Acetaldehyde disrupts tight junctions and adherens junctions in human colonic mucosa: protection by EGF and L-glutamine

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Basuroy, S., P. Sheth, C. M. Mansbach, and R. K. Rao. Acetaldehyde disrupts tight junctions and adherens junctions in human colonic mucosa: protection by EGF and L-glutamine. Am J Physiol Gastrointest Liver Physiol 289: G367–G375, 2005. First published February 17, 2005; doi:10.1152/ajpgi.00464.2004.—Acetaldehyde, a toxic metabolite of ethanol oxidation, is suggested to play a role in the increased risk for gastrointestinal cancers in alcoholics. In the present study, the effect of acetaldehyde on tyrosine phosphorylation, immunofluorescence localization, and detergent-insoluble fractions of the tight junction and the adherens junction proteins was determined in the human colonic mucosa. The role of EGF and L-glutamine in prevention of acetaldehyde-induced effects was also evaluated. Acetaldehyde reduced the protein tyrosine phosphatase activity, thereby increasing the tyrosine phosphorylation of occludin, E-cadherin, and β-catenin. The levels of occludin, zonula occludens-1, E-cadherin, and β-catenin in detergent-insoluble fractions were reduced by acetaldehyde, while it increased their levels in detergent-soluble fractions. Pretreatment with EGF or L-glutamine prevented acetaldehyde-induced protein tyrosine phosphorylation, redistribution from intercellular junctions, and reduction in the levels of detergent-insoluble fractions of occludin, zonula occludens-1, E-cadherin, and β-catenin. These results demonstrate that acetaldehyde induces tyrosine phosphorylation and disrupts tight junction and adherens junction in human colonic mucosa, which can be prevented by EGF and glutamine.

alcohol; barrier function; human colon; epidermal growth factor

ACETALDEHYDE IS A HIGHLY TOXIC, MUTAGENIC, AND CARCINOGENIC REACTIVE MOLECULE GENERATED BY OXIDATION OF ETHANOL BY ALCOHOL DEHYDROGENASE. ACETALDEHYDE APPEARS TO PLAY A CRUCIAL ROLE IN THE ALCOHOL-INDUCED RISK FOR GASTROINTESTINAL CANCERS (15). A SIGNIFICANT BODY OF EVIDENCE INDICATES THAT ACETALDEHYDE IS ACCUMULATED IN THE COLONIC LUMEN DUE TO ENTERIC MICROBIAL FERMENTATION (22, 23). INTRACOLONIC ACETALDEHYDE LEVEL IS DRAMATICALLY INCREASED BY ALCOHOL CONSUMPTION (6, 20, 27). MANY DIETARY AND PHARMACOLOGICAL AGENTS MAY FURTHER INCREASE THE LEVELS OF INTRACOLONIC ACETALDEHYDE (27). THEREFORE, COLONIC MUCOSA IS EXPOSED TO ACETALDEHYDE AT HIGH CONCENTRATION, ESPECIALLY IN ALCOHOLICS. A NUMBER OF DIFFERENT MECHANISMS MAY BE INVOLVED IN ACETALDEHYDE-INDUCED MUCOSAL INJURY AND INCREASED RISK FOR CARCINOGENESIS. ONE SUCH MECHANISM MAY INVOLVE LOSS OF CELL-CELL ADHESION.

 Junctional complexes, such as tight junctions (TJ) and adherens junctions (AJ), mediate the cell-cell adhesion in epithelial tissues. Cell-cell adhesion is an anti-carcinogenic property of the epithelial tissue. TJ is formed by the organization of specific proteins such as occludin (1), claudins (28), and zonula occludens (ZO)-1, ZO-2, and ZO-3 (8, 30), whereas AJ is organized by the interactions between E-cadherin, α-catenin, β-catenin, and γ-catenin (1, 5, 7, 14). These proteins in turn interact with the actin cytoskeleton to stabilize the TJ and AJ at the apical end of the cell (12, 30). Many TJ and AJ proteins are considered tumor suppressor proteins, as they are responsible for cell-cell adhesion. Carcinogenesis and tumor metastasis are associated with the loss of cell-cell adhesion that promotes cell proliferation and cell migration. Previous studies indicated that acetaldehyde disrupts TJ and AJ in Caco-2 cell monolayer by a tyrosine kinase-dependent mechanism (2, 17, 25, 26). Epidermal growth factor (EGF) and L-glutamine, the important gastrointestinal mucosal protective factors (4, 16, 21), prevent acetaldehyde-induced disruption of TJ and AJ and reduce the paracellular permeability in Caco-2 cell monolayer (25, 26). The protective effect of L-glutamine on acetaldehyde-induced permeability is mediated by EGF-receptor tyrosine kinase activity (25).

