Alcohol Metabolism Increases the Replication of Hepatitis C Virus and Attenuates the Antiviral Action of Interferon

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The interactions between hepatitis C virus (HCV) and alcohol metabolism are not well understood. To determine the effect that alcohol metabolism has on HCV replication and the antiviral action of interferon (IFN), Huh-7 cells that harbor HCV replication and metabolize ethanol via the introduced expression of cytochrome P450 2E1 (Cyp2e1) were treated with ethanol and IFN-α. Treatment of these cells with ethanol (0–100 mmol/L) significantly increased HCV replication. This effect was dependent on Cyp2e1 expression and alcohol-metabolized oxidative stress (OS), because the antioxidant N-acetylcysteine blocked this effect. Furthermore, the anti-HCV action of IFN-α was attenuated in the presence of ethanol metabolism, most likely via attenuation of Stat1 tyrosine-701 phosphorylation. These in vitro results mimic what is often noted clinically, and further dissection of this model system will aid in our understanding of interactions between HCV and alcohol metabolism.

Hepatitis C virus (HCV) is the leading cause of chronic hepatitis and liver disease–related morbidity worldwide [1]. Chronic alcohol consumption has been identified as a significant cofactor in exacerbation of HCV-related liver disease [2]. Patients who consume alcohol are particularly vulnerable to HCV-induced liver injury, with daily consumption of >50 g of alcohol resulting in a greater degree of inflammation, more rapidly progressive fibrosis, and a higher incidence of liver decompensation and hepatocellular carcinoma [3]. Even moderate doses of alcohol correlate with increased progression of fibrosis [4–6]. Furthermore, alcohol consumption greatly impairs the efficacy of interferon (IFN) therapy and is considered to be contraindicated during treatment for HCV [7].

Despite these clinical observations, the mechanisms by which alcohol accelerates liver injury in chronic hepatitis C (CHC) are unknown. Although the process clearly is multifactorial, several explanations have been postulated; these include modification of the immune system [8], direct enhancement of viral replication [9, 10], and synergistic enhancement of oxidative stress (OS). OS has been specifically associated with the pathogenesis of both CHC [11–14] and alcohol-induced liver disease [15]. Furthermore, HCV replication or expression of HCV proteins alters antioxidant status and promotes lipid peroxidation both in vitro and in vivo [16–18]. We therefore postulate that concurrent HCV infection and alcohol metabolism in the liver may result in either an additive or synergistic increase in OS, an increase that, in CHC, acts as a common pathway leading to liver injury and modulation of HCV replication.

Investigations of interactions between HCV and alcohol have been inconclusive because of the lack of a small-animal model of HCV pathogenesis. In addition, in vitro studies are compounded by the fact that hepatocyte-derived cell lines in culture do not express the alcohol-metabolizing enzymes, alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (Cyp2e1). To overcome these limitations, we have produced novel hepatocyte-derived...
cell lines constitutively expressing Cyp2e1 and harboring HCV replication. In the present study, we show that alcohol metabolism by Cyp2e1 results in an HCV-replication increase that is dependent on Cyp2e1-mediated production of OS. Furthermore, we also demonstrate that, via attenuation of the Jak/Stat signaling pathway, metabolism of alcohol by Cyp2e1 results in blocking of the anti-HCV action of IFN-α2b. These in vitro results form the rational basis for observations made in HCV-infected individuals and provide a model system for study of the molecular interactions between HCV replication, alcohol, the innate immune-system components, and the hepatocyte.

**MATERIALS AND METHODS**

**Establishment of Cell Lines and Culture Conditions**

Genomic (i.e., NNeo/C-5B) and subgenomic (i.e., NNeo/3-5B) HCV replicon–bearing Huh-7 cell lines were provided by Stanley Lemon (University of Texas Medical Branch, Galveston) [19] and were cultured as described elsewhere [20]. The replicon-containing cells were transfected with pcDNA-Cyp2e1, the plasmid expressing Cyp2e1, by use of Fugene 6 transfection reagent (Roche), and stable transformants were selected after treatment with blasticidin (5 μg/mL) (Invitrogen). pcDNA-Cyp2e1 [21] contains the cDNA encoding human Cyp2e1 (provided by A. I. Cederbaum, Mt. Sinai School of Medicine, New York, NY). Individual clones were selected and expanded.

