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Mechanism of action of ethanol in the down-regulation of Galβ1, 4GlcNAc α2,6-sialyltransferase messenger RNA in human liver cell lines

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Abstract

Galβl, 4GlcNAc α2,6-sialyltransferase (2,6-ST) mediates the addition of α2,6-linked sialic acid to glycoproteins in the Golgi compartment. Down-regulation of its gene and consequent impaired activity of 2,6-ST seems to be the major cause for the appearance of asialoconjugates in the blood of long-term alcoholics. Therefore, mechanism(s) involved in the regulation of 2,6-ST gene is important and clinically relevant. Our previous work showed that long-term ethanol feeding in rats caused a marked 59% decrease of 2,6-ST activity as well as 2,6-ST messenger RNA (mRNA) level in liver that were due to the decreased stability of its mRNA. We now mimic these actions of ethanol using (*a*) human liver HepG2 cells stably transfected with ethanol-inducible human cytochrome P4502E1 (CYP2E1 cells), or (*b*) with high alcohol dehydrogenase (HAD cells) but not in wild-type HepG2 cells lacking either of the above 2 enzymes as models. Incubation of these cells for 72 hours with 100 mmol/L ethanol caused decreases (up to 76%, $P < .05$) of 2,6-ST mRNA levels in CYP2E1 and HAD cells but not in the wild type. However, incubation of wild-type cells with acetaldehyde at concentrations of 50 and 100 μmol/L showed a dramatic decrease (up to 69%, $P < .02$) in the 2,6-ST mRNA levels. Furthermore, exposure of CYP2E1 cells to 4-hydroxy-2-nonenal, an endogenous lipid peroxidation product of reactive oxygen species, strongly decreased 2,6-ST mRNA level by 61% ($P < .02$). These results demonstrate that 2,6-ST gene is highly sensitive to ethanol action in human liver cells either via its oxidation product, acetaldehyde, or via reactive oxygen species leading to the generation of a more reactive aldehyde such as 4-hydroxy-2-nonenal. Thus, this study assumes major importance and clinical relevance because 2,6-ST gene regulation in a human liver cell model is demonstrated within a few days of ethanol exposure, whereas its in vivo regulation in liver generally takes prolonged period of ethanol exposure.

1. Introduction

Galβl, 4GlcNAc α2,6-sialyltransferase (2,6-ST) mediates the addition of α2,6-linked sialic acid to glycoproteins in the Golgi compartment. Down-regulation of its gene and consequent impaired activity of 2,6-ST seems to be the major cause for the appearance of asialoconjugates in the blood of long-term alcoholics. Therefore, mechanism(s) involved in the regulation of 2,6-ST gene is important and clinically relevant. 2,6-ST is strongly expressed in liver and can be either up- or down-regulated by a number of factors. In our rat alcohol-feeding model, rat 2,6-ST expression was reduced to as much as 59% by long-term alcohol treatment compared with the pair-fed control group in a dose-dependent manner [1]. It will be of major importance and clinical significance if the regulation of 2,6-ST gene can be mimicked in a human liver cell model, especially if it can be achieved within a few days of ethanol exposure in a human liver culture system rather than in vivo that may take prolonged period of ethanol exposure.

To define the significance and molecular mechanisms of aberrant sialylation in alcoholics, we focused attention on sialidases and sialyltransferases [2], 2 of the key enzymes involved in the metabolism of glycoproteins and glycolipids. Our studies have shown that long-term ethanol administration decreases the hepatic activity of β-galactoside-α2, 6-sialyltransferase (2,6-ST) in the rat liver via the down-regulation of the 2,6-ST gene [3]. In human beings, the vast majority of ethanol is oxidized to acetaldehyde by the hepatocytes of the liver [4]. However, whether or not ethanol metabolism by the liver is a prerequisite for its action in down-regulating 2,6-ST gene has not been established.

