Delivery of the Cu/Zn–Superoxide Dismutase Gene With Adenovirus Reduces Early Alcohol-Induced Liver Injury in Rats

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Background & Aims: Alcohol-induced liver injury is associated with an increase in oxidants from a variety of possible sources. Therefore, it was hypothesized that increased and stable expression of the antioxidant enzyme Cu/Zn–superoxide dismutase (SOD1) would diminish oxygen free radicals and reduce alcohol-induced liver injury. Methods: To test this hypothesis, rats were given recombinant adenovirus containing Cu/Zn–superoxide dismutase (Ad.SOD1) or β-galactosidase (Ad.lacZ) and fed ethanol enterally for 3 weeks. Results: SOD was increased significantly 3–5-fold over endogenous levels in both hepatocytes as well as Kupffer cells 3 weeks after infection. Serum transaminase levels and pathology were elevated significantly in Ad.lacZ-treated animals by using an intragastric feeding model. This effect was blunted significantly in Ad.SOD1-infected animals. Importantly, electron spin resonance–detectable free-radical adducts caused by ethanol were also decreased by SOD1 overexpression. Moreover, the increase in nuclear factor κB (NFκB), tumor necrosis factor α (TNF-α), and interleukin 1 messenger RNA (mRNA) caused by ethanol was blunted in animals treated with Ad.SOD1. Conclusions: These data support the hypothesis that oxidant production is critical in early alcohol-induced liver injury and that gene delivery of antioxidant enzymes may be useful in prevention and treatment.

The evidence that oxidative stress contributes to the pathogenesis in early alcohol-induced liver injury is extensive. Early work suggested that lipid peroxidation resulted from chronic ethanol exposure.1–3 With the introduction of the spin trapping technique, many groups showed that an α-hydroxyethyl free radical from ethanol was detected in vitro4,5 and in vivo.6 Later, this radical was detected in the bile from rats given ethanol chronically via enteral gastric feeding,7 a model that produces early changes in the liver that closely resemble the early pathology observed in human alcoholics.

Oxidants are produced from a variety of sources in the liver. Kupffer cells, which contain the superoxide-generating enzyme reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, have been shown to be involved in free radical production in the liver after chronic ethanol.8 However, hypoxia in parenchymal cells could also lead to the production of superoxide through xanthine oxidase and other reductive pathways.5,10 CYP2E1 in parenchymal cells is yet another potential source of oxidants,11–13 but may not be critically important in alcohol-induced liver injury because pathology was equally severe in wild-type mice and mice deficient in CYP2E1.16 However, mice deficient in p47phox, a regulatory subunit of NADPH, have been shown to be resistant to ethanol-induced liver injury,17 supporting the hypothesis that Kupffer cell–derived oxidants predominate. Although the precise source of oxidants in alcohol-induced liver injury is not completely clear, it is known that oxidant generation by any of these sources could activate nuclear factor κB (NFκB), which is a potent transcription factor responsible for the production of tumor necrosis factor α (TNF-α) and key adhesion molecules.18,19 Moreover, TNF-α has been shown to be a critical cytokine in this model, causing peripheral lipolysis and an increase in fatty acids, increases in leukocyte adhesion molecules, and necrosis of parenchymal cells.20,21 It was recently shown by using TNF-receptor knockout mice that TNF-α is necessary for alcohol-induced liver injury, at least in the mouse.22

Although normal livers have high antioxidant enzyme levels, glutathione and superoxide dismutase (SOD) levels were diminished in rats after chronic ethanol exposure.

Abbreviations used in this paper: Ad, adenovirus; Ad.lacZ, adenovirus β-galactosidase; EGTA, ethylene glycol-bis[β-aminoethyl ether]-N,N,N′,N′-tetraacetic acid; EMSA, electrophoretic mobility shift assays; ESR, electron spin resonance; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NFκB, nuclear factor κB; pfu, plaque-forming units; POBN, α-(4-pyridyl-1-oxide)-N-tert-butyl nitronate; RNase, ribonuclease; SOD, superoxide dismutase; TNF-α, tumor necrosis factor α.

