Deoxycholic acid causes DNA damage while inducing apoptotic resistance through NF-κB activation in benign Barrett’s epithelial cells

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GASTROESOPHAGEAL REFLUX DISEASE (GERD) and its sequel, Barrett’s esophagus, are well-established risk factors for esophageal adenocarcinoma (31). The gastroesophageal reflux of acid is thought to contribute to the development of this lethal tumor (36), but the incidence of esophageal adenocarcinoma continues to rise at an alarming rate, despite the widespread use of potent acid-suppressive medications, such as proton pump inhibitors (PPIs), for the treatment of GERD (25). This suggests that refluxed hydrochloric acid might not be the sole factor contributing to esophageal carcinogenesis in GERD.

Indeed, there are data to suggest a carcinogenetic role for refluxed bile acids.

Barrett’s-like intestinal metaplasia of the esophagus and esophageal adenocarcinoma develop in rat models of reflux esophagitis in which a surgeon creates an esophageojjunostomy to induce the reflux of acid and bile into the esophagus (6, 28). In one study, rats subjected to esophageojjunostomy combined with total gastrectomy developed esophageal adenocarcinomas at the same rate as those subjected to esophageojjunostomy without gastrectomy, showing that the reflux of bilious intestinal juice alone (without acid) is sufficient to cause esophageal cancer in rats (10). Patients with Barrett’s esophagus have significantly more esophageal exposure to bile and significantly higher esophageal luminal concentrations of bile salts than patients who have GERD without Barrett’s esophagus (23, 35). For patients with cancers in Barrett’s esophagus, a carcinogenetic role for bile reflux has been proposed (20).

Bile acids have been shown to cause DNA damage in cultures of Barrett’s epithelial cells and Barrett’s-associated esophageal adenocarcinoma cells and in biopsy specimens of Barrett’s epithelium maintained ex vivo (8, 9, 11, 17, 19). Severe DNA damage often triggers apoptosis (programmed cell death), which is an important mechanism to prevent the replication of cells with mutations that might result in cancer development. Indeed, to become malignant, cells must develop the ability to resist apoptosis (12). Ex vivo cultures of Barrett’s mucosa are more resistant to bile acid-induced apoptosis than normal esophageal squamous mucosa (9). This observation suggests that Barrett’s epithelial cells might be able to resist apoptosis in the setting of bile acid-induced DNA damage, an ability that might enable dangerous mutations to persist and facilitate neoplastic progression.

In earlier studies, we showed that nonneoplastic, telomerase-immortalized Barrett’s epithelial (BAR-T) cell lines exposed to UV-B radiation developed DNA injuries, but the cells resisted apoptosis by activating the NF-κB pathway (13). The present study was designed to explore DNA damage and the contribution of the NF-κB pathway to apoptotic resistance in BAR-T cell lines treated with deoxycholic acid (DCA), a toxic, hydrophobic bile acid, or ursodeoxycholic acid (UDCA), a hydrophilic bile acid. We also sought to confirm the validity of our in vitro findings by looking for DNA damage and for activation...
of NF-κB pathway proteins in biopsy specimens of Barrett’s metaplasia taken from patients whose Barrett’s esophagus was perfused with DCA or UDCA during endoscopic examinations.

METHODS

Cell culture. We used two telomerase-immortalized Barrett’s epithelial cell lines (BAR-T and BAR-T10) that were created from endoscopic biopsy specimens of nonglandular specialized intestinal metaplasia taken from patients with long-segment (>3 cm) Barrett’s esophagus (15, 36–38). As a control for cell phenotype, we used normal rat intestinal epithelial (IEC-6) cells (American Type Culture Collection, Manassas, VA). IEC-6 cells were cultured in DMEM with 10% FBS, and BAR-T cell lines were cocultured with a fibroblast feeder layer, as previously described (15, 26). All cell lines were maintained in monolayer culture at 37°C in humidified air with 5% CO2 in growth medium, as previously described. For individual experiments, the BAR-T cell lines were equally seeded into collagen IV-coated wells (BD Biosciences, San Jose, CA). Cellular morphology was documented using the Metamorph imaging system (Universal Imaging, Downingtown, PA).