**Western Blots**

Western blotting was performed as described elsewhere [20] and used the following antibodies, at a dilution of 1:1000: rabbit anti–human Cyp2e1, phospho-Stat1 (Tyr701), phospho-Stat1 (Ser727), phospho-Stat2 (Tyr690), Stat-1, and Stat-2. Rabbit anti–human β-actin (Sigma) was used to control loading of protein. Appropriate secondary antibodies labeled with horseradish peroxidase were used, and bound protein was visualized by use of chemiluminescence (Amersham).

**Immunofluorescence Microscopy**

Visualization of HCV antigens in the replicon-bearing cells expressing Cyp2e1 was performed by indirect immunofluorescence microscopy, essentially as described elsewhere [17], with the exception that the cells were incubated in a 1:300 dilution of HCV-positive human serum (pooled from 5 patients). A goat anti–human IgG Alexa 488–conjugated secondary antibody (Molecular Probes) was used. The cells were visualized by use of an Olympus Provis AX70 microscope.

**Treatment of Cells**

**Alcohol.** Cells were seeded, at a density of 2 × 10^5 cells/well, in 6-well cell-culture plates and were cultured for 24 h. The following day, the medium was replaced by medium containing various concentrations of ethanol (10–100 mmol/L) (Sigma). As in procedures used in studies published elsewhere, alcohol at a concentration of 100 mmol/L (the upper limit of what is noted clinically) was used in some experiments, to maximize the ability to observe differences, because our in vitro model has concentrations of both ADH and Cyp2e1 that are considerably lower than those seen in liver. RNA was harvested at 48 h and was extracted by use of TRIzol Reagent. Experiments using dialyl sulfide (DAS) (Sigma), an inhibitor of Cyp2e1 activity, were performed essentially in the same manner, with the exception that they included pretreatment with DAS (10 μmol/L) for 60 min before treatment with ethanol.

**IFN.** Cells that did or did not express Cyp2e1 were seeded, at a density of 7 × 10^4 cells/well, onto 12-well plates in Dulbecco’s minimal essential medium (DMEM) supplemented with 2% fetal calf serum and were incubated for 24 h before addition of IFN-α2b (Schering-Plough) and/or ethanol. Total RNA was isolated at 24 or 48 h after treatment, for cDNA synthesis and semiquantitative real-time polymerase chain reaction (PCR).

**Acetaminophen.** Cells were treated with acetaminophen, as described elsewhere [22]. In brief, 2 × 10^5 cells were treated with acetaminophen at a concentration of 1 or 5 mmol/L (Sigma Aldrich) for 48 h. Cell viability was then determined by trypan-blue exclusion assay.

**RNA extraction, cDNA synthesis, and real-time PCR.** Total RNA was extracted from the cultured cells by use of TRIzol reagent (Invitrogen), and first-strand cDNA was synthesized, according to protocols published elsewhere [23]. Real-time PCR analysis was used to quantitate the relative levels of HCV RNA in the replicon-bearing cells expressing Cyp2e1, according to protocols published elsewhere [20]. Each reaction was performed in duplicate, and all samples were standardized on the basis of the control ribosomal gene RPLPO [23].

**Cell-viability assay.** Viability of the cells after treatment with alcohol was determined by use of a Cell Titre Blue assay kit (Promega). The cells were seeded, at a density of 1 × 10^4, onto each well of a 96-well plate and were incubated for 48 h with 100 μL of DMEM/F12 containing ethanol at a concentration of 100 mmol/L. At the end of this treatment, 20 μL of Cell Titre Blue reagent was added, the cells were left for 2 h, and a reading was performed at 570 nm, with the reference reading at 600 nm, in a 96-well plate reader.