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Ethanol levels in the diet were gradually increased to isocalorically with ethanol. This diet has been reviewed elsewhere and vitamins. In the ethanol-containing diet, 35% of mL) contained corn oil as fat (37% of total calories), protein as described previously. The high-fat control diet (1.3 kcal/g) was adjusted to a final volume of 50 mmol/L KCl, 0.1 mmol/L Na2 EDTA, 0.5 mg/mL cytochrome c, and 0.25 mg/mL xanthine. Superoxide was generated by the addition of 10 μL of 0.4 U/mL xanthine oxidase, and the reduction of cytochrome c was measured spectrophotometrically at 550 nm. Assays were also performed in the presence of sodium azide (5 μmol/L), an inhibitor of cytochrome oxidase, catalase (1 U) to inhibit oxidation of cytochrome c, or KCN (1.5 mmol/L) to inhibit Cu/Zn-SOD activity. SOD activity was calculated based on the molar extinction coefficient of 18.5 and a standard curve generated by using purified bovine erythrocyte superoxide dismutase (Boehringer Mannheim).

Catalase Activity

Catalase activity was measured in liver homogenate as described by Aebi with some modifications. Briefly, homogenate (10 μg) was adjusted to a final volume of 50 μL with phosphate buffer. The reaction was initiated by adding 3.0 mL of 12.5 mmol/L H2O2 in phosphate buffer, and the change in absorbance at 240 nm was measured at 25°C for 1 minute. Based on a millimolar extinction coefficient for H2O2 of 34.9,
catalase activity was defined as μmol of H₂O₂ consumed/ min/mg protein.

**Glutathione Peroxidase Activity**

Glutathione peroxidase activity was determined by adding 10 μL of liver homogenate to 850 μL of buffer containing 0.1 mmol/L NaPO₄, 4 mmol/L reduced glutathione, 0.1 mmol/L NADPH, and 2 U of glutathione reductase. After 30 seconds, 10 μL of 1.2 mmol/L t-butyl-hydroperoxide was added to initiate the reaction. The rate of decrease in absorption of NADPH at 340 nm was measured, and the amount of NADPH consumed was calculated by using a millimolar extinction coefficient of 6.22.

**Spin Trapping Technique and Electron Spin Resonance**

Ethanol concentrations in the breath were analyzed by gas chromatograph to verify that levels were near 200 mg/dL when experiments were initiated. Animals were anesthetized with pentobarbital (75 mg/kg), the spin trapping agent 4-POBN (1 g/kg) was administered intravenously, and bile was collected at 30-minute intervals for 3 hours into 35 μL of 0.5 mmol/L deferoxamine mesylate to prevent ex vivo radical production. The electron spin resonance (ESR) spectra of radical adducts were obtained by using a Bruker ESP-300 spectrometer (Billerica, MA) operating at 9.8 GHz with 100 kHz modulation frequency. Instrument conditions were as follows: 20 mW microwave power, 0.8 G modulation amplitude, 80 G scan width, 677-second scan, and 1.3-second time constant.

**Electrophoretic Mobility Shift Assays**

For studies in whole liver, nuclear extracts were isolated as described by Dignam et al. with minor modifications. Binding conditions for NFκB were characterized and electrophoretic mobility shift assays (EMSA) were performed as described elsewhere. Briefly, nuclear extracts (20 μg) from liver tissue were preincubated for 10 minutes on ice with 1 μg poly (dI-dC) and 20 μg bovine serum albumin (BSA; Pharmacia Biotech, Piscataway, NJ), and 2 μL of a 32P-labeled DNA probe (10,000 cpm/μL, Cerenkov) containing 1 ng of double-stranded oligonucleotide in a total volume of 20 μL. Mixtures were incubated for 20 minutes on ice and resolved on 5% polyacrylamide-urea gel. After drying, the gel was visualized by autoradiography.

