In Benign Barrett’s Epithelial Cells, Acid Exposure Generates Reactive Oxygen Species That Cause DNA Double-Strand Breaks

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Abstract

Cells that sustain double-strand breaks (DSB) can develop genomic instability, which contributes to carcinogenesis, and agents that cause DSBs are considered potential carcinogens. We looked for evidence of acid-induced DNA damage, including DSBs, in benign Barrett’s epithelial (BAR-T) cell lines in vitro and in patients with Barrett’s esophagus in vivo. In BAR-T cells, we also explored the mechanisms underlying acid-induced DNA damage. We exposed BAR-T cells to acid in the presence of a fluorescent probe for reactive oxygen species (ROS) and in the presence or absence of disodium 4,4′-disothiocyanostilbene-2,2′-disulfonate (which prevents intracellular acidification) and N-acetylcysteine (a scavenger of ROS). DSBs were detected by Western blotting for histone H2AX phosphorylation and by CometAssay. During endoscopy in patients with Barrett’s esophagus, we took biopsy specimens from the metaplastic mucosa before and after esophageal perfusion with 0.1 N HCl for 3 min and sought DSBs by Western blotting for histone H2AX phosphorylation. In BAR-T cells, acid exposure resulted in ROS production and caused a time-dependent increase in levels of phospho-H2AX that continued for at least 48 h. Pretreatment with disodium 4,4′-disothiocyanostilbene-2,2′-disulfonate or N-acetylcysteine prevented the acid-induced increase in phospho-H2AX levels. DSBs also were detected in biopsy specimens of Barrett’s epithelium following esophageal acid perfusion in all of 6 patients with Barrett’s esophagus. Acid exposure causes DSBs in Barrett’s epithelial cells through ROS produced as a consequence of intracellular acidification. These findings suggest that acid can be considered a carcinogen in Barrett’s esophagus. [Cancer Res 2009;69(23):9083–9]

Introduction

In the last three decades, the incidence of esophageal adenocarcinoma has increased >6-fold in the United States (1). The major risk factors for this lethal cancer are gastroesophageal reflux disease and Barrett’s esophagus, the condition in which a metaplastic columnar epithelium replaces esophageal squamous epithelium that has been damaged by gastroesophageal reflux disease. Carcinogenesis in Barrett’s metaplasia involves the accumulation of genetic and epigenetic abnormalities that cause genomic instability (2–6). It has been proposed that acid reflux promotes carcinogenesis in Barrett’s esophagus, but it is not clear how, or even if, acid exposure contributes to genomic instability in Barrett’s epithelial cells (7).

Several studies have found that cells exposed to acid can develop DNA damage that might result in genomic instability (8–13). Double-strand breaks (DSB) are among the most pernicious forms of DNA damage (14, 15). Cells with persistent DSBs have been found to develop chromosomal abnormalities, such as translocations and deletions, which can contribute to genomic instability and cancer formation (14, 15). Indeed, agents that cause DSBs, such as ionizing radiation and UV light, can be considered carcinogens (15, 16). Clemons and colleagues have reported that protracted acid exposure results in DSBs in certain Barrett’s cell lines and SEG-1 cancer cells, which originally were alleged to be a Barrett’s cancer cell line but which recently were discovered to be nongenosphageal in origin (17). In the single nondysplastic Barrett’s cell line (QhTERT) used in that study, however, it is not clear whether the DSBs were the direct result of acid injury to DNA or merely a consequence of the marked (60%) increase in cell death induced by the protracted acid exposure (17). This distinction is critically important in determining whether acid is a potential carcinogen in Barrett’s esophagus. During apoptosis, the cell destroys itself by compelling severe DNA damage, which includes DSBs. If the sequence of events induced by acid in Barrett’s cells is apoptosis followed by DSBs, then acid would not be considered a carcinogen because DSBs in a dying cell cannot contribute to carcinogenesis. If, on the other hand, acid causes DSBs directly, without causing cell death, then the DSBs could persist and acid could well be a carcinogen in Barrett’s esophagus.