**Reactive oxygen species (ROS) measurements.** ROS measurements were performed essentially as described elsewhere [18]. In brief, the production of ROS was measured by flow cytometry using the fluorogenic dye dichlorofluoroscein diacetate (DCF-DA) (Molecular Probes). The cells were seeded, at 4 × 10^5 cells/dish, in triplicate, in 60-mm2 cell-culture dishes and were incubated for 24 h. The next day, for each cell line, the medium was replaced by serum-free medium, with either ethanol at a concentration of 0 or 100 mmol/L or (as a control) with...
hydrogen peroxide at a concentration of 1 mmol/L, and was left for another 5 h. Last, medium was replaced by 1 × PBS containing 1% glucose, and DCF-DA dye was added at a final concentration of 10 μmol/L. The cells were kept in a humidified incubator at 37°C for 30 min and then were harvested and resuspended in 1 × PBS supplemented with 1% fetal calf serum. DCF emission was measured at 525 ± 20 nm, by flow cytometry.

Promoter-reporter studies. Reporter-gene studies were performed by transfecting the cells with both pISRE-Luc, the IFN-stimulated response element driving the luciferase gene (400 ng/well), and pRL-TK (5 ng/well), to normalize transfection efficiency. The cells were treated with ethanol for 24 h, followed by treatment with IFN-α for 5 h, and luciferase activity was measured immediately thereafter by use of the Luciferase Stop and Glow Assay System (Promega).

Statistics
Results are expressed as means ± SEs. Student’s t test was used for statistical analysis. P < .05 was considered to be significant.

RESULTS

Establishment of HCV replicon–bearing cell lines expressing Cyp2e1. Because it lacks the enzyme Cyp2e1, the Huh-7 cell line does not metabolize ethanol [24]. We therefore transfected pcDNA-Cyp2e1 into the genomic HCV replicon–bearing and the subgenomic HCV replicon–bearing Huh-7 cells. Multiple G418-resistant clones were recovered (figure 1A) that express Cyp2e1 and maintain HCV replication (figure 1B). There was no significant difference, in either HCV replication, morphology, or toxicity, between the cells that expressed Cyp2e1 and those which did not (results not shown). Growth kinetics for the Cyp2e1-transfected cells were similar to those of their parent lines, although, in general, the Cyp2e1-expressing cells proliferated at a slightly slower rate (figure 1C).

Cyp2e1 activity was confirmed by assessment of cellular toxicity after the cells had been treated with the Cyp2e1-metabolized toxin acetaminophen [22]. Both the genomic replicon–bearing and the subgenomic replicon-bearing cells (results not shown) expressing Cyp2e1 showed significant rates of cell death after treatment with acetaminophen (figure 2A). This result indicates that, in the model system used in the present study, Cyp2e1 is metabolically active. Moreover, similar Cyp2e1-expressing Huh-7 cells using exactly the same Cyp2e1-expressing construct metabolize ethanol [21]. Expression of Cyp2e1 and incubation with ethanol did not lead to a cytotoxic effect in these cells (figure 2B and 2C).

Increase in HCV replication, by Cyp2e1-mediated metabolism of ethanol. In contrast to previous studies [24, 25], the present study showed no significant modulation of HCV replication after the genomic replicon–bearing and the subgenomic replicon–bearing cell lines had been stimulated with physiological concentrations of ethanol (figure 3A and 3B). This discrepancy could be due to many variables (see the Discussion section); however, the most obvious factor is that previous studies were conducted in the absence of alcohol metabolism.

When the replicon-bearing cell lines expressing Cyp2e1 were incubated with ethanol, there was a significant increase in HCV-RNA levels. For the subgenomic replicon–bearing cells, ethanol increased HCV replication in a concentration-dependent manner, to a maximum of ~3.5-fold at a concentration of 100 mmol/L (figure 3C). In the genomic replicon–bearing cells also, HCV replication in response to ethanol increased as much as 4-fold (figure 3D), although this increase occurred at a lower concentration (50 mmol/L) of ethanol. At ethanol concentrations >50 mmol/L, HCV replication was reduced, although it was still above baseline levels. This decrease was not related to alcohol-induced cellular toxicity as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (results not shown). It is possible that expression of the HCV proteins core/E1/E2/P7 and NS2, which are unique to the genomic replicon–bearing cells, may play a role in this threshold response to alcohol. Moreover, we have shown that the core protein can induce OS via interactions with mitochondria [21, 26], and it is not inconceivable that, after a threshold level of OS has been reached, there is no effect on HCV replication. Further studies are necessary to investigate this possibility. The ethanol-induced increase in HCV replication in the presence of Cyp2e1, in both the genomic replicon–containing and the subgenomic replicon–containing cell lines, was found in multiple selected clones, excluding clonal variation as a basis for these findings.

