Research Article

Cancer Prevention Research

Clinical Study of Ursodeoxycholic Acid in Barrett's Esophagus Patients

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Abstract

Prior research strongly implicates gastric acid and bile acids, two major components of the gastroesophageal refluxate, in the development of Barrett's esophagus and its pathogenesis. Ursodeoxycholic acid (UDCA), a hydrophilic bile acid, has been shown to protect esophageal cells against oxidative stress induced by cytotoxic bile acids. We conducted a pilot clinical study to evaluate the clinical activity of UDCA in patients with Barrett's esophagus. Twenty-nine patients with Barrett's esophagus received UDCA treatment at a daily dose of 13 to 15 mg/kg/day for 6 months. The clinical activity of UDCA was assessed by evaluating changes in gastric bile acid composition and markers of oxidative DNA damage (8-hydroxydeoxyguanosine), cell proliferation (Ki67), and apoptosis (cleaved caspase-3) in Barrett's esophagus epithelium. The bile acid concentrations in gastric fluid were measured by liquid chromatography/mass spectrometry. At baseline, UDCA (sum of unchanged and glycine/taurine conjugates) accounted for 18.2% of total gastric bile acids. After UDCA intervention, UDCA increased significantly to account for 93.4% of total gastric bile acids ($P < 0.0001$). The expression of markers of oxidative DNA damage, cell proliferation, and apoptosis was assessed in the Barrett's esophagus biopsies by IHC. The selected tissue biomarkers were unchanged after 6 months of UDCA intervention. We conclude that high-dose UDCA supplementation for 6 months resulted in favorable changes in gastric bile acid composition but did not modulate selected markers of oxidative DNA damage, cell proliferation, and apoptosis in the Barrett's esophagus epithelium. *Cancer Prev Res; 9(7); 528–33. ©2016 AACR.*

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Introduction

Barrett's esophagus is a condition where normal squamous epithelium is replaced by metaplastic intestinal-like columnar epithelium containing goblet cells (intestinal metaplasia). This lesion is linked to the development of esophageal adenocarcinoma, a cancer with poor prognosis, and a median survival of less than 1 year (1, 2).

Animal and human studies strongly implicate gastric acid and bile acids, two major components of the gastroesophageal refluxate, in the development of Barrett's esophagus and its pathogenesis (3–5). It has been shown that patients with Barrett's esophagus have higher acid and bile acid exposure in their esophagus than patients with erosive esophagitis or controls (4, 5). Hydrophobic bile acids, such as deoxycholic acid (DCA), are thought to play a major role in the development of gastrointestinal malignancies (6). In humans, the incidence of cancers of the laryngopharyngeal tract, esophagus, stomach, pancreas, small intestine (near the Ampulla of Vater), and colon are all positively associated with intestinal levels of hydrophobic bile acids (6). Preclinical studies demonstrated that a combination of a cytotoxic bile acid cocktail and low pH induces oxidative stress and oxidative DNA damage in cultured esophageal cells and in Barrett's esophagus biopsies *ex vivo* (7, 8). Similarly, Huo and colleagues showed that DCA induces the production of reactive oxygen species in Barrett's cells, which causes DNA damage and induces activation of the NF-κB pathway to prevent apoptosis in Barrett's cells (9).

Ursodeoxycholic acid (UDCA), the most hydrophilic of the bile acids, was shown to protect against bile acid and low pH–induced oxidative stress and oxidative DNA damage and modulate expression of enzymes associated with protection against oxidative stress in cultured esophageal cells (10). Furthermore, in a rat model of Barrett's esophagus, treatment with a combination of UDCA and aspirin resulted in fewer esophageal adenocarcinomas (11). Peng and colleagues (12) have recently shown that UDCA treatment (10 mg/kg) for 8 weeks increased the levels of two antioxidant enzymes (glutathione peroxidase 1 and catalase) in esophageal biopsies collected from patients with Barrett's esophagus. The treatment also prevented DNA damage and NF-κB activation induced by esophageal DCA perfusion in patients with Barrett's esophagus. However, it is unknown whether UDCA treatment will decrease the extent of DNA damage under physiologic condition (i.e., without esophageal DCA perfusion).