Cellular DNA damage—sensing molecules, such as Chk2 and p53, become activated in response to DNA injury. That activation halts cell cycle progression to allow the cell to repair the damaged DNA before it is replicated (14, 18, 19). We have reported that, in response to acid exposure, nonneoplastic Barrett’s epithelial cell lines activate Chk2 and p53 and exhibit a delay in cell cycle progression (20, 21). We also showed that a 10-min acid exposure did not affect the viability of BAR-T cells and produced only a trivial (1.1%) increase in apoptosis (20). Those observations suggest that acid directly damages DNA in Barrett’s esophagus, in which case acid might be a carcinogen. To explore that possibility, we looked for evidence of acid-induced DNA damage, including DSBs, in nonneoplastic Barrett’s epithelial cell lines and in biopsy specimens of Barrett’s metaplasia that were taken from patients whose Barrett’s esophagus had been perfused with acid during an endoscopic examination. We also explored the molecular mechanisms underlying acid-induced DNA injury in our Barrett’s cell lines using agents that inhibit intracellular acidification and agents that scavenge reactive oxygen species (ROS).

Materials and Methods

Patients and acid exposures. This study was approved by the institutional review board of the Dallas VA Medical Center. Patients with...
long-segment Barrett’s esophagus (specialized intestinal metaplasia involving ≥3 cm of the distal esophagus) without dysplasia who were scheduled for elective endoscopic examinations were invited to participate in the study. All patients were male, their average ± SE age was 64.8 ± 2.4 years, and all were on treatment with proton pump inhibitors. During endoscopy, six biopsy specimens of Barrett’s metaplasia were taken using a jumbo biopsy forceps (Olympus FB-50K-1) before and after perfusion of the distal esophagus with 10 mL of 0.1 N HCl over 3 min as described previously by our laboratory (22).

Cell culture. We used three nonneoplastic, telomerase-immortalized Barrett’s epithelial cell lines (BAR-T, BAR-T9, and BAR-T10) that were created in our laboratory from endoscopic biopsy specimens of nondysplastic Barrett’s specialized intestinal metaplasia taken from 3 patients with long segment Barrett’s esophagus (20, 23, 24). All of the BAR cell lines were co-cultured with a fibroblast feeder layer as described previously (25). Cells were maintained in monolayer culture at 37°C in humidified air with 5% CO₂ in growth medium as described previously (23). For individual experiments, cells were equally seeded into collagen IV-coated wells (BD Biosciences) and maintained in growth medium. We selected to use the BAR-T line for all experiments (unless otherwise indicated) because this line has been extensively characterized by our laboratory (20, 21, 23, 24, 26).

Acid exposure and inhibition of intracellular acidification. For individual experiments, the cells were cultured either in neutral full growth medium (pH 7.2) or in acidic full growth medium (brought to a pH of 4.0 with 1 mol/L HCl). Neutral or acidic medium was added for 10 min to equally seeded wells of BAR cells and then removed and replaced with neutral pH medium for the remainder of the experiment unless otherwise indicated. The pH levels and durations of acid exposure were chosen to simulate typical episodes of gastroesophageal reflux in gastroesophageal reflux disease patients (27). In experiments designed to prevent the intracellular acidification of cells exposed to acidic medium, cells were pretreated with 500 μmol/L disodium 4,4′-disothiocyamtostilbene-2,2′-disulfonate (DIDS; Sigma). The DIDS was dissolved in 0.1 mol/L KHCO₃ as a 100× stock solution, and cells were treated for 15 min before acid exposure.

Detection of intracellular ROS. Equally seeded wells of cells were washed twice with HBSS (Invitrogen) and incubated with 10 μmol/L of a 5-(and -6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (Molecular Probes) probe for 20 min at 37°C in the dark. Cells were exposed to neutral or acidic medium containing the probe for 10 min, after which the medium was removed and replaced with neutral pH medium containing the probe. After 30 min, cells were then washed twice with HBSS to remove any excess probe and placed in PBS, and fluorescent intensity was immediately detected using flow cytometry. To determine the specificity of the probe for ROS, cells were pretreated for 30 min with 10 mmol/L N-acetyl-cysteine (NAC; Sigma), which increases cellular pools of free radical scavengers, before incubation with 5-(and -6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate.