In the present study, we evaluated the effect of acetaldehyde on the tyrosine phosphorylation and cytoskeletal association of TJ and AJ proteins in normal human colonic mucosal biopsies. We also evaluated the effect of EGF and L-glutamine on these effects of acetaldehyde. Results show that 1) acetaldehyde inhibits protein tyrosine phosphatase (PTPase) activity and slightly elevates the activation of p60Src and p125FAK, resulting in an increased protein tyrosine phosphorylation in human colonic mucosa; 2) acetaldehyde increases tyrosine phosphorylation of occludin, E-cadherin, and β-catenin; 3) acetaldehyde reduces the levels of detergent-insoluble fractions of TJ and AJ proteins, leading to redistribution of these proteins from the intercellular junctions; and 4) both EGF and L-glutamine prevent the acetaldehyde-induced changes in protein tyrosine phosphorylation, the levels of detergent-insoluble TJ and AJ proteins, and redistribution of these proteins from the intercellular junctions.

MATERIALS AND METHODS

Human colonic biopsies. Mucosal biopsies from the left colon (4 forceps biopsies from visibly normal area of mucosa in each subject) were collected from subjects admitted for colonoscopy for the purpose of cancer surveillance. Both men and women, irrespective of race, were included in this study. These studies were conducted under the approval of the Institutional Review Board for human subjects, and written consents were taken from all patients before the procedure. The confidentiality was maintained by identifying the specimen with a code number that included identity of age, gender, and race.

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Fig. 1. Acetaldehyde inhibits protein tyrosine phosphatase (PTPase) activity and increases protein tyrosine phosphorylation. A: human colonic mucosal biopsies were incubated with varying concentrations of acetaldehyde, and Triton-insoluble and Triton-soluble fractions were prepared as described in MATERIALS AND METHODS. Triton-insoluble and -soluble proteins were separated on 4–12% gradient gel and were immunoblotted (IB) for phospho-tyrosine (p-Tyr). B: after incubation with or without (control) acetaldehyde (300 μM), native protein extracts were assayed for PTPase activity using 32P-labeled Raytide as substrate. Units are nanomoles of phosphate released in 1 h. Values are means ± SE (n = 3). *Significantly different from the value for control tissues, P < 0.05. C: after incubation with or without varying concentrations of acetaldehyde, denatured protein extracts from Triton-insoluble and -soluble fractions were immunoblotted for c-Src(pY418) and focal adhesion kinase (FAK) (pY397) by using specific antibodies.

For this study, biopsies were collected from 25 subjects, including 13 men and 12 women. All subjects had a normal colon; subjects with colonic polyps and symptoms of inflammatory bowel disease or other pathological diagnosis were excluded from this group.

Acetaldehyde treatment. Biopsies, immediately after collection, were transferred to vials containing PBS (Dulbecco’s saline containing 1.2 mM CaCl2, 1 mM MgCl2, 10 mM glucose, and 0.6% bovine serum albumin) and incubated for 45 min at room temperature. Each biopsy was incubated in PBS without or with acetaldehyde in the absence or presence of EGF (30 nM) or l-glutamine (2 mM), with or without 4-(3-chloroanilino)-6,7-dimethoxyquinazoline (AG1478) (3 μM). For immunoprecipitation of phospho-tyrosine (p-Tyr), two punch biopsies from the same patient were used for the control and acetaldehyde groups. Biopsies were exposed to vapor-phase acetaldehyde, as described previously (2, 17), to achieve acetaldehyde concentration of 100–600 μM in the buffer bathing the tissue. Briefly, biopsies in 24-well culture plates were treated with vapor-phase acetaldehyde by placing stock acetaldehyde solution (0.1–0.6%) in the reservoir wells and sealing the lid to the plate with tapes. Plates were incubated at 37°C for 3–6 h. In a previous study, we demonstrated that acetaldehyde absorbed by the buffer incubating the cell monolayer varied from 100–800 μM when 0.1–1.0% acetaldehyde solutions were placed in the reservoir wells (17). EGF and l-glutamine were administered 10 min before acetaldehyde treatment.