UDCA is an attractive candidate for chemoprevention because of its long-term safety record. It has been used safely at the dose of 8 to 10 mg/kg/day in patients with gallstone disease in the United

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States since 1987 and later in patients with primary biliary cirrhosis (PBC) at the dose of 13 to 15 mg/kg/day. In the clinical trial setting, it has demonstrated potential for risk reduction for colorectal cancer with a good safety profile. UDCA treatment at a dose of 8 to 10 mg/kg/day for a mean of 32 months was associated with a statistically significant 39% reduction in recurrence of colorectal adenomas with high-grade dysplasia (13). A study of 52 patients with ulcerative colitis and primary sclerosing cholangitis showed that treatment with UDCA (at a dose of 13 to 15 mg/kg/day for a median duration of 42 months) significantly reduced the risk of colorectal dysplasia or cancer compared with placebo (14).

We conducted a pilot clinical study to assess the clinical activity of UDCA in patients with Barrett's esophagus. The central hypothesis to be tested in the clinical study is that supplementation with UDCA would alter bile acid composition in the refluxate and subsequently decrease oxidative DNA damage, and cell proliferation and increase apoptosis in the Barrett's esophagus epithelium.

Materials and Methods

Study design

The study was an open label, single-arm intervention trial conducted at the University of Arizona (UA; Tuscon, AZ), University of North Carolina (UNC, Chapel Hill, North Carolina), and Southern Arizona VA Health Care System (SAVAHCS; Tuscon, AZ). The study was approved by the Institutional Review Board at each institution. The study endpoints were changes in oxidative DNA damage [measured by 8-hydroxydeoxyguanosine (8OHdG) levels], cell proliferation (measured by Ki67 expression), and apoptosis (measured by cleaved caspase-3, CC3) in the Barrett's esophagus epithelium and changes in gastric bile acid composition.

Study drug

Ursodiol (300 mg) capsules were supplied by the National Cancer Institute, Division of Cancer Prevention. The initial supply was manufactured by CorePharma LLC for Rising Phamaceuticals. Following expiration of the initial supply in August 2010, the replacement supply for the remainder of the trial was manufactured by Watson Pharma Private Limited and distributed by Watson Pharma, Inc. The study capsules were stored at room temperature and protected from environmental extremes.

Study population

We recruited healthy women and men $\geq$18 years of age with a diagnosis of Barrett's esophagus with histologically confirmed intestinal metaplasia anywhere in the tubular esophagus either with $\geq$2 cm of involvement or with a minimum of circumferential Barrett's esophagus length of 1 cm. Participants were required to have normal liver and renal function. Study exclusion criteria included Barrett's esophagus with high-grade dysplasia or carcinoma, medical conditions which would make completing endoscopies or completing the trial difficult, use of other investigational agents within 1 month, use of NSAIDs for more than 5 days a month within 1 month [except low dose aspirin (81 mg every day)], history of allergic reactions attributed to UDCA, uncontrolled acute and chronic diseases, pregnant and breast feeding women, major upper gastrointestinal surgery within 6 months, erosive esophagitis at baseline endoscopy, chemotherapy, radiotherapy, or cancer-related hormonal or immunotherapy within the last 18 months, current or planned use of anticoagulant drugs, or use of cyclosporine. Written informed consent was obtained from all participants.

Study procedure

During the initial visit, consented study subjects underwent medical and surgical history evaluation and had a blood sample collected for complete blood count (CBC) and comprehensive metabolic panel (CMP). Following the initial eligibility evaluation, subjects underwent upper endoscopy with biopsies. Prior to any mucosal irrigation, gastric fluid was aspirated through the endoscope and collected. The circumferential and maximum extents of metaplasia were determined using the Prague C&M criteria (15). Systematic biopsies, one in each of four quadrants every 2 cm in the appropriate areas of the Barrett's esophagus, were taken. These biopsies were processed for histopathology based on the institutional standards. One additional Barrett's esophagus biopsy was collected close to the distal end of the Barrett's esophagus segment and flash frozen.

Eligible subjects then initiated the 6 months of UDCA treatment at 13 to 15 mg/kg per day. Subjects returned to the clinic after 3 months of agent intervention to return unused pills for a pill count, receive a new supply of agents, have a blood sample collected for CBC/diff and CMP, and review the side effects with study staff. At the end of the 6-month intervention, subjects returned to the clinic to return unused pills, have a blood samples collected for CBC/diff and CMP, review the side effects with study staff, and undergo the postintervention endoscopy to obtain gastric fluid and biopsies of the Barrett's esophagus as described for the baseline endoscopy.

