Cytotoxicity and metabolic stress induced by acetaldehyde in human intestinal LS174T goblet-like cells

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Acetaldehyde, the first metabolite of ethanol, is known to be mutagenic. Intracolonic production and accumulation of acetaldehyde have been associated with colorectal carcinogenesis (41). Intracolonic production of acetaldehyde may lead to increased plasma acetaldehyde levels and subsequent hepatotoxicity (38). Although evidence on effects of acetaldehyde on goblet cells is relevant to acetaldehyde-intestinal barrier dysfunction, studies investigating effects of acetaldehyde on intestinal goblet cells are lacking. Therefore, we aimed to assess short-term effects of acetaldehyde in concentrations expected to be present in the colon lumen after moderate ethanol consumption, on functional characteristics of the intestinal goblet-like cells LS174T, including cellular redox status, mitochondrial calcium ($Ca^{2+}$) homeostasis and ATP production, viability, and the implications for MUC2 expression.

EXPERIMENTAL PROCEDURES

Chemicals. Cell culture reagents were purchased from Life Technologies (Bleiswijk, the Netherlands) and Lonza Benelux (Breda, the Netherlands). Dichlorodihydrofluorescein diacetate (DCF-DA), N-acetyl-L-cysteine (NAC), N-acetyl-L-cysteine (NAC), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyterazolium bromide (MTT), and caspase3/7 activity and percentage of cells expressing cleaved CK18 were tested Liver Physiol 307: G286–G294, 2014. First published June 5, 2014. Copyright © 2014 the American Physiological Society http://www.ajpgi.org.
based ELISA assay kits were purchased from RayBiotech (Norcross, GA). BAPTA-AM was purchased from Sigma. Rhod 2-AM was purchased from Molecular Probes (Eugene, OR). ATP, lactate dehydrogenase (LDH), and caspase 3/7 assay kits were purchased from Promega.

Antibodies. Mouse monoclonal antibody M30 and rabbit polyclonal anti-human MUC2 were purchased from Roche (Mannheim, Germany) and Abcam (Cambridge, UK), respectively.

Cell line and culture conditions. LS174T cells, kindly provided by Dr. Sara Linden, University of Gothenburg, Sweden, were grown in either T25 or T75 flasks and were maintained in DMEM (Lonza Benelux) containing 4.5 g/l glucose and 4 mM l-glutamine. The medium was supplemented with 10% (vol/vol) FCS (Invitrogen, Breda, the Netherlands), 1% nonessential amino acids (Invitrogen), and 1% antibiotic-antimycotic mixture (10,000 U of penicillin, 10,000 μg of streptomycin, and 25 μg of amphotericin B per ml; Invitrogen) at 37°C and in a 5% CO2 atmosphere.

Measurement of reactive oxygen species. The generation of reactive oxygen species (ROS) was monitored by using DCF-DA (Sigma Chemical), which is nonfluorescent unless oxidized by intracellular ROS. LS174T cells were cultured in 96-well plates and were pre-loaded with 100 μM DCF-DA for 1 h at 37°C. Measurement of intracellular ROS was performed by incubating LS174T cells with either medium only as negative controls or acetaldehyde (25, 50, 75, 100 μM) for 3 h with or without 1 h pretreatment with 100 μM of the antioxidant N-acetylcysteine (NAC). H2O2 (30 mM) was used as a positive control. Cells were then washed twice in Hank’s balanced salt solution buffer (HBSS; Invitrogen), and the fluorescence (485 excitation/530 emission) was measured by a SpectraMax M2 spectrophotometer (Molecular Devices). Data were reported as percentage of the negative controls.

Measurement of mitochondrial function. Mitochondrial function was assessed using MTT (Sigma-Aldrich) that is cleaved into insoluble formazan by active mitochondrial succinate dehydrogenases in living cells (48). Briefly, LS174T cells were cultured in 96-well plates and exposed to acetaldehyde (25–100 μM) for 3 h. Medium only and H2O2 (30 mM) were used as negative and positive controls, respectively. The cells were then washed with PBS and incubated with 5 mg/ml MTT in PBS, 200 μl/well for another 3 h at 37°C. Thereafter, the solution was aspirated, and the insoluble formazan product was solubilized with 0.1 M HCl in isopropanol (100 μl/well) and incubated at room temperature (RT) for 30 min. The optical density was measured at a wavelength of 560 nm using a spectrophotometer. Values are presented as percentage of medium only treated cells.

