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Beer Is Less Harmful for the Liver than Plain Ethanol: Studies in Male Mice Using a Binge-Drinking Model

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Abstract

Aims: Mechanisms involved in the less damaging effects of beer in comparison to hard spirits have not yet been fully understood. The aim of the study was to determine if the effect of beer intake on the liver differs from that of plain ethanol and if so to determine mechanisms involved.

Methods: Male C57BL/6J mice received either ethanol, beer (ethanol content: 6 g/kg body weight) or iso-caloric maltodextrin solution. Markers of steatosis, lipogenesis, activation of the toll-like receptor-4 signaling cascade and lipid export in liver and tight junction proteins in duodenum were measured 6 and 12 h after acute ethanol or beer intake.

Results: Alcohol ingestion resulted in a significant increase of hepatic triglyceride accumulation 6 and 12 h after ingestion, respectively, being markedly lower in mice fed beer. Expression of sterol regulatory element-binding protein-1c mRNA was significantly lower 12 h after alcohol or beer exposure, while fatty acid synthase mRNA expression was induced in livers of ethanol-fed mice and to a lesser extent in mice fed beer 6 h after acute alcohol ingestion. Protein levels of tight junction proteins in the small intestine were similar between groups while expression of myeloid differentiation primary response gene 88 in livers was significantly induced in ethanol- but not in beer-fed mice. Concentrations of 4-hydroxynonenal protein adducts and inducible nitric oxide synthase protein were also only induced in livers of mice fed ethanol. Protein levels of apolipoprotein B were induced in livers of beer-fed mice only.

Conclusion: Our data suggest that beer is less harmful on the development of acute alcohol-induced liver damage than plain ethanol in male mice.

INTRODUCTION

Chronic alcohol abuse is still one of the leading causes of liver associated diseases but also mortality worldwide (Rehm *et al.*, 2013). However, results of epidemiological studies also suggest that the impact of alcoholic beverages on the liver might differ. Indeed, results of epidemiological studies suggest that chronic consumption of hard spirits might be more harmful to the liver than that of fermented alcoholic beverages (Kerr *et al.*, 2000; Ponicki and Gruenewald, 2006). In line with these findings, beer constituents like xanthohumol but also iso-acids have been shown to at least in part attenuate hepatic inflammation and fibrosis (Stevens and Page, 2004; Dorn *et al.*, 2010). Furthermore, studies using alcohol-free beer also found beneficial effects

on markers associated with liver health (i.e. markers of lipid peroxidation) (Martinez Alvarez *et al.*, 2009). In a recent study of our own group we found that in female mice, shown to be more susceptible to alcohol-induced liver damage, the damaging effects of beer on the liver were less pronounced when compared to equal doses of plain ethanol (Kanuri *et al.*, 2014); however, molecular mechanisms underlying this less damaging effect of beer on the liver but also eventual gender-specific differences have not been clarified yet.

To study molecular mechanisms of early stages of alcohol-induced liver damage, animal-based models have been found to be useful tools, as they resemble many alterations found in humans with alcoholic liver disease (for overview see Bergheim *et al.*, 2011). As rodent models of acute alcohol ingestion and chronic intake have been shown before (Enomoto *et al.*, 2000; Bergheim *et al.*, 2006) to share similar mechanisms, mouse models of acute alcohol consumption are useful tools to mimic the very early alterations associated with high alcohol intake (i.e. like the activation of nuclear factor kappa B (NFκB) and formation of reactive oxygen species (ROS) as well as an increased translocation of bacterial endotoxin from the gut).

Starting from this background the aim of the present study was to determine if beer possesses a less harmful effect on livers of male mice in a mouse binge-drinking model, and if so, to further unravel molecular mechanisms involved.

MATERIALS AND METHODS

Animals and treatments

Seven weeks old male C57BL/6J mice ($n = 6$ per group; Janvier S.A.S, France) were housed in a standard pathogen-free barrier facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care with free access to standard pellet chow (Ssniff, Germany) and tap water at all times. All procedures were approved by the local Institutional Animal Care and Use Committee (V297/12EM; V277/10 EM). Mice received one single dose of either isocaloric and iso-alcoholic (6 g ethanol/kg body weight (b. w.)) ethanol solution or beer (EKU Pils, Kulmbacher Brauerei AG, Germany) or an iso-caloric maltodextrin control solution and were sacrificed 6 and 12 h later, respectively. As alcohol concentration in beer was too low to achieve concentrations of 6 g/kg b. w. with volume tolerated by mice, beer was enriched with 96% v/v plain ethanol to adjust beer to 20% v/v ethanol. This dosage was based on previous work of our group (Kanuri *et al.*, 2014). Behavior of animals was closely monitored during the first 30 min after alcohol exposure and then at least every 30 min until mice were killed. With this dose of alcohol, which did not cause mortality, mice were sluggish, but conscious and regained normal behavior within ~5–6 h after alcohol feeding. Animals were anesthetized with 80 mg ketamine and 6 mg xylazin/kg b. w. *i. p.* either 6 or 12 h after ethanol, beer or maltodextrin ingestion. Portions of liver and small intestinal tissue were frozen immediately in liquid nitrogen, while others were fixed in neutral-buffered formalin or frozen-fixed in OCT mounting media (Medite, Germany) for later sectioning and mounting on microscope slides. Blood was taken prior to sacrifice.