Bile acid exposure. For individual experiments, the cells were cultured in collagen IV-coated wells 24 h before treatment with experimental media (all at pH 7.2) containing DCA (50 or 250 μM; Sigma, St. Louis, MO) or UDCA (50 or 250 μM; Sigma) or control medium without bile acids. Media were added for 5, 10, or 30 min to equally seeded wells of subconfluent cells and then removed, and the samples were collected for analyses (see below). Exposure durations, bile acid concentrations, and pH were chosen to simulate typical episodes of gastroesophageal reflux in GERD patients who take PPIs (4, 7, 23, 27).

Detection of apoptosis. Apoptosis rates were assessed qualitatively by optic morphology and quantitatively using the cell death detection ELISAPLUS assay (Roche Applied Science, Indianapolis, IN) according to the manufacturer’s instructions. In this assay, monoclonal antibodies against DNA and histones are used to detect the presence of mono- and oligonucleosomes in the cell cytoplasm. Cells were equally seeded into a 24-well collagen IV-coated plate. After 5, 10, or 30 min of treatment, the medium was removed and replaced with neutral-pH medium for 24 h. The medium was removed, the plate was washed with 1× PBS, and the cells were incubated with 500 μl of cell lysis buffer at room temperature for 30 min. Equal amounts of cell lysate (20 μl) were added on a streptavidin-coated microplate along with 80 μl of immunoreagent containing incubation buffer, anti-histone-biotin, and anti-DNA MAb conjugated with peroxidase. Samples were covered with foil and incubated on a shaker for 2 h at room temperature. Samples were washed three times with 300 μl of incubation buffer, 100 μl of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) solution were added, and the plate was incubated for another 10–20 min with shaking while color development was monitored. Once the wells turned blue, 100 μl of ABTS stop solution were added, and the samples were analyzed using a DTX 880 Multimode Detector (Beckman Coulter, Brea, CA). Absorbance was measured at 450 nm; 490 nm served as the reference wavelength. Background absorbance values were based on measurements from incubation buffer and ABTS stop solution alone at 405 nm. The background absorbance value was subtracted from the absorbance value of each sample to generate a "corrected" absorbance value at 405 nm. The corrected absorbance value minus the absorbance value at 490 nm was generated for each sample, and the results were averaged. All assays were performed in duplicate.

Detection of intracellular reactive oxygen and/or nitrogen species. Reactive oxygen and/or nitrogen species (ROS/RNS) were detected using the ROS detection dye reacts with hydrogen peroxide, peroxynitrite, and hydroxyl radicals. Equally seeded wells of cells (with cover glass on the bottom) were washed twice with wash buffer and then incubated with the ROS detection solution for 30 min at 37°C while protected from light. Thereafter, cells were exposed to medium containing 250 μM DCA or UDCA for 5, 10, or 30 min; then the medium was removed and replaced with wash buffer containing the ROS detection solution. After 20 min, cells were washed with wash buffer to remove any excess solution; then the cover glasses were mounted to slides, and fluorescence was immediately detected using fluorescence microscopy. The slides were counterstained with 4′,6-diamidino-2-phenylindole for analysis.

Inhibition of intracellular ROS/RNS. In experiments designed to inhibit the generation of ROS/RNS by cells exposed to DCA, cells were pretreated for 30 min with 10 mM N-acetylcysteine (NAC; Sigma), which increases cellular pools of free radical scavengers. Thereafter, cells were treated with control medium containing 10 mM NAC or experimental medium containing 250 μM DCA and 10 mM NAC for 5 min; then the media were removed, and the cells were prepared for further analysis.