Assessment of mitochondrial Ca2+. For mitochondrial Ca2+ monitoring, LS174T cells were trypsinized and transferred from cell culture flasks to eight-well chamber slides (Lab-Tek; Nunc, Rochester, NY) at a density of 2.5 × 105 cells in 500 μl cell culture medium. After a period of 3 days, cells were incubated with acetaldehyde (25, 50, 75, 100 μM) for 3 h. Medium only and H2O2 (30 mM) were used as negative and positive controls, respectively. Cells were rinsed two times in serum-free culture medium and loaded with a mixture of 5 μM Rhod 2-AM (Molecular Probes), a fluorescent probe specific for mitochondrial Ca2+, with 0.1% pluronic acid for 30 min at 37°C as described previously (47). Medium was removed, replaced with dye-free culture medium, and incubated for an additional 60 min at 37°C. Thereafter, 1 μM of the fluorescent mitochondria-specific dye MitoTracker (green fluorescence; Molecular Probes) was added to each well for the last 30 min of incubation. Cells were visualized using a Leica TCS SPE confocal laser scanning microscope (Leica Microsystems, Mannheim, Germany). Excitation wavelength was 488 nm and fluorescence emission was recorded at 543 nm (for Rhod 2) and 516 nm (for MitoTracker). Ten images were randomly chosen in selected microscopic fields from three independent experiments, and fluorescence intensity was quantified by dividing the pixel intensity by the area of the spot (μm2) using Image J software (1).

Luminescent ATP assay. The amount of ATP produced by metabolically active cells was quantified based on the luciferase reaction (CellTiter-Glo Luminescent Cell Viability Assay; Promega). The amount of ATP is directly proportional to the number of living cells present in culture. Briefly, LS714T cells were cultured in 96-well plates and exposed to acetaldehyde (25–100 μM) for 3 h. In separate experiments, cells were pretreated for 1 h with 100 μM NAC before exposure to acetaldehyde. Medium only and H2O2 (30 mM) were used as negative and positive controls, respectively. Thereafter, plates were equilibrated at RT for 30 min and processed as described previously (17). Intracellular ATP was calculated from the luminescence values and presented as percentage of the medium only treated cells.

LDH assay. Cell plasma membrane integrity was evaluated by measuring LDH release. The assay (CytoTox-ONE Homogeneous Membrane Integrity Assay; Promega) was performed according to the manufacturer’s instructions. Briefly, LS714T cells were cultured in 96-well plates (Corning) and incubated with acetaldehyde (25–100 μM) with or without pretreatment for 1 h with 100 μM NAC. Next, plates were incubated at 37°C for 3 h and equilibrated at RT for 20 min. Thereafter, plates were processed, and LDH was measured as described previously (17). LDH release was calculated as the percentage of maximum LDH fluorescence values (i.e., the fully lysed cells using cell lysis buffer included in the kits) after subtracting culture medium background using the following equation:

%LDH release = [(100 × (experimental – culture medium background)) / (maximum LDH release – culture medium background)]

Assessment of caspase 3/7 activity. Caspase 3/7 activity was measured as a marker of apoptosis using a luminescent assay (ApoTox-Glo Triplex Assay; Promega). Briefly, the assay provides a lumogenic caspase-3/7 substrate, which contains the tetrapeptide sequence DEVD, in a reagent optimized for caspase activity, luciferase activity, and cell lysis. Addition of the Caspase-Glo 3/7 reagent results in cell lysis, followed by caspase cleavage of the substrate and generation of luminescent signals produced by luciferase, which will be proportional to the amount of caspase activity present. LS714T grown on the 96-well plates were incubated with 25, 50, 75, 100 μM acetaldehyde or 30 mM H2O2 at 37°C for 3 h, and then, 10 μl of Caspase-3/7 reagent assay was added to each well, briefly mixed by orbital shaking (300–500 revolutions/min for ~30 s), and incubated at RT for 30 min. Negative (HBSS buffer solution) control wells were also included and treated similarly as described above. The luminescence was measured by using SpectraMax M2 (Molecular Devices, Sunnyvale, CA). The caspase-3/7 activity was expressed as a ratio between treated and untreated cells.

