

Occasional Alcohol Intake Promotes Nonalcoholic Steatohepatitis in Obese Rats

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Abstract

The pathogenesis of nonalcoholic steatohepatitis (NASH) has been hypothesized to be a two-stage process in which steatosis is the first insult, and an unknown second "hit". In order to investigate whether occasional alcohol intake could be a second hit to develop NASH from simple steatosis caused by obesity, 30 weeks old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats (620 ± 10 g), which spontaneously develop steatosis and diabetes from 30 weeks old, were administered 10 ml of 10% ethanol or water using gavage tube five times, thrice, twice or once per week for 3 weeks. As control, male Otsuka Long-Evans Tokushima (OLET) rats (460 ± 15 g) were administered the same amount of ethanol or water. Both OLETF and OLET rats were sacrificed at the age of 10, 20 and 30 weeks to evaluate the induction of cytochrome P4502E1 (CYP2E1) through ageing. Massive steatohepatitis was observed in the livers of OLETF rats treated with ethanol once a week as well as five times, thrice and twice a week, while slight fatty degeneration was observed in OLETF rats received water. There was no fatty degeneration in OLET rats administered either ethanol or water. Both serum and hepatic triglyceride levels were significantly higher in all the groups of OLETF rats treated with ethanol compared to control OLETF rats received water. There was no difference in serum and hepatic triglyceride levels between OLET rats received ethanol or water. Immunohistochemical staining for CYP2E1 demonstrated dramatic increases in par with the increase of body weight in OLETF rats. 4-hydroxy-2-nonenal, a marker for reactive oxygen species (ROS) depicted remarkable staining in the hepatic tissue of OLETF rats treated with ethanol compared to OLETF rats received water. Our data indicates that occasional alcohol intake could be a second "hit" for the development of NASH in obese individuals.

Background and Aims

In 1980, Ludwig et al. coined the term "non-alcoholic steatohepatitis" (NASH) and subsequently the more embracing term "non-alcoholic fatty liver disease" (NAFLD) has been established to cover the full spectrum of hepatic steatosis associated with insulin resistance and the metabolic syndrome. Alcoholic liver disease and NAFLD are histologically indistinguishable. In order to distinguish between the two conditions, a cut-off limit for alcohol consumption has been introduced. In general, an intake of less than 140 g ethanol per week is considered for the diagnosis as NAFLD.

The molecular mechanisms involved in the pathogenesis of NASH are not clear. In NASH, diverse etiologies can give rise to the same histological features as of alcoholic hepatitis. In both human and experimental animals, ethanol induces hepatic cytochrome P4502E1 (CYP2E1). The increased expression of CYP2E1 in alcoholic hepatitis triggers the production of highly reactive oxygen species (ROS) and metabolites which in turn contribute to the development of steatohepatitis. In both human and rat liver, CYP2E1 is expressed predominantly in acinar zone 3. Induction of CYP2E1 by ethanol is associated with increased expression of the enzyme in zone 3, and also spread into zones 2 and 1. It was observed that CYP2E1 is also upregulated in NASH associated with diabetes mellitus and obesity. These data suggest that the molecular mechanisms involved in the pathogenesis of NASH may be similar to that of alcoholic steatohepatitis involving induction of CYP2E1 and subsequent events.

The aim of the present investigation was to study whether binge drinking could be a second hit to develop NASH from simple steatosis induced by obesity. In order to examine this, we used Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which spontaneously develop steatosis and diabetes.

Experimental protocol

Thirty weeks old male OLETF rats (620 ± 15 g), which spontaneously develop steatosis and diabetes beginning from 30 weeks of age, were administered 10 ml of 10% ethanol or water using intragastric tube for 5, 3, 2, or 1 day per week for 3 consecutive weeks. As the control, male Otsuka Long-Evans Tokushima (OLET) rats (460 ± 10 g) were administered the same amount of ethanol or water. At the end of treatment period, the animals were sacrificed and blood and livers were collected immediately. Untreated OLETF and OLET rats were sacrificed at the age of 10, 20 and 30 weeks to evaluate the auto-induction of CYP2E1 through ageing.

Serum levels of ALT, total cholesterol, triglycerides (TG) and glucose as well as hepatic TG contents were measured. The liver sections were stained for H & E and evaluated for steatohepatitis. Immunohistochemical staining was carried out for CYP2E1 and 4-hydroxy-2-nonenal (4-HNE).