Hepatic lipid accumulation and ethanol, alanine transaminase (ALT) and aspartate transaminase (AST) levels in plasma

Liver histology was assessed by hematoxylin and eosin (H&E) staining (both Sigma, Germany), and representative pictures were taken (400×). Furthermore, pictures of Oil Red O staining at 200× magnification were taken and a quantitative analysis of the staining was performed using a software included into the Leica microscope (DM4000 B LED, Leica, Germany). Oil Red O staining as well as isolation and measurement of hepatic triglycerides were performed as previously detailed (Kanuri *et al.*, 2009). Alcohol levels as well as activity of ALT and AST were measured in heparinized plasma as described previously (Wagnerberger *et al.*, 2013).

RNA isolation and real-time RT–PCR

For the detection of myeloid differentiation primary response gene 88 (MyD88), fatty acid synthase (FAS), sterol regulatory element binding protein (SREBP) 1c, insulin receptor substrate (IRS) 1 and 2 and 18S in liver tissue, RNA was isolated with Trizol (Peqlab, Germany), reverse transcribed (cDNA synthesis kit, Promega, Germany), and relative mRNA expression was quantified using a MX QPCR System (Agilent Technologies, Germany) as previously described (Kanuri *et al.*, 2014). The comparative C_T method was used to determine the amount of target genes, normalized to an endogenous reference (18S) and relative to a calibrator ($2^{-\Delta\Delta Ct}$). Primers were designed using Primer 3 software and synthesized by Eurofins MWG Operon (Eurofins, Germany). Primer sequences are listed in Table 1.

Immunostaining of 4-hydroxynonenal protein adducts and levels of inducible nitric oxide synthase protein in liver tissue

Staining of 4-hydroxynonenal (4-HNE) protein adducts as well as inducible nitric oxide synthase (iNOS) protein was performed as previously described (Kanuri *et al.*, 2009) using polyclonal primary antibodies (4-HNE: AG Scientific, USA; iNOS: Affinity BioReagents, USA). The extent of labeling in liver lobules was defined as percent of the field area within the default color range determined by the software using an image acquisition and analysis system incorporated in the microscope (DM4000 B LED, Leica, Germany and Axio Vert 200M, Zeiss, Germany). Data from 8 fields of each tissue section (200× magnification) were used to determine means.

Table 1. Primer sequences used for real-time RT–PCR

	Forward 5′–3′	Reverse 5′–3′
FAS	GGGGGTGGGAGGACAGAGAT	CACATGGGCTGACAGCTTGG
IRS-1	GCTCTAGTGCTTCCGTGTCC	GTTGCCACCCCTAGACAAAA
IRS-2	GAAGCGGCTAAGTCTCATGG	GACGGTGGTGGTAGAGGAAA
MyD88	CAAAAGTGGGGTGCCTTTGC	AAATCCACAGTGCCCCCAGA
SREBP-1c	ACCGGCTACTGCTGGACTGC	AGAGCAAGAGGGTGCCATCG
18S	CCATCCAATCGGTAGTAGCG	GTAACCCGTTGAACCCCATT

Western blot

Western blot analyses were performed as previously detailed (Spruss *et al.*, 2011). The resulting blots were then probed with antibodies against the protein kinase B (Akt) and pAkt and inhibitor kappa B alpha (IκBα) as well as pIκBα (all Cell Signaling Technology, Germany). Bands were visualized using Super Signal Western Dura kit (Thermo Scientific, Germany). To ensure equal loading, all blots were stained with Ponceau Red (Roth, Germany). Protein bands were detected and analyzed densitometrically using a ChemiDoc MP System (BioRad Laboratories, Germany).

Apolipoprotein B and tumor necrosis factor α protein levels

Using commercially available enzyme-linked immunosorbent assay kits apolipoprotein (Apo) B and tumor necrosis factor (TNF) α levels in liver lysates were determined following the instructions of the manufacturer (ApoB: Bioo Scientific, USA; TNFα: LOXO GmbH, Germany).

Statistical analyses

All results are expressed as means ± standard error of mean (SEM). Data were analyzed using analysis of variances with the *post hoc* test of Tukey and Pearson product-moment correlation test (GraphPad Prism Software, USA). Differences were considered significant at a P-value ≤ 0.05. Grubbs test was used to identify outliers (GraphPad Prism Software, USA).