Safety of UDCA intervention was assessed by reported adverse events and clinical labs. Adverse events were graded using NCI Common Terminology Criteria for Adverse Events version 3.0.

Analysis of bile acid concentrations in gastric fluid

Bile acid concentrations in the gastric fluid were analyzed by high performance liquid chromatography (HPLC) tandem mass spectrometry (MS). Briefly, an aliquot of gastric fluid was mixed with the internal standards (deoxycholic acid-d4 and glycoursodeoxycholic acid-d4) and then alkalized with 1 N NaOH. The mixture was extracted with hexane. The aqueous phase was collected and acidified with 5 N HCl and extracted with ethyl acetate. The organic layer was dried and reconstituted with 10 mmol/L ammonium acetate/methanol (50/50) and injected onto the LC/MS system. The chromatographic separation was achieved using a gradient system of methanol and 10 mmol/L ammonium acetate on an Ultrasphere XL column. Mass spectrometry was run in negative ion mode using electrospray ionization. Detection of five bile acids [UDCA, DCA, cholic acid (CA), chenodeoxycholic acid (CDCA), and lithodeoxycholic acid (LCA)] and their respective glycine and taurine conjugates was achieved by multiple reaction monitoring.

IHC for tissue biomarkers

Immunohistochemical assays were used to assess markers of cell proliferation (Ki67), apoptosis (CC3), and oxidative DNA damage (8OHdG) in Barrett's esophagus epithelium tissue sections. The Ki67 and CC3 IHC was performed on a Discovery XT Automated Immunostainer (VMSI; Ventana Medical Systems)

using VMSI-validated reagents, including deparaffinization, antigen retrieval with a borate-EDTA buffer, primary antibody staining, detection, and amplification, and hematoxylin counterstaining. A biotin-free diaminobenzidine (DAB) detection system was used for CC3 and a biotinylated-streptavidin-horseradish peroxidase and DAB system was used for Ki67. For Ki67, mouse mAb (clone: MIB-1; Dako) was diluted 1:100. Human tonsil carcinoma was used as a positive control. For CC3, anti-CC3 rabbit polyclonal antibody (Cell Signaling Technology #9661L) was diluted 1:8,000. Human tonsil carcinoma was used as a positive control. The 8OHdG IHC was performed as described previously (8) with minor modifications. Briefly, the slides were baked at 65°C for 1 hour, followed by deparaffinization with xylene, isopropanol, and water. Slides were then treated with 10% H_2O_2, 4 N HCl, 0.1 mol/L borax, and 5% horse serum sequentially prior to incubating with the mouse mAb for 8OHdG [QED Bioscience, #12501 (clone 15A3), diluted 1:1,000]. Slides were then incubated with secondary biotinylated rabbit anti-mouse IgG antibody, Vectastain Elite ABC reagent, and DAB prior to counterstaining with hematoxylin. Human esophageal carcinoma and tonsil carcinoma were used as positive controls. On the IHC slides, longitudinally sectioned crypts opening to the lumen were selected for scoring. The percent of nuclei stained positive for Ki67, CC3, and 8OHdG in the selected regions was quantified by Aperio Spectrum software and confirmed by a trained pathologist. Slides with fewer than 500 total nuclei in the selected regions were excluded for the statistical analysis. The marker expression from different segments was averaged for participants with tissue sections from multiple esophageal segments.

Statistical analysis

Descriptive statistics were calculated to summarize the demographic characteristics and disease characteristics at baseline and postintervention. The primary endpoint was the effect of UDCA intervention on 8OHdG levels in Barrett's esophagus epithelium. Signed rank test was performed to assess pre- to postintervention change in percentage of nuclei stained strongly and moderately for 8OHdG. The secondary endpoints were measurements of changes in gastric bile acid composition and Ki67 and CC3 expression. Signed rank test was performed to assess the change for each of the secondary endpoints. Spearman correlation coefficients were calculated to assess the relationship between changes in gastric bile acid composition and changes in 8OHdG, Ki67, and CC3, respectively.