Nuclear/cytoplasmic fractionation and Western blotting. Cells were immediately washed twice with ice-cold PBS and then harvested by scraping into 200 μl of 1× cell lysis buffer (Cell Signaling Technology) containing 0.5 mM PMSF in a 1.5-ml microcentrifuge tube. The biopsy specimens were lysed in buffer containing 150 mM NaCl, 1% NP-40, 1% DCA, 20 mM/tris-HCl (pH 7.5), 1 mM/l EDTA, 1 mM/l EGTA, 2.5 mM/l sodium pyrophosphate, 1 mM/l β-glycerophosphate, 1 mM/l sodium orthovanadate, 1 µg/ml leupeptin, 1 mM/l PMSF, and one protease inhibitor cocktail tablet per 50 ml of lysis buffer (Roche Applied Science). For nuclear expression of total and phosphorylated p65 (p-p65), nuclear extracts were isolated from cell lines using the NE-PER nuclear and cytoplasmic extraction kit (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer’s instructions. Protein concentrations were determined using a protein assay kit (BCA-200, Pierce, Rockford, IL). For Western blotting, equal amounts of protein were separated by SDS-PAGE. The proteins were separated and transferred to nitrocellulose membranes, which were incubated with 1:3,000 dilutions of the rabbit polyclonal antibodies and 1:1,000 dilution anti-total p65 (Santa Cruz), 1:2,000 dilutions of mouse monoclonal anti-total IκB (Cell Signaling Technology), 1:1,000 dilutions of rabbit polyclonal antibodies, anti-human phosphorylated Ser199/219 (p-p219), anti-phosphorylated (Ser530) p65 (p-p65), or rabbit monoclonal anti-Bcl-2, or a 1:500 dilution of mouse anti-human phosphorylated Ser122/124 IkBα (p-IkBα; Cell Signaling Technology). Horseradish peroxidase secondary antibody (Cell Signaling Technology) was used at 1:1,000 or 1:500 dilution, and chemiluminescence was detected using an enhanced chemiluminescence detection system (ECL, Pierce). Membranes were then stripped using Restore stripping buffer (Pierce) and reprobed with anti-NF-κB/p65 for total p65 expression or with β-tubulin (Sigma) to confirm equal loading. All Western blots were performed in duplicate.

NF-κB pathway inhibitors. Cells were treated with 5 μM BAY 11-7085 (Sigma), a pharmacological inhibitor that blocks IκBα phosphorylation, for 2 h prior to exposure to experimental or control medium (14). To confirm the role of NF-κB in mediating effects on apoptosis, we also used an IκBα superrepressor construct (with a mutant form of IκBα) (35). Cells were infected with viral medium containing recombinant-deficient adenovirus Ad5IκBα-SR 2 h before treatment with the experimental or control medium (14, 33). Cells were then exposed for 5 min to 250 μM DCA or control medium, which were removed and replaced with medium containing BAY 11-7085 or the Ad5IκBα-SR superrepressor overnight. Cells were then collected, and apoptosis rates were determined.

Study in patients with Barrett’s esophagus. This study was approved by the Institutional Review Board of the Dallas Veterans Affairs Medical Center. Ten patients with Barrett’s esophagus [9 men,
64.5 ± 2.8 (SE) yr old, who were taking PPIs] who had specialized intestinal metaplasia without dysplasia involving ≥2 cm of the distal esophagus and were scheduled for elective endoscopic examinations were invited to participate. During endoscopy, using a jumbo biopsy forceps (Olympus FB-50K-1) and a technique similar to that previously described by our laboratory (7, 23, 30), we obtained six biopsy specimens of Barrett’s metaplasia before and after perfusion of the distal esophagus with 10 ml of unconjugated DCA (5 patients) or conjugated UDCA (5 patients) at 250 µM over 5 min. For the in vivo perfusions, DCA and UDCA were dissolved in alcohol, resulting in solutions with pH 5.0 and 4.7, respectively. These pH levels are within the range of weakly acidic reflux episodes (pH 4–7), which commonly occur in patients who take PPIs (4).

**Statistical analyses.** The data were collected from at least two independent experiments. Quantitative data are expressed as means ± SE. Statistical analysis was performed using ANOVA and the Student-Newman-Keuls multiple-comparison test with the Instat for Windows statistical software package (GraphPad Software, San Diego, CA). *P* < 0.05 was considered significant for all analyses.

**RESULTS**

**DCA causes DNA damage but does not induce apoptosis in Barrett’s epithelial cells.** BAR-T and BAR-T10 cells were grown to 70% confluence and then exposed to 50 or 250 µM DCA for 5–30 min. Cells were collected immediately after exposure, and DNA damage was assessed by expression of p-H2AX (5). Apoptosis was determined by optic morphology and cell death ELISA at 24 h after treatment with DCA. In both BAR-T cell lines, 50 and 250 µM DCA caused substantial increases in p-H2AX expression, even after only 5 min of exposure (Fig. 1). However, BAR-T and BAR-T10 cells treated with 250 µM DCA for up to 30 min exhibited no significant changes in their rates of apoptosis (Fig. 2). These findings demonstrate that DCA causes DNA damage in Barrett’s epithelial cells, but the cells resist apoptosis, despite their genotoxic injuries.

**DCA does not cause DNA damage or induce apoptosis in normal intestinal cells.** In contrast to the BAR-T cell lines, IEC-6 cells (normal rat small intestinal epithelial cells) did not exhibit phosphorylation of H2AX after exposure to 250 µM DCA for ≤30 min (Fig. 3A). IEC-6 cells also exhibited no significant changes in their rates of apoptosis after exposure to DCA (Fig. 3, B and C).