Furthermore, we also showed that the increase in HCV replication was Cyp2e1 dependent, because the Cyp2e1 inhibitor DAS blocked this increase in HCV replication (figure 3E). Collectively, these results suggest that Cyp2e1-mediated metabolism of ethanol results in an increase in HCV replication.

OS and potentiation of HCV replication. It is well documented that Cyp2e1-mediated metabolism of alcohol results in an increase in cellular OS. Furthermore, HCV replication has been shown to induce OS [27, 28]. It is therefore possible that the combination of HCV replication and Cyp2e1-mediated metabolism of alcohol may mediate both the exacerbation of liver disease and increased viral replication via OS-dependent mechanisms. To determine whether OS plays a role in HCV replication, we first used our model system to investigate whether Cyp2e1-mediated ethanol metabolism results in an increase in cellular OS. The oxidant-sensitive dye DCF-DA and flow cytometry were used to measure OS in the subgenomic replicon–bearing and the genomic replicon–bearing cell lines expressing Cyp2e1 after they had been treated with ethanol (figure 4A and 4B). When incubated in the presence of ethanol at a concentration of 100 mmol/L, both cell lines produced an increase in ROS, compared with the cells incubated in media alone.

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We next determined the effect that OS has on HCV replication. The genomic replicon–bearing (NNeo/3-5B-RG) and genomic replicon–bearing (NNeo/C-5B-RG) HCV cell lines expressing Cyp2e1, as shown by Western blotting (lanes 1) and reverse-transcriptase polymerase chain reaction (lanes 2). Note that neither the parent nor the antisense Huh-7 cell lines express detectable Cyp2e1 message or protein. B, Immunofluorescence staining of HCV antigens in genomic replicon–bearing cells expressing Cyp2e1 (1). HCV antigens were detected by use of pooled human anti-HCV serum. HCV-specific staining is not noted in parent Huh-7 cells (2). C and D, Growth rate of subgenomic replicon–bearing (C) and genomic replicon–bearing (D) cell lines expressing Cyp2e1, compared with that of parent replicon-bearing cells not expressing Cyp2e1, by the trypan blue exclusion assay. Replicon-bearing cells expressing Cyp2e1 and those not expressing it showed no statistically significant difference, in cell growth. GAPDH, D-glyceraldehyde-3-phosphate dehydrogenase. AS, antisense.

Figure 1. Characterization of hepatitis C virus (HCV) replicon–bearing cell lines expressing cytochrome P450 2E1 (Cyp2e1). A, Multiple subgenomic replicon–bearing (NNeo/3-5B-RG) and genomic replicon–bearing (NNeo/C-5B-RG) HCV cell lines expressing Cyp2e1, as shown by Western blotting (lanes 1) and reverse-transcriptase polymerase chain reaction (lanes 2). Note that neither the parent nor the antisense Huh-7 cell lines express detectable Cyp2e1 message or protein. B, Immunofluorescence staining of HCV antigens in genomic replicon–bearing cells expressing Cyp2e1 (1). HCV antigens were detected by use of pooled human anti-HCV serum. HCV-specific staining is not noted in parent Huh-7 cells (2). C and D, Growth rate of subgenomic replicon–bearing (C) and genomic replicon–bearing (D) cell lines expressing Cyp2e1, compared with that of parent replicon-bearing cells not expressing Cyp2e1, by the trypan blue exclusion assay. Replicon-bearing cells expressing Cyp2e1 and those not expressing it showed no statistically significant difference, in cell growth. GAPDH, D-glyceraldehyde-3-phosphate dehydrogenase. AS, antisense.

