INTRODUCTION

Barrett’s esophagus (BE) is a premalignant condition in which the squamous epithelium, that occurs normally in the esophagus, has been replaced by an abnormal columnar epithelium containing the goblet cells (1). This condition is the only known precursor of esophageal and esophagogastric junction adenocarcinoma (Barrett's adenocarcinoma - BA) which develops secondary to long-standing gastroesophageal reflux. Compared with individuals in the general population, patients with BE have a 30- to 125-fold higher risk for developing BA. Alarmingly, the incidence of BA has been significantly increased over the last 3 decades. BA develops via a multistep process recognized phenotypically as the histologic sequence of metaplasia-low grade dysplasia-high grade dysplasia-adenocarcinoma (2).

The pathomechanism responsible for the development of metaplastic changes in the distal part of esophagus due to long-standing reflux is still poorly understood. At present it is well established that one of the most important risk factors responsible for the development of BE represents a chronic gastroesophageal reflux disease (GERD). GERD is a common disorder and the Barrett’s esophagus is found in 5-10% of GERD patients undergoing endoscopy due to heartburn or other esophagitis-associated symptoms (3). A better understanding of the molecular events underlying the progression from BE to BA might lead to novel therapies to prevent the development of this malignant disease.

Predominant role in the initiation of esophageal injury and inflammation play gastric acid and pepsin-major components of the refluxate. Prolonged exposition of the esophageal mucosa to low pH and digestive enzymes promotes injury of the esophageal mucosa followed by regeneration with appearance of columnar epithelium instead of normal squamous epithelium (4).

Another important harmful component of the refluxate represent bile acids. Clinical studies using Bilitec 2000® apparatus demonstrated that the bile reflux is more common and intraesophageal bile acid concentrations are significantly higher in patients with BE than in patients with uncomplicated GERD (5). Initially, the role of bile salts was underestimated but recent in vitro and in vivo studies indicate that they also might be implicated in the carcinogenesis of BE (6, 7). Different bile acids have been shown to have different biological effects in terms of carcinogenesis which may be related to their chemical structure and hydrophobic...

Bile salts play an important pathogenic role in the development of Barrett adenocarcinoma (BA). However, the precise role of different bile salts in this process is still unknown. The aim of the present study was to compare the effects of two different bile salts, deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA) on the expression of COX-2, CDX-2 and DNA repair enzymes (MUTYH, OGG-1) in the Barrett epithelial cancer cells (OE-19). OE-19 cells were incubated with DCA or UDCA (100 µM or 300 µM at pH=7.0) over 24 h. To investigate the involvement of NFκB, in separate experiments cells were incubated with DCA in the presence of proteosome inhibitor (MG-132). Cells cycle and apoptosis were analyzed by FACS analysis. After incubation of OE-19 cells with bile salts, the expression of mRNA of COX-2, DNA repair enzymes (MUTYH, OGG-1) and caudal-related homebox transcription factor CDX-2 were measured by quantitative RTPCR. OE-19 cell were also transfected with siRNA-RelA (p65) to asses effect of NFκB inactivation on COX-2 and CDX2 expression. DCA caused a stronger reduction in cell survival of OE-19 cells than UDCA. In addition, DCA stimulated directly the translocation of NFκB p65 (active form) in the nuclei of OE-19 cells. DCA caused stronger than UDCA stimulation of the COX-2 mRNA expression in these cells and this effect was significantly attenuated by the addition of inhibitor of NFκB activity (proteosome inhibitor MG-132). siRNA-RelA reduced expression not only of NFκB but also expression of COX-2 as well as CDX-2 mRNA. DCA caused stronger downregulation of mRNA for DNA repair enzymes MUTYH and OGG-1 than UDCA. In contrast, UDCA induced stronger CDX-2 mRNA expression than DCA in OE-19 cells. We conclude that bile salts are involved in the carcinogenesis of Barrett adenocarcinoma via inhibition of DNA repair enzymes and induction of COX-2 and this last effect is, at least partly, mediated by NFκB. DCA shows carcinogenic potential due to high upregulation of COX-2, CDX-2 and downregulation of DNA repair enzymes.