Results

The study opened to accrual in April 2010 and closed to accrual in November 2013. Eighty potentially eligible participants were consented, 39 from UA, 26 from UNC, and 15 from SAVAHCS. Forty-four consented individuals did not meet all the eligibility criteria. Thirty-six met all eligibility criteria to initiate agent intervention; of these 29 completed agent intervention, 1 was taken off agent intervention due to grade 2 diarrhea, an adverse event (AE) probably related to the study agent, that did not resolve within the protocol-specified timeframe, 3 were taken off agent intervention due to AEs deemed unlikely to be related or not related to the study agent, and 3 withdrew consent. UDCA treatment was well tolerated in our study cohort. Twelve subjects experienced related grade 1 or grade 2 AEs, including diarrhea, constipation, bloating, flatulence, nausea, vomiting, burping, rash, joint pain, and stomachache.

Table 1. Demographic and disease characteristics of participants who completed agent intervention ($n = 29$)

Variable	
Age, years (mean ± SD)	62.5 ± 9.8
BMI, kg/m^2 (mean ± SD)	28.3 ± 5.1
Gender	
Male/female	23/6
Race	
White/Multiracial	28/1
Ethnicity	
Non-Hispanic/Hispanic	25/4
Smoking history	
Current/former/never	3/14/12
Alcohol intake Heavy/moderate/ low/occasional/former/never	0/4/10/7/5/3
Length of circumferential involvement, cm, median (range)	
Baseline	4.0 (1–11)
Postintervention	3.8 (1–12)
Length of circumferential involvement, <3 cm / ≥ 3 cm	
Baseline	13/16
Postintervention	16/13
Change in circumferential length, decreased/no change/increased	7/18/4
Pathology grade, ND/LGD/HGD	
Baseline	20/9/0
Postintervention	24/4/1
Change in pathology grade, improved/no change/worsened	5/22/2

Abbreviations: ND, no dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia.

The demographic and disease characteristics of participants who completed the intervention are summarized in Table 1. The average age was 62.5 ± 9.8 years. The average BMI from these participants was 28.3 ± 5.1 kg/m^2. Eighty percent were male. The majority were White (97%) and 14% were Hispanic. Current smokers accounted for 10% of these participants. Fourteen percent had heavy or moderate alcohol intake. Twenty-eight of the 29 participants who completed agent intervention were treated concomitantly with proton pump inhibitor (PPI). Twenty-three of the 28 participants who used PPI had been treated with PPI for more than 6 months prior to initiating the UDCA intervention. Twelve of the 29 participants who completed agent intervention were taking daily 81 mg aspirin.

For disease characteristics of participants who completed the intervention, the median length of circumferential involvement was 4.0 cm at baseline, 13 participants with length <3 cm, and 16 participants with length ≥3 cm. The median circumferential involvement was 3.8 cm postintervention, 16 participants with length <3 cm, 13 participants with length ≥3 cm. The circumferential length decreased in 24% of participants, was unchanged in 62% of participants, and increased in 14% of participants. Biopsies from 69% of participants were not dysplastic at baseline whereas 31% of participants had at least one biopsy with low-grade dysplasia. Postintervention, biopsies from 83% participants were not dysplastic and at least one biopsy from 14% and 3% of participants had low-grade and high-grade dysplasia, respectively. The pathology grade improved in 17% of participants but worsened in 7% of participants.

There were 28 participants with gastric fluid collected at both the baseline and end of study endoscopies for bile acid analysis. Because of the large variation in gastric bile acid concentrations, the individual bile acids were expressed as the percent of total bile

Table 2. Gastric bile acid composition at baseline and postintervention ($n = 28$)

	Baseline (% of total bile acid)	Postintervention (% of total bile acid)	*P*[a]
UDCA and glycine/taurine conjugates	18.2 (26.1)[b]	93.4 (31.7)	<0.0001
CDCA and glycine/taurine conjugates	11.0 (10.2)	1.01 (3.90)	<0.0001
DCA and glycine/taurine conjugates	38.9 (44.0)	4.18 (7.82)	<0.01
CA and glycine/taurine conjugates	16.9 (18.9)	1.72 (7.21)	<0.0001
LCA and glycine/taurine conjugates	0.66 (1.30)	0.17 (0.37)	<0.001

[a]Derived from signed rank test.
[b]Median (interquartile range).