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On the other hand, CYP2E1-mediated oxidation of ethanol also produces a state of oxidative stress by generating reactive oxygen species (ROS) within the cells that is responsible for the progression of alcoholic liver disease or cell damage [5-7]. Therefore, it is possible that another mechanism of action of ethanol in regulating 2,6-ST gene expression may be mediated via ROS. One of the key metabolites generated because of oxidative stress is the α,β-unsaturated aldehyde, 4-hydroxy-2-nonenal (HNE), which may be more harmful than ROS because it has a longer half-life and can easily diffuse into cellular membranes [8].

In the present report, we have taken advantage of cultured human wild-type HepG2 liver cells that do not metabolize ethanol because they lack the key ethanol-metabolizing enzymes, namely, cytochrome P4502E1 (CYP2E1: ethanol-inducible) and alcohol dehydrogenase (ADH). However, when these wild-type liver cells are stably transfected with either CYP2E1 gene or high alcohol dehydrogenase (HAD) gene, they are efficient in metabolizing ethanol and truly reflect ethanol metabolism by human liver in situ. Thus, the 3 HepG2 cell types, the wild-type cells, CYP2E1 cells, and HAD cells are ideal models to clearly define the true action of ethanol in regulating 2,6-ST gene. It will be shown that ethanol causes the down-regulation of 2,6-ST gene only in the ethanol-metabolizing liver cells, but not in the wild type. In contrast, acetaldehyde, the immediate product of ethanol oxidation caused the down-regulation of 2,6-ST gene even in the wild type. Furthermore, we will also show that HNE strongly down-regulates 2,6-ST gene in CYP2E1 cells. Thus, it is unequivocally demonstrated that ethanol oxidation leading to generation of acetaldehyde and/or ROS is obligatory for its regulatory action on 2,6-ST gene in human liver.

2. Materials and methods

2.1. Chemicals

Tissue culture materials, including Dulbecco modified Eagle medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, L-glutamine, penicillin-streptomycin, and hygromycin B were procured from Invitrogen Corporation (Carlsbad, Calif). Geneticin was purchased from Strategene (La Jolla, Calif). Acetaldehyde was obtained from Sigma Chemicals (St Louis, Mo). Ethanol was purchased from Pharmco Products Inc (Brookfield, Conn). HNE was purchased from Cayman Chemical, Co (Ann Arbor, Mich).

2.2. Cell lines

Three human HepG2 liver cell sublines, wild-type cells, CYP2E1 cells, and HAD cells, were used as cell culture models in this study. Wild-type HepG2 (ATCC no. HB-8065), which is a cell line derived from human hepatocellular carcinoma, was acquired from the American Tissue Culture Collection (ATCC; Manassas, Va). CYP2E1 cells (CYP2E1 cells) derived from the wild-type cells stably transfected and constitutively expressing CYP2E1 [9] were a generous gift of Dr Arthur I. Cederbaum, Department of Pharmacology and Biological Chemistry, Mount Sinai School of Medicine, New York. Dr Dahn L. Clemens, Liver Study Unit, Department of Veterans Affairs Medical Center, Omaha, Neb, kindly provided us with HAD cells derived from wild-type cells stably transfected with alcohol dehydrogenase [10].

2.3. Cell cultures

All cell cultures were maintained in a humidified incubator in an atmosphere of 5% CO_2 and 95% air (vol/vol) at 37°C in DMEM medium supplemented with 10% FBS, 2 mmol/L glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin with or without additional specific antibiotics for each cell line as described below. Wild-type HepG2 cells were cultured in the basal DMEM medium described above without additional antibiotic. CYP2E1 cells were cultivated in the medium supplemented with an additional 0.4 mg/mL geneticin, whereas HAD cells were grown in the medium supplemented with 200 μg/mL hygromycin B. When the cells reached 80% to 90% confluency, they were harvested by trypsinization and replated in 75 cm^2 flasks (Corning) with the respective medium. All the cell lines were selected for at least 2 weeks before setting up the experiments and seeded at a density of 10.0×10^6 cells per flask. In all the studies, cell viability was determined by counting the cells in 0.4% trypan blue.