Key words: Barrett's carcinoma, bile salts, apoptosis, cell proliferation, cyclooxygenase-2, DNA repair
properties (8, 9). Increased incidence of mixed reflux episodes (acid and bile reflux) in BE patients suggests that bile acids exhibit their deleterious effects mainly in acid environment.

Studies involving endoscopy and biopsy analysis from BE demonstrated up-regulation of pro-inflammatory genes like COX-2, PPAR-γ, pro-inflammatory cytokines in the epithelial cells and enhanced production of reactive oxygen species (ROS) by inflammatory cells (10, 11, 12). ROS and other free radicals can damage cell membrane and cause DNA mutations leading to development of cancer cells. The implication of bile acids in the promotion of inflammation and destruction of the esophageal cells is poorly understood and requires further studies to better establish the pathology of Barrett’s adenocarcinoma and its prevention.

The present study was undertaken to investigate the molecular effects of two different bile acids deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA) in neutral and acidic environment; 1) on the expression of cyclooxygenase-2 (COX-2), DNA repair enzymes (MUTYH, OGG-1) and homeobox protein CDX-2 gene, 2) on the viability and rate of apoptosis in esophageal adenocarcinoma cells OE-19 and 3) to analyze the possible involvement of NfκB in the Barrett’s carcinogenesis.

MATERIAL AND METHODS

Cell culture and chemicals

The human cell line derived from esophageal adenocarcinoma (OE-19) was purchased from ECACC- the European Collection of Cell Cultures (Sigma-Aldrich, Germany). Cells were cultured in RPMI 1640 medium at pH~7 containing 10% fetal bovine serum (FBS) and antibiotics at 37°C in water saturated atmosphere of 95% air and 5% CO2. Deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA) were obtained from Sigma-Aldrich (Germany). Both acids were dissolved in RPMI 1640 medium at concentrations of 100 µM and 300 µM for 24 h. Cells were collected, washed twice with PBS, and lysed in 0.4 mL of lysis buffer (0.06 mol/L Tris-HCl, pH 6.8, 2% SDS, 5% beta-mercaptoethanol, 0.0025% bromophenol blue). DNA was sheared by a needle, the solution heated at 95°C for 5 min and centrifuged at 15000 g for 2 min. The total protein was loaded for 2 min. The total protein was loaded on SDS-polyacrylamide gel, run at 40 mA and transferred to nitrocellulose (Protran, Schleicher & Schuell, Germany). Analysis of labeled nuclei was performed on a FACS Calibur fluorescence-activated cell sorter (FACS) using CELLQuest software (both from Becton Dickinson). The percentage of apoptotic cells was determined by measuring the fraction of nuclei with a sub-diploid DNA content. Ten thousand events were collected for each analyzed sample.

RNA isolation and RT-PCR

For RNA isolation the cells were scraped from culture dishes and suspended in 250 µl of TRIZOL (Total RNA Isolation Reagent; Invitrogen, Germany). Further steps of RNA isolation was performed according to manufactur’s instruction. Isolated RNA was dissolved in 25 µl DEPC treated water.

Complementary DNA (cDNA) was generated by reverse transcription of 5 µg total RNA extracted from OE-19 cells, using Moloney murine leukemia virus reverse transcriptase kit (MMLV-RT) (Stratagene, Germany) and oligo dT primes. The cDNA (1 µl) was amplified in 25 µl volume samples containing 1 U Taq polymerase, dNTP (200 µM each) (Eppendorf, Germany), 1.5 mM MgCl2, 2.5 µl 10× polymerase chain reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH=8.3) and specific primers for β-actin, CDX-2, COX-2, OGG-1 and MUTYH (Table 1). The polymerase chain reaction mixture was amplified in a DNA thermal cycler (MJ-Research; Biozym, Germany). Polymerase chain reaction products were detected by ethidium bromide on a 1.5% agarose gel containing ethidium bromide. Location of predicted products was confirmed by using 100-bp ladder (Takara, Shiga, Japan) as a standard size marker.