Inhibition of anti-HCV action of IFN-α by alcohol. Alcohol consumption greatly impairs the efficacy of IFN-α therapy, and concurrent use of alcohol is considered to be contraindicated during treatment for hepatitis C [7]. However, the mechanism(s) responsible for this inhibition have not been identified. To determine the effect that ethanol metabolism has on IFN-induced changes in HCV replication, the genomic replicon–bearing cells expressing Cyp2e1 were incubated in the presence or absence of alcohol at a concentration of 100 mmol/L and/or IFN-α at a concentration of 5 or 10 U/mL (figure 5). Consistent with results reported elsewhere, IFN-α treatment of the replicon-bearing cells resulted in a significant decrease in
HCV replication [29–31]. However, when the genomic replicon–bearing cells expressing Cyp2e1 were incubated with ethanol and IFN-α/H9251, the anti-HCV effect of IFN-α/H9251 was significantly attenuated 24 and 48 h after treatment with ethanol (figure 5). These results indicate that, at least in vitro, alcohol impairs IFN-α-mediated inhibition of HCV-RNA replication.

The binding of IFN-α to its cognate receptor results in the activation of the Jak/Stat signaling pathway [32], culminating in the expression of IFN-stimulated genes (ISGs) that act to limit HCV replication. To investigate whether ethanol exerts its suppressive effect on IFN efficacy at the level of Jak/Stat signaling, we used the IFN-α promoter-reporter (i.e., ISRE-Luc) system. As can be seen in figure 6A, ISRE promoter activity was significantly reduced in the presence of ethanol, strongly suggesting involvement of the Jak/Stat pathway.

We next investigated the phosphorylation status of Stat1 and Stat2, both of which are essential for activation of the Jak/Stat pathway. Activation of Stat1 and Stat2 involves phosphorylation of conserved tyrosines at amino acid positions 701 and 690, respectively. In addition, Stat1 serine-727 phosphorylation is essential for maximum transcription activity [33]. In the presence of ethanol (100 mmol/L), Stat1 tyrosine-701 phosphorylation was significantly inhibited (figure 6B) at 5 h; in contrast, Stat1 serine-727 phosphorylation and Stat2 tyrosine-690 phosphorylation were not affected, suggesting that this inhibitory effect is specific to Stat1 tyrosine-701. Attenuation of Stat phosphorylation by ethanol was dependent on Cyp2e1 expression (figure 6C) and was noted in multiple Cyp2e1-expressing clones.

**DISCUSSION**

Despite the strong clinical and epidemiological evidence implicating alcohol as a synergistic toxin in patients with CHC, the mechanism(s) by which alcohol consumption accelerates liver disease in persons with CHC and impairs the response to IFN is not well understood. Although it is most certainly a multifactorial process, what is becoming increasingly clear is that hepatic OS plays a role in the pathogenesis of both alcohol-induced liver disease [34] and CHC [35]. Metabolism of alcohol by Cyp2e1 in hepatocytes generates OS, whereas expression of HCV proteins and/or HCV replication can result in an increase in cellular OS [16, 21, 27, 28, 36]. It is therefore likely that the combined effect of OS due to these 2 insults may be the primary mechanism of synergistic liver injury and of modulation of HCV replication.

To study the effects that alcohol metabolism has on HCV replication and hepatocyte homeostasis, we engineered HCV repi-
con–bearing cells to constitutively express Cyp2e1. Using our model system and physiological concentrations of ethanol, we have shown that Cyp2e1-mediated metabolism of alcohol results in a significant increase (3–4-fold) in HCV replication. This increase in HCV replication could be blocked in the presence of the antioxidant NAC, strongly suggesting that OS plays a central role in HCV replication. Further supporting this notion is our finding that NAC also reduced HCV replication by 50% in Huh-7 cells harboring the genomic replicon only. Given that HCV can generate OS, it is conceivable that HCV uses OS to its replicative advantage. The precise mechanism whereby OS modulates HCV replication is not known; however, OS is a potent second messenger in the activation of cellular transcription factors such as activating protein-1 and nuclear factor-κB [37].