Tissue extraction. At the end of the experiment, tissues were washed in PBS and placed in hot lysis buffer D (0.3% SDS in 10 mM Tris buffer, pH 7.4, containing 1 mM vanadate and 0.33 mM PMSF), heated at 100°C for 5 min, and sonicated to homogenize the tissue. The homogenate was centrifuged at 300 g for 15 min at 4°C, and the supernatant was used for immunoprecipitation of p-Tyr and direct immunoblot analysis. For PTPase assay, tissues were homogenized by sonication in PTPase buffer (50 mM HEPES, pH 7.2, 60 mM NaCl, 60 mM KCl, 0.2 mM PMSF, 10 μg/ml aprotinin and bestatin, 2 μg/ml leupeptin), and directly used for the assay.

Preparation of actin cytoskeleton. Biopsy tissues were lysed in lysis buffer CS (Tris buffer, pH 7.4, containing 1.0% Triton X-100, 2 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml bestatin, 10 μg/ml pepstatin A, 1 mM vanadate, and 1 mM PMSF). Using a miniature Dounce homogenizer, the tissue was broken down manually to ensure complete dissolution of membranous structures. Extracts were centrifuged at 15,600 g for 4 min at 4°C to sediment high-density actin cytoskeleton. The pellet was suspended in 200 μl of lysis buffer CS. After withdrawal of aliquots for protein assay, cytoskeletal fractions were mixed with an equal volume of Laemmli’s sample buffer (2× concentrated) and heated at 100°C for 5 min.

PTPase assay. Tissue extracts were incubated in 60 μl of PTPase buffer containing 32P-Raytide (50K counts/min). 32P-Raytide was prepared by phosphorylating Raytide with [γ-32P]ATP and c-Src (13). The assay mixture was incubated at 30°C for 10 min, and the reaction was terminated by spinning down beads and placing 50 μl of supernatant onto P81 filter disk. Filters were washed with 0.5% phosphoric acid, and the radioactivity was counted. For control, the assay was conducted in the presence of 1 mM pervanadate.

Immunoprecipitation of p-Tyr. Proteins were extracted in lysis buffer D under denaturing conditions (heating at 100°C for 5 min). The clear tissue extract containing 0.4 mg protein in 0.8 ml of IP
buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% Nonidet P-40, 0.33 mM PMSF, 1 mM vanadate, 2 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml bestatin, 10 μg/ml pepstatin A) was incubated with 2 μg of biotin-conjugated anti-p-Tyr antibody. Immune complexes were isolated by precipitation using streptavidin-agarose (for 1 h at 4°C). Washed beads were suspended in 25 μl of Laemmli’s sample buffer and heated at 100°C for 5 min. Extracts were immunoblotted for occludin, ZO-1, E-cadherin, or β-catenin, as described below, using specific primary and horseradish peroxidase (HRP)-conjugated secondary antibodies.

**Immunoblot analysis.** Proteins (20 μg from each sample) were separated by SDS-polyacrylamide gel electrophoresis (4–12% or 7% gels) and transferred to polyvinylidene difluoride membranes. Blots were probed for p-Tyr, c-Src(pY418), focal adhesion kinase (FAK) (pY397), occludin, ZO-1, E-cadherin, or β-catenin by using HRP-conjugated recombinant anti-p-Tyr antibody, mouse monoclonal anti-occludin, anti-E-cadherin, or anti-β-catenin, or rabbit polyclonal anti-ZO-1, anti-c-Src(pY418), and anti-FAK(pY397) antibodies in combination with HRP-conjugated anti-mouse IgG or HRP-conjugated anti-rabbit IgG.

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**Fig. 3.** Acetaldehyde reduces detergent-insoluble fractions of tight junction (TJ) and adherens junction (AJ) proteins. A: human colonic biopsies were incubated with varying concentrations of acetaldehyde for 5 h. Triton-insoluble and Triton-soluble fractions were prepared and immunoblotted for different proteins. Actin was probed as a loading control.

