Role of protein tyrosine phosphorylation in acetaldehyde-induced disruption of epithelial tight junctions

K. J. ATKINSON1 AND R. K. RAO1,2

1Department of Pediatrics and 2Department of Cell Biology, Medical University of South Carolina, Charleston, South Carolina 29425

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Atkinson, K. J., and R. K. Rao. Role of protein tyrosine phosphorylation in acetaldehyde-induced disruption of epithelial tight junctions. Am J Physiol Gastrointest Liver Physiol 280: G1280–G1288, 2001.—Acetaldehyde-induced cytotoxicity is an important factor in pathogenesis of alcohol-related diseases; however, the mechanism of this toxicity is unknown. We recently showed that acetaldehyde increases epithelial paracellular permeability. We asked whether protein tyrosine phosphorylation via modulation of tyrosine kinases and/or PTPases is a mechanism involved in acetaldehyde-induced disruption of the tight junctions in the Caco-2 cell monolayer. Immunofluorescence localization of occludin and ZO-1 showed disruption of the tight junctions in acetaldehyde-treated cell monolayer. Administration of genistein prevented acetaldehyde-induced permeability. Acetaldehyde increased tyrosine phosphorylation of three clusters of proteins with molecular masses of 30–50, 60–90, and 110–150 kDa; three of these proteins were ZO-1, E-cadherin, and β-catenin. Acetaldehyde reduced PTPase activity in plasma membrane and soluble fractions, whereas tyrosine kinase activity remained unaffected. Treatment with acetaldehyde resulted in a 97% loss of protein tyrosine phosphatase (PTP)1B activity and a partial reduction of PTP1C and PTP1D activities. These results strongly suggest that acetaldehyde inhibits PTPases to increase protein tyrosine phosphorylation via modulation of tyrosine kinase activity (31, 32). In the present study, oxidant-induced increase in paracellular permeability to macromolecules in both human subjects (5) and experimental animals (7, 45).

Evidence indicates that ethanol is oxidized to acetaldehyde in the gastrointestinal tract (34) and suggests that acetaldehyde may contribute to the pathogenesis of alcohol-related diseases (22, 33). In addition to mucosal alcohol dehydrogenases, intestinal bacteria seem to play a significant role in the oxidation of ethanol to acetaldehyde (34). Studies in rats demonstrated that ethanol administration at conditions that result in 0.11% intracolonic ethanol generates up to 0.5 mM acetaldehyde (43). As high as 3 mM acetaldehyde has been reported at 2 h after administering a dose of 1.5 g/kg ethanol (17). A study by Lieber and co-investigators (4) suggested that luminal bacteria play a role in the oxidation of ethanol to acetaldehyde in the jejunal lumen. Although bacterial content of small intestine is low under normal conditions, it is increased in alcoholics (6). In a recent study, our laboratory demonstrated that acetaldehyde at concentrations ranging from 99 to 760 μM increases the paracellular permeability of Caco-2 cell monolayer (30), suggesting that acetaldehyde may disrupt the tight junctions (TJ) and the adherens junctions (AJ), increasing the intestinal permeability to endotoxins. The significance of acetaldehyde-induced intestinal permeability is associated with the fact that endotoxemia plays a critical role in pathogenesis of alcoholic liver disease (1, 11, 15, 29, 35) and that ethanol consumption increases intestinal permeability to macromolecules in both human subjects (5) and experimental animals (7, 45).

Studies reported during the past decade indicate that epithelial TJ and AJ are regulated by intracellular signaling systems (2). Our laboratory recently showed that oxidant-induced increase in paracellular permeability in Caco-2 and T84 cell monolayers requires tyrosine kinase activity (31, 32). In the present study, we posed the question whether acetaldehyde disrupts TJ and AJ and whether this effect of acetaldehyde involves protein tyrosine phosphorylation. This study shows that acetaldehyde treatment 1) disrupts TJ of Caco-2 cell monolayer in a tyrosine kinase-dependent mechanism, 2) induces tyrosine phosphorylation of a number of proteins that are primarily located at the intercellular junctions, 3) increases tyrosine phosphorylation of a cluster of proteins with molecular masses of 30–50, 60–90, and 110–150 kDa, and 4) reduces PTPase activity in plasma membrane and soluble fractions, whereas tyrosine kinase activity remained unaffected.