RESULTS

Effect of acute alcohol and beer intake on blood alcohol levels and the liver

In line with previous findings (Kanuri *et al.*, 2014), endogenous alcohol synthesis contributed to plasma ethanol levels of 70–75 nmol/µl in control mice, despite no exposure to ethanol at any time. In contrast, 6 h after the acute ethanol or beer challenge, blood ethanol levels of mice ingesting ethanol or beer were significantly higher ~1.9- and ~2.2- fold, respectively, than those of control mice. Twelve hours after the acute ingestion of ethanol or beer, blood ethanol levels of both were at the level of controls (Table 2). As expected, the ingestion of a bolus dose of ethanol was associated with significantly ~5-fold higher triglyceride levels in the livers 6 h after ingestion which was still found 12 h after the ethanol ingestion (approximately +8-fold in comparison to controls; see Fig. 1a). In contrast, in livers of mice fed beer, triglyceride content was only ~2.7-fold and ~3.1-fold higher than in control animals (not significant) 6 and 12 h after intake, respectively (Fig. 1d). Quantitative analysis of Oil Red O staining showed significantly elevated levels of total lipids in the liver 6 h after ingestion of ethanol and beer, respectively, in comparison to controls, whereas 12 h after ingestion only Oil Red O staining in livers of ethanol-fed mice reached the level of significance in comparison to controls. H&E staining revealed that fat accumulation was rather evenly distributed among liver lobes in both alcohol fed groups 6 and 12 h after ingestion (Fig. 1). While ALT and AST levels did not differ between groups 6 h after the ethanol ingestion, both ALT and AST levels were significantly higher in plasma of mice fed beer 12 h after ingestion in comparison to controls (Table 2). A similar effect was not found in mice fed plain ethanol; however, data varied considerably in some groups.

Table 2. Ethanol concentration, ALT and AST levels in plasma 6 and 12 h after ethanol or beer ingestion

	Control	Ethanol	Beer
Ethanol concentration (nmol/µl)			
6 h	75 ± 3	140 ± 22[a]	167 ± 7[a]
12 h	70 ± 6	72 ± 6	59 ± 4
ALT levels (U/l)			
6 h	31 ± 6	37 ± 3	54 ± 12
12 h	19 ± 1	22 ± 1	40 ± 2[a,b]
AST levels (U/l)			
6 h	56 ± 9	62 ± 2	76 ± 8
12 h	38 ± 1	46 ± 1	53 ± 5[a]

Data are expressed as means ± SEM.
ALT, alanine transaminase; AST, aspartate transaminase.
[a]$P < 0.05$ when compared to the respective control group fed maltodextrin.
[b]$P < 0.05$ when compared to the respective animals fed plain ethanol.

Markers of lipogenesis and triglyceride export in livers 6 and 12 h after ethanol or beer ingestion

Six hours after alcohol ingestion mRNA expression of FAS and SREBP-1c was markedly induced in livers of mice fed ethanol or beer; however as data varied considerably between mice, differences only reached the level of significance for FAS in livers of ethanol-fed mice. In contrast, 12 h after the beer or ethanol ingestion, expression of FAS did not differ between groups while that of SREBP-1c was markedly lower in livers of both, mice fed ethanol and beer, respectively (approximately −75% in comparison to controls, Table 3). Protein levels of ApoB were significantly higher in liver tissue of mice fed beer in comparison to controls 6 h after ingestion. A similar effect on ApoB protein levels was not found in livers of mice fed ethanol. In contrast, 12 h after ingesting ethanol or beer, ApoB protein levels in liver tissue were significantly lower in both alcohol-treated groups when compared to controls (Fig. 2a). When correlating hepatic Oil Red O staining or triglyceride levels and FAS mRNA expression, we found a significant correlation for data obtained 6 h after acute alcohol ingestion (Oil Red O: $r = 0.62$, $P = 0.014$; triglycerides: $r = 0.51$, $P = 0.036$), whereas a similarly positive correlation was not found for data obtained 12 h after treatment (Oil Red O: $r = 0.16$, $P = 0.52$; triglycerides: $r = 0.02$, $P = 0.95$).

Effect of acute alcohol or beer ingestion on tight junction proteins in the duodenum and markers of the hepatic toll-like receptor-4 signaling cascade as well as lipid peroxidation and NFκB signaling

While neither protein levels of the tight junction proteins occludin nor ZO-1 differed between groups at the time points studied (see Table 4, representative pictures of staining are shown in Supplementary Figure S1), mRNA expression levels of MyD88 were significantly higher in livers of mice fed ethanol in comparison to controls (Fig. 2b). A similar effect was not found in livers of mice fed beer. 12 hours after the acute exposure to beer or alcohol no differences for MyD88 mRNA in liver tissue were found between groups. Furthermore, 6 h after the alcohol ingestion, iNOS protein levels, shown before to be regulated by toll-like receptor (TLR4) and MyD88-dependent signaling pathways (Spruss *et al.*, 2011), were significantly induced in livers of mice fed ethanol in comparison to controls (approximately +4.3-fold) (see Fig. 3a and b). In mice fed beer, protein levels were only by ~1.9-fold higher than in controls ($P < 0.05$). A similar effect on iNOS protein levels was still found 12 h after the alcohol challenge. In line with these findings, levels of 4-HNE protein

