

## Review

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# Therapeutic and Chemopreventive Effects of Ursodeoxycholic Acid (UDCA): Potential Role in Patients with Barrett's Esophagus

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## ABSTRACT

Ursodeoxycholic Acid (UDCA) is widely used for the treatment of cholestatic liver diseases. Major mechanisms of this action are protection of cholangiocytes against cytotoxicity of hydrophobic bile acids, stimulation of hepatobiliary secretion, and protection of hepatocytes against bile acid induced apoptosis. UDCA has also been shown to modulate mitochondrial transmembrane potential and prevent increases in mitochondrial Reactive Oxygen Species (ROS) production. Moreover, UDCA has been shown to have a chemopreventive role in mouse models of colon cancer and in patients with primary sclerosing cholangitis and concomitant Inflammatory Bowel Disease (IBD), low dose UDCA lowers the risk of IBD-associated colorectal neoplasia. Our studies suggest that oral UDCA may protect against DNA damage induced by hydrophobic bile acids such as Deoxycholic acid (DCA) in the metaplastic mucosa of patients with Barrett's esophagus. We have found that DCA induces carcinogenic DNA damage in Barrett's metaplasia. UDCA which counters the DNA damaging effects of DCA therefore might have a role as a chemopreventive agent in Barrett's metaplasia. Future clinical studies on the use of UDCA for chemoprevention in patients with Barrett's esophagus should be considered.

**KEYWORDS:** Esophagus; Hepatocytes; Ursodeoxycholic acid; Chemopreventive.

**ABBREVIATIONS:** UDCA: Ursodeoxycholic Acid; ROS: Reactive Oxygen Species; IBD: Inflammatory Bowel Disease; DCA: Deoxycholic acid; FDA: Food and Drug Administration; PBC: Primary Biliary Cirrhosis; GR: Glucocorticoid Receptor; TGF: Transforming Growth Factor; AOM: Azoxymethane; PPIs: Proton Pump Inhibitors.

## INTRODUCTION

Ursodeoxycholic acid (UDCA; 3 $\alpha$ ,7 $\beta$ -dihydroxy-5 $\beta$ -cholanolic acid) was first identified by Olof Hammarsten, an academic biochemist from Sweden, in the gall bladders of Polar bears and several other arctic mammals, which were brought to him by Swedish and Danish explorers in 1900-1901.<sup>1</sup> Hammarsten isolated the previously unknown bile acid from the gallbladder of the Polar bear naming it "Ursochoeinsaure".<sup>1</sup> Twenty-five years later, Shodawas able to crystallize this bile acid from a commercial preparation of Black bear bile and renamed it UDCA.<sup>1</sup> UDCA, a major primary bile acid in Black bears, has been used in Chinese traditional medicine for the treatment of biliary stone disease and liver disease.<sup>1,2</sup> In human bile, UDCA is present in low concentrations comprising about 1-3% of the total bile acid pool and it is thought that this small percentage of UDCA in human bile arises from bacterial conversion of chenodeoxycholic acid.<sup>1,2</sup> Unlike Black bears in which UDCA is a primary bile acid, UDCA is a secondary bile acid in humans. Currently, UDCA is US Food and Drug Administration (FDA) approved to treat patients with Primary Biliary Cirrhosis (PBC) that have abnormal liver tests.<sup>2</sup> Although UDCA has been used for the treatment of cholestatic hepatopathies beyond PBC, no studies

have clearly demonstrated a survival benefit in these other conditions.<sup>2</sup>