ACETALDEHYDE IS GENERATED in many tissues by bacterial fermentation and metabolism of exogenous ethanol by tissue alcohol dehydrogenases (34). The toxic effects of acetaldehyde have been shown in cells derived from liver (10, 19, 39), gastrointestinal tract (17, 27, 30, 36), gonads (25), immune system (20), and lung (3, 37). The mechanism of acetaldehyde-induced cytotoxicity involves its ability to form acetaldehyde-protein adducts (21). Acetaldehyde reacts with lysine amino groups to form unstable Schiff-base adducts (41, 42), and its reaction with thiols generates thiolzolidines (10, 24). Formation of such acetaldehyde-protein adducts results in inhibition of lysine-dependent enzymes (28), inhibition of mitochondrial functions (10), modulation of receptor and ligand activities (8, 16, 18), and prevention of microtubule formation (38).

Address for reprint requests and other correspondence: R. K. Rao, Dept. of Physiology, Univ. of Tennessee, 894 Union Ave., Memphis, TN 38163 (E-mail: rkrao@physio1.utmem.edu).

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ylation of ZO-1, E-cadherin, and β-catenin, and 4) reduces the activity of protein tyrosine phosphatase (PTP)1B, PTP1C, and PTP1D.

MATERIALS AND METHODS

Chemicals. Cell culture medium and related reagents were purchased from Gibco-BRL (Grand Island, NY). Acetaldehyde was from Fisher Scientific (Tustin, CA), and Raytide was from Oncogene Sciences (Gaithersburg, MD). Genistein, genistin, Poly (Glu-Tyr), ATP, p-nitrophenyl phosphate, and cyanamide were from Sigma Chemical (St. Louis, MO). Tyrosine phosphopeptide and serine phosphopeptide were purchased from Upstate Biotechnology (Lake Placid, NY). All other chemicals were of analytical grade purchased from Sigma Chemical or Fisher Scientific.

Antibodies. FITC-conjugated antiperoxidase antibody, horse radish peroxidase-conjugated antirabbit IgG, and Cy3-conjugated antirabbit IgG were purchased from Sigma Chemical. Rabbit polyclonal anti-ZO-1 antibody was from Zymed Laboratories (South San Francisco, CA). Horseradish peroxidase-conjugated-antiphosphotyrosine, biotin-conjugated antiperoxidase, mouse monoclonal anti-E-cadherin, mouse monoclonal anti-β-catenin, mouse monoclonal anti-PTP1B, mouse monoclonal anti-PTP1C, and mouse monoclonal anti-PTP1D antibodies were from Transduction Laboratories (Lexington, KY). Rabbit polyclonal anti-PTP1B was from Upstate Biotechnology. Oregon green-labeled rabbit antirat IgG was from Molecular Probes (Eugene, OR). Rabbit polyclonal antioccludin antibody was from Zymed Laboratories and rat monoclonal anti-ZO-1 antibody was from Chemicon International (Temecula, CA).

Cell culture and acetaldehyde treatment. A subclone of Caco-2 cell line obtained from Dr. Jeffrey Fields (Iowa City, IA) was maintained under standard cell culture conditions at 37°C in DMEM containing 10% (vol/vol) fetal bovine serum. Cells were grown on polycarbonate membranes in Transwells (6.5 mm or 24 mm; Costar, Cambridge, MA). Experiments were performed 12–15 days (6.5-mm wells) or 19–22 days (24-mm wells) after seeding cells onto Transwells. Under these conditions, confluent monolayers attained steady-state resistance to passive transepithelial ion flow, and the cells were adjoined by TJ and AJ.

Monolayers were bathed in PBS containing 1.2 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, 0.6% bovine serum albumin, and 0.1 mM cyanamide, 0.2 ml (2 ml to 24-mm wells) and 1.0 ml (2.5 ml to 24-mm wells) to apical and basal wells, respectively. After 2-h equilibration, cell monolayers were exposed to acetaldehyde vapor generating 650 μM acetaldehyde in the incubation buffer as described previously (30). Genistein (30–300 μM) was administered directly to the incubation buffer both before and during acetaldehyde treatment. Genistein (inactive isomer of genistein) was used as a negative control.