**Fig. 4.** Epidermal growth factor (EGF; A) and l-glutamine (Gln; B) reduce acetaldehyde-induced protein tyrosine phosphorylation. Colonic mucosal biopsies were incubated with or without acetaldehyde (300 μM) in the absence or presence of EGF (30 nM) for 5 h. Triton-insoluble fractions were prepared and immunoblotted for p-Tyr.

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**Fig. 5.** EGF reduces acetaldehyde-induced reduction of detergent-insoluble fractions of TJ and AJ proteins. Colonic mucosal biopsies were incubated with or without acetaldehyde (300 μM) in the absence or presence of EGF (30 nM) for 5 h. Triton-insoluble (A) and Triton-soluble (B) fractions were prepared. Proteins were separated in 7% gel and immunoblotted for different proteins.

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**Fig. 6.** EGF reduces acetaldehyde-induced reduction of detergent-insoluble fractions of TJ and AJ proteins. Colonic mucosal biopsies were incubated with or without acetaldehyde (300 μM) in the absence or presence of EGF (30 nM) for 5 h. Triton-insoluble and -soluble fractions were prepared and immunoblotted for different proteins. The intensity of protein bands was evaluated by densitometry, and the arbitrary density units were normalized for corresponding values for positive controls. A: occludin density; B: ZO-1 density; C: β-catenin density. Values are means ± SE (n = 8). *Significantly different from corresponding value for control group, P < 0.05. #Values are different from corresponding values for acetaldehyde group.
Chemicals. Acetaldehyde, EGF, l-glutamine, AG1478, bovine DNase (type IV), streptavidin-agarose, and protein A Sepharose were purchased from Sigma Aldrich Chemicals (St. Louis, MO). All other chemicals were of analytic grade, purchased either from Sigma Aldrich Chemicals (St. Louis, MO), and rabbit polyclonal anti-β-catenin was from Chemicon International (Temecula, CA). AlexaFluor 488-conjugated anti-mouse IgG was purchased from Molecular Probes (Eugene, OR).

Antibodies. Mouse monoclonal anti-occludin, rabbit polyclonal anti-occludin, and rabbit polyclonal anti-ZO-1 antibodies were from Zymed Laboratories (South San Francisco, CA). Mouse monoclonal anti-β-catenin, recombinant HRP-anti-p-Tyr, mouse monoclonal anti-β-catenin, HRP-conjugated anti-mouse IgG, and HRP-conjugated anti-rabbit IgG antibodies. The blot was developed by using the enhanced chemiluminescence method (Amersham, Arlington Heights, IL).

Confocal immunofluorescence microscopy. Biopsy tissues were washed in PBS, blotted in tissue paper, and embedded in optimum cutting temperature compound by freezing it in liquid nitrogen. Cryosections of 10-µm thickness were prepared and fixed with acetone-methanol (1:1) at 0°C for 5 min. Cell monolayers were blocked in 3% nonfat milk and incubated with primary antibodies (rabbit polyclonal anti-ZO-1, mouse monoclonal anti-occludin, mouse monoclonal anti-E-cadherin, or rabbit polyclonal anti-β-catenin antibodies) for 1 h, followed by incubation with the secondary antibodies (AlexaFluor 488-conjugated anti-mouse IgG and cy3-conjugated anti-rabbit IgG goat antibodies) for 1 h, followed by incubation with the secondary antibodies (AlexaFluor 488-conjugated anti-mouse IgG and cy3-conjugated anti-rabbit IgG goat antibodies) for an additional 1 h. Fluorescence was analyzed by using a confocal laser scanning microscope (Biorad MRC1024) as a series of images from 1-µm XY-sections using Comos (confocal microscope operating system). Images were stacked by using the software Image J and processed by using Adobe Photoshop (Adobe Systems, San Jose, CA).