For quantification effects of siRNA transfection, PCR reactions were performed on qI5 Multicolor Real-Time Detection System; Bio-Rad (Bio-Rad, Germany) contained qI SYBR green mix (Bio-Rad, Germany; according to manufacturer’s instruction) and specific gene primers: β-actin; NfκB (p-65), COX-2 and CDX-2 (Table 1). Samples without templates were included as negative control. Gene expression was normalized to the β-actin. All samples were performed in duplicates.

Western blot

OE-19 cells were incubated with DCA and UDCA at concentrations of 100 µM and 300 µM for 24 h. Cells were collected, washed twice with PBS, and lysed in 0.4 mL of lysis buffer (0.06 mol/L Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5% beta-mercaptoethanol, 0.0025% bromophenol blue). DNA was sheared by a needle, the solution heated at 95°C for 5 min and centrifuged at 15000 g for 2 min. The total protein was loaded on SDS-polyacrylamide gel, run at 40 mA and transferred to nitrocellulose (Protran, Schleicher & Schuell, Germany) by electroblotting. Filters were blocked with 5% non fat milk (Roth, Germany) in TBS/Tween-20 buffer (137 mM NaCl, 20 mM Tris-HCl, pH 7.4, 0.1% Tween-20) before incubation with antibodies against p21 (mouse monoclonal, 1:500 dilution; Dunn

| Table 1. Primers used for polymerase chain reaction and amplification conditions (Ta = annealing temperature). |
|-----------------|----------------|----------------|
| Gene           | Primer sequence | Ta [°C]       |
| β-actin        | sense: 5′-CAC TCT TCC AGC CTT CCT TC-3′  | 58             |
|                 | antisense: 5′-GGA GTA ACG CAA CTA AGT CAT AG-3′ |               |
| COX-2          | sense: 5′-TGG TGC CTG GTC TGA TGA TGT ATG C-3′  | 56             |
|                 | antisense: 5′-ATC TGC CTG CTC TGG TCA ATG GAA G-3′ |               |
| MUTYH          | sense: 5′-GGA AGT GGT CAC AGG AAG-3′  | 57             |
|                 | antisense: 5′-AAG GCG ATA GAG GCA ATG-3′    |               |
| RelA (p65)     | sense: 5′-AGCACAGATACACCAAGAGCC-3′  | 59             |
|                 | antisense: 5′-TCAGCCTTATAGAAAAGCATCC-3′ |               |
| OGG1           | sense: 5′-AGG AGG TGG AGG AAT TAA G-3′  | 57             |
|                 | antisense: 5′-CAG TGG TGA TAC AGT TGA G-3′ |               |
| CDX-2          | sense: 5′-CGG GCA GGC AAC AAG GTT TAC-3′  | 57             |
|                 | antisense: 5′-AAC AGC AGC AAC AAC AC-3′    |               |
The night in cells culture incubator under 5% CO2, 37°C and (400000 per well) in RPMI 1640, 10% FBS and cultured over RNA interference experiments recommendation (Sigma Aldrich, Germany). 20 µg proteins were measured by BCA method according to the manufacture’s 1:30000; Promega, WI, USA). The nuclear isolated proteins were measured by BCA method according to the manufacturer’s recommendation (Sigma Aldrich, Germany). 20 µg proteins were loaded on SDS-polyacrylamide gel. The further steps of Western blot analysis were performed as described above.