Measurement of transepithelial electrical resistance. Transepithelial electrical resistance (TER) was measured according to the method of Hidalgo et al. (14) using a Milli-cell-ERS Electrical Resistance System (Millipore, Bedford, MA) and was calculated as ohms per square centimeter by multiplying it with the surface area of the monolayer (0.33 cm²). The resistance of the supporting membrane in Transwells (which is usually ~30 Ω/cm²) was subtracted from all readings before calculations.

Measurement of unidirectional fluxes of [3H]-mannitol. d-[2-3H]-mannitol (15 Ci/mmol; ICN Biomedicals, Costa Mesa, CA), 0.2 μCi/ml, was administered to the basal well. At the end of acetaldehyde treatment, 100 μl each of apical and basal buffers were withdrawn and counted for radioactivity in a scintillation counter. The flux into the apical well was calculated as the percentage of total isotope added into the basolateral well per square centimeter.

Immunofluorescence staining. After treatment with PBS with or without acetaldehyde and genistein, cell monolayers were fixed in acetone-methanol (1:1) at ~20°C for 5 min. After blocking in 10% bovine calf serum (for phosphotyrosine) or 3% nonfat milk suspension (for occludin-ZO-1 double labeling), cell monolayer was incubated with FITC-labeled antiphosphotyrosine antibody (1:100 dilution) or a mixture of rat monoclonal anti-ZO-1 (1:200 dilution) and rabbit polyclonal antioccludin (1:200 dilution) antibodies. For occludin and ZO-1 labeling, cell monolayers were incubated with the following secondary antibodies: Oregon green-labeled rabbit antirat IgG (1:150 dilution) and Cy3-labeled goat antirabbit IgG (1:100 dilution) antibodies, respectively. After washing with PBS, monolayers were mounted on slides using 90% glycerol containing 1,4-diazabicyclo[2.2.2]octane (20 mg/ml). Slides were viewed with a confocal laser scanning microscope (BioRad MRC500). Images from multiple sections were gathered by using a confocal microscope operating system. Images were processed by using NIH Image and Adobe Photoshop 5.0 (Adobe Systems, San Jose, CA) computer softwares.

Western blot analysis. After treatment with acetaldehyde or PBS, cells were washed with 1 ml of cold PBS, and proteins were extracted in 100 μl boiling lysis buffer-D, 50 mM Tris, pH 8.0, containing 0.5% SDS, 0.1 mM vanadate, and 0.1 mM phenylmethylsulfonyl fluoride. Homogenate was heated at 100°C for 5 min and then passed through a 26-gauge needle six times. Proteins were separated by SDS-PAGE and transferred to nitrocellulose PVDF membranes. A horseradish peroxidase-conjugated antiphosphotyrosine antibody was blotted onto the membrane to detect phosphotyrosyl proteins. The blot was then developed by using the enhanced chemiluminescence method (Amersham, Arlington Heights, IL). For Western blot analysis of ZO-1, rabbit polyclonal anti-ZO-1 and horse radish peroxidase-conjugated goat antirabbit IgG were used.

Tyrosine phosphorylation of junctional proteins. After treatment with acetaldehyde or PBS alone for 5 h, proteins were extracted in lysis buffer-D as described above. Tyrosine-phosphorylated proteins were immunoprecipitated by using biotin-conjugated antiphosphotyrosine antibodies. Immunoprecipitation was continued as described below by use of streptavidin-agarose. Immune complexes were then Western blotted for ZO-1, E-cadherin, and β-catenin by using specific antibodies.