**Ribonuclease Protection Assay**

Total RNA was isolated from liver tissue by using RNA STAT 60 (Tel-Test). Ribonuclease (RNase) protection assays were performed by using the Ribo-Quant multiprobe assay system (Pharmingen, Franklin Lanes, NJ). Briefly, [32P]RNA probes were transcribed with T7 polymerase by using the multiprobe template set rCK-1. RNA (20 μg) was hybridized with 4 × 10⁶ cpm of probe overnight at 56°C. Samples were then digested with RNase followed by proteinase K treatment, phenol/chloroform extraction, and ethanol precipitation. Samples were resolved on a 5% acrylamide-bisacrylamide (19:1) urea gel. After drying, the gel was visualized by autoradiography.

**Results**

**SOD Expression Caused by Ad.SOD1**

Before testing the effect of Cu/Zn-SOD (SOD1) delivery in an enteral ethanol feeding model, it was first necessary to evaluate if Cu/Zn-SOD expression was elevated by Ad-SOD1 treatment for 3 weeks. In pilot experiments, animals were infected with Ad.lacZ (1 × 10⁹ pfu), and transgene was examined by immunostaining against β-galactosidase. Importantly, 3 weeks after infection, immunostaining revealed that approximately 60% of the liver expressed adenoviral transgene (Figure 1). Moreover, expression of transgene was panlobular and was observed mostly in hepatocytes; however, some sinusoidal cells were positively stained, indicating that adenovirus transduces both parenchymal and nonparenchymal cells in vivo.

To test the hypothesis that recombinant Cu/Zn-SOD was expressed, animals were infected with Ad.SOD1 (1 × 10⁹ pfu) and Ad.lacZ and were killed 3 weeks later. The expression of Cu/Zn-SOD was evaluated in whole liver homogenate by Western blot. SOD expression was increased in whole liver nearly 3-fold in animals infected with Ad.SOD1 compared with Ad.lacZ-infected controls (data not shown). To address whether or not Kupffer cells are transduced, Kupffer cells and hepatocytes were isolated from animals infected with Ad.SOD1 and Ad.lacZ, and the expression of β-galactosidase and Cu/Zn-SOD was evaluated by Western blot analysis (Figure 2). Both hepatocytes and Kupffer cells from animals infected with Ad.lacZ expressed β-galactosidase. By using an antibody that is cross-reactive with both endogenous rat SOD (∼17 kilodaltons) as well as recombinant human SOD (∼19 kilodaltons), it was shown that both hepatocytes and Kupffer cells isolated from Ad.SOD1-infected animals expressed 3–5-fold more Cu/Zn-SOD than controls, which expressed only the endogenous enzyme (Figure 2B). Extracts from Ad.lacZ- and Ad.SOD1-infected tissue culture embryonic kidney (HEK 293) cells were used.
as positive controls. The data clearly indicate that adenovirus transduces Kupffer cells as well as hepatocytes in vivo.

Superoxide dismutase activity in liver extracts was measured by reduction of ferricytochrome c. In animals infected with Ad.lacZ and given either high-fat control diet or diet containing ethanol for 3 weeks, SOD activity was not different from naive animals (data not shown). However, in animals that were infected with Ad.SOD1 and fed either high-fat control diet or ethanol for 3 weeks, SOD activity was increased nearly 3-fold (Table 1). These data are consistent with data from immunoblots, indicating that Cu/Zn-SOD expression results in significant 2–3-fold increases in SOD activity above endogenous levels (Figure 2B). SOD activity was also evaluated in the presence of catalase (1 U) and NaN₃ (5 µmol/L), an inhibitor of both catalase and cytochrome oxidase, to rule out interference of endogenous enzymes with the measurement of superoxide dismutase activity. No changes in SOD were detected in Ad.lacZ- or Ad.SOD1-infected animals when tested in the presence of either catalase or NaN₃. To evaluate the contribution of endogenous mitochondrial SOD to total SOD activity, the assay was performed in the presence of KCN (1.5 mmol/L), an inhibitor of the cytosolic Cu/Zn-SOD isoform. Addition of KCN nearly completely inhibited SOD activity in cytosolic extract from both Ad.lacZ- and Ad.SOD1-infected animals. Activities of catalase and glutathione peroxidase, enzymes that reduce H₂O₂, were also measured in whole liver. No significant changes were observed in any of the treatment groups studied (Table 1).