2.4. Protein extraction and Western blot

When the cells reach about 80% confluency, the cells were washed 3 times with cold phosphate buffered saline. Cells were then harvested into 3 mL radio-immunoprecipitation assay buffer and homogenized. The supernatant was collected after centrifuging at 3000 rpm at 4°C for 15 minutes. The protein concentration was determined by Bradford method. Twenty micrograms of total proteins were separated on a 4% to 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membrane (Bio-Rad, Hercules, Calif). A standard Western blot protocol was then followed to carry out the blot with either antihuman CYP2E1 antibody (Research Diagnostics, Flanders, NJ) or antihuman alcohol dehydrogenase antibody (Biodesign International, Saco, Me), respectively.

2.5. Studies on the effects of ethanol, acetaldehyde, and HNE

A typical protocol was as follows: 10 million cells were plated in each 75 cm^2 flask and allowed to grow with a respective selection of antibiotics. The next day the medium was replaced with the same medium except that they were incubated with 50 to 100 mmol/L ethanol or 50 to 100 μmol/L acetaldehyde for indicated time intervals. The flasks were then sealed with parafilm to prevent evaporation of ethanol or acetaldehyde. Similar experiments with 32, 64, and

96 μmol/L HNE were carried out for 72 hours except that FBS was omitted from the incubation medium. The medium was changed every 24 hours. At the end of the indicated experimental period, the cells were rinsed with cold phosphate-buffered saline and immediately lysed in Tri-reagent for RNA extraction.

2.6. RNA isolation

The total RNA was isolated from all the cultured cell lines using the Tri-reagent (Molecular Research Center [MRC], Cincinnati, Ohio) following the manufacturer's instructions. Adequate measures were undertaken to ensure high quality of RNA extracted in our samples. Briefly, the treated cells were homogenized in 1 mL of Tri-reagent. Samples were left for 5 minutes at room temperature followed by addition of 0.2 mL of bromochloropropane (MRC) and shaken vigorously for few seconds and again left at room temperature for 15 minutes. After centrifugation (12 000*g* for 20 minutes) at 4°C, the upper aqueous phase was carefully pipetted out into a sterile tube. The RNA was precipitated by addition of 0.5 mL of isopropanol and incubated at room temperature for 5 to 10 minutes. RNA was pelleted by centrifuging again at 12 000*g* at 4°C for 15 minutes. The precipitated RNA was washed in 70% ethanol, briefly air-dried, and then solubilized in formazol (MRC). Total RNA concentrations were measured by absorbance reading at 260 nm using SpectraMAX 190 (Molecular Devices Co, Sunnyvale, Calif). The purity of total RNA samples was examined by determining the A_{260}/A_{280} ratio. Isolated RNA was used immediately or stored at −80°C until use.

2.7. Northern blot analyses

RNA electrophoresis and Northern transfer onto the nylon membranes were performed as described by Ausubel et al [11].Total RNAs (20 μg) from control and ethanol- or acetaldehyde- or HNE-treated cells were electrophoresed in formaldehyde agarose gels. RNA was transferred onto the Hybond-N^+ membrane (Amersham BioScience, Piscataway, NJ) by overnight transferring in 10× SSC (1× SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate) buffer. Later, the wet nylon membrane was exposed to UV light for cross-linking the RNA onto the membrane, followed by air-drying. The membrane was either directly used in Northern hybridization or stored at −20°C until use.

2.8. Northern hybridization

The probe for human 2,6-ST was a 780–base pair *Bst*EII cleaved fragment of the coding sequence of 2,6-ST, which was a generous gift from Dr Joseph Lau, Roswell Park Memorial Cancer Institute (Buffalo, NY). A complementary DNA probe for human glyceraldehydes 3-phosphate dehydrogenase (GAPDH) was procured from ATCC (ATCC no. 81141). The probes were labeled using Prime-It II Random Primer labeling kit (Strategene) and [α-^{32}P]dCTP. Briefly, the dried membrane was first soaked in 10× SSC for 5 minutes. Prehybridization was performed in 10 mL prehybridization buffer (MRC) at 42°C for 60 minutes, followed by hybridization with the labeled complementary DNA probes also at 42°C for 16 to 18 hours in 10 mL of hybridization buffer (MRC). Probes were added at 1×10^6 cpm/mL of the hybridization buffer. Later, blots were washed and taken for autoradiography. Quantitative measurement of the radioactive spots was carried out using a Cyclone Packard Storage Phosphor System (Packard Bioscience Co, Downers Grove, Ill).

