liver disease.