Detection of caspase activity with M30 cytoDEATH. Caspase activity was further investigated with the commercially available monoclonal antibody M30 cytoDEATH (Roche). After incubation with acetaldehyde, LS513 (passage 28) and LS714T (passage 30) cells grown on Lab-Tek eight-chamber glass slides (Nalge Nunc International, Naperville, IL) were washed with PBS and fixed for 30 min in methanol (Merck) at −20°C. Fixed cells were washed twice with buffer consisting of PBS, 1% (wt/vol) BSA, and 0.1% (vol/vol) Tween 20 (Sigma, St. Louis, MO) and were subsequently incubated with M30 cytoDEATH for 30 h at RT. Thereafter, the cells were incubated with Alexa-488-conjugated goat anti-mouse (Invitrogen) [1:100 dilution in 3% (wt/vol) BSA in PBS, pH 7.4] at RT for 30 min. After another washing in HBSS, cells were stained for 20 min with diamidino-2-phenylindole (DAPI; 1:10,000 dilution in PBS; Sigma). Finally, the chambers were removed, and slides were mounted in Vectashield mounting medium containing DAPI (Vector Laboratories), cover slipped, and sealed with a clear nail polish. The slides were examined under a Leica TCS SPE confocal laser scanning microscope (Leica Microsystems). Confocal images obtained were processed and analyzed using Image J software (1).
Assessment of MUC2 protein expression. MUC2 protein expression was assessed using cell-based ELISA kits (RayBio cell-based protein phosphorylation ELISA kit; RayBiotech) with minor modifications. Briefly, LS174T cells (20 × 10^3) were seeded in 96-well plates and incubated overnight at 37°C and 5% CO₂. Cells were then exposed to medium only or acetaldehyde (25, 50, 75, 100 μM) for 3 h and processed for cell-based ELISA using rabbit anti-human MUC2 (1:100 dilution in the blocking solution; Abcam) as we described previously (17). Finally, 25 μL of stop solution were added to each well, and the optical density was read at 450 nm with a spectrophotometer.

Statistical analysis. All data analyses were conducted using the GraphPad Prism software package (GraphPad Software). Data are expressed as means ± SD of triplicate experiments. One-way ANOVA and Tukey’s post hoc tests were performed to compare between different experimental conditions, considering difference of P value < 0.05 as statistically significant.

RESULTS

Effects of acetaldehyde on cellular redox state. Because acetaldehyde is a potent oxidative stress inducer (46), we used DCF-DA to investigate whether acetaldehyde can increase mitochondrial ROS production in LS174T goblet-like cells and whether induction of oxidative stress can be inhibited by pretreatment with 100 μM of the antioxidant NAC. We found that exposure to acetaldehyde at concentrations ranging from 25 to 100 μM significantly increased intracellular ROS contents in LS174T cells compared with the negative controls (all P < 0.001; Fig. 1A). As expected, the increase of ROS was higher after incubation with the positive control (H₂O₂) compared with the negative controls (P < 0.001; Fig. 1A). Pretreatment with 100 μM NAC significantly prevented acetaldehyde-induced oxidative stress (compared with each acetaldehyde concentration in the absence of NAC, P < 0.001; Fig. 1A).

Effects of acetaldehyde on mitochondrial function. Cells were incubated with various doses of acetaldehyde, and mitochondrial function was assessed with the MTT assay that determines the mitochondrial complex II enzyme activity succinyl dehydrogenases. Our results revealed that exposure to 25–100 μM acetaldehyde for 3 h significantly reduced the mitochondrial activity compared with negative controls (P < 0.05; Fig. 1B). Mitochondrial function was also significantly reduced after exposure to the positive control 30 mM H₂O₂ compared with the negative controls (P < 0.05).