Staining apoptotic cells. Apoptosis was detected by labeling DNA nick ends (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling technology) using In Situ Cell Death Detection kit (Roche Diagnostics, Penzberg, Germany). Cryosections of biopsies incubated without or with acetaldehyde were fixed in 3% paraformaldehyde and permeabiliized with 0.2% Triton X-100 in PBS. Sections were stained with terminal deoxynucleotidyl transferases and fluorescein-conjugated UTP, according to the vendor’s instructions. For a positive control, cryosections of untreated biopsies were incubated with 0.1 U/ml of DNase for 10 min at 25°C before staining. Sections were also costained for actin by using AlexaFluor 350-conjugated phalloidin. Fluorescence images were collected by using the confocal microscope.

Acetaldehyde induces tyrosine phosphorylation of TJ and AJ proteins in human colonic mucosa. Disruption of TJ and AJ by acetaldehyde in Caco-2 cell monolayer is associated with tyrosine phosphorylation of ZO-1 and β-catenin by a tyrosine kinase-dependent mechanism (2). The present study shows that exposure of human colonic mucosa to acetaldehyde (100–600 μM) in the absence or presence of Gln (2 mM) for 5 h. Triton-insoluble and -soluble fractions were prepared and immunoblotted for different proteins. Actin was probed as a loading control. Bands in blots from different patients were analyzed by densitometry. Arbitrary units were normalized to corresponding bands for positive control.

Results

Acetaldehyde induces tyrosine phosphorylation of TJ and AJ proteins in human colonic mucosa. Disruption of TJ and AJ by acetaldehyde in Caco-2 cell monolayer is associated with tyrosine phosphorylation of ZO-1 and β-catenin by a tyrosine kinase-dependent mechanism (2). The present study shows that exposure of human colonic mucosa to acetaldehyde (100–600 μM) in the absence or presence of Gln (2 mM) for 5 h. Triton-insoluble and -soluble fractions were prepared and immunoblotted for different proteins. The intensity of protein bands was evaluated by densitometry, and the arbitrary density units were normalized for corresponding values from positive controls.

Fig. 7. Gln reduces acetaldehyde-induced reduction of detergent-insoluble fractions of TJ and AJ proteins. Colonic mucosal biopsies were incubated with or without acetaldehyde (300 μM) in the absence or presence of Gln (2 mM) for 5 h. Triton-insoluble (A) and Triton-soluble (B) fractions were prepared and immunoblotted for different proteins. Actin was probed as a loading control. Bands in blots from different patients were analyzed by densitometry. Arbitrary units were normalized to corresponding bands for positive control.

Fig. 8. Gln reduces acetaldehyde-induced reduction of detergent-insoluble fractions of TJ and AJ proteins. Colonic mucosal biopsies were incubated with or without acetaldehyde (300 μM) in the absence or presence of Gln (2 mM) for 5 h. Triton-insoluble and -soluble fractions were prepared and immunoblotted for different proteins. The intensity of protein bands was evaluated by densitometry, and the arbitrary density units were normalized for corresponding values from positive controls.
μM) increased the tyrosine phosphorylation of a wide spectrum of proteins in a dose-dependent manner (Fig. 1A). Acetaldehyde-induced increase in protein tyrosine phosphorylation was associated with a significant reduction in the PTPase activity (Fig. 1B). Acetaldehyde increased the levels of c-Src(pY418) and FAK(pY397) in Triton-soluble fractions, but not in Triton-insoluble fractions, indicating a slight activation of c-Src and FAK (Fig. 1C). Immunoprecipitation of p-Tyr followed by immunoblot analysis showed that low levels of tyrosine phosphorylated occludin, ZO-1, E-cadherin, and β-catenin are present in untreated colonic mucosal tissues (Fig. 2A). Acetaldehyde treatment significantly increased tyrosine phosphorylation of occludin, E-cadherin, and β-catenin (Fig. 2B), while phosphorylation of ZO-1 remained unaffected.

**Acetaldehyde reduces the levels of detergent-insoluble TJ and AJ proteins.** Previous studies indicated that occludin, ZO-1, E-cadherin, and β-catenin associated with the detergent-insoluble fraction (F-actin-rich fraction) correlates well with the integrity of TJ and AJ (18). Incubation of colonic tissues with acetaldehyde reduced the Triton-insoluble fractions of occludin, ZO-1, E-cadherin, and β-catenin with a concomitant increase in the levels of these proteins in Triton-soluble fractions (Fig. 3). The effect of acetaldehyde on the levels of Triton-insoluble fractions of TJ and AJ proteins was dose dependent.