Results

Massive steatohepatitis was observed in the livers of all OLETF rats treated with ethanol viz. 5, 3, 2 and 1 day per week, while slight fatty degeneration was observed in OLETF rats received water. There was no fatty degeneration in OLET rats administered either ethanol or water. Both serum and hepatic triglyceride levels were significantly higher in all the groups of OLETF rats treated with ethanol compared to control OLETF rats received water. There was no difference in serum and hepatic triglyceride levels between LETO rats received ethanol or water. Immunohistochemical staining for CYP2E1 demonstrated dramatic increases in par with the increase of body weight in OLETF rats. Staining for 4-HNE, a marker for reactive oxygen species (ROS) depicted marked increases in the hepatic tissue of OLETF rats treated with ethanol compared to similar rats received water.

Figure 1

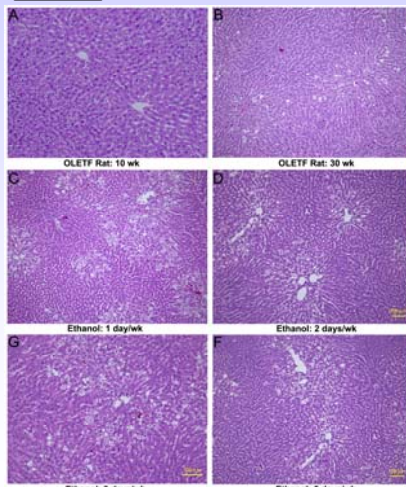


Figure 1. H & E staining of the liver tissue of OLETF rats at the age of 10 and 30 weeks and also after oral administration of 10 ml of 10% ethanol (x100). Ethanol was administered 1, 2, 3 or 5 days per week for 3 weeks beginning at the age of 30 weeks. There was no pathological alteration in the liver tissue of 10 weeks old OLETF rats. Fatty degeneration and simple steatosis were observed in the livers of 30 weeks old OLETF control rats. Massive steatohepatitis was present in the livers of all OLETF rats treated with ethanol.

Figure 2

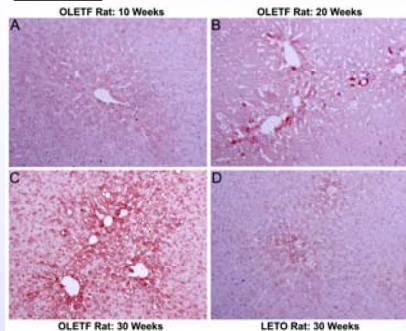


Figure 2. Immunohistochemical staining for CYP2E1 in the liver tissue of 10, 20 and 30 weeks old OLETF rats and 30 weeks old LETO rats (x100). There was dramatic increase in CYP2E1 expression in par with ageing and increase of body weight in OLETF rats.

Figure 3

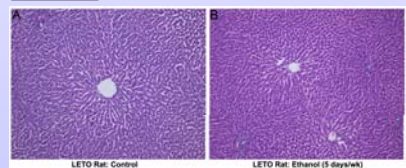


Figure 3. H & E staining of the liver tissue of LETO rats (x100). Thirty weeks old LETO rats were administered 10 ml of 10% ethanol 5 days/week for 3 weeks. There was no pathological alteration or fatty degeneration in LETO rats treated with ethanol compared to normal rats.

Figure 4

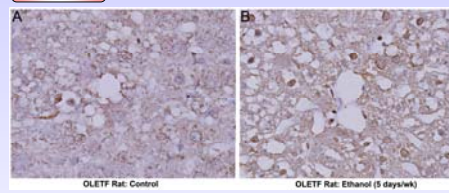


Figure 4. Immunohistochemical staining of 4-HNE in the liver tissue of OLETF rats (x100). Marked increases in 4-HNE staining in rats treated ethanol for 5 days/week compared with that of control rats.

Figure 5

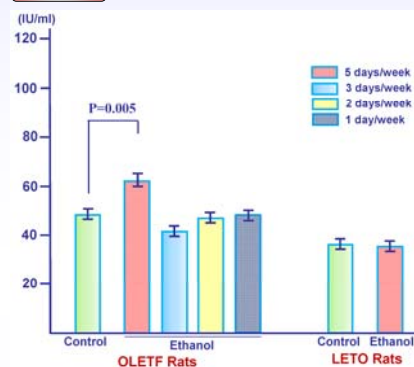


Figure 5. Serum ALT levels in OLETF and LETO rats treated with ethanol. Serum ALT level in control OLETF rats was significantly higher (P=0.0001) than that of control LETO rats. Serum ALT level in OLETF rats treated with ethanol 5 days/week was significantly higher (P=0.005) than that of control, while there was no difference in serum ALT levels in LETO rats treated with ethanol compared to LETO controls. The values are Mean ± S.D. (n=10).