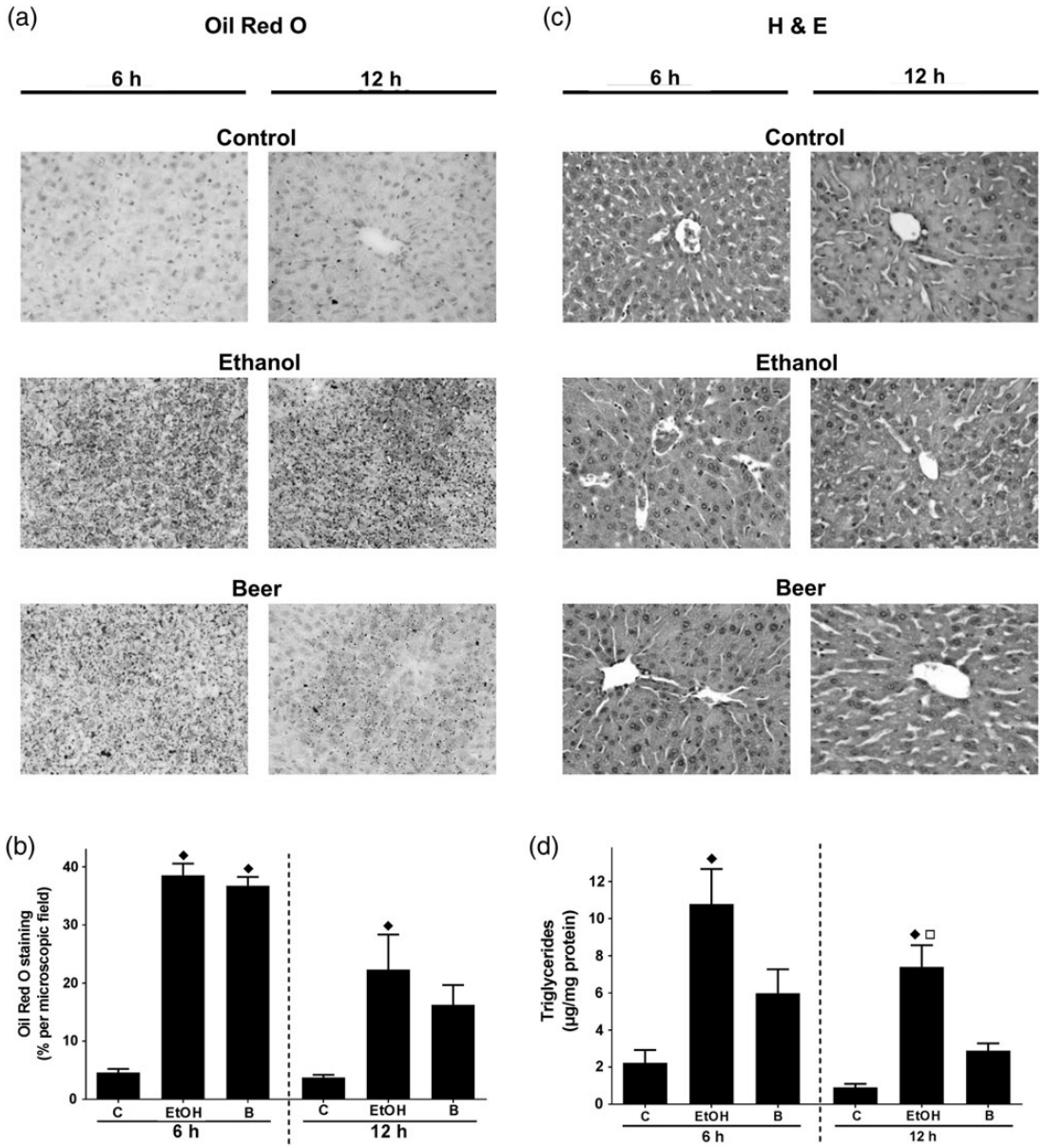

Fig. 1. Effect of acute alcohol ingestion on hepatic lipid accumulation after 6 and 12 h. (**a**) Representative pictures (200×) as well as (**b**) quantitative analysis of the Oil Red O staining and (**c**) representative pictures of a hematoxylin and eosin (H&E) staining in the liver (400×). (**d**) Quantification of the hepatic triglyceride levels in liver. Data are shown as means ± SEM. ♦$P < 0.05$ when compared to the respective control animals fed maltodextrin (=C). □$P < 0.05$ when compared to the respective animals fed beer (=B). Ethanol-fed mice (=EtOH).

adducts were also higher in livers of mice fed beer or plain ethanol 6 and 12 h after ingestion (Fig. 3c and d). However, as data varied considerable in the ethanol group 12 h after intake, differences only reached the level of significance at the 6 h time point. Phosphorylation levels of IκB were not different between groups 6 h after alcohol ingestion (Fig. 4a). Twelve hours after ingestion, pIκB concentration was markedly higher in livers of mice fed ethanol in comparison to controls and mice fed beer. Again, as data varied considerably within the ethanol group, differences only reached the level of significance for the comparison of the ethanol and beer group. Furthermore, protein levels of TNFα in the liver did not differ between groups at any of the time points studied (Table 3).