Effects of acetaldehyde on intracellular ATP levels. Production of energy in the form of ATP via oxidative phosphorylation and oxygen consumption is one of the main functions of the mitochondria (10). We therefore assessed the amount of ATP in LS174T cells exposed to either acetaldehyde or H₂O₂ (positive control) for 3 h. As shown in Fig. 2A, acetaldehyde significantly induced a dose-dependent decrease in intracellular ATP compared with negative controls (P < 0.05).

ROS generation and acetaldehyde-induced ATP depletion. To establish whether acetaldehyde induces ATP depletion via oxidative stress, LS174T cells were exposed for 3 h to either acetaldehyde or H₂O₂ (positive control) with or without pretreatment with NAC. As shown in Fig. 2B, exposure to acetaldehyde dose-dependently decreased intracellular ATP levels as did H₂O₂ compared with the negative controls (all P < 0.001, Fig. 2B), whereas pretreatment with NAC partially prevented acetaldehyde-induced ATP depletion (compared with corresponding acetaldehyde-exposed cells, P < 0.05; Fig. 2B).

Effects of acetaldehyde on mitochondrial Ca²⁺ in LS174T cells. Next we tested whether acetaldehyde caused a change in mitochondrial Ca²⁺ in LS174T cells using the positively charged and cell-permeant Ca²⁺ indicator Rhod 2-AM, which accumulates predominantly in the negatively charged matrix of the mitochondria (47). The dye MitoTracker was used to confirm the mitochondrial localization of Rhod 2. As presented in Fig. 3A, acetaldehyde treatment of LS174T cells induced an increase in Rhod 2 fluorescence that appears predominantly in the mitochondria as demonstrated by the yellow spots of strong intensity found in the merged images. In contrast, the distri-

Fig. 1. Effects of acetaldehyde on oxidative stress and mitochondrial function. LS174T cells were incubated with either acetaldehyde at the indicated concentrations or 30 mM H₂O₂ to serve as positive control for 3 h. A: reactive oxygen species (ROS) generation was assayed using 2’,7’-dichlorodihydrofluorescein diacetate (DCF-DA) and expressed as a percentage of negative controls (−ve). NAC, N-acetylcysteine; ACh, acetaldehyde. Values are presented as means ± SD of 3 experiments. *P < 0.001 vs. negative controls and **P < 0.001 vs. corresponding acetaldehyde-exposed cells. B: mitochondrial function was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and absorbance was measured at a wavelength of 560 nm. Data were reported as percentages of −ve controls. Values are presented as means ± SD of 3 experiments. *P < 0.05 and **P < 0.01 vs. −ve controls.
To examine whether acetaldehyde induces cytotoxicity via oxidative stress, LDH released in medium was measured following exposure of LS174T cells to either acetaldehyde or H$_2$O$_2$ (positive control) for 3 h with or without pretreatment with NAC. Pretreatment with NAC significantly prevented acetaldehyde-induced LDH release (compared with corresponding acetaldehyde-exposed cells, $P < 0.001$; Fig. 4A).

**Effects of acetaldehyde on caspase-3/7 activity.** The observation that acetaldehyde induces cell necrosis as indicated by loss of membrane integrity led us to investigate cell apoptosis by determining activation of caspase-3/7 in LS174T cells (11). As shown in Fig. 4B, exposure to acetaldehyde at a concentration range of 25, 50, 75 and 100 µmol/l significantly increased caspase-3/7 activity compared with negative controls. Incubation with positive control H$_2$O$_2$ also significantly induced 3/7 caspase activity vs. the negative controls ($P < 0.05$; Fig. 4B).