2.9. Statistical analysis

Data are presented as the mean ± SEM. Statistical significance was determined by 1-way analysis of variance test followed by the Tukey test using SAS Software (SAS Institute, Cary, NC).

3. Results

3.1. Expression of CYP2E1 and ADH in the respective HepG2 cell lines

To confirm that CYP2E1 and ADH were expressed in the stably transfected CYP2E1 and HAD cells, respectively, but not in the wild type, Western blot analysis was carried out with the extracts of the respective cells and the wild-type cells using antihuman CYP2E1 and antihuman ADH. As shown in Fig. 1A, a 55-kDa band corresponding to CYP2E1 was identifiable only in the extracts of CYP2E1 cells but not in wild-type cells. Furthermore, CYP2E1 activity, as measured by *p*-nitrophenol oxidation rate, was

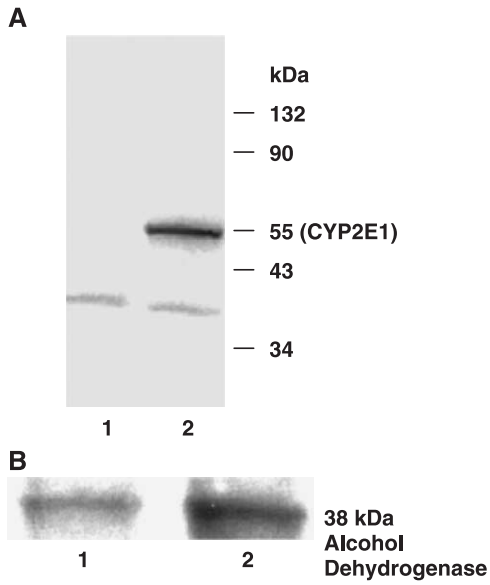

Fig. 1. Western blot analysis of CYP2E1 and HAD cells. Twenty micrograms equivalent of total protein extracts from CYP2E1 and HAD cells were subjected to Western blot analyses using the polyclonal CYP2E1 and ADH antibodies, respectively. A, lane 1 indicates wild-type cell extract; lane 2, CYP2E1 cell extract. B, lane 1 indicates wild-type cell extract; lane 2, HAD cell extract.

found to be 53.2 pmol/min per milligram of protein in the CYP2E1 cells, thus agreeing with the results of Yang and Cederbaum [12]. CYP2E1 activity was undetectable in the wild-type cells. Similarly, it can be seen in Fig. 1B that a strong 39-kDa band corresponding to human ADH subunit was identifiable in the extracts of HAD cells but only a faint one in the wild type. Furthermore, ADH activity, as measured by ethanol oxidation was found to be 216.2 nmol/h per milligram of protein similar to the results reported by Clemens et al [10]. Negligible ADH activity was detectable in the wild-type cell extracts.

3.2. Effect of ethanol on 2,6-ST mRNA expression in CYP2E1 cells

The time course effect of 100 mmol/L ethanol on 2,6-ST messenger RNA (mRNA) level in CYP2E1 cells showed 5% (NS) decrease at 24 hours, 8% (NS) at 48 hours, and 39% at 72 hours. Therefore, the concentration effect of ethanol was carried out at the 72-hour time point. A representative Northern blot analysis of 2,6-ST mRNA from the total RNA extracts of control and ethanol-treated cells is shown in Fig. 2A. Fig. 2B shows the effect of ethanol concentration on the relative levels of 2,6-ST mRNA in CYP2E1 cells after 72 hours of incubation. It can be seen from the figure that the level of 2,6-ST mRNA was decreased by 46% ($P < .05$) at 100 mmol/L ethanol compared with untreated CYP2E1 cells. The housekeeping gene GAPDH expression was not affected by ethanol treatment under identical conditions.