Effect of acute beer or alcohol intake on markers of insulin signaling and plasminogen activator inhibitor 1 protein levels

As it has been shown before that in settings of acute and chronic alcohol intake, endotoxin exerts its damaging effects on the liver at least in part through inducing TNFα subsequently leading to alterations of the insulin and plasminogen activator inhibitor 1 (PAI-1) signaling cascade in the liver (Bergheim *et al.*, 2006), we determined expression of IRS-1 and 2 as well as phosphorylation status of AKT and PAI-1 protein levels. While expression of IRS-2 did not differ between groups at any of the time points studied, expression of IRS-1 was significantly higher in livers of mice fed beer in comparison to controls 6 h after ingestion. A similar effect on hepatic IRS-1 expression was not found in

Table 3. Markers of lipogenesis and inflammation 6 and 12 h after ethanol or beer ingestion

	Control	Ethanol	Beer
FAS mRNA (% over control)			
6 h	100 ± 13	819 ± 171[a]	433 ± 52
12 h	100 ± 19	108 ± 23	103.5 ± 27
SREBP-1c mRNA (% over control)			
6 h	100 ± 27	212 ± 60	154 ± 31
12 h	100 ± 19	24 ± 3[a]	23 ± 2[a]
TNFα protein (% over control)			
6 h	100 ± 14	95 ± 13	122 ± 18
12 h	100 ± 15	77 ± 14	130 ± 30
IRS-1 mRNA (% over control)			
6 h	100 ± 27	127 ± 23	238 ± 45[a]
12 h	100 ± 10	79 ± 12	59 ± 7[a]
IRS-2 mRNA (% over control)			
6 h	100 ± 38	138 ± 30	178 ± 12
12 h	100 ± 11	114 ± 34	71 ± 8
PAI-1 protein (% over control)			
6 h	100 ± 8	87 ± 9	101 ± 7
12 h	100 ± 5	89 ± 11	76 ± 8

Data are expressed as means ± SEM.

[a]$P < 0.05$ when compared to the respective control group fed maltodextrin.

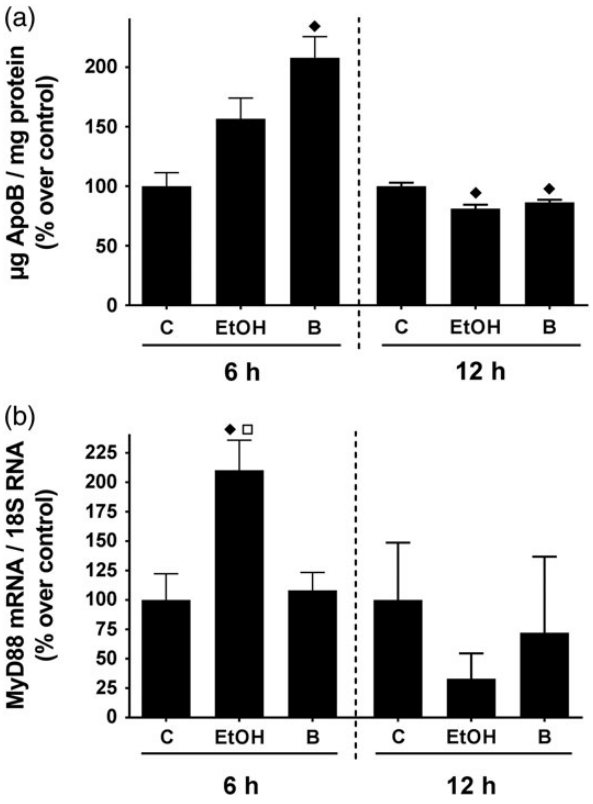

Fig. 2. Effect of acute alcohol ingestion on lipid export and activation of the TLR-4 signaling cascade in the liver after 6 and 12 h. (**a**) ApoB Protein concentration and (**b**) relative mRNA expression of the TLR-4 adapter molecule MyD88 in the liver normalized to 18S RNA expression after 6 and 12 h. Data are shown as means ± SEM and are expressed as percent over control. ♦$P < 0.05$ when compared to the respective control animals fed maltodextrin (=C). □$P < 0.05$ when compared to the respective animals fed beer (=B). Ethanol-fed mice (=EtOH).

Table 4. Effect of acute ethanol or beer consumption on occludin and ZO-1 protein staining 6 h after ingestion

	Control	Ethanol	Beer
Occludin	21 ± 2	22 ± 1.5	21.5 ± 1
ZO-1	20 ± 2	22 ± 2	21 ± 1

Data are expressed as percentile means per microscopic field ± SEM. Representative pictures of the staining are shown in Supplementary Figure S1.

mice fed ethanol. However, 12 h after ingestion, IRS-1 expression was significantly lower in liver of mice fed beer in comparison to controls (Table 3). Phosphorylation status of Akt and protein levels of total PAI-1 did not differ between groups at any time point studied (Fig. 4b and Table 3).

