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Acetaldehyde-derived Advanced Glycation End-products Promote Alcoholic Liver Disease

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Abstract

Background: Chronic ingestion of ethanol increases acetaldehyde and leads to the production of acetaldehyde-derived advanced glycation end-products (AA-AGE). We evaluated the cytotoxicity of AA-AGE on hepatocytes and examined the role of AA-AGE in the pathogenesis of alcoholic liver disease (ALD).

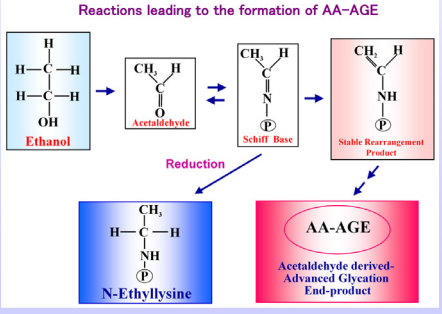
Methods: Rat hepatocyte cultures were treated with N-ethyllysine (NEL) or AA-AGE and the cell viability was evaluated using MTT assay. Male Wistar rats were fed with liquid diet containing 5% ethanol for 8 weeks followed with normal diet for 12 weeks. A group of animals were sacrificed at 4, 6, and 8th week and the remaining animals at 12, 14, 16, 18 and 20th week. The liver sections were stained for AA-AGE and 4-hydroxynonenal (4-HNE). Liver biopsy obtained from ALD patients was also stained for AA-AGE and 4-HNE.

Results: The hepatocyte viability was significantly reduced in cultures treated with AA-AGE compared to control cultures or with NEL. Severe fatty degeneration was observed during the chronic administration of ethanol increasing from 4-8 weeks. The staining of AA-AGE and 4-HNE was strongly correlated with the degree of ALD in both rat and human. In rats, hepatic fatty degeneration was completely disappeared and the staining for both AA-AGE and 4-HNE returned to normal at 12th week of abstinence. Staining for AA-AGE and 4-HNE was completely absent in normal human liver.

Conclusions: The data demonstrated that AA-AGE is toxic to hepatocytes, but not NEL. Chronic ethanol ingestion produces AA-AGE and reactive oxygen species (ROS) contributing to the pathogenesis of ALD. Abstinence of alcohol results in complete disappearance of both AA-AGE and 4-HNE suggesting that AA-AGE may play a significant role in the pathogenesis of ALD.

Introduction

The pathological role of nonenzymatic modification of proteins by reduced sugars such as glucose, a process which is known as glycation, has become increasingly evident in different diseases. It is now well established that early glycation products undergo progressive modification in vivo over time to form irreversible cross-links, after which these molecules are termed advanced glycation end-products (AGEs). AGEs have been implicated in the development of many of the pathological sequelae of diabetes and aging, such as atherosclerosis and renal insufficiency. It has become evident that AGEs also have a role in nuero-degenerative disorders such as Alzheimer's disease, cancer, and non-alcoholic steatohepatitis (NASH). Based on our studies, we proposed a pathway for the formation of acetaldehyde-derived advanced glycation end-products (AA-AGE) by the Maillard reaction in vivo. We hypothesize that NEL pathway for reaction of Amadori compounds could serve as a physiologically relevant mechanism for averting potentially damaging consequences of the AA-AGE formation.



Scheme 1. Pathway of reactions leading to the formation of NEL and AA-AGE during chronic ingestion of ethanol.

Figure 1

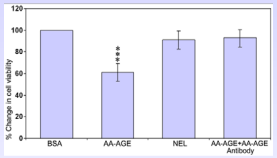


Figure 1. Effect of AA-AGE or NEL and AA-AGE antibody on viability of cultured rat hepatocytes. The 24 hr old hepatocyte cultures were treated with media containing 25 µg/ml of AA-AGE or NEL and incubated for 48 h. Viability of hepatocytes cultured with AA-AGE was significantly decreased compared with hepatocytes cultured with NEL. ***P < 0.001 compared to the NEL treated cultures by Student's *t*-test. Neutralization of AA-AGE with purified AA-AGE antibody resulted in complete prevention hepatocyte cell death induced by AA-AGE.