Whether or not superoxide dismutase activity was increased in Kupffer cells was also addressed. SOD activity was determined in Kupffer cells isolated from animals infected with Ad.lacZ or Ad.SOD1 (1 × 10⁹ pfu). SOD activity in Kupffer cells from Ad.SOD1-infected animals was increased to 2.1 ± 0.2 U/mg protein, compared with 0.6 ± 0.1 U/mg protein in Kupffer cells from control animals (P < 0.05, Student t test).

**Adenoviral Expression of SOD Protects Against Early Alcohol-Induced Liver Injury**

To test the hypothesis that adenoviral-mediated delivery of Cu/Zn-SOD would minimize or prevent alcohol-induced liver injury, male Wistar rats (300–325 g) were given either Ad.SOD1 (1 × 10⁹ pfu) or Ad.lacZ
as control 3 days before ethanol diet. Subsequently, rats were given either high-fat control diet or diet containing ethanol. During feeding, body weight and urine alcohol concentration were monitored daily. Animals in all groups had an average rate of body weight gain of about $16/11006 \times 2$ g/wk, indicating that nutrition was adequate. The urine alcohol concentrations in the ethanol-fed animals displayed the reported cyclic pattern41 and urine alcohol averaged 215/11006 $16$ and 226/11006 $21$ mg/dL in the Ad. lacZ- and Ad.SOD1-treated animals, respectively.

Blood samples were collected for serum transaminase measurements (Figure 3). Serum alanine transaminase levels in the Ad. lacZ-treated animals that received ethanol diet were increased significantly compared with the high-fat controls. However, alanine transaminase levels in the Ad.SOD1-treated animals given ethanol were blunted by nearly 60%, indicating that overexpression of Cu/Zn-SOD protects the liver from alcohol-induced liver injury. The levels of serum aspartate transaminase were also elevated by ethanol (167/11006 $21$ U/L) and significantly blunted in Ad.SOD1-infected animals given ethanol (102/11006 $13$ U/L, $P < 0.05$).

Pathology caused by chronic ethanol was evaluated for steatosis (scale 0 – 4), inflammation (0 – 2), or necrosis (0 – 2). In the Ad.lacZ animals that received ethanol diet, significant panlobular steatosis (3.5 ± 0.5), massive lymphocytic infiltration (1.6 ± 0.4), and necrosis (0.8 ± 0.2) was observed in pericentral regions of the liver lobule (Figure 4C). However, in the Ad.SOD1-treated animals after ethanol exposure (Figure 4D), inflammation (0.2 ± 0.2) was nearly completely prevented, necrosis was absent, and steatosis (1.8 ± 0.5) was reduced by nearly 50%. These data clearly indicate that Cu/Zn-SOD overexpression protects the liver from early alcohol-induced injury.