Neutral and alkaline single-cell gel electrophoresis (CometAssay) for DNA damage. We detected DNA damage using an alkaline and neutral CometAssay (Trevigen) per the manufacturer’s instructions. The alkaline assay detects single-stranded breaks, DSBs, apurinic and apyrimidinic sites, and alkali labile DNA adducts. If the assay is done at neutral conditions (without the alkaline buffer), then it mainly detects DSBs (28). We mixed acid-exposed or control cells (100,000/mL) with melted LMAgarose at 37°C in a ratio of 1:10 and then layered 75 μL of the mixture onto a CometSlide. The slide was maintained at 4°C for 10 min for gelling and then immersed in Lysis Solution at 4°C for 45 min. For the alkaline assay, the slide was placed in Alkaline Unwinding Solution for 45 min at room temperature in the dark; this step was eliminated for the neutral CometAssay. The slide was then placed in alkaline solution or TBE (for the neutral CometAssay) and electrophoresis was conducted for 80 min at 1 V/cm and 315 mA at 4°C for the alkaline CometAssay or 20 min at 1 V/cm and 6 mA at 4°C for the neutral CometAssay. After the electrophoresis, slides were dipped in 70% ethanol for 5 min and allowed to air dry. Slides were then stained with silver staining or SYBR Green I solution and comet “tails” were visualized with a light microscope (Olympus) or fluorescence microscope (Nikon), respectively. All CometAssays were done in duplicate. The comet extent tail moment values were quantitated from slides stained with SYBR Green using the CometScore software (TriTek) from a minimum of 50 individual cells. This parameter measures the smallest detectable size of migrating DNA, as indicated by the tail length, and the number of broken DNA pieces, as indicated by the intensity of the DNA stain in the tail (28). BAR-T cells growing in culture served as negative controls; BAR-T cells treated with 200 μmol/L hydrogen peroxide served as positive controls.

Western blotting and immunofluorescence. For the phospho-H2AX Western blots, cells were exposed to neutral or acidic medium for 1.5, 3, or 10 min, after which the acidic medium was removed and the cells washed in cold PBS and immediately lysed. For the phospho-H2AX time-course Western blots, cells were exposed to neutral or acidic medium for 10 min, after which the acidic medium was removed and replaced with neutral medium for 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 24 h, or 48 h. Cells were then washed in cold PBS followed by immediate cell lysis. Cells were lysed in 1× cell lysis buffer (Cell Signaling Technology). The biopsy specimens were lysed in buffer containing 150 mmol/L NaCl, 1% NP-40, 1% deoxycholate, 20 mmol/L Tris-HCl (pH 7.5), 1 mmol/L EDTA, 1 mmol/L EGTA, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L β-glycerophosphate, 1 mmol/L sodium orthovanadate, 1 μg/mL leupeptin, 1 or 0.1 mmol/L phenylmethylsulfonl fluoride, and one Protease Inhibitor Cocktail Tablet per 50 mL lysis buffer (Roche Applied Science). Equal amounts of protein were separated by SDS-PAGE; protein concentrations were determined using the BCA-200 Protein Assay Kit (Pierce). After separation and transfer to nitrocellulose membranes, the membranes were incubated with primary antibodies (1:1,000 dilutions) to phospho-H2AX (Cell Signaling Technology). Horseradish peroxidase secondary antibodies were used and chemiluminescence was determined using the Super Signal West Dura Detection System (Pierce); β-actin (Sigma) was used to confirm equal loading. All Western blots were done in duplicate.

For the phospho-H2AX immunofluorescence assays, cells were exposed to neutral or acidic medium for 10 min after which the medium was removed and the cells were immediately fixed with cold methanol (−20°C) for 10 min at -20°C followed by incubation in 3% bovine serum albumin in PBS for 45 min to block the nonspecific binding sites. Primary antibody to phospho-H2AX (1:500 dilution) was added for 1 h at room temperature. Secondary antibodies were conjugated with Alexa Fluor 488 and used at 1:1,000 dilutions of goat anti-rabbit for phospho-H2AX. Specificity was determined by omitting the primary antibody from the incubation. The slides were counterstained with 4′,6-diamidino-2-phenylindole for analysis. BAR-T cells treated with 200 μmol/L hydrogen peroxide for 10 min served as a positive control for phospho-H2AX.

Statistical analyses. The data were collected from at least three independent experiments. Quantitative data are expressed as mean ± SE. Statistical analysis was done using ANOVA and the Student’s-Newman-Keuls’ multiple-comparison test with the Instat for Windows statistical software package (GraphPad Software). P values < 0.05 were considered significant for all analyses.