acid concentrations in the gastric fluid. The sum of each individual bile acid and its respective glycine and taurine conjugates is summarized in Table 2. At baseline, UDCA, CDCA, DCA, CA, LCA, and their respective glycine and taurine conjugates accounted for 18.2%, 11.0%, 38.9%, 16.9%, and 0.66% of total gastric bile acids. Postintervention, UDCA and its glycine and taurine conjugates increased significantly to account for 93.4% of total gastric bile acids, whereas the composition of the other bile acids decreased significantly. Glycine conjugates constituted the majority of each of the five bile acid groups in the gastric fluid. We performed exploratory stratified analysis on the gastric bile acid composition by PPI use (<6 months vs. ≥6 months), aspirin use (yes vs. no), smoking status (never smokers vs. current or former smokers), alcohol intake (no current intake vs. any current intake), baseline Barrett's esophagus length (<3 cm vs. ≥3 cm), change in Barrett's esophagus length (shortened vs. no change or increased), baseline pathology grade (ND vs. LGD), and change in pathology grade (improved vs. no change or worsened). There was no difference in the baseline gastric bile acid composition in most of the stratified analysis (data not shown) except that baseline DCA and its glycine and taurine conjugates accounted for a higher fraction of total gastric bile acid in aspirin users than that in nonusers [median (interquartile range): 45.0 (29.5)% ($n = 12$) vs. 15.7 (37.4)% ($n = 16$), respectively, $P = 0.04$]. Postintervention, the DCA and its glycine and taurine conjugates composition was similar between the aspirin users and nonusers [median (interquartile range): 2.87 (2.75)% vs. 5.88 (15.4)%, respectively, $P = 0.12$]. The stratified analysis showed that the pre- to postintervention change in bile acid composition was different between the aspirin users and nonusers [median change (interquartile range) of UDCA/conjugates: +75.7 (17.9) vs. +54.5 (57.1), $P = 0.04$; DCA/conjugates: −43.6 (44.6) vs. −8.43 (25.6), $P < 0.05$; and CA/conjugates: −15.7 (9.68) vs. −4.22 (12.1), $P = 0.02$]. LCA and its glycine and taurine conjugates accounted for a small fraction of the bile acid composition. Stratified analysis showed that those who did not currently consume alcohol had a larger fraction of LCA/conjugates than those who consumed alcohol [median (interquartile range): 0.52 (1.01)% ($n = 7$) vs. 0.12 (0.25)% ($n = 20$), respectively, $P = 0.03$]. Nevertheless, the stratified analysis will need to be interpreted with caution due to the small sample size and multiple comparisons.

The tissue biomarker data are summarized in Table 3. Adequate baseline and postintervention data on 8OHdG, Ki67, and CC3 expression were obtained from 25, 29, and 27 participants, respectively. Because of the concern of nonspecific 8OHdG staining, only the percent of strongly and moderately stained nuclei was used for the statistical analysis. The median (interquartile range) baseline 8OHdG expression was 39.90 (39.14)%. The median (interquartile range) baseline Ki67 and CC3 expression, assessed as positively stained nuclei, was 35.92 (13.95)% and 1.62 (2.86)%, respectively. The expression of these markers did not change following 6 months of UDCA intervention. We performed similar exploratory stratified analysis on the tissue biomarkers. There was no difference in the baseline and postintervention tissue biomarker expression in the stratified analysis (data not shown).

Table 4 summarizes the correlation between changes in gastric bile acid composition and changes in tissue biomarker expression. The changes in tissue biomarker expression were not correlated with the changes in gastric bile acid composition.

Discussion

Our single-arm pilot clinical study was designed to evaluate the clinical activity of UDCA in patients with Barrett's esophagus. We evaluated the clinical activity of UDCA by assessing changes in gastric bile acid composition and markers of oxidative DNA damage, cell proliferation, and apoptosis in the Barrett's esophagus epithelium because prior research suggested that these markers could be modulated with UDCA intervention (10, 12). The study showed that supplementation with UDCA at a daily dose of 13 to 15 mg/kg/day for 6 months in patients with Barrett's esophagus increased proportions of cytoprotective bile acids and decreased proportions of cytotoxic bile acids in the gastric fluid. Despite the favorable change in the bile acid composition, we did

Table 3. Tissue biomarker expression[a]

	Baseline (% positive)	Postintervention (% positive)	*P* value[b]
8OHdG ($n = 25$)	39.9 (39.1)[c,d]	34.9 (30.7)	0.52
Ki67 ($n = 29$)	35.9 (14.0)[e]	36.9 (18.3)	0.44
CC3 ($n = 27$)	1.62 (2.86)[e]	1.00 (2.11)	0.25

Abbreviations: 8OHdG, 8-hydroxydeoxyguanosine; CC3, cleaved caspase 3.
[a]Longitudinally sectioned crypts opening to the lumen were selected for scoring. Slides with fewer than 500 total nuclei in the selected regions were excluded for the statistical analysis. The marker expression from different segments was averaged for participants with tissue sections from multiple esophageal segments.
[b]Derived from signed rank test.
[c]Median (interquartile range).
[d]% of strongly and moderately stained nuclei.
[e]% of positively stained nuclei.