**SOD Overexpression Blunts Ethanol-Induced Free Radical Formation**

To provide direct evidence whether or not overexpression of antioxidant Cu/Zn-SOD prevented oxidant production in vivo, Ad.lacZ- and Ad.SOD1-infected an-

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**Table 1. Summary of Liver Antioxidants in Ad.lacZ- and Ad.SOD1-Infected Animals After Ethanol**

<table>
<thead>
<tr>
<th></th>
<th>Ad.lacZ/ control</th>
<th>Ad.lacZ/ ethanol</th>
<th>Ad.SOD1/ control</th>
<th>Ad.SOD1/ ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total SOD (U/mg protein)</td>
<td>3.5 ± 0.4</td>
<td>3.1 ± 0.3</td>
<td>7.3 ± 0.7a</td>
<td>8.2 ± 0.4a</td>
</tr>
<tr>
<td>+ Catalase (1 U)</td>
<td>3.1 ± 0.6</td>
<td>3.1 ± 0.9a</td>
<td>7.8 ± 1.1a</td>
<td></td>
</tr>
<tr>
<td>+ NaN3 (5 μmol/L)</td>
<td>3.4 ± 0.5</td>
<td>0.7 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ KCN (1.5 mmol/L)</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH peroxidase (U/mg protein)</td>
<td>523 ± 50</td>
<td>428 ± 93</td>
<td>486 ± 60</td>
<td>563 ± 40</td>
</tr>
<tr>
<td>Catalase (μmol/mg/min)</td>
<td>3.0 ± 0.4</td>
<td>4.2 ± 0.6</td>
<td>3.0 ± 0.4</td>
<td>2.3 ± 0.4</td>
</tr>
</tbody>
</table>

NOTE. SOD activity was measured as described in the Materials and Methods section in postmitochondrial supernatants from livers of rats infected with Ad.lacZ or Ad.SOD1 (1 × 109 pfu) and fed either high-fat control diet or diet containing ethanol for 3 weeks. The assay was also performed in the presence of catalase (1 U), NaN3 (1 μmol/L), or KCN (1.5 mmol/L). Glutathione peroxidase and catalase activities were also measured in liver homogenates as described in the Materials and Methods. Data are expressed as mean ± SEM of 4 individual experiments. $aP < 0.05$, 2-way ANOVA followed by the Tukey post hoc analysis.

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**Figure 3.** Increases in serum transaminases caused by ethanol are blunted by Ad.SOD1. Serum alanine transaminase levels were measured by standard serum enzyme kits (Sigma, St. Louis, MO). Data are expressed as mean ± SEM of 5 individual experiments in each group. $^aP < 0.05$ compared with high-fat control; $^bP < 0.05$ compared with Ad.lacZ, ethanol-treated samples by 2-way ANOVA followed by the Tukey post hoc analysis. Con, high-fat control diet; EtOH, ethanol-containing diet.
Animals were fed either high-fat control diet or diet containing ethanol for 3 weeks, and bile was collected for 1 hour immediately after the administration of the spin trapping agent α-(pyridyl-1-oxide)-N-tert-butylnitrone (POBN) (1 g/kg, intravenously). This dose of POBN is used because it has recently been shown to be the dose for radical adduct formation with optimal signal-to-noise ratio. Free-radical adducts in the bile were then measured by ESR spectroscopy (Figure 5). The observed free-radical adducts caused by chronic ethanol exhibited ESR hyperfine coupling constants ($\alpha^N = 15.70$ G and $\alpha^H_B = 2.62$ G) typical of either lipid-derived or α-hydroxyethyl radical adducts based on spectral simulations. Earlier studies and current work with $^{13}$C-ethanol show that the α-hydroxyethyl radical adduct contributes about 60% of ESR signal intensity under these conditions, with the remainder mostly caused by the lipid-derived radical adducts. Chronic ethanol caused a marked increase in radical adduct formation in the Ad.lacZ-infected animals (Figure 5B) compared with high-fat control animals. The signal intensity was significantly blunted nearly to control levels in animals infected with Ad.SOD1 (Figure 5D) after 3 weeks of enteral ethanol.