#### POTENTIAL MECHANISMS FOR UDCA THERAPEUTIC EFFECTS IN CHOLESTASIS

The precise mechanisms whereby UDCA improves liver function are continuing to be revealed. Three major mechanisms of action of UDCA have been proposed: 1) protection of cholangiocytes against toxic effects of hydrophobic bile acids, 2) stimulation of bile acid secretion by the biliary system, and 3) anti-apoptotic effects on hepatocytes that are exposed to hydrophobic bile acids.<sup>3</sup> One way in which UDCA has been shown to protect cholangiocytes against bile acid toxicity in cholestasis is to positively modulate ductular bile flow by regulating hepatocellular transporters that are involved in bile formation.<sup>4</sup> A common feature in cholestasis is the endocytosis of canalicular export pumps that normally are involved in generating the flow of bile. The internalization of these export pumps therefore decreases bile flow and impairs biliary output of choleric compounds.<sup>4</sup> UDCA has been shown to prevent the endocytic internalization of these canalicular transporters thus enhancing bile flow and preserving the integrity of cholangiocytes.<sup>4</sup> More recently studies suggest that stabilization of the “biliary HCO<sub>3</sub> – umbrella” may be a crucial mechanism of action of UDCA in cholestatic liver diseases.<sup>2</sup> The “biliary HCO<sub>3</sub> – umbrella” allows for the maintenance of an alkaline pH near the apical surface of the hepatocytes and cholangiocytes which prevents toxic, protonated, glycine-conjugated bile acids from penetrating the cell membrane.<sup>2</sup> UDCA has been shown to stimulate biliary HCO<sub>3</sub> – secretion allowing for the stabilization of the “umbrella”.<sup>2</sup> UDCA has also been described to act as a Glucocorticoid Receptor (GR) agonist leading to GR activation, nuclear translocation, and promoter binding in the absence of any glucocorticoid hormone.<sup>5</sup> Interaction of UDCA with the GR has been linked to its anti-apoptotic effect in Transforming Growth Factor (TGF)- $\beta$ 1-induced apoptosis of primary rat hepatocytes.<sup>6</sup>

In recent years, experimental data have shown that UDCA not only exerts cytoprotective effects through inhibiting apoptosis but also acts as an antioxidant.<sup>7,8</sup> It has been shown that UDCA modulates mitochondrial transmembrane potential and prevents increases in mitochondrial reactive oxygen species (ROS) production.<sup>7</sup> In an animal model of indomethacin-induced ileitis, UDCA was found to protect against indomethacin-induced mucosal ulceration, intestinal permeability and ROS generation.<sup>8</sup> Its effects such as these that have led to investigations into the chemopreventive potential of UDCA in carcinogenesis.

#### POTENTIAL MECHANISMS FOR UDCA CHEMOPREVENTIVE EFFECTS

UDCA can protect against injuries caused by hydrophobic bile acids such as deoxycholic acid (DCA) and might

even protect against cancers whose development can involve exposure to hydrophobic bile acids such as colon cancer.<sup>9</sup> In animal models, UDCA has been shown to prevent colon cancer induced by Azoxymethane (AOM) and by N-methylnitrosourea.<sup>10,11</sup> In a recent meta-analysis of patients with primary sclerosing cholangitis and concomitant Inflammatory Bowel Disease (IBD), it appears that low dose UDCA (8-15 mg/kg/day) lowers the risk of IBD-associated colorectal neoplasia (colon cancer and/or high grade dysplasia).<sup>12</sup> One potential mechanism for the chemoprotective effect of UDCA on colon cancer maybe due to a reduction in the concentrations of secondary bile acids in the colon.<sup>13,14</sup> Other potential mechanisms include altering cell signaling pathways that have been linked to colon carcinogenesis. For example, UDCA has been shown to block alterations in protein kinase C isoform expression and to reduce activity and expression levels of group II phospholipase A2 expression which are involved in AOM-induced colon carcinogenesis in animal models.<sup>15,16</sup> In humans, alterations in both of these signaling pathways have been linked with the formation of adenomas and colorectal cancers.<sup>17-19</sup> As noted above, UDCA also acts as an antioxidant by stabilizing the mitochondrial membrane and reducing the generation of oxidative radicals, which can induce DNA damage in the cell and perhaps lead to carcinogenesis.

#### POTENTIAL CHEMOPREVENTIVE EFFECTS OF UDCA IN BARRETT'S ESOPHAGUS

Barrett's esophagus, the condition in which a metaplastic columnar epithelium predisposed to malignancy replaces squamous epithelium of the distal esophagus, is the major risk factor for esophageal adenocarcinoma. The primary strategy for cancer prevention in patients with Barrett's esophagus has been to treat the underlying GERD with Proton Pump Inhibitors (PPIs), and to perform endoscopic surveillance for dysplasia.<sup>20</sup> Although the use of PPIs has been extremely effective for decreasing gastric acid production and healing reflux esophagitis, the incidence of esophageal adenocarcinoma continues to increase despite the widespread use of PPIs.<sup>21</sup> This finding suggests that substances in refluxed gastric juice other than acid, such as bile acids, might have an important role in carcinogenesis in Barrett's esophagus. In fact, patients with Barrett's esophagus frequently reflux bile acids into the esophagus in concentrations <200  $\mu$ M.<sup>22,23</sup> Thus an effective chemopreventive agent that reduces bile reflux-induced damage would be extremely beneficial for patients with Barrett's esophagus. There are emerging data that suggest that UDCA might be one such agent for patients with Barrett's esophagus.