3.3. Effect of ethanol on 2,6-ST mRNA expression in HAD liver cells

Because ethanol effect on 2,6-ST mRNA was not evident before the 72-hour point (data not shown), the concentration effect of ethanol was carried out at the 72-hour time point. Fig. 3 shows the effect of ethanol concentration on the relative levels of 2,6-ST mRNA in HAD cells after 72 hours of incubation. It can be seen from the figure that the level of 2,6-ST mRNA was decreased by 70% ($P < .05$) at 50 mmol/L ethanol and by 76% ($P < .05$) at 100 mmol/L ethanol as compared with untreated HAD cells. The housekeeping gene GAPDH expression was not affected by ethanol treatment under identical conditions.

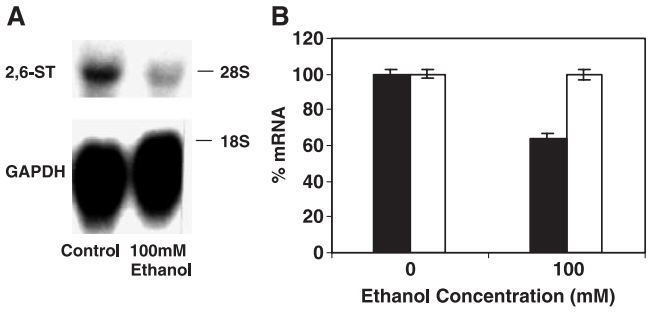

Fig. 2. Effect of ethanol on 2,6-ST mRNA level in CYP2E1 cells. CYP2E1 cells were incubated without or with 100 mmol/L ethanol for 72 hours and the total RNA was extracted and subjected to Northern blot analysis. A, A representative Northern blot analysis of 2,6-ST mRNA from total RNA extracts of control and 100 mmol/L ethanol-treated cell extracts. Lane 1, RNA from CYP2E1 cells without ethanol exposure. Lane 2, RNA from the same cell line after exposure to 100 mmol/L of ethanol. B, Relative levels of 2,6-ST mRNA. Each value is the mean ± SEM of determinations from 5 independent experiments, each of which was run in duplicate cell cultures. Black solid bars indicate 2,6-ST mRNA; white open bars, GAPDH mRNA.

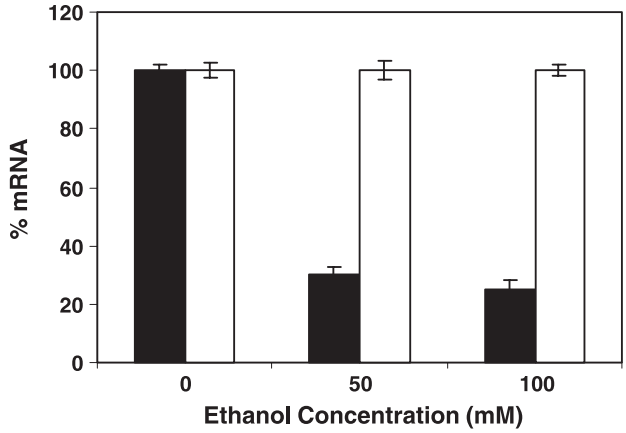

Fig. 3. Effect of ethanol exposure time on 2,6-ST mRNA level in HAD cells. HAD cells were incubated without or with 50 and 100 mmol/L ethanol for 72 hours and the total RNA was extracted and subjected to Northern blot analysis. Lane 1 is RNA from untreated HAD cells, lane 2 is RNA from HAD treated with 50 mmol/L ethanol for 72 hours, and lane 3 is RNA from same cell line treated with 100 mmol/L ethanol for 72 hours. Expression of human 2,6-ST dramatically down-regulated in treated cells. Each value is the mean ± SEM of determinations from 5 independent experiments, each of which was run in duplicate cell cultures. Black solid bars indicate 2,6-ST mRNA; white open bars, GAPDH mRNA.