Overexpression of SOD1 Reduces Activation of NFκB

Oxidants are known to activate NFκB in several models, including the enteral ethanol model described here. Therefore, to test the hypothesis that Cu/Zn-SOD prevents activation of NFκB by preventing oxidant production in the liver, whole liver nuclear extracts from animals infected with Ad.SOD1 and given either high-fat control diet or ethanol diet for 3 weeks were analyzed by EMSA (Figure 6). Supershift of the NFκB complex by using antibodies against the p50 and p65 subunits of active NFκB dimer confirms that ethanol causes activation and nuclear localization of NFκB.
which is comprised of both p50 and p65 subunits (Figure 6A). Chronic ethanol caused nearly a 4-fold activation of NFκB in animals infected with Ad.lacZ (Figure 6B and C). However, NFκB activation was blunted nearly to control levels in animals given Ad.SOD1. Importantly, these data indicate that Cu/Zn-SOD overexpression prevents activation and translocation of NFκB caused by chronic ethanol, most likely by scavenging radicals known to induce the transcription factor.

**Overexpression of SOD Prevents TNF-α and Interleukin 1 Messenger RNA Production Caused by Chronic Ethanol**

Oxidants activate NFκB leading to an increase in cytokine production.\(^{19}\) Thus, it was hypothesized that overexpression of Cu/Zn-SOD would decrease oxidants, causing less NFκB activation, and thereby diminish cytokine messenger RNA (mRNA) synthesis. To test this hypothesis, critical cytokine mRNA levels were analyzed by RNase protection assay (Figure 7). Chronic ethanol caused an increase in TNF-α and interleukin 1 mRNAs.
in animals infected with Ad.lacZ. However, cytokine mRNA expression was blunted to control levels (i.e., Ad.lacZ and high-fat diet) in animals given Ad.SOD1 and exposed to ethanol. These data indicate that SOD1 overexpression blunts TNF-α and interleukin 1 expression caused by chronic ethanol, which is consistent with the hypothesis that oxidant production caused by ethanol activates NFκB, leading to increased transcription of TNF-α and other critical inflammatory cytokines.

Discussion

Adenoviral Transgene Expression Can Be Achieved In Vivo

Although adenovirus provides robust, transient expression of a transgene that is very useful in experimental situations in which short-term expression can be achieved, the lack of stable transgene expression could limit its use in long-term experimental protocols, such as in the enteral ethanol model. However, here adenoviral transgene expression persisted at high levels for at least 3 weeks (Figure 1) and is still detectable at 8 weeks (data not shown), considerably longer than what is commonly reported in the literature. Thus, proof of principle experiments to test the therapeutic effects of SOD overexpression by using adenovirus could be performed. The increased persistence of transgene expression in these studies is most likely caused by the viral titer. Viral titers greater than $10^{10}$ to $10^{11}$ pfu per animal are commonly used, whereas in this study, a much lower dose of virus was used. Indeed, in pilot studies using high titers of adenovirus (i.e., $3 \times 10^{10}$ pfu), transgene was removed rapidly, consistent with these reports. Others have suggested that viral load is a key component of the cytotoxic T-cell response against adenoviral-infected cells. These data are significant because they support the idea that low-dose adenoviral-mediated gene delivery may be used in long-term experimental, and possibly clinical, applications. It is also shown here that adenovirus transduces Kupffer cells (Figure 2). SOD expression and activity was significantly increased in Kupffer cells isolated from Ad.SOD1-infected animals, indicating that adenovirus transduces Kupffer cells as well as hepatocytes in vivo.