![Figure 1. Results of ROS production in BAR-T cells after a single, 10-min acid exposure. Mean ± SE of at least three individual experiments. *P < 0.001, compared with non–acid-treated controls; +, P < 0.001, compared with acid-treated cells.](image-url)
Acid exposure generates ROS in Barrett’s epithelial cells. In earlier experiments, we found that a single, 3-min acid exposure (pH 4.0) induces ROS production in BAR-T cells (29). To determine the effect of a single 10-min acid exposure (pH 4.0) on ROS production, we incubated BAR-T cells with 5-(and -6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate and determined fluorescence after acid exposure using flow cytometry. We found that a 10-min acid exposure significantly increased ROS production in BAR-T cells (Fig. 1).

Acid exposure causes DNA damage in Barrett’s epithelial cells. BAR-T cells were treated with a single, 10-min acid exposure, and DNA damage was assessed using the CometAssay at neutral conditions, which detects primarily DSBs. We found that acid exposure induced a significant increase in damaged DNA fragments detected in the comet “tails,” suggesting that acid causes DSBs in BAR-T cells (Fig. 1).

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Recently, the detection of histone H2AX phosphorylation has been found to be more sensitive assay than the CometAssay for detecting DSBs in live cells (16, 30, 31). To confirm our findings from the CometAssay, we treated the BAR-T cells with a single, 1.5-, 3-, or 10-min acid exposure and performed Western blotting for phospho-H2AX expression. We found that acid exposure induced a time-dependent increase in phospho-H2AX expression (Fig. 4A). We then determined the effect of a single, 10-min acid exposure on phospho-H2AX expression in two additional telomerase-immortalized metaplastic Barrett’s cell lines (BAR-T9 and BAR-T9).

Results

Acid exposure generates ROS in Barrett’s epithelial cells. In earlier experiments, we found that a single, 3-min acid exposure (pH 4.0) induces ROS production in BAR-T cells (29). To determine the effect of a single 10-min acid exposure (pH 4.0) on ROS production, we incubated BAR-T cells with 5-(and -6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate and determined fluorescence after acid exposure using flow cytometry. We found that a 10-min acid exposure significantly increased ROS production in BAR-T cells (Fig. 1). To confirm this finding, BAR-T cells incubated with 5-(and -6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate were treated with a single, 10-min exposure to acidic medium in the presence of 10 mmol/L NAC, a general scavenger of ROS. NAC treatment significantly decreased ROS levels (Fig. 1).

Acid exposure causes DNA damage in Barrett’s epithelial cells. Having found that acid exposure generates ROS in BAR-T cells, we next determined whether acid induces DNA damage. BAR-T cells were treated with a single, 10-min exposure to acidic medium, and DNA damage was assessed using the CometAssay at alkaline conditions, which detects single-strand breaks and DSBs, apurinic and apyrimidinic sites, and alkali-labile DNA adducts by the presence of comet “tails” (28). We found that acid exposure induced a significant increase in damaged DNA fragments detected in the comet “tails” in BAR-T cells (Fig. 2A and B).

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BAR-T10). As in the BAR-T cells, a single 10-min acid exposure increased the expression of phospho-H2AX in the BAR-T9 and BAR-T10 cell lines (Fig. 4B).

We next sought to determine the duration for which H2AX remains phosphorylated in response to acid exposure. We found that phospho-H2AX expression persisted for up to 48 h after a single, 10-min acid exposure in BAR-T cells (Fig. 4C and D). In addition to an increase in expression, phospho-H2AX also localizes to the sites of damaged DNA and forms nuclear foci. Using immunofluorescence, we observed phospho-H2AX–containing nuclear foci in acid-treated, but not in untreated, BAR-T cells (Fig. 5). These findings show that acid exposure causes DSBs in Barrett’s epithelial cells.

**DIDS prevents DSBs in Barrett’s epithelial cells exposed to acid.** Acid exposure has been shown to cause intracellular acidification via a DIDS-inhibitable mechanism in esophageal epithelial cells (including BAR-T cells; refs. 32, 33). To explore whether intracellular acidification is responsible for causing DSBs in

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**Figure 4.** A, representative Western blots showing expression of phospho-H2AX in BAR-T cells following exposure to acid for 1.5, 3, or 10 min. B, representative Western blots showing expression of phospho-H2AX in BAR-T9 and BAR-T10 cells following a single, 10-min acid exposure. C and D, representative Western blots showing the time course for phospho-H2AX expression in BAR-T cells following a single, 10-min acid exposure. β-Actin served as a loading control.