**Effects of acetaldehyde on caspase-mediated cytokeratin 18 cleavage.** During apoptosis, cytoplasmic cytokeratin 18 (CK18), a major component of cytoplasmic cytokeratin filaments in epithelial cells, is cleaved by caspases, mainly caspase 3 or 7, resulting in exposure of a neoepitope that is detectable with the monoclonal antibody M30 (20). To complement our data on 3/7 caspase activity, we examined CK18 cleavage using immunofluorescent staining. As shown in Fig. 5A, acetaldehyde induced CK18 cleavage as indicated by increased immunoreactivity to M30 in LS174T cells compared with controls. Furthermore, the percentages of M30 reactive cells was significantly increased after incubation with acetaldehyde compared with controls (Fig. 5B).

**Effects of acetaldehyde on MUC2 protein expression.** To establish whether acetaldehyde modulates mucin production, LS174T cells were exposed to acetaldehyde at concentrations 25 and 100 µM, and protein levels of MUC2 were assessed by a cell-based ELISA. As shown in Fig. 6A, protein levels of MUC2 were significantly increased up to 14-fold after exposure to acetaldehyde in a dose-dependent manner compared with the controls ($P < 0.001$). To complement these data, dot blotting was performed. Data revealed that exposure to acetaldehyde results in substantially increased MUC2 protein expression (Fig. 6B).

**DISCUSSION**

In the present study, effects of acetaldehyde on cellular redox status, mitochondrial function, cell viability, and MUC2 expression were investigated using LS174T goblet-like cells. It was shown that acetaldehyde: 1) increases ROS production; 2) induces intramitochondrial Ca$^{2+}$ accumulation; 3) dose-dependently decreases intracellular ATP levels; and 4) dose-dependently induces loss of cell membrane integrity, caspase 3/7 activation, and increases in cleaved CK18 and MUC2 protein expression.

Acetaldehyde is considered to be involved in various ethanol-related gastrointestinal disorders, including barrier dysfunction and colorectal carcinogenesis (18). The perturbations of goblet cells, especially in colon epithelium by acetaldehyde, are relevant since acetaldehyde can reach high concentrations within the colon. Incubation of human colonic contents with ethanol at concentrations achievable in vivo after moderate ethanol consumption (i.e., 22 and 44 mM) can result in gen-

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Fig. 2. Effects of acetaldehyde on intracellular ATP levels. LS174T cells were treated with or without various concentrations of acetaldehyde at 37°C for 3 h in the presence or absence of pretreatment with NAC. A: intracellular ATP levels were determined by a bioluminescence-based assay. Data were reported as percentages of negative controls. Data present mean ± SD of 3 experiments. *$P < 0.05$ and **$P < 0.01$ vs. −ve controls. B: acetaldehyde-induced ATP depletion is mediated by oxidative stress. ***$P < 0.001$ vs. −ve controls and *$P < 0.05$, *$P < 0.01$, and **$P < 0.001$ vs. corresponding acetaldehyde-exposed cells.
eration of 58 and 238 μM acetaldehyde, respectively (24). Furthermore, blood acetaldehyde levels of 20–50 μM have been reported after moderate ethanol ingestion (40). Therefore, acetaldehyde concentrations used in the present study reflect concentrations that can be reached in human colon lumen following moderate ethanol consumption. Because intestinal microbiota are less capable of metabolizing acetaldehyde by aldehyde dehydrogenase (ALDH) (34) and levels of ALDH in colonic mucosa are rather low (29), higher concentrations of acetaldehyde are expected to accumulate in the lumen of the large intestine (38). Previously, intracolonic production and accumulation of acetaldehyde have been linked to colorectal carcinogenesis (41) and have been considered as important determinants for blood acetaldehyde levels and subsequent hepatotoxicity (38). However, little is known about effects of acetaldehyde on intestinal goblet cells. We used the goblet-like cell line LS174T to examine their response to acetaldehyde exposure. The LS174T cell line was established from a moderately well-differentiated primary colon adenocarcinoma (27) that constitutively produces and releases large amounts of mucus glycoproteins in the culture medium (8). Furthermore, LS174T cells express genes for mucins that are predominant in the gastrointestinal tract, including MUC2 and mucin 3 (21). Therefore, LS174T cells provide a convenient in vitro model for the study of human intestinal goblet cell biology.