Figure 2

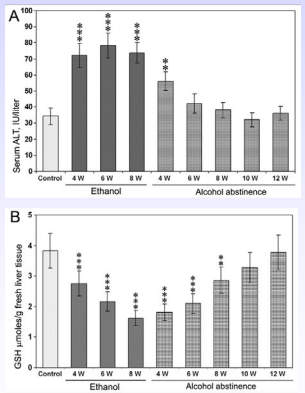


Figure 2A. Aspartate transaminase levels in rat serum after the start of chronic administration of alcohol up to 8 weeks and also after abstinence of alcohol. **P < 0.01 and ***P < 0.001 compared to the control group by ANOVA (n=5).

Figure 2B. Reduced glutathione (GSH) levels in liver during chronic administration of ethanol and after alcohol abstinence in rats. **P < 0.01 and ***P < 0.001 compared to the untreated control by ANOVA (n=5).

Figure 3

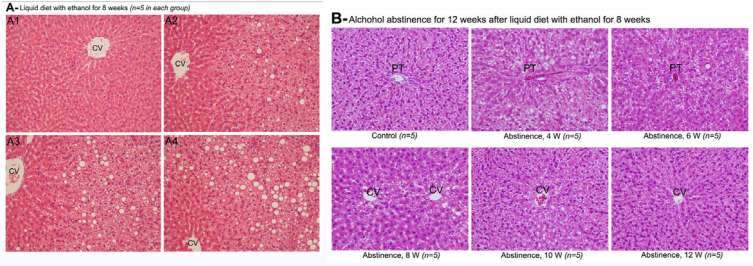


Figure 3. (A) Histopathological changes in rat liver during chronic administration of ethanol (H&E x100). (A1) No histopathological alteration was observed in rats received control liquid diet. (A2), (A3) & (A4) Animals received liquid diet containing ethanol for 4, 6 and 8 weeks, respectively. Hepatic fatty degeneration was observed in all animals in increasing order from 4–8 weeks. **(B) Alcohol abstinence 4–12 weeks (H&E x100).** There was no alteration in the liver of rats received control liquid diet for 20 weeks. Fatty degeneration was ameliorated from 4–12 weeks with complete disappearance of steatosis at 12 weeks. CV- central vein, PT- portal triad.

Figure 4

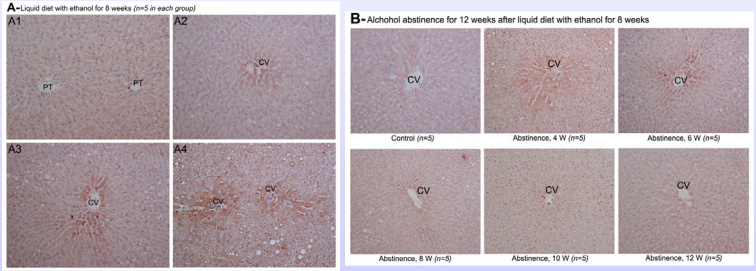


Figure 4. (A) Immunohistochemical staining for AA-AGE in rat liver during chronic administration of ethanol (x100). (A1) AA-AGE staining was completely absent in rats received control liquid diet. (A2), (A3) & (A4) Animals received liquid diet containing ethanol for 4, 6 and 8 weeks, respectively. Marked staining for AA-AGE in perivenular areas in increasing order from 4–8 weeks. **(B) Staining for AA-AGE during alcohol abstinence, 4–12 weeks (x100).** Staining for AA-AGE was absent in the liver of rats received control liquid diet for 20 weeks. Marked staining for AA-AGE was present in pericentral areas from 4–10 weeks of abstinence in sequentially decreasing order. The staining for AA-AGE was completely absent at 12 weeks of abstinence. CV- central vein, PT- portal triad.

