Adrenic acid induces oxidative stress in hepatocytes

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学位論文の内容要旨

Adrenic acid induces oxidative stress in hepatocytes

肝細胞においてAdrenic acidが酸化ストレスを誘導する

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Background:

Nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver diseases worldwide. As its progressive phenotype, nonalcoholic steatohepatitis (NASH) is characterized by inflammation, hepatocellular damage, and varying degrees of fibrosis, and may progress to cirrhosis in up to 25 % of patients. Despite the increasing prevalence and severe prognosis of NASH, its pathogenesis has not been fully understood, and the effective therapeutic strategies are still limited. Nowadays, lipotoxicity, which is defined as the accumulation of intracellular toxic lipids leading to cellular dysfunction and death, is widely considered as a pivotal mechanism of NASH, and drive liver cell injury through activating oxidative stress, apoptosis, ER stress and inflammation. In our previous studies, we examined the lipid profiles in NAFLD patients, and it was found that the level of adrenic acid (22:4n6) (ADA) was significantly increased in plasma of NAFLD patients compared with the corresponding controls. In addition, the same alterations in ADA levels were observed in NAFLD model mice. These results suggest the associations between ADA and NAFLD. In the cells, ADA, which is an endogenously synthesized polyunsaturated free fatty acid, is formed through a 2-carbon chain elongation of arachidonic acid (20:4n6) (AA) by elongase enzymes referred to as elongation of very long chain fatty acids protein (ELOVL) 2 or 5. The current study was designed to examine ADA-induced lipotoxicity in human hepatocarcinoma HepG2 cells, and further to investigate the relationship between ADA and AA in hepatocytes.

Methods:

HepG2 cells were treated with ADA or AA, and then were subjected to measurement of fatty acids by liquid chromatography/mass spectrometry. mRNA were collected to test oxidative stress markers such as Gpx1, SOD2 and HO-1 using qRT-PCR. Cell viability and endogenous ROS were also assessed by Cell Counting Kit-8 and DCFDA cellular ROS detection assay kit, respectively. In the transfection experiments involving siRNA targeting ELOVL 2 and 5, HepG2 cells were incubated with medium containing 10 nM siRNA- Lipofectamine RNAiMAX mixture diluted with Opti-MEM.

Results :

1.ADA decreased cell viability and induced oxidative stress in HepG2 cells.

ADA decreased cell viability in a dose-dependent manner. Viable cell amounts reduced dramatically at the concentration of ADA with more than 50 μM, particularly to only around 30% of the cells surviving at 500 μM ADA treatment. The ADA treatment significantly increased the ROS production. NAC, which is a commonly used antioxidant, could mitigate ADA-induced cell damage and oxidative stress.

2.AA decreased cell viability and induced oxidative stress in HepG2 cells.

AA caused cell damage in a dose-dependent manner, and remarkably stimulated the ROS production. NAC could decrease the AA-induced cell death and ROS production.

3.AA stimulated the ADA production through ELOVL2 or ELOVL5 in HepG2 cells.

In HepG2 cells treated with AA, the intracellular ADA levels were increased to 36.2 fold, and the increased ratio of ADA was more than that of AA. At the knockdown experiments, the abundance of ADA was decreased by 44% in ELOVL2 knockdown, 63% in ELOVL5 knockdown, and 61% in knockdown of both ELOVL2 and 5. These results indicate that ADA is produced by ELOVL2 and 5 in HepG2 cells.

4.ADA modulated antioxidant enzymes, but not NF-E2-related factor 2 (Nrf2) and Kelch-like ECH-associated protein 1 (Keap1).

The treatment with 500 μM ADA increased mRNA levels of SOD2 and HO-1 to 1.5 and 1.7 folds, respectively. On the contrary, the Gpx1 mRNA level was decreased to 60%, and the NAC treatment inhibited the ADA-caused downregulation of Gpx1 mRNA. The Nrf2-Keap1 pathway is a major regulator of cytoprotective responses to oxidative stress, but mRNA levels of Nrf2 and Keap1 were not altered by the ADA treatment.

Discussion:

In our previous report, the levels of ADA was increased in NAFLD patients and NAFLD model mice, but it remains to elucidate how ADA is related to the pathogenesis of NAFLD/NASH. AA, from which ADA is produced by ELOVL2 and 5, also reported to induce oxidative stress. In this study, our goal was to clarify the physiological effects of ADA and AA on HepG2 cells.

Oxidative stress, which probably plays a primary role as the starting point of liver cell injury in NAFLD, is induced via an imbalanced condition between ROS and antioxidant defenses. ROS attacks lipid membranes to initiate membrane lipid peroxidation, resulting in structural and functional cellular damages including alterations in membrane permeability and fluidity, dysfunctionality of membrane receptors, and decreasing activity of membrane-bound enzymes. SOD and Gpx are first line defense antioxidants preventing or suppressing the formation of intracellular ROS. SOD acts to break down superoxide radical or singlet oxygen radical into hydrogen peroxide, and subsequently Gpx converts hydrogen peroxide to water. HO carries out antioxidant effects by not only enhancing the production of biliverdin and bilirubin, which are potent scavengers of singlet oxygen, but also increasing the reduced glutathione, SOD and catalase levels. In this study, exposure to ADA or AA notably caused the ROS production in HepG2 cells, indicating induction of oxidative stress. ADA also upregulated SOD2 and HO-1 expressions, suggesting that upregulations of antioxidant enzymes were induced to scavenge the produced ROS leading to protection of the cells from oxidative stress. In contrast to SOD2 and HO-1, the Gpx1 expression was downregulated, and this may mean that the ADA-increased ROS overwhelmed the antioxidant defenses. The Nrf2-Keap1 pathway is a major regulator of cytoprotective responses to oxidative stress. In this study, ADA did not affect Nrf2 and Keap1 mRNA expressions, suggesting that ADA's functions may not be involved in Nrf2 and Keap1. NAC, which is a precursor of glutathione, is a greatly applied antioxidant due to its ability to protect cells against oxidative stress. In this study, the NAC pretreatment could remarkably reverse the ROS production and cell death induced by ADA and AA, suggesting that ADA and AA enhance oxidative stress in hepatocytes.

Apoptosis is another important factor in the progression of NASH, and several inhibitors of apoptosis have been suggested as potential treatments for NASH. The ADA and AA treatment significantly reduced the number of viable cells in HepG2 cells. However, further researches are needed to clarify whether the ADA's and AA's cell death functions is due to apoptosis.

ADA is formed through a 2-carbon chain elongation of AA by elongase enzymes referred to as ELOVL2 or ELOVL5. AA treatment in HepG2 cells increased ADA abundance, and knockdown of ELOVL2 and 5 decreased

the ADA production, which suggest that ADA can be synthesized from AA through ELOVL2 or 5. Consistent with previous studies, AA stimulated oxidative stress followed by cell death. Based on these findings, we speculated that the ROS production and cell death induced by AA may be not only due to AA itself but also due to ADA synthesized from AA.

Conclusion:

In summary, we proved that ADA as well as AA promoted oxidative stress and cell death in HepG2 cells, which could be reversed by the NAC pretreatment, and ADA modulated antioxidant enzymes. Based on these results, this study provides new insights into the mechanisms underlying toxic fatty acid-based cell damages in hepatocytes. Hopefully, this will help us have a better understanding of lipotoxicity, which plays an important role in the pathogenesis of NAFLD/NASH.