Because oxidative stress occurring during ethanol metabolism is a major cause of cell injury, we examined effects of its oxidative metabolite acetaldehyde on cellular redox status. Not surprisingly, we observed that acetaldehyde in the range of concentrations of 25–100 μM increased ROS generation in LS174T cells, which could be inhibited by the antioxidant NAC. These results are consistent with a recent observation by our group that acetaldehyde at concentrations as low as 25 μM enhances intracellular oxidative stress in the enterocytes-like Caco-2 cell monolayers, with subsequent induction of epithelial to mesenchymal transition and, consequently, barrier dysfunction (16). Ethanol-induced oxidative stress in intestinal epithelium has been implicated in loss of epithelial integrity (4, 39).

Fig. 3. Influence of acetaldehyde on mitochondrial Ca\(^{2+}\) homeostasis in LS174T cells. Top: representative fluorescence images of LS174T cells treated with medium as controls, acetaldehyde, and H\(_2\)O\(_2\) after prior loading with the mitochondrial dye MitoTracker (green) and the mitochondrial Ca\(^{2+}\) indicator rhod 2 (red). Merged images indicate colocalization of the 2 dyes in the mitochondria. Mitochondrial Ca\(^{2+}\) accumulation is indicated by arrows. Scale bar = 10 μm. Bottom: quantification of Rhod 2 fluorescence intensity in LS174T cells subjected to acetaldehyde or H\(_2\)O\(_2\) treatment. Values are presented as means ± SD of 3 experiments. *P < 0.05 and **P < 0.01 vs. →ve controls.
The mitochondrion is one of the major sources of oxidants and target for their injurious effects (33). However, little is known about its response to acetaldehyde in goblet cells. Previously, accumulation of oxidants in mitochondria has been shown to induce metabolic stress and to decrease ATP production (32), which can contribute to intestinal epithelial barrier dysfunction (30, 49). Because cytotoxic effects of acetaldehyde might include impairment of energy generation by mitochondria, we assessed mitochondrial function and measured cellular ATP levels. Our data revealed that incubation of LS174T cells

![Graph A](http://example.com/graphA.png)

**A:** values are presented as means ± SD of 3 independent experiments. ***P < 0.001 vs. -ve controls. *P < 0.001 vs. corresponding acetaldehyde-exposed cells.

![Graph B](http://example.com/graphB.png)

**B:** activity of 3/7 caspase activity as a marker of cell apoptosis was evaluated using a luminescent-based assay. Values are reported as means ± SD of 3 independent experiments. *P < 0.01 vs. -ve controls.

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![Image A](http://example.com/imageA.png)

**A:** representative images of distribution of cytokeratin after staining with M30 (green) as reproduced in 3 independent experiments. Nuclei were counterstained with diamidino-2-phenylindole (blue). Scale bar, 10 μm. **B:** percentage number of M30 reactive cells. Data bars represent means ± SD of 3 independent experiments. *P < 0.01 vs. -ve controls.
with acetaldehyde decreased mitochondrial function and induced a marked decrease in ATP levels. The effects of acetaldehyde on mitochondrial function and ATP were comparable with effects of the potent oxidant H_2O_2_. These observations are in line with previous investigations showing that exposure to H_2O_2_ decreases cellular ATP levels in the mucus-secreting goblet-like HT-29 cells (19). Because NAC could largely inhibit acetaldehyde-induced ATP depletion in our study, it seems plausible that the reduction in ATP synthesis is largely related to the observed increase in oxidative stress and subsequent decrease in mitochondrial metabolic activity. However, future work will be required to define the precise regulatory mechanism(s).

In addition to ATP synthesis, mitochondria also regulate Ca^{2+} signals through rapid accumulation or release of Ca^{2+} (22). Although intracellular Ca^{2+} plays a crucial role in cellular metabolism, mitochondrial Ca^{2+} overload has been demonstrated to trigger ROS generation, mitochondrial dysfunction, and ATP depletion. In the present study, our results show that acetaldehyde is able to induce accumulation of mitochondrial Ca^{2+}. Together with the aforementioned observations, this may eventually lead to induction of apoptosis (22), which has also been observed in this study.