Oxidants Contribute to Early Alcohol-Induced Liver Injury

Alcoholic liver disease has long been known to be associated with oxidative stress; however, neither the role of oxidants nor their primary source is known. In studies using knockout mice deficient of CYP2E1, ethanol induced early pathologic changes and increased radical adducts similar to wild-type mice, suggesting that other sources of oxidants predominate. Destruction of the Kupffer cell with GdCl₃ prevented free-radical formation in the ethanol model, implicating Kupffer cell NADPH oxidase. Moreover, mice deficient in NADPH oxidase are resistant to ethanol-induced increases in serum transaminases, pathology, and free-radical formation, suggesting that superoxide production by NADPH oxidase in the Kupffer cell is important. Here, it is shown that SOD expression and activity is significantly increased in Kupffer cells from Ad.SOD1-infected animals compared with cells from control animals. Thus, data presented here are consistent with the hypothesis that oxidant production primarily from Kupffer cells activates NFκB, leading to increased cytokine production. Indeed, Kupffer cells are the major source of TNF-α in the liver. Additionally, mice deficient in TNF-α receptor 1 are also resistant to ethanol-induced liver injury, yet TNF-α receptor 1 knockout mice fed ethanol generate free radicals (Yin and Thurman, unpublished data). Despite multiple possible sources of oxidants in liver, the evidence for Kupffer cell NADPH oxidase is strong. Moreover, these data suggest that oxidants most likely act as important signaling molecules, leading to an inflammatory response involving NFκB and TNF-α production. However, the direct effects of oxidants on lipids, proteins, or DNA cannot be ruled out as contributors to liver injury induced by ethanol.

Overexpression of SOD Prevents Alcohol-Induced Liver Injury

The use of the adenoviral delivery system allows transient expression of an antioxidant Cu/Zn-SOD1 that persists for longer than 3 weeks (Figures 1 and 2). An increase in Cu/Zn-SOD expression protected the liver from alcohol-induced liver injury, assessed by serum transaminases and pathology (Figures 3 and 4). These data are consistent with the hypothesis that oxidants are involved in alcoholic liver disease and suggest that antioxidant status is critically important in the pathogenesis of the disease. Direct evidence to support this hypothesis is that Cu/Zn-SOD overexpression blunted free-radical adduct formation caused by ethanol (Figure 5). Antioxidants have been shown both in vitro and in vivo to blunt redox-sensitive NFκB activation and subsequent NFκB-dependent cytokine production. In fact, Cu/Zn-SOD blunted NFκB activation and cytokine production caused by ethanol (Figures 6 and 7). Interestingly, Cu/Zn-SOD overexpression markedly reduced fat accumulation in liver caused by ethanol (Figure 4), suggesting a role for oxidants in the development of alcohol-induced
stearosis. Prevention of NFκB activation in Kupffer cells is most likely the reason for the reduction in alcohol-induced stearosis because NFκB induces TNF-α production, and TNF-α stimulates peripheral adipose tissue lipolysis and fatty acid mobilization. Work with TNF receptor 1 knockout mice also suggests that fat accumulation in the liver caused by ethanol is dependent on TNF-α signaling because steatosis caused by alcohol was reduced in the TNF receptor 1 knockout.

Because H₂O₂, a potent oxidant and activator of NFκB, is a by-product of SOD reduction of superoxide, it is also interesting that overexpression of SOD did not exacerbate oxidant-induced injury. Moreover, antioxidant mechanisms such as glutathione and glutathione peroxidase have been shown to be decreased by ethanol.53 However, others have clearly shown that SOD delivery alone, either through direct infusion of recombinant enzyme or transgenic overexpression, was protective against ischemia-reperfusion in heart and liver and that additional antioxidants such as catalase or glutathione were not required to contend with H₂O₂.54–56 Catalase in the liver is extremely abundant, and the rate of production of H₂O₂ from SOD (3–10⁵) is much slower than the catalytic rate constant for catalase (10⁶).57

In conclusion, Cu/Zn-SOD overexpression in liver can be achieved through adenoviral gene delivery, and antioxidant overexpression protects against alcohol-induced liver injury. Moreover, these data are consistent with the hypothesis that oxidant production is an important factor. It is also important that long-term expression of an adenoviral transgene can be achieved by carefully reducing the viral load. Because long-term daily treatment with drugs may be difficult in the prevention of alcoholic liver disease, delivery of antioxidant genes via adenovirus may be useful clinically.

References