The challenge now will be to identify which OS-sensitive cellular transcription factor or activation of downstream cellular genes is involved in enhancement of HCV replication. Interestingly, it recently has been reported that OS induced by HCV replication in vitro activates the transcription factor Stat-3, which, in turn, stimulates HCV replication [28].

There are conflicting reports about the effects that alcohol and OS have on HCV replication in vitro. Plumlee et al. [24] have shown that a single instance of acute exposure to ethanol inhibits replication, whereas Zhang et al. [25] have reported that alcohol-
treated subgenomic replicon–bearing cells show increased HCV replication. In addition, Choi et al. have shown that exogenous application of hydrogen peroxide to replicon-bearing cells increases cellular OS and causes the release of calcium from the endoplasmic reticulum, which secondarily inhibits replication [38, 39]. In the present study, we have shown that alcohol increases HCV replication and that this effect depends on OS generation via Cyp2e1. We cannot discount the possibility that alcohol may have effects that are not related to the generation of OS; regardless, the results of the antioxidant experiments and the Cyp2e1-dependent nature of the increase in HCV replication indicate that OS plays the predominant role.

There are several methodological issues that can explain these apparent discrepancies. First, the addition of exogenous hydrogen peroxide to replicon-bearing cells increases cellular OS and causes the release of calcium from the endoplasmic reticulum, which secondarily inhibits replication [38, 39]. In the present study, we have shown that alcohol increases HCV replication and that this effect depends on OS generation via Cyp2e1. We cannot discount the possibility that alcohol may have effects that are not related to the generation of OS; regardless, the results of the antioxidant experiments and the Cyp2e1-dependent nature of the increase in HCV replication indicate that OS plays the predominant role.

There are several methodological issues that can explain these apparent discrepancies. First, the addition of exogenous hydrogen peroxide may produce either a degree of OS or a subcellular distribution of ROS that differs from that induced by endogenous ethanol metabolism [40]. Interestingly, using our model system, we also have demonstrated that, in response to exogenous addition of hydrogen peroxide (results not shown), there is a decrease in HCV replication, a decrease that is consistent with...
results reported by Choi et al. [38, 39]. Our findings in the present study show that there is a possible biphasic effect of alcohol in genomic replicon–bearing cells (figure 4), which suggests that low levels of OS could stimulate replication whereas high levels of OS could repress it. In the present study, OS was generated in situ as a product of alcohol metabolism by Cyp2e1, whereas Huh-7 cells themselves do not express this alcohol-metabolizing enzyme. These results could explain some other studies’ failure to see stimulation. It is therefore intriguing to speculate how the Huh-7 cells used by Zhang et al. show that HCV replication increases in response to ethanol [25]. It may be possible that there are metabolism-independent effects of alcohol or that these Huh-7 cells retain residual Cyp2e1 and/or ADH activity; however, their expression was not investigated.

Continued alcohol abuse during IFN-α/ribavirin combination therapy adversely affects the response to HCV treatment and results in reduced rates of antiviral response. However, the molecular mechanism(s) responsible is not well understood. We therefore investigated, in replicon–bearing cells expressing Cyp2e1, the anti-HCV activity that IFN-α has in the presence or absence of alcohol. We found that, in the presence of alcohol, the anti-HCV action of IFN-α was significantly impaired, a result that supports our hypothesis that alcohol has a direct effect, at the cellular level, on the antiviral activity of IFN-α. IFN-α exerts its antiviral effect via activation of the Jak/Stat signaling pathway, resulting in expression of IFN-stimulated genes, many of which are required for viral replication. We found that alcohol metabolism inhibited IFN-α signaling by reducing the expression of key IFN-stimulated genes.

Figure 5. Ethanol metabolism’s effect on the anti-HCV action of interferon (IFN). Hepatitis C virus (HCV) genomic replicon–bearing cells expressing cytochrome P450 2E1 were incubated in the presence of IFN–α2b (5 or 10 IU) with or without ethanol (100 mmol/L); total RNA was recovered; and HCV RNA was quantitated by real-time polymerase chain reaction at 24 h (A) and 48 h (B) after treatment with IFN. HCV RNA levels are expressed as the fold change relative to the levels in the absence of treatment. *P < .05.