DISCUSSION

Worldwide, beer is still among the most frequently consumed alcoholic beverages (World Health Organization, 2014). Results of epidemiological studies suggest that chronic intake of fermented alcoholic beverages like wine and beer is associated with lower odds of overall and liver related mortality when compared to the intake of distilled alcoholic beverages (Kerr *et al.*, 2000; Jiang *et al.*, 2014). It has further been shown that chronic intake of elevated amounts of beer and wine in comparison to spirits may have less harmful effects on the liver (Kerr *et al.*, 2000; Ponicki and Gruenewald, 2006). The results of others (Martinez Alvarez *et al.*, 2009) even suggest that alcohol-free beer may have beneficial effects on lipid profile and pro-inflammatory markers related to liver diseases. It was further shown that some beer constituents like xanthohumol might have inhibitory effects on hepatic inflammation and fibrosis (Van *et al.*, 2009; Dorn *et al.*, 2012). However, in many of these *in vivo* studies beer constituents were given isolated at concentrations that could most of the time not be reached by moderate or even elevated beer consumption. Using a binge-drinking mouse model where female mice were challenged with one bolus dose of beer (e.g. stout and beer, respectively), we recently showed that they were markedly protected from the onset of alcohol-induced liver damage when compared to mice fed the same amount of plain ethanol (Kanuri *et al.*, 2014). However, neither mechanism involved in the less damaging effect of beer nor if this 'beneficial' effect is only found in female mice has yet been clarified. Using a binge-drinking model in male mice, i.e. 6 g alcohol/kg b. w. given by oral gavage, we determined in the present study if beer has also a less harmful effect on the liver of male mice and further studied molecular mechanisms involved. In line with earlier findings of our group (Wagnerberger *et al.*, 2013), acute ingestion of ethanol resulted in a significant accumulation of lipids in the liver both 6 and 12 h after ingestion, which was associated with a slight but, due to the inter-individual variation, not significant increase in ALT and AST levels in plasma. In mice ingesting beer despite having a similar ethanol and caloric content, this increase in hepatic triglyceride but also total lipid accumulation was markedly lower after 6 h and already almost resolved 12 h after ingestion. However, despite this marked protection from the alcohol-induced liver damage, for so far unknown reasons, ALT and AST levels were also markedly increased in mice ingesting beer. Furthermore, blood alcohol levels but also differences in behavior following ethanol or beer exposure were similar between groups, suggesting that ethanol absorption and metabolism was similar between ethanol and beer fed groups. Alcohol found in the plasma of control mice not being challenged with alcohol solution probably