Table 4. Spearman correlation coefficient between changes in gastric bile acid composition of changes in tissue biomarker expression

Bile acid	8OHdG ($N = 25$)	Ki67 ($N = 28$)	CC3 ($N = 26$)
Total UDCA	0.04; $P = 0.85$	0.01; $P = 0.94$	0.29; $P = 0.15$
Total CDCA	−0.16; $P = 0.43$	−0.20; $P = 0.31$	0.24; $P = 0.24$
Total DCA	−0.14; $P = 0.49$	−0.19; $P = 0.34$	−0.09; $P = 0.65$
Total CA	0.24; $P = 0.26$	−0.04 $P = 0.28$	−0.23; $P = 0.26$
Total LCA	−0.07; $P = 0.74$	0.01; $P = 0.96$	0.06; $P = 0.78$

not observe any significant changes in markers of oxidative DNA damage, cell proliferation, and apoptosis in the Barrett's esophagus epithelium.

In our study, all but one participant were treated with PPI for symptom control with most treated for more than 6 months prior to initiation of the UDCA intervention. The PPI treatment may have contributed to the lack of UDCA effects on tissue markers of oxidative DNA damage, cell proliferation, and apoptosis. In a multicenter prospective cohort study of 540 patients with Barrett's esophagus, PPI use was associated with a reduced risk of neoplastic progression (16). High-dose PPI treatment in patients with Barrett's esophagus that results in effective esophageal acid suppression has been shown to decrease the markers of cell proliferation and inflammation and increase apoptosis (17). PPI treatment reduces the acidity and the volume of the refluxate, which may diminish the exposure of esophagus to cytotoxic bile acids (18). Therefore, modulation of bile acid composition with the UDCA intervention may not result in any further improvement in histology and the selected tissue biomarkers. Furthermore, bile acids that are cytotoxic to the mucosa in an acidic environment may lose their damaging activity at neutral pH from PPI treatment. Bozikas and colleagues (19) evaluated the effect of 6 months of UDCA (600 mg twice a day) intervention in nine Barrett's esophagus patients treated with high dose PPI. Similarly, UDCA intervention did not lead to significant changes in histology and markers of proliferation, differentiation, and inflammation in this study with limited sample size.

An alternative explanation for the lack of change in the selected tissue biomarkers is that cytotoxic bile acid reflux may not be a causative factor in the pathogenesis of progression in Barrett's esophagus. It was recently demonstrated in an animal model that cytotoxic bile acids and not gastric acid were pathogenic in the development of Barrett's-like metaplasia (20); however, the progression to dysplasia may be caused by other, unknown factors. The development of Barrett's esophagus results in a more durable epithelium that may be more resistant to insult by refluxate. Thus, UDCA treatment may be more effective to prevent the development of Barrett's esophagus than to prevent the pathogenesis of Barrett's esophagus.

It is important to note that the null findings in the tissue biomarkers from this single-arm pilot study will need to be interpreted with caution as the study is limited by the lack of a control arm and the small sample size. The study selected an intervention duration of 6 months to coincide with the recommended interval for surveillance endoscopy for patients with Barrett's esophagus with low-grade dysplasia at the time of the study protocol development. Based on prior research (10, 12), it was anticipated that 6 months of UDCA intervention would be sufficient to modulate the selected tissue biomarkers. It is not known whether the selected tissue biomarkers would be modulated with a longer intervention duration.

The tissue biomarkers used in this study have been correlated with the histologic grade of Barrett's esophagus (21–24) and used as intermediate biomarkers to assess preventive interventions in patients with Barrett's esophagus (17, 19, 25, 26). However, these markers have not been proven in large, well-designed study to predict the risk of development of high-grade dysplasia or adenocarcinoma. Multiple studies have shown that esophageal adenocarcinomas have extensive chromosomal instability, high levels of chromosome copy-number alterations, and frequent catastrophic chromosomal events (27–30). Li and colleagues showed that esophageal adenocarcinoma risk predicted by somatic chromosome alterations outperformed risk predicted by TP53 mutation, flow cytometric DNA content, and histopathologic diagnosis of dysplasia (31). This line of research may offer unique opportunities to identify exposures that lead to the mutation signatures in esophageal adenocarcinoma to better develop preventive strategies to target mutagens leading to the genomic alterations.