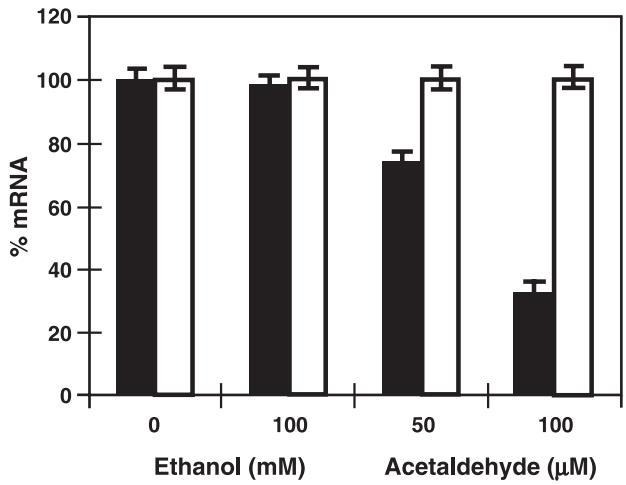

Fig. 4. Effect of ethanol and acetaldehyde on 2,6-ST mRNA level in wild-type cells. Wild-type cells were incubated without or with 100 mmol/L ethanol or 50 and 100 μmol/L acetaldehyde for 72 hours and the total RNA was extracted and subjected to Northern blot analysis. Each value is the mean ± SEM of determinations from 5 independent experiments, each of which was run in duplicate cell cultures. Black solid bars indicate 2,6-ST mRNA; white open bars, GAPDH mRNA.

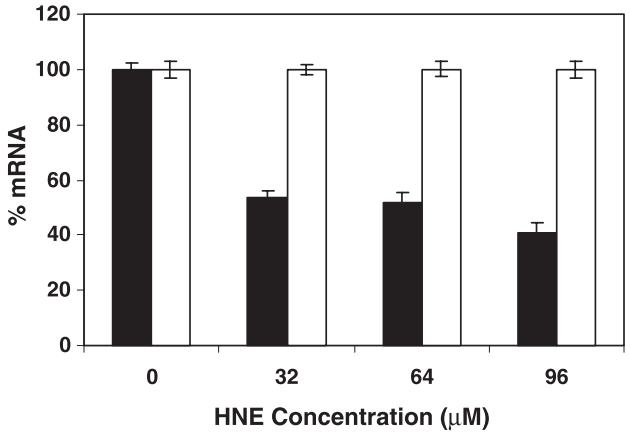

Fig. 5. Effect of HNE on 2,6-ST mRNA level in CYP2E1 cells. CYP2E1 cells were incubated without or with indicated concentrations of HNE for 72 hours and the total RNA was extracted and subjected to Northern blot analysis. Each value is the mean ± SEM of determinations from 5 independent experiments, each of which was run in duplicate cell cultures. Black solid bars indicate 2,6-ST mRNA; white open bars, GAPDH mRNA.

3.4. Effect of ethanol and acetaldehyde on 2,6-ST mRNA expression in wild-type HepG2 liver cells

The concentration effects of ethanol and acetaldehyde were carried out at the 72-hour time point. Fig. 4 shows that the effect of ethanol concentration on the relative levels of 2,6-ST mRNA in wild-type cells decreased negligibly after 72 hours of incubation with 100 mmol/L ethanol. In contrast, the relative level of 2,6-ST mRNA was decreased by 35% ($P < .05$) at 50 μmol/L acetaldehyde and by 69% ($P < .05$) at 100 μmol/L acetaldehyde compared with untreated wild-type cells. The housekeeping gene GAPDH expression was not affected by ethanol or acetaldehyde exposure under identical conditions.

3.5. Effect of HNE on 2,6-ST mRNA expression in CYP2E1 cells

The concentration effect of HNE was carried out at the 72-hour time point. Fig. 5 shows the effect of HNE concentration on the relative levels of 2,6-ST mRNA in CYP2E1 cells after 72 hours of incubation. It can be seen from the figure that the level of 2,6-ST mRNA was decreased by 46% ($P < .02$) at 32 μmol/L HNE, by 49% ($P < .02$) at 64 μmol/L HNE, and by 61% ($P < .02$) at 96 μmol/L HNE compared with untreated CYP2E1 cells. The housekeeping gene GAPDH expression was not affected by HNE treatment under identical conditions.