**UDCA does not cause DNA damage or induce apoptosis in Barrett’s epithelial cells.** We treated BAR-T and BAR-T10 cells with 50 or 250 µM UDCA for 5–30 min. In contrast to treatment with DCA, UDCA did not cause DNA damage at any dose or duration of exposure (Fig. 4). In addition, the cells exhibited no significant changes in their rates of apoptosis after exposure to UDCA (Fig. 5).
**NF-κB pathway is activated by DCA, but not by UDCA, in Barrett’s epithelial cells.** In Barrett’s-associated adenocarcinoma cells, DCA has been shown to activate NF-κB and to increase expression of NF-κB target genes, including IL-8 and IkB (1, 18). We sought to determine whether bile acids activate NF-κB pathway proteins in nonneoplastic Barrett’s epithelial cells. BAR-T or BAR-T10 cells were treated with 250 μM DCA or UDCA for 5 min, and Western blotting for p-IκBα and p-p65 was performed. DCA increased the expression of p-IκBα and p-p65 in both Barrett’s epithelial cell lines (Fig. 6A). In contrast, UDCA had no effect on the expression of these phosphoproteins. Activation and phosphorylation of p65 lead to its nuclear translocation. As shown in Fig. 6B, DCA increased nuclear expression of total p65 and p-p65 in both Barrett’s epithelial cell lines. DCA also increased expression of Bcl-2, a survival protein that is a downstream target of NF-κB, by 24 h (Fig. 6C). Taken together, these data suggest that the NF-κB pathway is activated by DCA in Barrett’s epithelial cells.

**DCA, but not UDCA, generates ROS/RNS in Barrett’s epithelial cells.** In Barrett’s-associated adenocarcinoma cells, treatment with antioxidants has been shown to prevent DCA-induced DNA damage and expression of NF-κB-dependent genes, which suggests a role for ROS/RNS in triggering these events (17, 19). Using fluorescence microscopy, we determined the effect of 250 μM DCA or UDCA on ROS/RNS production by our nonneoplastic Barrett’s epithelial cells. In agreement with the data on Barrett’s cancer cells, we found that exposure to DCA for 5, 10, or 30 min induced ROS/RNS production in BAR-T and BAR-T10 cells (Fig. 7A; data not shown for BAR-T). In contrast, exposure to 250 μM UDCA for up to 30 min did not induce the production of ROS/RNS in either Barrett’s epithelial cell line (Fig. 7A; data not shown for BAR-T).

**NAC prevents DNA damage and activation of the NF-κB pathway in Barrett’s epithelial cells exposed to DCA.** To explore whether the DCA-induced production of ROS/RNS was responsible for DNA damage and activation of the NF-κB pathway we observed in BAR-T and BAR-T10 cells, we treated the cells with 250 μM DCA for 5 min in the presence of 10 mM NAC. NAC prevented the DCA-induced increase in p-H2AX and p-p65 expression, suggesting that ROS/RNS produced in response to DCA exposure are responsible for the genotoxic injury and activation of the NF-κB pathway in Barrett’s epithelial cells (Fig. 7B).

**In patients with Barrett’s esophagus, esophageal perfusion with DCA, but not UDCA, causes DNA damage and activation of the NF-κB pathway.** Having found that exposure to DCA, but not UDCA, increases phosphorylation of H2AX and activation of NF-κB pathway proteins in our Barrett’s epithelial cell lines, we sought to confirm that these same effects occur in patients with Barrett’s esophagus in vivo by taking endoscopic...
biopsy specimens of Barrett’s epithelium before and after a 5-min perfusion of the esophagus with 250 μM DCA or UDCA. In agreement with our in vitro data, we found increased p-H2AX, p-IκBα, and p-p65 expression in biopsy specimens of Barrett’s metaplasia from all five patients whose esophagus was perfused with DCA (Fig. 8A), but in none of the five patients whose esophagus was perfused with UDCA (Fig. 8B).

