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Journal of Hepatology 35 (2001) 130–133

Journal of Hepatology

www.elsevier.com/locate/jhep

Editorial

Alcoholic liver disease: a matter of hormones?

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Women are more likely than men to develop alcoholic liver disease. Although this has been known for a long time, the mechanisms underlying this gender difference are only now being elucidated [1,2]. The past decade has seen major advances in our understanding of the increased susceptibility of women to develop alcoholic liver disease. First, the group of C.S. Lieber in New York demonstrated a gastric first-pass metabolism of orally ingested ethanol, which was shown to be less in women than in men [1,3]. The lower effectiveness in women is due to a lower alcohol dehydrogenase (ADH) activity in the gastric mucosa [3]. Therefore, consumption of the same amount of ethanol results in higher blood ethanol levels in women than in men. The gastric mucosa contains several ADH isozymes, one of which is the type IV or σ-ADH (*ADH7*) which is not present in the liver and has a high capacity for ethanol metabolism [1]. Since estrogens increase the expression of ADH isozymes [4–6], the reason for the lower gastric σ-ADH activity in females remains to be elucidated.

Another major advance in the understanding of the gender differences in susceptibility to ethanol-induced liver injury was contributed by the group of R.G. Thurman [2,7–10]. They demonstrated that estrogens contribute importantly to ethanol-induced liver injury. Administration of ethanol to female rats resulted in more hepatic steatosis, inflammation and necrosis compared to male rats, despite equal ethanol intake, metabolism and excretion [7]. Plasma endotoxin levels were also significantly higher in female rats than in male rats [7]. In another study they demonstrated that treatment of female rats with estrogens increased the sensitivity to endotoxin: estrogen pre-treatment significantly increased mortality after a sublethal dose of endotoxin [8]. The increased sensitivity to endotoxin of estrogen-treated rats is explained by increased expression of the endotoxin receptor CD14 and the pro-inflammatory cytokine TNF-α in Kupffer cells [8]. The sensitizing effect of estrogen was prevented by prior elimination of the Kupffer cell population [8]. Likewise, ethanol ingestion resulted in a stronger activation of the inflammation-associated transcription factor NF-κB and a higher expression of CD14 and TNF-α in livers of female rats compared to male rats [9]. Lowering estrogen levels, by subjecting female rats to ovariectomy, resulted in a significant reduction of ethanol-induced liver injury including steatosis and inflammation [10]. In addition, hepatic CD14 expression as well as plasma endotoxin levels were reduced [10]. Taken together, these results indicate that females are more susceptible to ethanol-induced liver damage because: (1) gastric ADH-dependent ethanol metabolism is lower, resulting in higher blood ethanol levels; (2) plasma endotoxin levels are higher after ethanol ingestion; (3) the inflammatory response in the liver is more severe due to an estrogen-dependent sensitization to endotoxin.

In this issue of the *Journal of Hepatology*, Järveläinen et al. [11] further contribute to our understanding of the role of estrogens in the higher susceptibility of females to ethanol-induced liver injury. Using female rats they confirmed previous results that ethanol induced hepatic steatosis, inflammation and necrosis as well as increased expression of CD14 and TNF-α in Kupffer cells [11]. Moreover, they demonstrate that the estrogen antagonist toremifene reduced ethanol induced hepatic inflammation and necrosis. Unexpectedly, toremifene failed to reduce hepatic steatosis and had no effect on the ethanol-induced hepatic expression of CD14 and TNF-α mRNA [11]. Toremifene suppressed the ethanol-induced increase in the activity of the ethanol-metabolizing enzyme CYP2E1 and it counteracted the ethanol-induced reduction in selenium-dependent glutathione-peroxidase activity, an important anti-oxidant enzyme [11]. The findings of Järveläinen et al. differ from previous findings in that the estrogen antagonist toremifene failed to reduce the expression of ethanol-induced inflammation-related genes like CD14 and TNF-α [10,11]. The reason for this discrepancy remains to be elucidated but could be related to specific differences between anti-estrogens

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PII: S0168-8278(01)00115-5

(toremifene) [11] and ovariectomy [10]. Methodological differences should not be overlooked: Järveläinen et al. used oral ingestion of ethanol avoiding cyclical changes in blood ethanol levels observed during intragastric ethanol administration [10] and their study lasted for 6 weeks versus 4 weeks in Thurman's studies. However, the overall message is the same: estrogens aggravate the inflammatory response to ethanol.

An important aspect of the study by Järveläinen et al. is that estrogens regulate enzymes involved in ethanol metabolism and in the generation and protection against oxidative stress. As mentioned earlier, estrogens increase the expression of the major ethanol metabolizing enzyme ADH [4–6]. Järveläinen et al. report that anti-estrogens reduce the ethanol-induced expression of the ethanol metabolizing enzyme CYP2E1. These results would suggest a more rapid metabolism of ethanol in females than in males and would implicate higher levels of the toxic metabolite acetaldehyde generated by ADH and CYP2E1. This prediction awaits experimental confirmation. Acetaldehyde is a very toxic and reactive compound, e.g. it promotes hepatic fibrogenesis [12] and it reacts with proteins resulting in the formation of neo-antigens and the induction of an inappropriate immune response to these neo-antigens [13,14].