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3 R.C. Orlando, personal communication.
Barrett’s epithelial cells, we studied the effects of DIDS on DSBs in acid-treated BAR-T cells. We treated BAR-T cells with a single, 10-min exposure to acidic medium in the presence of 500 μmol/L DIDS and assessed DSBs by phospho-H2AX expression. We found that DIDS prevented the acid-induced increase in phospho-H2AX, suggesting that intracellular acidification is the mechanism whereby acid exposure causes DSBs in Barrett’s epithelial cells (Fig. 6A).

**NAC prevents DSBs in Barrett’s epithelial cells exposed to acid.** To determine whether the acid-induced ROS caused the DSBs, we treated BAR-T cells with a single, 10-min exposure to acidic medium in the presence of 10 mmol/L NAC and assessed for DSBs by determining phospho-H2AX expression. NAC prevented the acid-induced increase in phospho-H2AX expression, suggesting that the ROS produced in response to acid exposure are responsible for inducing DSBs in BAR-T cells (Fig. 6A).

**Esophageal acid exposure causes DSBs in patients with Barrett’s esophagus.** Having found that acid exposure increases phosphorylation of H2AX in three Barrett’s epithelial cell lines, we sought to confirm that these same effects occur in vivo by obtaining endoscopic biopsy specimens of Barrett’s epithelium before and after esophageal acid perfusion in 6 patients with Barrett’s esophagus. In agreement with our in vitro data, we found that acid increased phospho-H2AX expression in biopsy specimens of Barrett’s metaplasia from all 6 patients (Fig. 6B).

![Figure 5](image-url)  
A representative experiment showing the results of phospho-H2AX nuclear foci formation in BAR-T cells after a single, 10-min acid exposure. Note the increase in phospho-H2AX–containing nuclear foci in the acid-treated cells but not in the untreated control cells. 4′,6-Diamidino-2-phenylindole (DAPI) staining shows the total number of cell nuclei in the same field. BAR-T cells treated with hydrogen peroxide served as a positive control. Magnification, x60.

![Figure 6](image-url)  
A, representative Western blots showing the results of NAC or DIDS treatment on acid-induced phospho-H2AX expression in BAR-T cells. Note that both NAC and DIDS prevent the acid-induced increase in phospho-H2AX expression. B, representative Western blot showing phospho-H2AX expression before and after esophageal acid perfusion in biopsy specimens of Barrett’s metaplasia taken from 6 patients with Barrett’s esophagus during endoscopic examinations. Note that acid increased phospho-H2AX expression in the metaplastic Barrett’s mucosa in all 6 patients. β-Actin served as a loading control.
Discussion

We have shown that acid exposure induces DNA damage, including DSBs, in Barrett’s epithelial cells both in vitro and in vivo. In BAR-T cells, we have shown evidence of acid-induced DNA damage by Comet Assays performed under neutral and alkaline conditions, by H2AX phosphorylation, and by the demonstration of nuclear foci containing phospho-H2AX. We have found that H2AX phosphorylation increases immediately after acid exposure and continues up to 48 h and that phosphorylation increases with the duration of acid exposure.

To explore whether the induction of DSBs by acid is a general feature of Barrett’s epithelial cells or a unique feature of BAR-T cells, we studied two additional telomerase-immortalized Barrett’s epithelial cell lines that had been established in our laboratory (24). We found that acid exposure caused an increase in the phosphorylation of H2AX in all three Barrett’s cell lines, suggesting that DNA vulnerability to acid is a common property of nonneoplastic BAR-T cells. In confirmation of these in vitro experiments, we found evidence of DSBs by H2AX phosphorylation in biopsy specimens of Barrett’s metaplasia taken from all of 6 patients who had the esophagus perfused with acid during endoscopic examinations. In cultures of esophageal cells (including rabbit esophageal squamous cells, primary cultures of Barrett’s epithelial cells, and BAR-T cells), extracellular acid has been found to cause intracellular acidification through activation of the Na+–independent Cl−/HCO3− exchanger, which can be inhibited by DIDS (32, 33).3 Our finding that DIDS prevents phospho-H2AX production in BAR-T cells exposed to acid suggests that it is intracellular acidification that mediates the generation of DSBs.