**EGF and L-glutamine prevent acetaldehyde-induced protein tyrosine phosphorylation.** Recent studies demonstrated that EGF and L-glutamine prevent acetaldehyde-induced increase in paracellular permeability in Caco-2 cell monolayers (25, 26). The present study shows that pretreatment of human colonic mucosa with EGF (Fig. 4A) or L-glutamine (Fig. 4B), 10 min before acetaldehyde treatment, significantly reduced the acetaldehyde-induced protein tyrosine phosphorylation. EGF and L-glutamine by themselves produced a slight increase in protein tyrosine phosphorylation. The extent of protein tyrosine phosphorylation varied among the biopsies from subjects due to individual variation, but the pattern of changes in phosphorylation due to acetaldehyde, EGF, and glutamine was consistent.

**EGF and L-glutamine prevent acetaldehyde-induced effects on the levels of detergent-insoluble fractions of TJ and AJ proteins.** EGF significantly ameliorated the acetaldehyde-induced decrease in Triton-insoluble occludin, ZO-1, E-cadherin, and β-catenin (Fig. 5A) and reduced the acetaldehyde-induced elevation of these proteins in Triton-soluble fractions (Fig. 5B). Densitometric analysis demonstrates that these effects of acetaldehyde on Triton-insoluble and Triton-soluble fractions of

![Fig. 9. Effect of EGF and Gln on acetaldehyde-induced reorganization of occluding (Ocl) and ZO-1. Colonic mucosal biopsies were incubated with or without acetaldehyde (300 μM) in the absence or presence of Gln (2 mM) for 5 h. Biopsies were frozen, and cryosections sections were fixed and stained for occludin and ZO-1 by the immunofluorescence method.](http://ajpgi.physiology.org/)

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Fig. 10. Effect of EGF and Gln on acetaldehyde-induced reorganization of E-cadherin (EC) and β-catenin (BC). Colonic mucosal biopsies were incubated with or without acetaldehyde (300 μM) in the absence or presence of Gln (2 mM) for 5 h. Biopsies were frozen, and cryosections sections were fixed and stained for E-cadherin and β-catenin by the immunofluorescence method.

Fig. 11. Acetaldehyde does not induce apoptosis in epithelial cells. A: cryosections of biopsies were incubated without or with acetaldehyde (300 μM) for 5 h and stained for apoptotic cells by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling technology (green fluorescence). B: tissue sections were colabeled for actin by using AlexaFluor 350-conjugated phalloidin (blue fluorescence). For DNase-positive controls, tissue sections from untreated biopsies were treated with DNase before staining.
occludin (Fig. 6A), ZO-1 (Fig. 6B), and β-catenin (Fig. 6C) are statistically significant. EGF, by itself, showed no significant effect on Triton-insoluble or Triton-soluble pools of occludin, ZO-1, and β-catenin, but it significantly prevented the effect of acetaldehyde. Due to rapid proteolytic cleavage of immunogenic E-cadherin, there was a wide variability in the values for E-cadherin and, therefore, could not be accurately assessed. Inclusion of a classic set of proteolytic inhibitors during sample preparations did not prevent such E-cadherin degradation in biopsy samples. Immunoblot analysis (Fig. 7) and densitometric evaluation show that pretreatment with t-glutamine also significantly prevents the changes in the levels of Triton-insoluble and Triton-soluble fractions of occludin (Fig. 8A), ZO-1 (Fig. 8B), and β-catenin (Fig. 8C). EGF and glutamine by themselves showed a slight change in the levels of TJ and AJ proteins in Triton-insoluble and Triton-soluble fractions in some samples (Figs. 5 and 7), but these effects were not statistically significant (Figs. 6 and 8).