Figure 6. Ethanol metabolism’s effect on the Jak/Stat pathway. A, Ethanol’s selective inhibition of pISRE-Luc, the interferon (IFN)–α-sensitive promoter driving the luciferase reporter gene. *P < .05. B, Hepatitis C virus (HCV) genomic replicon–bearing cells expressing cytochrome P450 2E1 (Cyp2e1), which were incubated in the presence or absence of ethanol (100 mmol/L) and were stimulated with IFN–α2b for up to 300 min. Total cell lysates were recovered, and immunoblots were probed with various phosphorylation-specific Stat antibodies. Note the significant decrease in Stat1 tyrosine-701 (pStat1-701) phosphorylation after treatment with IFN–α and ethanol. Neither phosphorylation at other Stat1 and Stat2 residues nor total Stat protein levels were affected by ethanol metabolism. C, Quantitation of Stat1 tyrosine-701 phosphorylation (Stat1 P 701), performed by use of NIH Image. D, Decreased Stat1 tyrosine-701 (pStat1-701) phosphorylation in HCV genomic replicon–bearing cells, which does not occur in the absence of Cyp2e1.
which possess antiviral activity. Consistent with our hypothesis that alcohol metabolism may negatively affect activation of the Jak/Stat pathway, we found that alcohol preferentially inhibits Stat1 tyrosine-701 phosphorylation but does not inhibit either Stat1 serine-727 phosphorylation or Stat2 tyrosine-690 phosphorylation, in a Cyp2e1-dependent manner. The implications of this inhibition would be a decrease in Stat1/Stat2 heterodimerization, resulting in reduced nuclear translocation and subsequent downstream ISG expression. Clearly, alcohol targets specific aspects of the Jak/Stat pathway, and further experiments are necessary to determine the downstream consequence of this inhibition. Interestingly, in vitro it also has been demonstrated that IFN-γ also inhibits Stat1 tyrosine-701 phosphorylation [41]. It is also possible that alcohol inhibits other components of the cellular innate response to HCV infection and to IFN, components such as cellular recognition of viral pathogen–associated molecular patterns and activation of the Toll-like receptor–3 and retinoic acid–induced gene–1 pathways. Alternatively, alcohol may modulate suppressor of cytokine signaling (SOCS) proteins such as SOCS–1 and –3, which, by inhibition of JAKs, inhibit IFN signaling. Hong et al.’s recent work using a cancanavalin A model of hepatitis in mice has shown that activated Stat3 plays an important role in inducing SOCS–3, a potent inhibitor of IFN signaling [42]. Interestingly, we have observed (results not shown) that, in our model system, activation of Stat3 is enhanced after alcohol stimulation, suggesting that Stat3 possibly plays a role in alcohol-induced suppression of IFN signaling. Clearly, the factors at play in alcohol-induced suppression of IFN function in the background of HCV replication are complex. However, the system described herein provides a model for further in-depth dissection of innate immune response and IFN signaling cascades in response to alcohol and HCV.

In summary, we have developed an in vitro cell-culture model to evaluate interactions between HCV and alcohol metabolism by Cyp2e1. Specifically, we have shown that metabolism of alcohol by Cyp2e1 results in enhancement of HCV replication, an enhancement that is dependent on alcohol-induced OS. Furthermore, we also have demonstrated that alcohol can impair the anti-HCV action of IFN-α, most likely via abrogation of the Jak/Stat signaling pathway. These observations are particularly important in that they are consistent with the known clinical effects of alcohol consumption in persons with CHC. The protective effect that the antioxidant NAC has on the reduction of HCV-replication levels implies that antioxidant supplementation potentially can play a therapeautic role to augment standard IFN-based antiviral therapies. Use of this model system to further dissect the interactions between alcohol, HCV, and hepatocytes will aid in our understanding not only of CHC and alcohol but also of alcohol-induced liver disease.

References