4. Discussion

In this study, we used a human hepatocyte HepG2 cell lines as a model to study the effects of ethanol on the regulation of 2,6-ST gene expression because ethanol metabolism takes place primarily in the liver. That the expression of 2,6-ST mRNA was affected by ethanol only in ethanol-metabolizing cells but not in the wild type (Figs. 2-4) clearly demonstrates the importance of ethanol metabolism for eliciting this regulatory response. The 2 major hepatic ethanol-metabolizing enzymes are CYP2E1 and alcohol dehydrogenase. Both of these enzymes oxidize ethanol to acetaldehyde, which is more toxic than ethanol itself. The fact that acetaldehyde, the immediate product of ethanol oxidation, is effective in down-regulating 2,6-ST mRNA confirms that ethanol may mediate this response via acetaldehyde.

On the other hand, CYP2E1-mediated oxidation of ethanol also produces a state of oxidative stress by generating ROS within the cells that can lead to the generation of a key metabolite, the α,β-unsaturated aldehyde, HNE. HNE may be more harmful than ROS because it has a longer half-life and can easily diffuse into cellular membranes [8]. Under normal conditions, intracellular HNE concentration is less than 1.0 μmol/L, but it can reach a level of as high as 100 μmol/L under oxidative stress [13]. The fact that HNE strongly down-regulates 2,6-ST mRNA even at 32 μmol/L (Fig. 5) strongly supports the concept that oxidative stress caused by ethanol oxidation via CYP2E1 could also play a key role in regulating this gene.

Sialyltransferases are a family of enzymes consisting of more than 18 glycosyltransferases that catalyze the transfer of sialic acid from cytidine monophospho-*N*-acetylneuraminic acid to the nonreducing terminal positions on the oligosaccharide chains of glycoproteins and glycolipids [14]. Terminal sialic acids are key determinants of carbohydrate structures involved in a variety of biologic events, such as viral-host recognition [15], cell-cell adhesion [16-18], and tumor cell invasiveness [19]. In particular, alteration of sialic acids, generally found in the nonreducing terminus of most glycoproteins and glycolipids, has been associated with long-term alcohol exposure [20]. A number of reports have provided evidences that long-term alcohol exposure interferes with the metabolism of the complex glycoconjugates of certain circulating as well as membrane-bound species in human and experimental animals [21,22], resulting in either the appearance of a number of carbohydrate-deficient glycoconjugates including carbohydrate-deficient transferrin [22], sialic acid–deficient apolipoprotein J [20], and α1-acid glycoprotein in the plasma of long-term alcohol consumers [23].

In conclusion, ethanol exposure down-regulates the expression of 2,6-ST mRNA in 2 ethanol-metabolizing human liver cell lines, the CYP2E1 cells and HAD cells but not in wild-type HepG2 cells, which do not metabolize ethanol. Thus, this study assumes major importance and clinical relevance because 2,6-ST gene regulation in a human liver cell model is demonstrated within a few days of ethanol exposure, whereas its in vivo regulation in liver generally takes prolonged period of ethanol exposure. The possible mechanism(s) of action ethanol seems to be mediated via acetaldehyde as well as via ROS. Further work is warranted to shed light as to how acetaldehyde and ROS regulate the expression of 2,6-ST and what signaling pathways are involved.

