Adrenic acid as an inflammation enhancer in non-alcoholic fatty liver disease

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学 位 論 文 の 内 容 要 旨

Adrenic acid as an inflammation enhancer in non-alcoholic fatty liver disease

アドレン酸は非アルコール性脂肪性肝疾患において炎症促進因子として作用する

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Epidemiological studies have indicated that the prevalence of non-alcoholic fatty liver disease (NAFLD) is rising in both Western countries and other regions of the world. A subset of NAFLD patients, which are considered to have non-alcoholic steatohepatitis (NASH), are at increased risk of liver cirrhosis, hepatocellular carcinoma, and liver-related mortality. Therefore, the early identification of NASH is very important for preventing liver complications. Disease progression in NAFLD is a complex process involving several factors, including lipid metabolism. Despite extensive investigation of the molecular mechanisms responsible for lipid-related liver damage in NAFLD, the available quantitative data about the use of lipid profiles for differentiating steatohepatitis from simple steatosis are still inconclusive. So far, lipidomic studies have suggested that at least 500 lipid species are present in plasma, and over 1,000 lipid species are found within cells. Hence, we hypothesized that there might be other lipid species that are pathologically related to NAFLD, and some of them might be useful for differentiating steatohepatitis from simple steatosis. Thus, our aim is to identify novel links between lipid species and the progression of NAFLD.

In this study, we first examined the lipid profiles of genetically obese db/db mice and used a choline deficient-L-amino acid defined-high fat diet (CDAHFD) to induce steatohepatitis in the mice. The CDAHFD induced hepatic steatohepatitis and fibrosis, which are comparable to the pathology of human NASH, in db/db mice. CDAHFD-fed db/db mice also showed obesity and hypercholesterolemia, two metabolic features commonly found in human NAFLD. The mRNA expression level of ACOX1 (peroxisomal beta-oxidation) was decreased in CDAHFD-fed db/db mice and there were no significant changes in mRNA expression of CYP2E1 and CYP4A14 (microsomal omega oxidation) compared to standard diet (SD)-fed db/db mice. The mRNA expression level of FAS (de novo lipogenesis), FATP2, FATP5 (fatty acid uptake) were also decreased but no significant changes in mRNA levels of MCAD, LCAD (mitochondria beta oxidation) and MTTP (triglyceride export). The mRNA levels of GPx1 (antioxidative enzyme) was decreased, while UCP2 (mitochondria uncoupling protein) increased. Overall, these data suggest that liver steatosis in our steatohepatitis model could be due to impaired peroxisomal beta-oxidation while liver injury in part related to reactive oxidative stress (ROS).

Lipid analysis was performed using liquid chromatography/mass spectrometry (LC/MS). LC/MS can profile lipid species accurately from a small minimally pre-treated sample within a short period of time. Based on LC/MS analysis, CDAHFD-fed db/db mice

showed significantly higher hepatic and plasma levels of free adrenic acid ($p<0.05$). Because adrenic acid is less examined in NAFLD, we next focused on elucidating its potential role in the progression of the disease. The higher free adrenic acid was not related to higher intake because the diet did not contain adrenic acid. Therefore, we analyzed mRNA expression of ELOVL2 and 5, two enzymes responsible for elongation of arachidonic acid to adrenic acid. Basal expressions of ELOVL2 and 5 were lower in SD-fed db/db mice but increased in CDAHFD-fed db/db mice. Combining these results with gene expression analysis of steatosis-related genes, we concluded that increased level of free adrenic acid in CDAHFD-fed db/db mice was due to impaired peroxisomal beta-oxidation and enhanced elongation of arachidonic acid.

The mRNA level of CD68, a marker for monocyte/macrophage, was increased in livers of CDAHFD-fed db/db mice. This suggests that there is a recruitment of inflammatory cells. Chemokines can attract immune cells to inflammatory sites. Therefore, we hypothesized that adrenic acid could influence disease progression by enhancing chemokine gene expressions. Using HepG2 as a hepatocyte model we found that under treatment with recombinant human (rh) TNFα, adrenic acid-pretreated HepG2 cells expressed higher mRNA levels of TNFα, IL8, MIP1β and MCP1. The similar findings were observed after treatment with rhIL1β, except for MCP1. Therefore, the upregulating effect of adrenic acid on MCP1 mRNA expression during inflammation is cytokine-specific. The inflammatory activities of TNFα and IL1β are mediated by type 1 TNF receptors (TNFR-1) and type 1 IL-1 receptors (IL1R-1), respectively. On the other hand, type 2 IL-1 receptors (IL1R-2) act as "decoy receptors" and inhibit IL1β activity The mRNA levels of TNFR-1 in adrenic acid-pretreated HepG2 cells was not upregulated, while mRNA levels of IL1R-1 were significantly decreased under cytokine stimulation. On the other hand, mRNA levels of IL1R-2 were increased. These results indicated that: (i) negative regulation by IL1R-2 inhibits IL1β-induced inflammation, and (ii) adrenic acid might modify TNFα or IL1β signaling pathways at the post-receptor level. Overall, from invitro experiment, we concluded that adrenic acid could enhance chemokine gene expressions.

We next confirmed our experimental findings in the setting of human NAFLD. The previous study showed that changes in the serum ALT levels of NASH patients were correlated with liver inflammation. Therefore, we divided our patients into three groups based on their ALT levels (different cut-off values were employed for males and females). In our data analysis, we found that the mean plasma adrenic acid level was higher in group 3 (ALT $\geq$60 IU/L for

males, ≥40 IU/L for females) than in group 1 (ALT <30 IU/L for males, <20 IU/L for females) and group 2 (30 IU/L ≤ ALT < 60 IU/L for males, 20 IU/L ≤ ALT < 40 IU/L for females). These intergroup differences and the associated linear trend were statistically significant. Overall, these findings support our in vitro results. We also obtained a similar finding with regard to the plasma level of docosapentaenoic acid (22:5n6) in our human data set. Since the formation of docosapentaenoic acid (22:5n6) involves β-oxidation in peroxisomes, this might indicate that our patients had mild disease that did not involve significant changes in peroxisome β-oxidation, as shown by the mouse model. The limitations of our human sample analysis include the small number of patients, the lack of histological examinations, and the fact that it only included patients from one medical center.

In conclusion, the combination of increased polyunsaturated fatty acids and elongase expression and impaired peroxisomal β-oxidation cause the accumulation of adrenic acid in experimental steatohepatitis, but not in simple steatosis. Under inflammatory conditions, adrenic acid might exacerbate inflammation by enhancing the expression of chemokine genes in hepatocytes. Increased plasma adrenic acid levels are associated with high ALT levels in NAFLD patients. Taken together, adrenic acid accumulation contributes to disease progression in NAFLD. A further study is needed to determine whether adrenic acid measurements could be used as a diagnostic biomarker of steatohepatitis in NAFLD.