Preparation of membrane and soluble fractions. After acetaldehyde treatment, cell monolayers (in 24-mm wells) were homogenized in lysis buffer-F (0.75 ml/well), which contains 50 mM β-glycerophosphate in PBS, pH 7.0, 2 μg/ml of leupeptin, 10 μg/ml of aprotinin, 10 μg/ml of bestatin, 10 μg/ml of pepstatin-A, 1 mM benzamidine, and 0.1 mM phenylmethylsulfonyl fluoride. For each value for each experimental condition, materials from two identical wells were pooled. Monolayers were disrupted by using a Dounce homogenizer (50 strokes) followed by sonication for 10 s. Homogenate was centrifuged at 3,000 g for 10 min at 4°C to remove cell debris and cytoskeleton. Supernatant was then centrifuged at 30,000 g for 30 min at 4°C to separate the plasma membrane and soluble fractions. These fractions were immediately utilized for tyrosine kinase and PTPase assays.

PTPase assay. Tyrosine-phosphorylated Raytide (26) was used as substrate for this assay. Raytide was phosphorylated by incubation with c-Src and [γ-32P]ATP and was purified as...
previously described (36). Membrane or soluble fractions were incubated with \(^{32}P\)-labeled PTPase in PTPase buffer (50 mM Hepes, pH 7.2, 60 mM NaCl, 60 mM KCl, and protease inhibitors as described above) at a final volume of 60 \(\mu\)l. After incubation at 30°C for 10 min, the reaction was ended by spotting 50 \(\mu\)l of the reaction mixture to P81 phosphocellulose filter discs. Discs were washed in 0.5% phosphoric acid, dried, and placed in a scintillation counter to measure radioactivity. A decrease in filter-bound radioactivity was considered as PTPase activity. Zero minutes of incubation or presence of 1 mM vanadate was used for control assay.

Tyrosine kinase assay. Ten microliters of either the membrane or soluble fractions were mixed with 10 \(\mu\)l of 3\(\times\) kinase buffer (150 mM imidazole, 750 mM NaCl, 3 mM MnCl\(_2\), 0.3% Triton X-100, pH 7.4) and 5 \(\mu\)l Poly(Glu-Tyr). The reaction was started by adding 5 \(\mu\)l ATP mix, containing 1.2 mM ATP, 72 mM MgCl\(_2\), 12 mM MnCl\(_2\), 0.6 mM vanadate. The reaction was stopped by placing 20 \(\mu\)l of the reaction mixture on to DE80 filter discs (Whatman). After washing with 10% trichloroacetic acid, filter discs were dried and counted for radioactivity (Beckman LS3801). An increase in radioactivity against the control assay was considered as tyrosine kinase activity. For controls, the reaction was terminated immediately after the addition of the ATP mix. These values were similar to the control values obtained by using heat-inactivated enzyme.

Immune complex PTPase assay. After acetaldehyde treatment, Caco-2 cell monolayers (24-mm wells) were washed with ice-cold 20 mM Tris (pH 7.4), and the proteins were extracted in lysis buffer-N (20 mM Tris, pH 7.4, containing 150 mM NaCl, 0.5% NP40, and protease inhibitors as described above for lysis buffer-F) at 4°C for 30 min. Each cell monolayer was extracted in 0.5 ml of lysis buffer-N, and extracts from three monolayers were pooled for each value for each experimental condition. Extracts were centrifuged at 3,000 g for 10 min at 4°C, and the supernatant containing 1.0–1.5 mg protein/ml was incubated with 2 \(\mu\)g of anti-PTP1B (rabbit polyclonal), anti-PTP1C (mouse monoclonal), or anti-PTP1D (mouse monoclonal) antibodies at 4°C overnight. Immune complexes were isolated by binding to Sepharose beads conjugated with protein-A or protein-G (for 1 h at 4°C). Washed beads were suspended in 30 \(\mu\)l of PTPase buffer and incubated with 5 \(\mu\)l of phosphopeptide substrate, TSTEPQpYQPGENL (5 \(\mu\)g), as described above, and free phosphate was assayed by using malachite green reagent (13) using a 96-well microtiter plate and a HTS 7000 Bio Assay Reader (Perkin-Elmer, Norwalk, CT). PTPase activity in complexes prepared using preimmune IgG (species and isotype selective) was used as control activity and subtracted from the activities associated with specific immune complexes. Specificity of PTPase activity in immune complexes was also evaluated by using phosphoserine peptide substrate.

Statistical analysis. The comparison between the two groups was made by the Student’s t-tests for grouped data. The significance in all tests was derived at the 95% or greater confidence level.