Figure 5

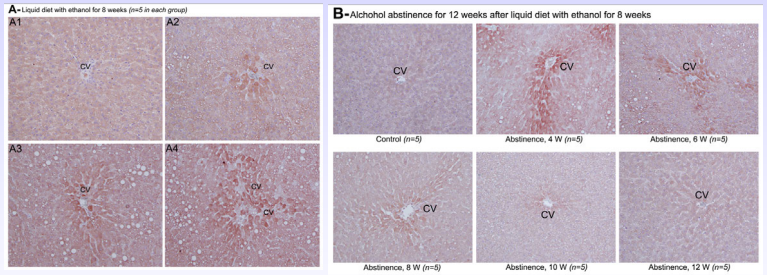


Figure 5. (A) Immunohistochemical staining for 4-HNE in rat liver during chronic administration of ethanol (x100). (A1) The staining for 4-HNE was completely absent in rats received control liquid diet. (A2), (A3) & (A4) Animals received liquid diet containing ethanol for 4, 6 and 8 weeks, respectively. Remarkable staining for 4-HNE in perivenular areas in increasing order from 4–8 weeks. **(B) Staining for 4-HNE during alcohol abstinence, 4–12 weeks (x100).** Staining for 4-HNE was absent in rats received control liquid diet for 20 weeks. There was marked staining for 4-HNE in pericentral areas from 4–10 weeks of abstinence in sequentially decreasing order. A staining for 4-HNE was completely absent at 12 weeks of abstinence. CV- central vein.

Figure 6

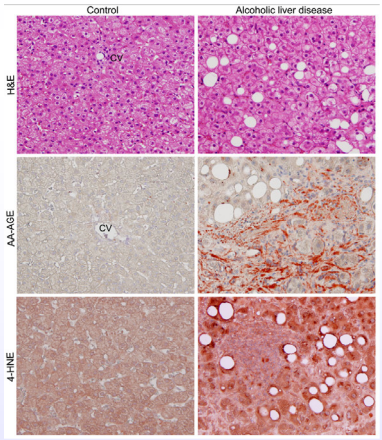


Figure 6. Histopathological (H&E) evaluation for steatosis and immunohistochemical staining for AA-AGE and 4-HNE in ALD patients liver biopsy (x200). H&E staining depicted marked fatty degeneration in all cases. Remarkable and conspicuous staining for AA-AGE and 4-HNE in the areas with severe fatty degeneration. Marked staining for AA-AGE and 4-HNE was present in areas without steatosis also. CV- central vein.

Figure 7

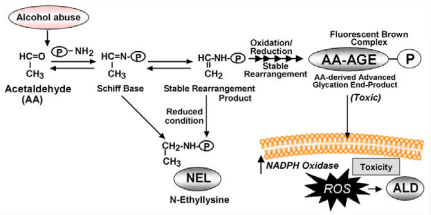


Figure 7. A schematic representation of formation of AA-AGE from acetaldehyde, production of ROS through membrane binding of RAGE and pathogenesis of ALD. This scheme was prepared based on our previous work and current data. *(J Neuropathol Exp Neurol 2003; 62:486-496).*

Conclusions

- **Treatment of rat hepatocyte cultures with AA-AGE resulted in 40% decrease of cell viability.**
- **Administration of liquid diet containing 5% ethanol for 8 weeks resulted in severe fatty degeneration in rat livers.**
- **Staining for AA-AGE and 4-HNE was strongly correlated with the degree of ALD in both rat and human livers.**
- **AA-AGE and 4-HNE levels along with fatty degeneration returned to normal at 12th week of alcohol withdrawal in rats.**
- **The complete disappearance of both AA-AGE and 4-HNE along with ALD after 12 weeks of abstinence suggests that AA-AGE and ROS play a significant role in the pathogenesis of ALD.**

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