Figure 6

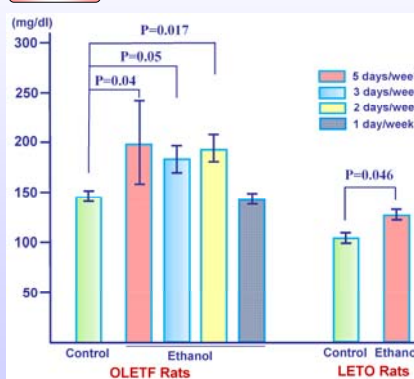


Figure 6. Fasting blood sugar (FBS) levels in OLETF and LETO rats treated with ethanol. FBS level in control OLETF rats was significantly higher (P=0.0005) than that of control LETO rats. FBS levels were significantly higher in OLETF rats treated with ethanol for 5, 3 or 2 days per week compared to the untreated control. A significant difference (P=0.046) was also observed in LETO rats treated with ethanol compared to normal LETO rats. The values are Mean ± S.D. (n=10).

Figure 7

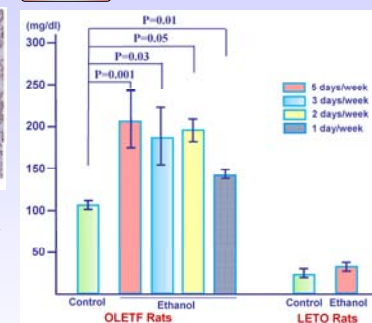


Figure 8

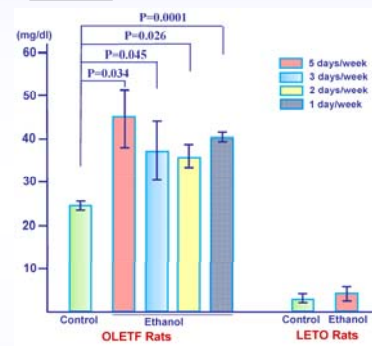
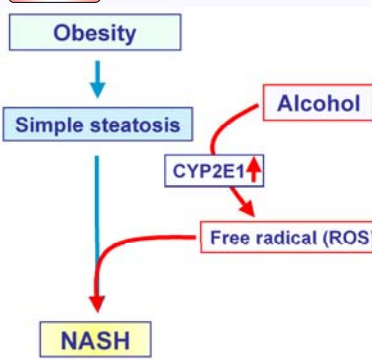


Figure 9



Conclusions

- Obesity accumulates triglycerides in the hepatic tissue and induces simple steatosis ("The First hit").
- Simple steatosis upregulates CYP2E1 and generates ROS.
- CYP2E1 metabolizes ethanol and produces ROS ("The Second hit").
- Binge increases serum and hepatic triglyceride contents in obese individuals.
- Binge promotes pathogenesis of NASH in obese individuals.

Figure 7. Serum triglyceride (TG) levels in OLETF and LETO rats treated with ethanol. Serum TG level in control OLETF rats was significantly higher (P=0.0001) than that of control LETO rats. Serum TG levels were significantly higher in OLETF rats treated with ethanol for 5, 3 or 2 days and 1 day per week compared with that of control. There was no difference in serum TG levels in LETO rats treated with ethanol compared to controls. The values are Mean ± S.D. (n=10).

Figure 8. Hepatic TG levels in OLETF and LETO rats treated with ethanol. Hepatic TG content in control OLETF rats was significantly higher (P=0.0001) than that of control LETO rats. Hepatic TG contents were also higher in OLETF rats treated with ethanol for 5, 3 or 2 days and 1 day per week compared with that of control. There was no difference in hepatic TG levels in LETO rats treated with ethanol compared to controls. The values are Mean ± S.D. (n=10).

Figure 9. Schematic representation of the mechanism of pathogenesis of NASH from simple steatosis. Obesity induces hepatic CYP2E1 and produces free radicals during ageing which induces the development of simple steatosis. So obesity is the "first hit" towards pathogenesis of NASH. During binge drinking, ethanol is metabolized by CYP2E1 and produces highly reactive metabolic intermediates and ROS that promote NASH from simple steatosis. Thus, binge could be the "second hit" towards pathogenesis of NASH in obesity.