We conclude that high-dose supplementation with UDCA for 6 months in patients with Barrett's esophagus increased proportions of cytoprotective bile acids and decreased proportions of cytotoxic bile acids in the gastric fluid. Despite of the favorable change in the bile acid composition in the gastric fluid, we did not observe any significant changes in markers of oxidative DNA damage, cell proliferation, and apoptosis in the Barrett's esophagus epithelium. Given recent research describing genomic alterations that develop in esophageal adenocarcinoma, future studies may consider determining the effects of UDCA on genomic alterations, and the effect of combining with PPI use, to determine its roles in prevention of neoplastic progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: N.J. Shaheen, H-H.S. Chow
Development of methodology: N.J. Shaheen, B.A. Gibson, H.-H.S. Chow
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B. Banerjee, N.J. Shaheen, H-H.S. Chow
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N.J. Shaheen, J.A. Martinez, C-H. Hsu, B.A. Gibson, H-H.S. Chow
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References

1. DeMeester SR. Management of Barrett's esophagus free of dysplasia. Semin Thorac Cardiovasc Surg 1997;9:279–84.
2. Drewitz DJ, Sampliner RE, Garewal HS. The incidence of adenocarcinoma in Barrett's esophagus: a prospective study of 170 patients followed 4.8 years. Am J Gastroenterol 1997;92:212–5.
3. Nehra D, Howell P, Williams CP, Pye JK, Beynon J. Toxic bile acids in gastro-oesophageal reflux disease: influence of gastric acidity. Gut 1999;44: 598–602.
4. Iftikhar SY, Ledingham S, Steele RJ, Evans DF, Lendrum K, Atkinson M, et al. Bile reflux in columnar-lined Barrett's oesophagus. Ann R Coll Surg Engl 1993;75:411–6.
5. Vaezi MF, Richter JE. Role of acid and duodenogastroesophageal reflux in gastroesophageal reflux disease. Gastroenterology 1996;111:1192–9.
6. Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. Mutat Res 2005;589:47–65.
7. Dvorak K, Fass R, Dekel R, Payne CM, Chavarria M, Dvorakova B, et al. Esophageal acid exposure at pH < or = 2 is more common in Barrett's esophagus patients and is associated with oxidative stress. Dis Esophagus 2006;19:366–72.
8. Dvorak K, Payne CM, Chavarria M, Ramsey L, Dvorakova B, Bernstein H, et al. Bile acids in combination with low pH induce oxidative stress and oxidative DNA damage: relevance to the pathogenesis of Barrett's oesophagus. Gut 2007;56:763–71.
9. Huo X, Juergens S, Zhang X, Rezaei D, Yu C, Strauch ED, et al. Deoxycholic acid causes DNA damage while inducing apoptotic resistance through NF-kappaB activation in benign Barrett's epithelial cells. Am J Physiol Gastrointest Liver Physiol 2011;301:G278–86.
10. Goldman A, Condon A, Adler E, Minnella M, Bernstein C, Bernstein H, et al. Protective effects of glycoursodeoxycholic acid in Barrett's esophagus cells. Dis Esophagus 2010;23:83–93.
11. Rizvi S, Demars CJ, Comba A, Gainullin VG, Rizvi Z, Almada LL, et al. Combinatorial chemoprevention reveals a novel smoothened-independent role of GLI1 in esophageal carcinogenesis. Cancer Res 2010;70: 6787–96.
12. Peng S, Huo X, Rezaei D, Zhang Q, Zhang X, Yu C, et al. In Barrett's esophagus patients and Barrett's cell lines, ursodeoxycholic acid increases antioxidant expression and prevents DNA damage by bile acids. Am J Physiol Gastrointest Liver Physiol 2014;307:G129–39.
13. Alberts DS, Martinez ME, Hess LM, Einspahr JG, Green SB, Bhattacharyya AK, et al. Phase III trial of ursodeoxycholic acid to prevent colorectal adenoma recurrence. J Natl Cancer Inst 2005;97:846–53.