**Inhibition of the NF-κB pathway sensitizes Barrett’s cells to DCA-induced apoptosis.** Having found that DCA activates the NF-κB pathway in Barrett’s epithelial cells in vitro and in vivo, we next sought to determine whether that activation contributes to their resistance to apoptosis. BAR-T and BAR-T10 cells were treated with BAY 11-7085 at 5 μM (a concentration that we previously found to inhibit NF-κB activity and induce apoptosis in BAR-T cells) and then exposed to 250 μM DCA for 5 min (13). We confirmed inhibition of the NF-κB pathway by performing Western blotting for p-IκBα in the presence and absence of DCA (Fig. 9A). DCA induced a significant increase in apoptosis in both cell lines treated with BAY 11-7085 (Fig. 9B). Moreover, cells treated with BAY 11-7085 and exposed to DCA became small and shrunken, a morphology that suggests an apoptotic phenotype (Fig. 9C). To confirm these findings,
we infected the cells with the AdIκB-SR construct (containing a mutant form of IκB) and determined the rate of apoptosis following exposure to DCA. Infection with AdIκB-SR alone caused no change in basal rates of apoptosis, but the infection significantly increased apoptosis after exposure to DCA (Fig. 10). These data suggest that the apoptosis induced by DCA in the infected cells was indeed due to inhibition of the NF-κB pathway and not a nonspecific effect of the AdIκB-SR.

**DISCUSSION**

We have shown that DCA causes DNA damage and induces phosphorylation of proteins in the NF-κB signaling pathway in Barrett’s epithelial cells in vitro and in vivo. In two Barrett’s epithelial cell lines, we have found that DCA-induced DNA damage and NF-κB pathway activation are mediated by the generation of ROS/RNS. In addition, we have demonstrated that DCA-mediated activation of the NF-κB pathway allows Barrett’s epithelial cells to resist apoptosis in the setting of DNA injury, events that might contribute to neoplastic progression in Barrett’s esophagus.

In an earlier study, ex vivo cultures of Barrett’s mucosa were found to be more resistant to bile acid-induced apoptosis than esophageal squamous mucosal cultures (9). From that study, however, it was not clear whether resistance to bile acid-induced apoptosis was a unique feature of Barrett’s cells or whether the differences in apoptotic resistance were merely due to differences in cell phenotypes (i.e., intestinal-type columnar cells vs. squamous cells). Barrett’s epithelial cells share phenotypic features with small intestinal epithelial cells. Therefore, we exposed normal rat intestinal epithelial (IEC-6) cells to DCA and assessed DNA damage and apoptosis. Unlike our Barrett’s cell lines, we found that IEC-6 cells did not develop DNA damage with DCA exposure. In the absence of DNA damage, it was not surprising that DCA also had no effect on apoptosis in IEC-6 cells. Thus, despite some similar phenotypic features, the Barrett’s cells and small intestinal cells responded very differently to bile acid exposure. Epithelial cells of the small intestine regularly are exposed to high concentrations of bile acids, yet small bowel cancers are uncommon. The differences we observed between small intesti-
tinal and Barrett’s cells in their susceptibility to DCA-induced DNA damage might contribute to the rarity of cancer in the small intestine and the frequency of cancer in Barrett’s esophagus.

In earlier studies, we demonstrated that Barrett’s epithelial cells resist apoptosis due to UV-B-induced DNA injury by activating the NF-κB pathway (13). Although UV-B is a well-established, potent inducer of genomic injury, it is not a physiologically relevant agent for the esophagus. In the current study, we exposed our Barrett’s cells to DCA at physiological concentrations (50 and 250 μM) for 5–30 min to simulate episodes of gastroesophageal reflux (7, 23, 35). We selected DCA on the basis of clinical data demonstrating that concentrations of DCA are especially high in the gastric refluxate of patients with Barrett’s esophagus who take PPIs (23, 29, 32).

In patients who take PPIs, 80% of reflux episodes are either weakly acidic (pH 4–7) or weakly alkaline (pH >7) (4). Thus we elected to use bile acid solutions at pH 4.0 for the in vitro and in vivo studies. DCA, at neutral and acidic pH, has been shown to induce DNA damage in Barrett’s-associated adenocarcinoma cells (8, 17). In agreement with these data in cancer cells, we found that DCA induced genomic injury, as determined by the increase in H2AX phosphorylation in Barrett’s epithelial cells in vitro and in vivo.

We used phosphorylation of H2AX as a marker for DNA damage. H2AX becomes phosphorylated in response to various types of DNA damage, including single- and double-stranded breaks (DSBs) (5). DSBs are among the most serious forms of DNA damage, because cells with persistent DSBs have been found to develop chromosomal abnormalities that can contribute to genomic instability and cancer formation (5, 22). In fact, agents that cause DSBs, such as ionizing radiation and UV light, are considered carcinogens (2, 22). If the DCA-induced DNA damage we observed in Barrett’s cells included DSBs, then this bile acid would be considered a carcinogen in Barrett’s esophagus.