RNA interference experiments

OE-19 cells were seeded in 6-well plate in confluency (400000 per well) in RPMI 1640, 10% FBS and cultured over the night in cells culture incubator under 5% CO2, 37°C and 90% humidity. Next day, 6 h before transfection medium was exchanged to RPMI 1640 without FBS. For each transfection reaction in two separate tubes, 25 or 50 nM siRNA against NFκB-p65 (Ambion) or siRNA negative control (Ambion) and 5 µL SilentFect reagent (Bio-Rad) were mixed with 250 µl serum free media and incubated for 5 min at room temperature. After this time, the contents of the two tubes were combined and allowed to form siRNA-SilentFect complexes for 20 min at room temperature. A 500 µl aliquot of siRNA-SilentFect was combined with cells growing in 1,5 ml RPMI 1640 medium and placed in 37°C, 5% CO2 incubator for 12 h. Then medium was changed for full culture medium contained 10% FBS. RNA interference experiments were performed according to the method described by Gerlag et al. (13). The expression of NFκB-p65 was analysed by Western blot using primary Ig rabbit polyclonal antibody (1:500, Santa Cruz, USA) and secondary anti-rabbit-IgG (dilution 1:30000; Promega, WI, USA). The nuclear isolated proteins were measured by BCA method according to the manufacture’s recommendation (Sigma Aldrich, Germany). 20 µg proteins were loaded on SDS-polyacrylamide gel. The further steps of Western blot analysis were performed as described above.

Data analysis

Data are presented as ±SEM. Statistical analysis was performed using Student’s t-test. P <0.05 was considered significant.

RESULTS

Modulatory effect of bile acids on mRNA expression of COX-2, MUTYH, OGG-1 and CDX-2

The effects of bile acids on gene expression of important genes involved in inflammation and carcinogenesis such as COX-2, MUTYH and OGG-1 (DNA repair enzymes) and CDX-2 (gene involved in the intestine development) were analyzed by means of RT-PCR. As shown in the Fig. 1a, the mRNA expression for COX-2 was low in control group. In contrast, the level of mRNA for this gene was significantly up-regulated in OE-19 cells incubated with DCA in neutral medium (Fig. 2). Significant accumulation of NFκB-p65 indicating the transactivation of NFκB was observed already after 30 min after the start of incubation with DCA. The increased levels of NFκB-p65 maintained over 2 h after start of incubation with DCA. No significant upregulation of NFκB-p65 was observed in OE-19 cells incubated with UDCA.

Effect of bile acids on transactivation of NFκB

To evaluate the influence of bile acids on NFκB activation, cells were incubated with DCA at the concentration of 300 µM for different time periods, between 30 min and 2 h. DCA was chosen in these experiments because of its stimulatory effect on COX-2 expression and because COX-2 gene expression is under control of this transcription factor. The inhibitory effect of DCA on the mRNA expression of DNA repair enzymes MUTYH and OGG-1 in OE-19 cells. Both, DCA and UDCA induced a significant down-regulation of gene expression for these important genes. The inhibitory effect of DCA on the mRNA expression of MUTYH and OGG-1 was much stronger when compared to that induced by UDCA.

Influence of bile acids on apoptosis and cell cycle

Cells treated over 24 h with DCA and UDCA at concentration of 300 µM were analyzed by flow cytometry (FACS). This procedure was used to measure the number of apoptotic cells and alterations in cell cycle. As shown in the Fig 3, the incubation with DCA was associated with a significant increase in apoptosis rate in OE-19 cells, whereas UDCA failed to cause any significant changes in apoptosis rate in these cells.

Additionally, DCA caused significant alteration in phases of cell cycle. As demonstrated in the Fig. 4, it promoted transition from G1 to G2 phase. Additionally, in cells incubated with DCA, the number of cells in S phase was significantly increased as compared to control. In contrast, incubation with UDCA promoted cell arrest in G1 phase. Thus, the number of cells in S and G2 stage was significantly decreased under UDCA as compared to control culture.

To confirm the impact of DCA on cells cycle, Western blot analysis of p21 protein, an inhibitor of CDK–cyclin complexes, was performed (Fig. 5). The immunobloting analysis demonstrated a significant reduction of p21 protein in OE-19
cells treated with both doses of DCA (100 µM and 300 µM). Much weaker down-regulation of p21 protein was observed in cells incubated with UDCA 300 µM.