RESULTS

Disruption of TJ by acetaldehyde. Treatment with acetaldehyde resulted in a time-dependent decrease in TER of Caco-2 cell monolayer, which was accompanied by an increase in unidirectional flux of D-[2-\(^{3}H\)]-mannitol (Fig. 1A). To determine whether this increase in paracellular permeability by acetaldehyde was caused by a disruption of epithelial TJ, we analyzed the effect of acetaldehyde on TJ by transmission electron microscopy and confocal immunofluorescence microscopy for occludin and ZO-1, the TJ proteins. Immunofluorescence stains for ZO-1 and occludin were colocalized at the intercellular junctions of control cell monolayer. In acetaldehyde-treated cell monolayer, ZO-1 stain appeared to be markedly reduced (Fig. 1B), whereas occludin stain was only slightly affected. Presence of genistein during the acetaldehyde treatment considerably prevented dissociation of ZO-1 and occludin from the intercellular junctions. Western blot analysis for ZO-1 showed no change in the total amount of ZO-1 protein present in acetaldehyde-treated cell monolayers (Fig. 1C).

Inhibition of acetaldehyde-induced paracellular permeability by a tyrosine kinase inhibitor. To determine whether protein tyrosine phosphorylation is involved in acetaldehyde-induced paracellular permeability, we evaluated the effect of genistein on acetaldehyde effect. Administration of genistein significantly inhibited the acetaldehyde-induced changes in TER (Fig. 2A) and mannitol flux (Fig. 2B). This effect of genistein on acetaldehyde-induced changes in TER and mannitol flux was concentration related (Fig. 2, C and D). Genistein, an inactive isomer of genistein, produced no significant effect on acetaldehyde-induced changes in TER or mannitol flux.

Acetaldehyde-induced protein tyrosine phosphorylation. On the basis of the above observation that acetaldehyde-induced increase in permeability requires tyrosine kinase activity, we asked whether acetaldehyde induces protein tyrosine phosphorylation in Caco-2 cell monolayer. Western blot analysis for phosphotyrosine showed that acetaldehyde treatment resulted in tyrosine phosphorylation of a number of proteins in Caco-2 cell monolayer (Fig. 3A). Acetaldehyde induced tyrosine phosphorylation of three clusters of proteins with the molecular masses of 30–50 kDa, 60–90 kDa, and 110–150 kDa. Treatment with genistein (300 \(\mu\)M) blocks this acetaldehyde-induced protein tyrosine phosphorylation. To further confirm our data, immunofluorescence staining of phosphotyrosine was performed. There was no fluorescence detected in cell monolayers incubated for 5 h in the absence of acetaldehyde (Fig. 3B). Fluorescence was detected in acetaldehyde-treated cell monolayers; fluorescence was predominantly localized at the intercellular junctions. No fluorescence was detected in cell monolayers treated with acetaldehyde in the presence of genistein.

Tyrosine phosphorylation of junctional proteins. To determine the effect of acetaldehyde on tyrosine phosphorylation of specific cellular proteins located at the TJ and AJ, we analyzed for tyrosine phosphorylation of ZO-1, E-cadherin, and \(\beta\)-catenin. Detectable levels of tyrosine-phosphorylated E-cadherin and \(\beta\)-catenin were present in control cell monolayers (Fig. 4), which were considerably increased by acetaldehyde treatment. Tyrosine-phosphorylated ZO-1 was not detectable in control cell monolayer, but acetaldehyde-
treated cell monolayers showed the presence of tyrosine-phosphorylated ZO-1. Acetaldehyde-treated cell monolayers also showed a 170-kDa tyrosine-phosphorylated band recognized by anti-ZO-1 antibody (Fig. 4).