Our finding that DCA induces DNA damage in Barrett’s epithelial cells has interesting clinical implications. In an effort to prevent cancer in Barrett’s esophagus, clinicians commonly prescribe PPIs. However, PPI therapy has been associated with increases in the concentration of toxic, unconjugated bile acids, such as DCA, in the gastric juice of Barrett’s patients (29, 32). Although PPIs clearly heal reflux esophagitis, an effect that would be expected to protect against cancer in Barrett’s esophagus, PPI effects on bile acids could conceivably contribute to cancer formation.

In Barrett’s-associated cancer cell lines, DCA has been shown to cause ROS/RNS production, DNA damage, and NF-κB activation, which can be prevented by treatment with antioxidants (16, 17, 19). However, it has not been clear that
these DCA effects in cancer cells are applicable to benign Barrett’s cells. We have found that DCA increases ROS/RNS production in nonneoplastic Barrett’s epithelial cells. We also have found that treatment with a ROS/RNS scavenger (NAC) prevents the development of DNA damage and the phosphorylation of p65 when BAR-T cells are exposed to DCA. These findings demonstrate that, as in cancer cells, DCA causes DNA damage and NF-κB pathway activation that is mediated by ROS/RNS production in benign Barrett’s epithelial cells.

The mechanism underlying the apoptotic resistance of Barrett’s mucosa is not known, but circumstantial evidence has suggested a role for the NF-κB pathway. For example, expression of NF-κB has been found in 40–60% of biopsy specimens of benign Barrett’s epithelium and in 61–80% of esophageal adenocarcinomas, but in only 13% of biopsy specimens of reflux-injured squamous epithelium (1, 24). DCA has been shown to activate NF-κB and increase expression of the NF-κB target genes IL-8 and IkB in Barrett’s-associated adenocarcinoma cells (1, 18). We have reported that activation of the NF-κB pathway allows benign Barrett’s epithelial cells to resist apoptosis in the setting of DNA injury induced by UV-B irradiation (13). In the present study, we have shown that DCA increases the expression of activated NF-κB proteins (p-IkBα and p-p65) in Barrett’s epithelial cell lines in vitro and in biopsy specimens from patients with Barrett’s esophagus in vivo. We also have found that DCA increases nuclear expression levels of total p65 and p-p65 in Barrett’s cell lines, which also express higher levels of Bcl-2. This indicates that DCA activates the NF-κB pathway. In addition, we have found that inhibition of the NF-κB pathway with BAY 11-7085 or an iκB superrepressor construct abolishes resistance to apoptosis after DCA exposure in BAR-T cells. Taken together, these data demonstrate that the NF-κB pathway mediates the apoptotic resistance of Barrett’s epithelial cells.

UDCA is a hydrophilic bile acid that has been used to treat cholestatic liver diseases (21) and has been reported to reduce bile salt-induced CDX2 expression differs in esophageal squamous cells (22) and to inhibit acid and bile salts-induced DNA damage and NF-κB pathway activation in Barrett’s cell lines in vitro and in patients with Barrett’s esophagus in vivo. Unlike DCA, UDCA does not increase the expression of p-H2AX, p-IkBα, or p-p65 in Barrett’s epithelial cells in vitro or in patients with Barrett’s esophagus in vivo. We also have found that UDCA does not induce ROS/RNS production in BAR-T cells, which may account for the absence of DNA damage and NF-κB activation. Although we did not explore a specific protective effect for UDCA in preventing bile acid damage, UDCA had no apparent ill effects in Barrett’s epithelial cells.

In conclusion, we have shown that DCA causes genotoxic injury in nonneoplastic, Barrett’s epithelial cell lines, which are able to resist the apoptosis that ordinarily follows severe DNA damage by activating the NF-κB pathway. Furthermore, we have verified our in vitro findings by demonstrating the development of DNA damage and phosphorylation of NF-κB pathway proteins in biopsy specimens of Barrett’s esophagus from patients who had DCA perfused into the esophagus for 5 min during endoscopic examinations. These findings suggest a molecular pathway whereby bile reflux, by inducing DNA damage in cells that can resist apoptosis, may contribute to carcinogenesis in Barrett’s esophagus.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

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