Another important consequence of the differences in regulation of CYP2E1 between females and males relates to the formation of reactive oxygen species by this enzyme. Part of ethanol-induced liver injury is the result of increased exposure to reactive oxygen species [1,2,15–17]. The ethanol-inducible enzyme CYP2E1 is an important source of these reactive oxygen species [18]. Indeed, inhibition of CYP2E1 activity significantly reduces ethanol-induced liver injury [19–21]. Thurman's group reported that ethanol-induced generation of reactive oxygen species is larger in female rats than in male rats [22]. However, they also reported that alcoholic liver injury and generation of reactive oxygen species were similar in CYP2E1 knockout mice and normal mice [23]. These findings argue against an important role of CYP2E1 in the pathogenesis of alcoholic liver injury, although it is possible that a compensatory increase in the expression of other ethanol-metabolizing P-450 isozymes occurs in CYP2E1 knockout mice [23]. Based on Järveläinen's study, the effect of blocking estrogen action on the generation of reactive oxygen species is twofold: (1) attenuation of the ethanol-induced rise in CYP2E1 activity, resulting in reduced generation of reactive oxygen species and (2) partial prevention of the reduction in the activity of the anti-oxidant enzyme glutathione-peroxidase, thus preserving the protection against reactive oxygen species. Together, these effects counteract the pro-inflammatory and damaging effects of reactive oxygen species. Unfortunately, Järveläinen et al. did not measure the generation of reactive oxygen species in their study [11].

Important questions remain. It needs to be established whether the concepts based on studies with experimental animals are valid in humans. This will be a challenging task since there is a large interindividual variation in the expression and regulation of genes involved in inflammation, ethanol metabolism and detoxification of reactive oxygen species due to polymorphisms. Furthermore, many parameters, including the amount and duration of ethanol intake, age, diet and body mass index, have to be taken into account. In addition, analysis of gene expression in human liver tissue is technically challenging. Human in vitro preparations, e.g. human liver cells or human liver slices, preserving the interaction between hepatocytes and Kupffer cells, could also be used to study gender differences in the response to ethanol [24,25]. Another important area of investigation is the elucidation of the mechanism by which estrogens modulate the response to ethanol. For example, the genes involved in inflammation, ethanol metabolism and generation and detoxification of reactive oxygen species should be analyzed for the presence of estrogen responsive elements in their promoters. Since the transcription factor NF-κB plays a central role in inflammation, the interaction between estrogens, ethanol and NF-κB also needs to be investigated. The literature is controversial here and opposing mechanisms could be at work: on the one hand, ethanol directly inhibits cytokine-induced activation of NF-κB and NF-κB-regulated genes [26–30]. On the other hand, ethanol ingestion causes increased plasma endotoxin levels, resulting in inflammation, NF-κB activation and production of cytokines [7,9,10,31]. Apparently, the latter mechanism dominates in animal models of ethanol administration. Likewise, estrogens have been reported to decrease the activation of NF-κB and the expression of NF-κB-regulated genes, e.g. iNOS [32–35], yet estrogens aggravate the ethanol-induced hepatic inflammation [9,10]. As suggested by Thurman's group [9], other effects of estrogens, e.g. on gut permeability and/or gut flora, resulting in increased translocation of endotoxin from the gut or a stimulatory effect of estrogens on Kupffer cell CD14 expression, could be involved as well. Together, these observations illustrate the need to resolve the complex interplay between estrogens, ethanol and NF-κB-controlled signal transduction pathways.

Finally, can we interfere with the action of estrogens or CYP2E1 to prevent or treat ethanol-induced liver injury. The results from the current study using an estrogen antagonist as well as previous studies demonstrating a beneficial effect of CYP2E1 inhibition in experimental alcoholic liver injury, suggest we can [11,19–21]. Can we also use these interventions to treat non-alcoholic steatohepatitis (NASH)? This is a more controversial issue. There appears to be a clear connection between estrogen and development of steatosis and in Järveläinen's study, the anti-estrogen toremifene decreased the mild fatty infiltration seen in control rat livers [11]. However, in humans, the long-term use of anti-estrogens, in particular tamoxifen, has been associated with hepatic steatosis [36,37]. Therefore, inhibition of CYP2E1 or other members of the P-450 family such as CYP4A could be more promising for the treatment of NASH, as recently reported by Leclercq et al. [38].

Studies like the one by Järveläinen et al. contribute importantly to our understanding of the gender difference in susceptibility to alcohol-induced liver injury. They provide novel insights and inspire future research.

Note Added in proof:

Very recently, results of the group of Thurman suggested that reactive oxygen species derived from NADPH oxidase in Kupfler cells contribute to ethanol-induced liver injury (Kono H et al., Am J Physiol 2001;280:G1005–G1012 and Kono H et al., J Clin Invest 2000;106:867–872).

Acknowledgements

The author would like to thank Dr R.J. de Knegt for valuable suggestions and critically reading the manuscript.

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