Several lines of evidence suggested to us that ROS would be involved in the DSBs induced by acid exposure in Barrett’s epithelial cells. For example, several studies have shown that esophageal epithelial cells exposed to acid and bile salts increased their production of ROS (13, 34–37). In an earlier study, we also found that a 3-min acid exposure significantly increased ROS production in BAR-T cells (29). ROS are well known to cause oxidative damage to DNA (38, 39), and biopsy specimens of Barrett’s metaplasia have been found to exhibit evidence of oxidative DNA injury (11–13). Moreover, ROS have been shown to cause DSBs. Cultures of fibroblasts from SOD1 transgenic mice, which have abnormally high levels of endogenous ROS production, develop more DSBs than fibroblasts from wild-type control mice (40).

In this study, we have found that a 10-min acid exposure increases fluorescence in BAR-T cells that were treated with 5- and 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate, indicating that acid induces ROS production. Using another benign Barrett’s epithelial cell line (QhTERT), Clemons and colleagues also found that a 10-min acid exposure increased the generation of intracellular ROS (17). That study did not determine whether the ROS caused the DSBs in the nonneoplastic cell line, however. Our finding that treatment with a ROS scavenger (NAC) prevents the development of DSBs after acid exposure indicates that acid-induced DNA damage is mediated by ROS production. Taken together, our results suggest that exposure of Barrett’s epithelial cells to acid results in intracellular acidification that induces the production of ROS, which cause DSBs.

DSBs are especially dangerous mutations because, if they persist, they can cause genomic instability and contribute to carcinogenesis (14, 15). In earlier studies, we showed that acid activates the Chk2 and p53 DNA damage response pathways and delays cell cycle progression in nonneoplastic Barrett’s epithelial cells (20, 21). DSBs are the primary stimuli that activate the Chk2 DNA damage response pathway, and p53 is one of its major downstream effectors (41, 42). In our nonneoplastic Barrett’s cells, we found an increase in H2AX phosphorylation (a sensitive marker of DSBs) immediately after acid exposure, suggesting that DNA damage is the likely trigger for the early, acid-induced activation of the Chk2 pathway that we observed in our previous studies (20). After a 10-min acid exposure, moreover, the increase in H2AX phosphorylation persisted up to 48 h, which is more than enough time to activate the p53 DNA damage response pathway that we also observed in our earlier studies. In nonneoplastic Barrett’s epithelial cells, therefore, we have shown that acid is a genotoxin that causes DSBs that persist for at least 48 h. These observations suggest that acid can be considered a carcinogen in Barrett’s esophagus.

Our study has potential clinical implications. Our finding that acid causes DSBs in nonneoplastic Barrett’s epithelial cells and therefore can be considered a carcinogen in Barrett’s esophagus supports the practice of aggressive acid suppression for patients with this condition. In an earlier study using BAR-T cells, we showed that acid exposure had potentially beneficial antiproliferative effects that were mediated by activation of the p53 and Chk2 pathways. In that study, however, it was not clear whether those pathways were activated directly by acid (which would be beneficial) or indirectly through DNA damage (which would be detrimental). Our present study showing that acid causes severe DNA damage suggests that the apparent antiproliferative effects of acid are merely a response to genetic injury. The molecular mechanisms elucidated by our study support the prescription of aggressive acid suppression as a chemotherapeutic strategy for patients with Barrett’s esophagus. This issue requires further investigation in prospective, controlled clinical trials.

In conclusion, we have shown that benign Barrett’s epithelial cells exposed to acid in vitro produce ROS and develop severe DNA damage, including DSBs, which persist for at least 48 h. We have also documented the development of DSBs in Barrett’s metaplasia following esophageal acid perfusion in vivo in patients with Barrett’s esophagus. Our finding that acid-induced DSBs can be prevented by treating Barrett’s epithelial cells with NAC or DIDS suggests that the DNA damage is caused by ROS produced as a consequence of intracellular acidification. Taken together, these observations suggest that acid should be considered a carcinogen in Barrett’s esophagus.

Disclosure of Potential Conflicts of Interest

S.J. Spechler: commercial research grant, AstraZeneca, Takeda, and BARRx Medical Inc.; consultant/advisory board, AstraZeneca, Takeda, and Procter and Gamble. R.F. Souza: commercial research grant, AstraZeneca; consultant/advisory board, Takeda and AstraZeneca. The other authors disclosed no potential conflicts of interest.

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