In earlier studies, we found that treatment of Barrett's epithelial cells in vitro with 250  $\mu$ M DCA caused the production of ROS leading to the induction of DNA damage and also caused activation of the NF- $\kappa$ B pathway, which prevented DCA-induced apoptosis.<sup>24</sup> In contrast, treatment of Barrett's epithelial cells with 250  $\mu$ M UDCA did not cause ROS production, DNA damage, or activation of the NF- $\kappa$ B pathway.<sup>24</sup> In light of these

findings, we designed a translational study using oral UDCA treatment in patients with Barrett's esophagus to explore a role in protecting the metaplastic mucosa from the damaging effects of DCA exposure.<sup>25</sup> In this study, patients with non-dysplastic Barrett's esophagus (specialized intestinal metaplasia involving >2 cm of the distal esophagus) were treated with PPIs (omeprazole 20 mg twice a day) for at least 4 week before undergoing upper endoscopy. During endoscopy, biopsies of Barrett's metaplasia were taken before and after perfusion of the distal esophagus with 10 cc of 250  $\mu$ M DCA or 10 cc of 250  $\mu$ M UDCA for 5 minutes. The selection of DCA or UDCA for the perfusion was randomly assigned using a sealed envelope strategy. Patients returned one year later and underwent endoscopy with biopsies before and after perfusion of the esophagus with the bile acid not used during the first endoscopy. After the second endoscopy, patients were placed on oral UDCA (10 mg/kg) for 8 weeks. After the 8 week UDCA course, a final endoscopy with biopsies was done before and after the esophagus was perfused with 250  $\mu$ M of DCA. Biopsies were evaluated for DNA damage and NF- $\kappa$ B activation by performing Western blotting for phospho-H2AX and phospho-p65, respectively.

Twenty-one patients completed the study. We found that esophageal perfusion with DCA caused a significant increase in DNA damage and in NF- $\kappa$ B activation. After the patients were treated with 8 weeks of oral UDCA, esophageal perfusion with DCA did not cause DNA damage or NF- $\kappa$ B activation. These results suggest that treatment with oral UDCA protects against DNA damage-induced by hydrophobic bile acids such as DCA in the metaplastic mucosa of patients with Barrett's esophagus.

One way in which oral UDCA may protect against DNA damage induced by hydrophobic bile acids is to decrease concentrations of toxic bile acids like DCA in refluxed gastric juice by increasing its concentration in gastric juice. In fact, the oral dose of UDCA (10 mg/kg) that we used in our study has been shown to replace the hydrophobic bile acids to comprise 40-50% of the bile salt pool.<sup>26</sup> Using Barrett's epithelial cells in vitro, we found that simply mixing equimolar concentrations of DCA and UDCA did not prevent DNA damage, however. Rather, Barrett's cells had to be pretreated with UDCA in order to observe a reduction in DCA-induced DNA damage suggesting that UDCA treatment must upregulate a protective factor in the Barrett's cells. In subsequent experiments, we found that UDCA activated signaling *via* the Nr2 pathway causing the upregulation of the antioxidants catalase and glutathione peroxidase (GPX) 1 in the Barrett's cells. In our biopsy tissues of Barrett's metaplasia taken from patients treated with oral UDCA, we also found upregulation of catalase and GPX1. Moreover, we found that the DNA damage induced by DCA included DNA double strand breaks, the most deleterious form of DNA damage because these lesions can cause genomic instability contributing to carcinogenesis.<sup>27</sup> In fact, agents that induce DNA double strand breaks are considered carcinogens,<sup>28,29</sup> thus hydrophobic bile acids like DCA can be considered carcinogens in Barrett's

metaplasia. Therefore, UDCA which counters the DNA damaging effects of DCA might have a role as a chemopreventive agent in Barrett's esophagus.

To our knowledge, there has been only one other study exploring the chemopreventive role of UDCA combined with PPIs in Barrett's esophagus.<sup>30</sup> This study examined inflammation, dysplasia, proliferation, differentiation, p53 and p16 abnormalities and found no effects of this therapeutic combination on any of these indices.<sup>30</sup> In contrast, our study examined mechanisms and markers whereby UDCA protects against oxidative DNA damage, a read-out not used in the aforementioned study.

## CONCLUSION

In the past three decade, a lot of progress has been made in understanding the mechanisms underlying UDCA therapeutic effects in treating patients with cholestatic liver disorders. More recently, attention has turned to understanding the mechanisms underlying UDCA's chemopreventive effects in colon carcinogenesis and even more recent, mechanisms involved in protecting against bile acid-induced oxidative injury in Barrett's esophagus. Perhaps clinical trials of UDCA for chemoprevention in patients with Barrett's esophagus will be in our future.

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