Effects of NFκB inactivation by siRNA

To better estimate the role of NFκB in Barrett’s metaplasia and its progression to cancer, series of experiments with the application of siRNA against NFκB mRNA were performed. Firstly, optimal concentration of siRNA for transfection and time for NFκB inactivation was assessed (Fig. 6a-b). Based on our experiment, concentration of 50 nM siRNA-RelA and 72 h after transfection were chosen for further experiments. Then OE-19 cells were incubated with DCA (at 300 µM) after siRNA-RelA transfection. Quantitative analysis of expression for RelA, COX-2 and CDX-2 mRNA a Western blotting for RelA and CDX-2 were performed (Fig. 7a-d).

Transfection of EO-19 cell line with siRNA-RelA caused significant inhibition of RelA(p65) expression and led to a significant reduction in expression for COX-2 and CDX-2. Because of known anti apoptotic properties of NFκB, apoptotic rate in cells transfected with siRNA-RelA was evaluated (Fig. 8). Cells transfected with siRNA-RelA showed significant rise of apoptosis compared to control (over tree times). Moreover, cells transfected with siRNA and treated with DCA presented highest apoptotic rate. Interestingly, in cells incubated only with transfection reagent and DCA, the number of apoptotic cell was also significantly increased probably due to easiest cell membranes permeability for DCA which can be side effect of transfection.

DISCUSSION

The present study shows an important modulatory effect of bile acids on expression of multiple genes involved in essential cellular processes such as cell division and apoptosis. Moreover, all these effects were observed in cells exposed to bile acids at neutral pH, suggesting that even after inhibition of acid reflux, the presence of the duodeno-gastroesophageal reflux containing bile acids might be responsible for the progression of carcinogenesis in the Barrett’s mucosa.
Previous studies demonstrated that the chronic inflammatory reaction in esophageal mucosa observed in patients with chronic gastroesophageal reflux and/or Barrett’s esophagus is associated with an increased production of reactive oxygen species (ROS) (14, 15). ROS contribute to oxidation of many essential cellular components, especially DNA. This last process has major impact on gene mutations playing a crucial role in the process of carcinogenesis. Therefore, the cells are equipped with numerous defensive mechanisms to counteract the oxidative damage of DNA. Especially two enzymes, MUTYH and OGG-1, seem to play important roles in cells avoiding the accumulation of oxidative DNA damage, thereby suppressing the carcinogenesis (16). MUTYH excises adenine opposite 8-oxoG in DNA, and, thus, suppresses 8-oxoG-induced mutagenesis. MUTYH also possesses a 2-OH-ADNA glycosylase activity for excising 2-OH-A incorporated into the cellular genomes. OGG-1 excises 8-oxoG in DNA as a DNA glycosylase and thus minimizes the accumulation of 8-oxoG in the cellular genome. The previous studies reported increased susceptibilities to spontaneous carcinogenesis in MUTYH- and OGG-1-null mice. The present study demonstrates that bile acids inhibit mRNA expression of both MUTYH and OGG-1 enzymes and this could contribute to formation and accumulation of DNA mutations (16, 17).
Exposure of BA cells (OE-19 cells) to bile acids induce the activation of NFκB, which seems to play a crucial role in the promotion of inflammatory reaction and carcinogenesis in the Barrett’s mucosa (11). Moreover, we found a link between exposure of OE-19 cells to bile acids and increase in COX-2 expression. Interestingly, the increase in COX-2 expression by bile acids was NFκB-dependent, while this effect was significantly attenuated by NFκB inhibitor (MG-132) or by NFκB-p65 siRNA. Taken together, the complex interaction between bile acids and BA cells involves the activation of nuclear transcription factor NFκB followed by COX-2 overexpression.