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References

[1] Rao MN, Lakshman MR. Chronic ethanol consumption leads to destabilization of rat liver 2,6-sialyltransferase mRNA. Metabolism 1999;48:797-803.
[2] Ghosh P, Okoh C, Lakshman MR. Effects of chronic ethanol on enzymes regulating sialation and desialation of transferrin in rats. Alcohol Clin Exp Res 1993;17:576-9.
[3] Rao MN, Lakshman MR. Chronic ethanol downregulates 2,6-sialyltransferase and 2,3-sialyltransferase mRNAs in rat liver. Alcohol Clin Exp Res 1997;21:348-51.
[4] Crabb DW, Bosron WF, Li T-K. Ethanol metabolism. Pharmacol Ther 1987;34:59-73.
[5] Hartley DP, Peterson DR. Co-metabolism of ethanol, ethanol-derived acetaldehyde and 4-hydroxynonenal in isolated hepatocytes. Alcohol Clin Exp Res 1997;21:298-304.
[6] Chen J, Clemens DL, Cederbaum AI, Gao B. Ethanol inhibits JAK-STAT signaling pathway in freshly isolated hepatocytes but not in cultured hepatocytes or HepG2 cells: Evidence for lack of involvement of ethanol metabolism. Clin Biochem 2001;34:203-9.
[7] Nagy Le. Molecular aspects of alcohol metabolism: Transcription factors involved in early ethanol-induced liver injury. Ann Rev Nutr 2004;24:55-78.
[8] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. Free Radic Biol Med 1991;11:81-128.
[9] Dai Y, Rashba-Step J, Cederbaum AI. Stable expression of human cytochrome P4502E1 in HepG2 cells: Characterization of catalytic activities and production of reactive oxygen intermediates. Biochemistry 1993;32(27):6928-37.
[10] Clemens DL, Halgard CM, Miles RR, Sorrell MF, Tuma DJ. Establishment of a recombinant hepatic cell line stably expressing alcohol dehydrogenase. Arch Biochem Biophys 1995;321:311-8.
[11] Ausubel FM, Brent R, Kingston RE, Moore DD, Siedman JG, Smith JA, editors. Current Protocols in Molecular Biology. New York (NY): Wiley; 1992. p. 4-22104-25.
[12] Yang M-X, Cederbaum AI. Glycerol increases content and activity of human CYP2E1 in a transduced HepG2 cell line by protein stabilization. Alcohol Clin Exp Res 1997;21:340-7.
[13] Burczynski ME, Sridhar GR, Palackal NT, Penning TM. The reactive oxygen species– and Michael acceptor–inducible human aldo-keto reductase AKR1C1 reduces the α,β-unsaturated aldehyde, 4-hydroxy-2-nonenal to 1,4-dihydroxy-2-nonene. J Biol Chem 2001;276:2890-7.
[14] Harduin-Lepers A, Vallejo-Ruiz V, Krzewinski-Recchi M, Samyn-Petit B, Julien S, Delannoy P. The human sialyltransferase family. Biochimie 2001;83:727-37.
[15] Gagneux P, Cheriyan M, Hurtado-Ziola N, van der Linden EC, Anderson D, McClure H, et al. Human-specific regulation of alpha2-6–linked sialic acids. J Biol Chem 2003;278(48):48245-50.
[16] Schauer R. Sialic acids and their role as biological masks. Trends Biochem Sci 1985;10:357-60.
[17] Pilatte Y, Bignon J, Lambre CR. Sialic acids as important molecules in the regulation of the immune systems: Pathophysiological implications of sialidases in immunity. Glycobiology 1993;3:201-17.
[18] Lin S, Kemmner W, Grigull S, Schlag PM. Cell surface alpha 2, 6 sialylation affects adhesion of breast carcinoma cells. Exp Cell Res 2002;276(1):101-10.
[19] Zhu Y, Srivatana U, Ullah A, Gagneja H, Berenson CS, Lance P. Suppression of a sialyltransferase by antisense DNA reduces invasiveness of human colon cancer cells in vitro. Biochemica et Biophysica Acta 2001;1536(2-3):148-60.
[20] Ghosh P, Hale EA, Lakshman MR. Plasma sialic acid index of apolipoprotein J (SIJ): A new alcohol intake marker. Alcohol 2001;25:173-9.
[21] Lakshman MR, Rao MN, Marmillot P. Alcohol and molecular regulation of protein glycosylation and function. Alcohol 1999;19:239-47.
[22] Stibler H, Borg S. Evidence of a reduced sialic acid content in serum transferrin in male alcoholics. Alcohol Clin Exp Res 1981;5:545-9.
[23] Tsutsumi M, Wang JS, Takada A. Microheterogeneity of serum glycoproteins in alcoholics: Is desialo-transferrin the marker of chronic alcohol drinking or alcoholic liver injury? Alcohol Clin Exp Res 1994;18:392-7.