Acetaldehyde is known to be cytotoxic to the intestinal epithelial cells, which may contribute to the development of oropharyngeal and colorectal cancers (18). In the present study, exposure of LS174T cells to acetaldehyde resulted in increased LDH release. This is a widely used marker of cytotoxicity (14), indicating loss of membrane integrity and early necrosis. LDH release did negatively correlate with ATP (r = −0.6; P < 0.05; data not shown), suggesting that the observed decrease in ATP content was reflecting reduced cell viability. Indeed, LS174T cells responded to acetaldehyde with an increase in LDH release, indicating loss of membrane integrity and early necrosis.

Furthermore, NAC attenuated acetaldehyde-induced LDH release, suggesting a potential role for oxidative stress in cytotoxicity and membrane injury in LS174T cells. Given that loss of membrane integrity can be followed by cell death, we extended our experiments to examine whether acetaldehyde can induce apoptosis. This was confirmed by increased caspase 3/7 activity and expression of cleaved CK18 in LS174T cells, indicating induction of early apoptosis. Intestinal epithelial cell apoptosis has been shown to disrupt intestinal barrier function (3, 6, 7). Because goblet cells contribute to the integrity of the epithelium (35), together with the enterocytes (31), our data suggest that acetaldehyde-induced goblet cell apoptosis might contribute to enhanced permeability and thereby eventually predispose host to ALD and large intestinal carcinogenesis.

MUC2, a glycoprotein synthesized by goblet cells, is the most abundantly present secretory mucin in the upper and lower intestines (45). Therefore, we examined effects of acetaldehyde on MUC2 protein expression. Our data revealed that exposure to acetaldehyde increased MUC2 protein levels. Previously, secretion of mucins has been reported to be increased in goblet cells in response to induction of ROS and exposure to various noxious substrates such as endotoxins (5, 52). Therefore, the observed increase in MUC2 protein expression in our study may be due to acetaldehyde-induced cellular oxidative stress. Increasing mucin synthesis and secretion by goblet cells contributes to homeostasis of intestinal epithelium and protection against intestinal injury (28). Nevertheless, overexpression of mucin by goblet cells is suggested to be related to a decrease in their viability (12). In line with this, the observed increase in MUC2 protein in our study can be explained by acetaldehyde-induced cell apoptosis. However, whether this phenomenon should be considered as a compensatory response to acetaldehyde toxicity or as a direct result of acetaldehyde effects on goblet cells cannot be answered based on our results and therefore remains to be determined. Recent studies have disclosed that altered or inappropriate mucin gene expression is related to tumor development, invasiveness, and prognosis in colorectal cancer (26). Therefore, this study, along with previous ones, strengthens the concept that intestinal goblet cells are also important targets for the toxic effects of acetaldehyde. Because intestinal mucus has been found to be modulated by ethanol (20, 36), it would be interesting to evaluate effects of acetaldehyde on gene and protein expression of mucins such as MUC2 and other protective peptides, including the trefoil factors (i.e., TFF3). Furthermore, alterations in colon mucin biochemistry, including decreased oligosaccharide chain length, reduced sulfation, and increased sialylation, have been observed in patients with inflammatory bowel disease (42). Future studies should also focus on the biochemical composi-
tion of the mucus secreted in response to acetaldehyde exposure.

In conclusion, our results demonstrate for the first time that exposure to acetaldehyde exerts distinct metabolic and cytotoxic effects on LS174T cells that can lead to induction of cellular apoptosis and modulation of MUC2 protein expression. These effects may contribute to acetaldehyde-induced intestinal barrier dysfunction and, consequently, liver injury. Further delineation of mechanisms involved may provide new therapeutic targets for prevention of acetaldehyde-induced intestinal goblet cell injury and its subsequent barrier disruption. Future studies evaluating intestinal barrier should more systematically take into account goblet cell function and mucus production.

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DISCLOSURES

The authors declare no actual or potential conflicts of interest with other people or organizations in the present work.

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