**EGF and t-glutamine prevent acetaldehyde-induced redistribution of TJ and AJ proteins.** Immunofluorescence staining of cryosections of human colonic biopsies and confocal microscopy show that occludin and ZO-1 are localized at the intercellular junctions, and acetaldehyde treatment caused a redistribution of these proteins into the intracellular compartments (Fig. 9). Pretreatment with EGF or t-glutamine attenuated this effect of acetaldehyde. E-cadherin and β-catenin were also organized at the intercellular junctions of epithelial cells, especially at the apical end of the lateral membranes (Fig. 10). Acetaldehyde treatment resulted in a loss of apical distribution of these proteins and rounding of cells. Pretreatment with EGF or t-glutamine completely prevented this effect of acetaldehyde (Fig. 10). Staining of tissue sections for apoptosis by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling technology showed few apoptotic cells in the lamina propria of control and acetaldehyde-treated tissues (Fig. 11). However, there was no sign of apoptosis in epithelial cells, both in control and acetaldehyde-treated tissues. On the other hand, DNase-treated sections from control tissue showed DNA breaks in all epithelial cells.

**Glutamine-mediated prevention of acetaldehyde-induced reduction of detergent-insoluble TJ and AJ proteins requires EGF receptor tyrosine kinase.** Our recent study demonstrated that glutamine-induced protection of TJ and AJ from acetaldehyde in Caco-2 cell monolayer is mediated by EGF receptor tyrosine kinase activity (25). Therefore, we analyzed the effect of AG1478, a selective inhibitor of EGF receptor tyrosine kinase, on glutamine-induced protection of human colonic biopsies from acetaldehyde. Pretreatment of colonic biopsies with AG1478 blocked the effect of glutamine on acetaldehyde-induced reduction of detergent-insoluble fractions of occludin, ZO-1, E-cadherin, and β-catenin (Fig. 12). AG1478 showed no considerable change in the intensity of occludin, ZO-1, E-cadherin, and β-catenin bands in acetaldehyde-treated cells.

**DISCUSSION**

Acetaldehyde is accumulated in the colonic lumen of alcoholics at high concentrations due to a high rate of oxidation of ethanol to acetaldehyde by mucosal and enterobacterial alcohol dehydrogenase and a low rate of oxidation of acetaldehyde into acetate by aldehyde dehydrogenase (10). Several experimental studies have demonstrated that acetaldehyde concentrations in the colonic lumen reach as high as 1 mM, and, therefore, acetaldehyde-induced cellular changes have significant implication for the integrity of colonic mucosa. Previous studies showed that exposure to acetaldehyde increases paracellular permeability by disruption of TJ and AJ (2) and promotes cell proliferation (11) in Caco-2 cells. The present study demonstrates that acetaldehyde, at 100–300 μM concentration, induces structural and functional modification of TJ and AJ proteins in the human colonic mucosa. The results indicate that acetaldehyde disrupts epithelial TJ by inducing tyrosine phosphorylation and dissociation from the cytoskeleton of TJ and AJ proteins. These effects of acetaldehyde may have significant implications for the loss of cell-cell adhesion and increased risk for colon cancer. A growing body of evidence indicates that alcohol increases the risk for colorectal cancer (24). This effect of alcohol is most likely mediated by the accumulation of acetaldehyde in the colonic lumen (15).

The present study shows that acetaldehyde induces tyrosine phosphorylation of a wide spectrum of proteins in the human colonic mucosa. This study also demonstrates that acetaldehyde increases tyrosine phosphorylation of TJ protein, occludin, and AJ proteins, E-cadherin and β-catenin. Previous studies showed that oxidative stress (18) increases the tyrosine phosphorylation of occludin, ZO-1, E-cadherin, and β-catenin, and acetaldehyde (2) increases tyrosine phosphorylation of ZO-1 and β-catenin in Caco-2 cell monolayer. It was suggested that tyrosine phosphorylation of TJ and AJ proteins may play a role in the disruption of TJ and AJ. Previous studies indicate that tyrosine phosphorylation of β-catenin prevents its interaction with E-cadherin, leading to the loss of cadherin-based cell-cell adhesion (3). Our recent study demonstrated that tyrosine phosphorylation of occludin prevents its interaction with ZO-1, ZO-2, and ZO-3 (9). Therefore, the results of the present study indicate that acetaldehyde-induced tyrosine phos-
occludin, ZO-1, E-cadherin, and β-catenin that are bound to the F-actin-rich, detergent-insoluble fraction of the cell are important in determining the integrity of TJ and AJ (18); the levels of membrane-associated and membrane cytoskeleton-associated TJ and AJ proteins do not correlate with the integrity of TJ and AJ. Therefore, the level of detergent-insoluble TJ and AJ proteins is an indicator of the integrity of TJ and AJ. The present study shows that acetaldehyde reduces the levels of detergent-insoluble fractions of occludin, ZO-1, E-cadherin, and β-catenin in human colonic mucosa, indicating that acetaldehyde disrupts the interaction of TJ and AJ proteins with the actin cytoskeleton. This loss of interaction with the actin cytoskeleton may be mediated by protein tyrosine phosphorylation. However, the possible association of these proteins in lipid rafts as a contaminant in detergent-insoluble fraction cannot be ruled out.