Effect of acetaldehyde on tyrosine kinase and PTPase activity. To determine whether protein tyrosine phosphorylation was increased by the activation of tyrosine kinases or inhibition of PTPases, we evaluated the effect of acetaldehyde on tyrosine kinase and PTPase activities. PTPase activity in plasma membrane and soluble fractions of acetaldehyde-treated cell monolayers was significantly lower than that in control cell monolayers (Fig. 5A); activity in acetaldehyde-treated cell monolayer was nearly 50% lower than that in control cell monolayer at 6 h. However, tyrosine kinase activity associated with plasma membrane and soluble fractions of acetaldehyde-treated cell monolayer was not significantly altered compared with that in control monolayers (Fig. 5B). To test whether acetaldehyde...
has any direct effect on PTPases in a cell-free system, we isolated plasma membrane and soluble fractions, which were then incubated with acetaldehyde. Incubation of plasma membrane and soluble fractions with acetaldehyde in vitro for 1 or 5 h also resulted in a time-dependent decrease in PTPase activity (Fig. 5C).

To determine the effect of acetaldehyde on activities of specific PTPases, we incubated Caco-2 cell monolayers with or without acetaldehyde for 6 h and prepared immune complexes of PTP1B, PTP1C, and PTP1D. For controls, complexes were prepared using species and isotype specific preimmune IgGs. A considerable level of PTP1B activity was present in control Caco-2 cell monolayers, whereas 7% of that activity was present in acetaldehyde-treated cell monolayers (Fig. 6A). Similarly, PTPase activity in immune complexes of PTP1C and PTP1D prepared from acetaldehyde-treated cell monolayers was 37 and 34%, respectively, of the corresponding activities in immune complexes from control cell monolayers. Western blot analysis of immune complexes of PTP1B, PTP1C, and PTP1D.
plexes for respective PTPase showed that similar amounts of each PTPase were precipitated from the extracts of control and acetaldehyde-treated cell monolayers (Fig. 6B).

DISCUSSION

This study shows that acetaldehyde at a pathophysiologically relevant concentration disrupts TJ of Caco-2 cell monolayers by a tyrosine phosphorylation-dependent mechanism. Evidence indicates that acetaldehyde generation plays a significant role in the pathogenesis of alcoholic liver disease (34) and alcohol-associated rectal carcinogenesis (36). Our laboratory recently demonstrated that acetaldehyde, but not ethanol directly, increases intestinal epithelial paracellular permeability without altering the cell viability (30). Increase in intestinal permeability to macromolecules has been shown in alcoholics (5), and this is suggested to be responsible for increased endotoxin absorption in alcoholics. It is well established that increased serum endotoxin in alcoholics plays a crucial role in alcoholic liver damage (35). Therefore, it is very important to understand the underlying biochemical mechanism of acetaldehyde-induced increase in intestinal epithelial permeability.

Our previous study suggested that increased paracellular permeability in Caco-2 cell monolayer (30) was caused by the disruption of TJ and AJ. In the present study, we provide evidence to acetaldehyde-induced disruption of TJ in Caco-2 cell monolayer by transmission electron microscopy and confocal immunofluorescence microscopy of TJ-specific proteins, occludin and ZO-1. Dramatic reduction in ZO-1 stain at the intercellular junctions of acetaldehyde-treated cell monolayers indicated that ZO-1 is dissociated from TJ, whereas a high level of occludin remained at the intercellular junctions. Because association of ZO-1 with occludin is critical for the integrity of TJ (12), the dissociation of ZO-1 from the junctions in acetaldehyde-treated cell monolayer may explain the disruption of TJ structure and the increase in paracellular permeability. Western blot analysis however, showed no change in the total amount of ZO-1 protein by acetaldehyde treatment. This observation suggests that acetaldehyde treatment results in release of ZO-1 from the TJ and redistribution throughout the cell. Such redistribution may have
Fig. 6. Effect of acetaldehyde on the activities of PTP1B, PTP1C, and PTP1D. Proteins were extracted under native conditions from cell monolayers incubated in the absence (solid bars) or presence (open bars) of acetaldehyde for 6 h. PTP1B, PTP1C, and PTP1D were immunoprecipitated and PTPase activity was measured in these immune complexes as described in MATERIALS AND METHODS. Values are means ± SE (n = 5 for PTP1B and n = 4 for PTP1C and PTP1D). *Significantly different (P < 0.05) from respective control values. For experiments in B, immune complexes used for PTPase assay were washed, and proteins were extracted in Laemmli’s sample buffer and Western blotted for respective PTPase.