14. Pardi DS, Loftus EV Jr, Kremers WK, Keach J, Lindor KD. Ursodeoxycholic acid as a chemopreventive agent in patients with ulcerative colitis and primary sclerosing cholangitis. Gastroenterology 2003;124:889–93.
15. Alvarez Herrero L, Curvers WL, van Vilsteren FG, Wolfsen H, Ragunath K, Wong Kee Song LM, et al. Validation of the Prague C&M classification of Barrett's esophagus in clinical practice. Endoscopy 2013;45:876–82.
16. Kastelein F, Spaander MC, Steyerberg EW, Biermann K, Valkhoff VE, Kuipers EJ, et al. Proton pump inhibitors reduce the risk of neoplastic progression in patients with Barrett's esophagus. Clin Gastroenterol Hepatol 2013;11:382–8.
17. de Bortoli N, Martinucci I, Piaggi P, Maltinti S, Bianchi G, Ciancia E, et al. Randomized clinical trial: twice daily esomeprazole 40 mg vs. pantoprazole 40 mg in Barrett's oesophagus for 1 year. Aliment Pharmacol Ther 2011;33:1019–27.
18. Dunbar KB, Souza RF, Spechler SJ. The effect of proton pump inhibitors on Barrett's esophagus. Gastroenterol Clin North Am 2015;44:415–24.
19. Bozikas A, Marsman WA, Rosmolen WD, van Baal JW, Kulik W, ten Kate FJ, et al. The effect of oral administration of ursodeoxycholic acid and high-dose proton pump inhibitors on the histology of Barrett's esophagus. Dis Esophagus 2008;21:346–54.
20. Sun D, Wang X, Gai Z, Song X, Jia X, Tian H. Bile acids but not acidic acids induce Barrett's esophagus. Int J Clin Exp Pathol 2015;8:1384–92.
21. Binato M, Gurski RR, Fagundes RB, Meurer L, Edelweiss MI. P53 and Ki-67 overexpression in gastroesophageal reflux disease—Barrett's esophagus and adenocarcinoma sequence. Dis Esophagus 2009;22:588–95.
22. Coban S, Ormeci N, Savas B, Ekiz F, Ensari A, Kuzu I, et al. Evaluation of Barrett's esophagus with CK7, CK20, p53, Ki67, and COX2 expressions using chromoendoscopical examination. Dis Esophagus 2013;26: 189–96.
23. Sikkema M, Kerkhof M, Steyerberg EW, Kusters JG, van Strien PM, Looman CW, et al. Aneuploidy and overexpression of Ki67 and p53 as markers for neoplastic progression in Barrett's esophagus: a case-control study. Am J Gastroenterol 2009;104:2673–80.
24. Dvorakova K, Payne CM, Ramsey L, Bernstein H, Holubec H, Chavarria M, et al. Apoptosis resistance in Barrett's esophagus: *exvivo* bioassay of live stressed tissues. Am J Gastroenterol 2005;100:424–31.
25. Chak A, Buttar NS, Foster NR, Seisler DK, Marcon NE, Schoen R, et al. Metformin does not reduce markers of cell proliferation in esophageal tissues of patients with Barrett's esophagus. Clin Gastroenterol Hepatol 2015;13:665–72 e661–4.
26. Lao-Sirieix P, Roy A, Worrall C, Vowler SL, Gardiner S, Fitzgerald RC. Effect of acid suppression on molecular predictors for esophageal cancer. Cancer Epidemiol Biomarkers Prev 2006;15:288–93.
27. Dulak AM, Schumacher SE, van Lieshout J, Imamura Y, Fox C, Shim B, et al. Gastrointestinal adenocarcinomas of the esophagus, stomach, and colon exhibit distinct patterns of genome instability and oncogenesis. Cancer Res 2012;72:4383–93.
28. Dulak AM, Stojanov P, Peng S, Lawrence MS, Fox C, Stewart C, et al. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. Nat Genet 2013;45: 478–86.
29. Nones K, Waddell N, Wayte N, Patch AM, Bailey P, Newell F, et al. Genomic catastrophes frequently arise in esophageal adenocarcinoma and drive tumorigenesis. Nat Commun 2014;5:5224.
30. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute quantification of somatic DNA alterations in human cancer. Nat Biotechnol 2012;30:413–21.
31. Li X, Paulson TG, Galipeau PC, Sanchez CA, Liu K, Kuhner MK, et al. Assessment of esophageal adenocarcinoma risk using somatic chromosome alterations in longitudinal samples in Barrett's Esophagus. Cancer Prev Res 2015;8:845–56.