EGF, a well-characterized gastrointestinal mucosal protective factor, is a peptide of 53 amino acids (4). EGF is secreted in saliva and other gastrointestinal secretions at high concentrations (16). EGF prevents oxidative stress (19) and acetaldehyde-induced (26) increase in paracellular permeability in Caco-2 cell monolayer. The present study shows that EGF reduces acetaldehyde-induced protein tyrosine phosphorylation and prevents the acetaldehyde-induced reduction in the levels of detergent-insoluble fractions of occludin, ZO-1, E-cadherin, and β-catenin. These results suggest that EGF released in gastrointestinal secretions may prevent the acetaldehyde-mediated disruption of epithelial junctions in alcoholics. Similarly, L-glutamine also prevents acetaldehyde-induced protein tyrosine phosphorylation and reduction in the levels of detergent-insoluble occludin, ZO-1, E-cadherin, and β-catenin in human colonic mucosa. L-Glutamine is another gastrointestinal mucosal protective factor (21) and was recently shown to prevent acetaldehyde-induced increase in paracellular permeability in Caco-2 cell monolayers (25). In Caco-2 cells, glutamine prevents acetaldehyde-induced paracellular permeability by an EGF receptor tyrosine kinase-dependent mechanism (25). The present study shows that AG1478, a selective inhibitor of EGF receptor tyrosine kinase, blocks the glutamine-mediated prevention of acetaldehyde-induced reduction of the levels of occludin, ZO-1, E-cadherin, and β-catenin in the detergent-insoluble fractions. This observation demonstrates that L-glutamine prevents the acetaldehyde-mediated modulation of TJ and AJ in human colonic mucosa, and this protection is also mediated by EGF receptor tyrosine kinase activity.

In the present study, the immunofluorescence confocal microscopy shows that acetaldehyde induces redistribution of TJ and AJ proteins, indicating that acetaldehyde-induced tyrosine phosphorylation and loss of interaction with the actin cytoskeleton are associated with the disruption of TJ and AJ. ZO-1 distribution was clearly limited to the apical end of the epithelial cells. Occludin, on the other hand, was distributed along the lateral membranes, but the intensity was much greater at the apical end, indicating the role of occludin and ZO-1 in the organization of TJ. Acetaldehyde treatment resulted in reduction in ZO-1 and occludin stain at the apical end of epithelial cells, with increase in stain in the intracellular compartments. Acetaldehyde did not induce apoptosis of the epithelial cells. Pretreatment with EGF or L-glutamine prevented this acetaldehyde on redistribution of occludin and ZO-1. E-cadherin and β-catenin were localized predominantly at the apical end of the lateral membranes of epithelial cells in control tissue. Acetaldehyde dramatically reduced apical localization of E-cadherin and β-catenin and resulted in a dramatic change in cell morphology. EGF and glutamine clearly prevented the acetaldehyde-induced effect on AJ. These observations indicate that EGF and L-glutamine protect the integrity of TJ and AJ from acetaldehyde in human colonic mucosa.

This study, for the first time, shows that acetaldehyde disrupts epithelial junctions in human colonic mucosa. This effect of acetaldehyde on colonic epithelium may be mediated by tyrosine phosphorylation of occludin, ZO-1, E-cadherin, and β-catenin and loss of interaction of TJ and AJ proteins with the actin cytoskeleton. EGF and L-glutamine may serve as mucosal protective factors against the toxic effects of acetaldehyde in human colon. These studies in human colonic mucosa not only validate the observations made in cell culture studies, but also demonstrate a possible tool to analyze the integrity of TJ and AJ in biopsy specimen.

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