TABLE 2

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity (nmol PMP/min/mg protein)</th>
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<tr>
<td>PTP1B</td>
<td>0.6 ± 0.1</td>
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<tr>
<td>PTP1C</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>PTP1D</td>
<td>0.6 ± 0.1</td>
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Values are means ± SE (n = 5 for PTP1B and n = 4 for PTP1C and PTP1D). *Significantly different (P < 0.05) from respective control values.

In this study, we show that acetaldehyde treatment resulted in an increase in tyrosine phosphorylation of ZO-1. Additionally, a second band of 170-kDa phosphotyrosine was recognized by anti-ZO-1 antibody. Such a 170-kDa ZO-1-like band was also detected in Western blot analysis of ZO-1 in whole homogenates (data not shown). This second ZO-1-immunoreactive band may correspond to ZO-2, a TJ plaque protein localized exclusively at the TJ. However, we could not confirm this speculation because of the lack of a specific antibody for human ZO-2. The present study also shows that acetaldehyde treatment considerably increased tyrosine phosphorylation of E-cadherin and β-catenin. Binding of β-catenin to transmembrane E-cadherin is known to be critically important in maintenance of cadherin-based cell-cell contact, an important mechanism in the function of the AJ. Evidence indicates that tyrosine phosphorylation of these proteins results in dissociation of these protein complexes and disruption of cadherin-based cell-cell contact. Increase in tyrosine phosphorylation of E-cadherin and β-catenin may play an important role in acetaldehyde-induced disruption of AJ.

Ethanol at high concentrations (5–10%) was previously shown to induce an increase in paracellular permeability in Caco-2 cell monolayer (23). This effect of ethanol was dependent on the activity of myosin light chain kinase, a serine/threonine kinase. Therefore, the mechanism of this ethanol effect appears to be different from the mechanism of acetaldehyde-mediated increase in permeability.

Protein tyrosine phosphorylation in cell is regulated dynamically by changes in activity and subcellular localization of both tyrosine kinases and PTPases. To determine the mechanism of acetaldehyde-induced protein tyrosine phosphorylation, we evaluated the effect of acetaldehyde on the activities of tyrosine kinases and PTPases. Acetaldehyde treatment produced no significant effect on tyrosine kinase activity in the plasma membrane or soluble fraction of Caco-2 cell monolayer. However, PTPase activity in both plasma
membrane and soluble fractions was significantly reduced in a time-dependent manner. Immune complex PTPase assay demonstrated that acetaldehyde reduced PTPase activity associated with PTP1B, PTP1C, and PTP1D by 93, 67, and 66%, respectively. The amounts of PTP1B, PTP1C, and PTP1D present in the cells or that was immunoprecipitated were not altered by acetaldehyde treatment.

Inhibition of PTPase activity by in vitro incubation of plasma membrane and soluble fractions of Caco-2 cells suggests that acetaldehyde may directly block the activity of PTPases. These data indicate that acetaldehyde modulates the activity of PTPases rather than that of tyrosine kinases to increase protein tyrosine phosphorylation. Inhibition of acetaldehyde-induced tyrosine phosphorylation by genistein suggests that basally active tyrosine kinases are involved in increased protein tyrosine phosphorylation. Although a number of studies suggested that receptor tyrosine kinases are important targets for ethanol action (9), there are no reports showing a direct modulation of cell signaling components by acetaldehyde. Formation of acetaldehyde-PTPase adduct may be responsible for the inhibition of PTPase activity. Acetaldehyde has been shown to react with both the e-amino group of lysine and the thiol side chain of cysteine of different proteins (10, 24, 41, 42). Results of the present study suggest that acetaldehyde may react with thiol side chain of Cys215 in PTPase signature motif to form thiolzolidines and hence prevent substrate binding and formation of phosphoenzyme intermediate (46).

This study therefore shows, for the first time, that acetaldehyde, the toxic oxidative product of ethanol, inhibits PTP1B, PTP1C, and PTP1D, resulting in an increase in protein tyrosine phosphorylation in Caco-2 cell monolayer. This inhibition of PTPases and increased protein tyrosine phosphorylation might be a mechanism for the disruption of TJ and AJ to increase paracellular permeability.

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