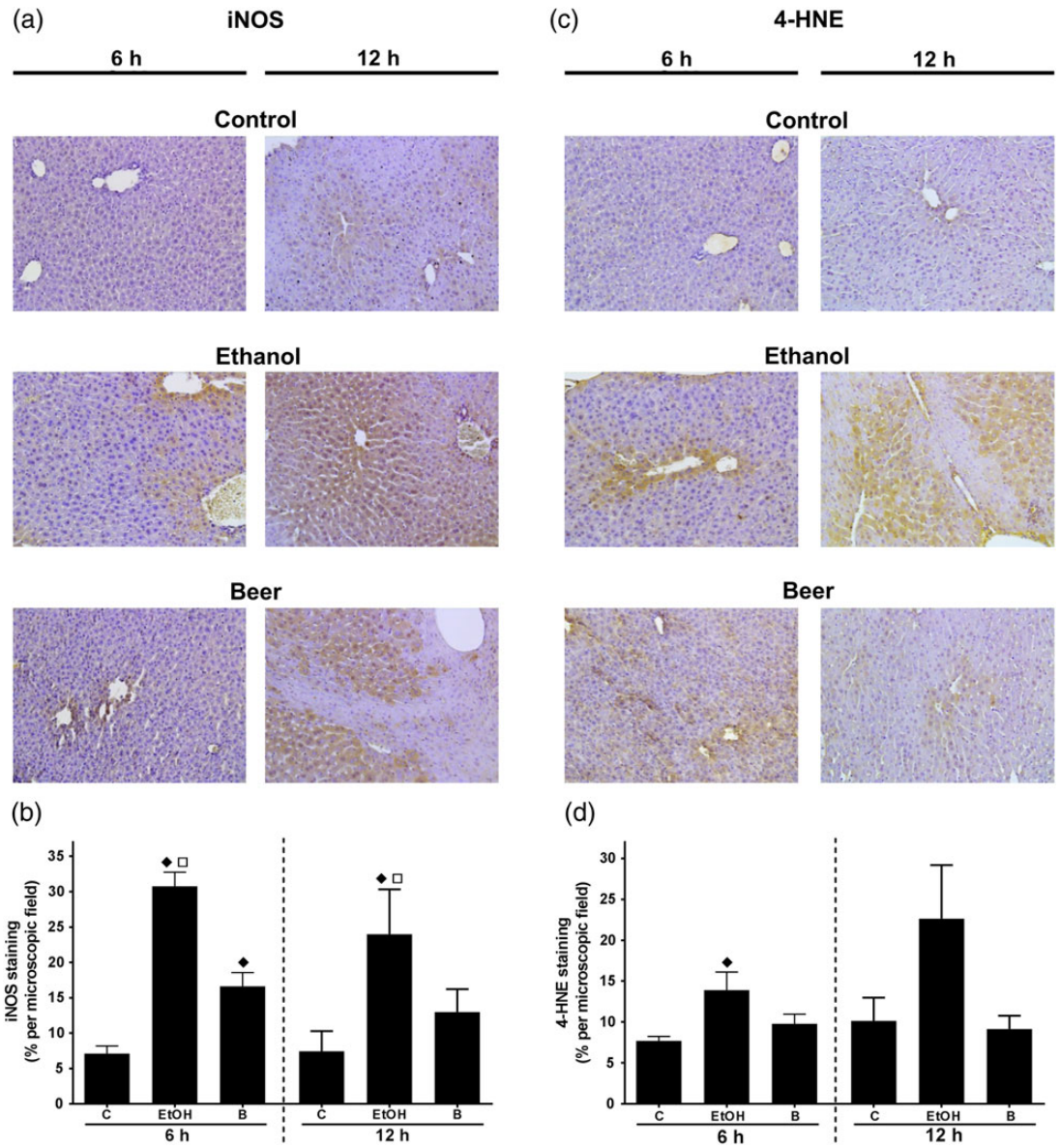

Fig. 3. Effect of acute alcohol or beer ingestion on iNOS protein levels as well as on lipid peroxidation in the liver after 6 and 12 h. (**a**) Representative pictures and (**b**) quantification of the staining of iNOS protein levels (brown staining) in the liver. (**c**) Representative pictures of the staining of 4-HNE protein adducts (brown staining) and (**d**) quantification of the staining. Data are shown as means ± SEM and are expressed as % per microscopic field. $^{\blacklozenge}P < 0.05$ when compared to the respective control animals fed maltodextrin (=C). $^{\square}P < 0.05$ when compared to the respective animals fed beer (=B). Ethanol-fed mice (=EtOH).

resulted from endogenous alcohol synthesis mediated by intestinal microbiota as suggested repeatedly by others (Cope *et al.*, 2000). The less damaging effects of beer on the liver were not associated with any marked effects the acute phase protein PAI-1 at the time points studied. However, as the ingestion of plain ethanol had no marked impact on protein levels of this pro-inflammatory marker in the liver, it may be that despite having studied two different time points, alterations of protein levels might have been missed. Indeed, in earlier studies of our group, no differences were found at the level of protein concentration PAI-1 in the liver of male mice, too (Wagnerberger *et al.*, 2013). Taken together, our data suggest that similar to the findings in female mice, acute ingestion of beer is associated with less harmful effects on the liver in male mice, too. Of course, models of acute alcohol exposure by no means resemble all effects of chronic alcohol consumption on the liver; however, our data suggest that despite leading to a less pronounced fat accumulation in the liver (increase in triglyceride levels 12 h after ingestion: ~8-fold in male vs. 11-fold in female mice in comparison to controls (Kanuri *et al.*, 2014)), beer indeed has less harmful effects on the liver when compared to plain ethanol in both male and female mice.

Alterations of lipid metabolism and herein especially *de novo* lipogenesis have been discussed to be critical in the development of alcoholic liver diseases (You and Crabb, 2004). SREBP-1c is a key regulator of lipid biosynthesis in the liver and an induction of SREBP-1c has been shown to be associated with an up-regulation of the expression of many enzymes involved in lipogenesis like FAS, in turn leading to an increased synthesis of fat in the liver and the development of fatty liver (You and Crabb, 2004). In support of the

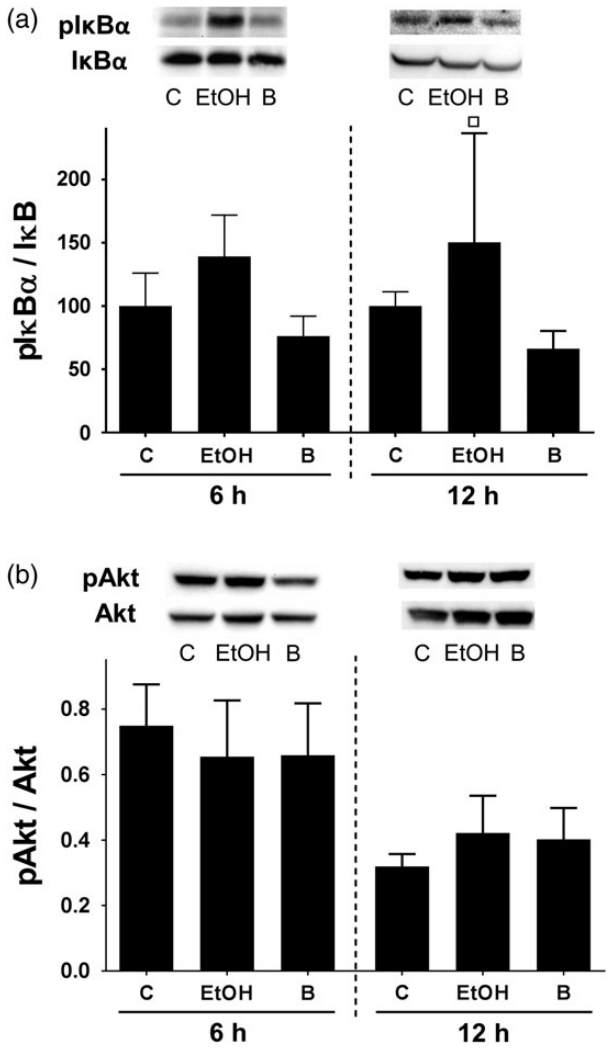

Fig. 4. Effect of acute alcohol treatment on the phosphorylation status of IκB and protein kinase B (Akt) in the liver after 6 and 12 h. Densitometric analysis and representative pictures of western blots of (**a**) pIκBα normalized to IκBα and (**b**) pAkt normalized to Akt 6 and 12 h after acute alcohol treatment. Data are shown as means ± SEM. □$P < 0.05$ when compared to the respective animals fed beer (=B). Ethanol-fed mice (=EtOH). Control animals fed maltodextrin (=C).

hypothesis that SREBP-1c may also play a critical role in the development of alcohol-induced liver damage, it was shown that chronic intake of alcohol is associated with marked alterations of SREBP-1c and -dependent signaling molecules and that SREBP1-1c knockout mice are markedly protected from the development of chronic alcohol-induced liver damage (You and Crabb, 2004; Ji *et al.*, 2006). In the present study, expression of SREBP-1c was slightly higher 6 h after acute alcohol ingestion in both alcohol groups, while 12 h after the alcohol binge, expression was markedly lower in livers of alcohol fed mice when compared to the control animals. In line with these findings, FAS mRNA expression was markedly higher 6 h after acute alcohol ingestion and at the level of controls 12 h after the acute alcohol intake. These findings are in line with earlier studies of our group that showed that 12 h after acute alcohol ingestion, SREBP-1c and FAS mRNA expression is decreased in livers of alcohol-treated mice (Kanuri *et al.*, 2014). Furthermore, our data also suggest that expression of FAS in liver and hepatic fat are at least shortly after alcohol ingestion positively related, while 12 h after ingestion, when alcohol-induced fat accumulation already starts to resolve, this association is no longer found. Our data also suggest that despite no marked differences in SREBP-1c expression, FAS expression may be differently affected by alcohol and beer, thereby maybe adding to the less pronounced damaging effects of beer on the liver. However, molecular mechanisms involved remain to be determined. Taken together, our data suggest that alterations at the level of FAS but not SREBP-1c may have been involved in the less harmful effect of beer ingestion on the liver of male mice found in the present study.

Chronic but also acute ingestion of alcohol has been shown to be associated with endotoxemia in animal models but also in humans (Bode *et al.*, 1987) leading to an activation of TLR-4-dependent signaling cascades in liver (e.g. MyD88-dependent) (Kanuri *et al.*, 2014). It has further been shown that an induction of iNOS and an increased formation of ROS, leading to an activation of NFκB are critical in mediating the damaging effects of the increased translocation of bacterial endotoxins on the liver (Spruss *et al.*, 2011). In the present study, we found that expression of MyD88 but also markers of lipid peroxidation, i.e. levels of 4-HNE protein adducts and IκB were markedly induced in livers of mice fed ethanol. Similar changes were not found in livers of mice fed beer. These results are in line with our findings in female mice (Kanuri *et al.*, 2014) and *in vitro* studies (Van *et al.*, 2009; Saugspier *et al.*, 2012) reporting that the protective effects of beer and beer constitutes are associated with a protection against the induction of iNOS and the formation of ROS. Somewhat contrary to the results found when measuring markers of lipid peroxidation and IκB, TNFα protein levels were not altered in mice either fed ethanol or beer. However, a lack of induction of TNFα in livers of mice who acutely ingested alcohol was reported before (Wagnerberger *et al.*, 2013; Kanuri *et al.*, 2014) and might have resulted from the time-dependent regulation of TNFα shown before by others (Marasliogl *et al.*, 2014). Taken together, these data suggest that the less harmful effects found in mice ingesting beer might stem at least in part from a protection against the induction of endotoxin-/TLR-4 and iNOS-dependent signaling cascades in the liver.

In summary, our results suggest that the acute ingestion of beer may be less harmful on the liver than the ingestion of plain ethanol in both male and female mice. Furthermore, the results of the present study suggest that beer, or beer compounds may attenuate alcohol-induced liver damage through mechanisms involving an attenuation of the induction of TLR-4- and iNOS-dependent signaling cascades subsequently leading to a protection from lipid peroxidation found in livers after acute ingestion of alcohol. However, molecular mechanisms and beer constituents involved but also the question if chronic intake of beer is also less harmful than the ingestion of plain ethanol will have to be addressed in future studies.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Alcohol and Alcoholism* online.

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CONFLICT OF INTEREST STATEMENT

None declared.

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