Previous studies including our own demonstrated that the COX-2 overexpression plays a crucial role in the initiation and progression of carcinogenesis in upper and lower GI tract (17, 18). COX-2 is an inducible isoform of rate-limiting enzyme in synthesis of prostaglandins (PGs), that are involved in carcinogenesis by multiple pathways including inhibition of apoptosis, stimulation of angiogenesis, increase in cellular proliferation and mutagenesis (18). The fact that bile acids increase COX-2 expression indicates that the biliary reflux may be responsible for the development of Barrett’s carcinogenesis. However, there was a significant difference between DCA and UDCA in terms of induction of COX-2 mRNA expression. DCA caused a significantly higher expression of COX-2 than UDCA, suggesting higher carcinogenic potential of DCA. Taken together our results indicate that the activation of NFκB and increased COX-2 expression represent the important molecular events responsible for the progression of the carcinogenesis in the Barrett’s esophagus. Our data are in keeping with the previous results obtained in in vitro experiments and in biopsies from Barrett’s esophagus and Barrett’s adenocarcinoma (2, 19).

Fig. 7. Quantitative RT-PCR evaluation of RelA (p65), COX-2 and CDX-2 mRNA expression in OE-19 cells treated with DCA at the concentration of 300 µM incubated 24 h in pH neutral medium following siRNA transfection. The representative Western blot analysis of NFkB-p65 in OE-19 cells whole lysate (panel (b)). 1) control, 2) SilentFect reagent alone, 3) SilentFect with DCA 300 µM, 4) siRNA-RelA, 5) siRNA-RelA with DCA 300 µM, 6) DCA 300 µM. Single cross indicates significant increase above the control value. Double crosses indicate significant increase as compared to cells without addition of RelA at the concentration of 50 nM. Single asterisk indicates significant decrease compared to control value.

Fig. 8. Assessment of apoptotic rate in cells transfected with siRNA-RelA treated with or without DCA at the concentration of 300 µM. Single cross indicates significant increase above the control value. Double crosses indicate significant increase as compared to the values obtained in respective controls with siRNA-RelA at 50 nM and DCA at 300 µM.

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It is well established that the modulation of apoptosis plays an important role in the cancer development (18). In FACS analysis, DCA and UDCA showed different effects on apoptosis. In contrast to UDCA, DCA caused a rise of apoptotic rate in OE-19 cells with simultaneous transition from G1 to G2 phase of cell cycle. The last effect can be, at least partly, explained by reduction of p21 protein (Waf1/Cip1- tumour suppressor) expression. p21 can directly bind to PCNA (proliferating cell nuclear antigen) and inhibit DNA
replication or can inhibit cyclin/CDK complexes, resulting in G2 or G1 phase arrest, respectively (20). In connection with previously described results, especially an inhibition of expression DNA repair enzymes (MUTYH and OGG-1), bile acids might favour replication of DNA containing mutations which in normal cells stop cellular divisions and initiate apoptosis.

The most important observation of the present study is that DCA, in contrast to UDCA, stimulates CDX-2 expression. CDX-2 is a transcription factor that plays a crucial role during development of gastrointestinal system as well as differentiation of gastrointestinal stem cells (21). Increased expression of this transcription factor was described in biopsies obtained from BE and BA (22, 23). Moreover, studies in transgenic mice demonstrated that CDX-2 favours the development of intestinal metaplasia (19). However, reports about the role of CDX-2 in carcinogenesis are contradictory, what can be associated with its tissue specific expression or mutation. For example, in colon mucosal cells an inverse relationship between CDX-1/CDX-2 mRNA levels and the extension of dysplasia and severity of clinical outcome in colorectal cancer was described. In cells overexpressing both CDX-1 and CDX-2 the tumorgenicity of CRC cells was significantly reduced (24). On the other side, in the esophagus or stomach, the level of expression was used as a marker of early metastatic changes (25). In addition, Kazumori et al. found that bile acids increase the expression of CDX-2 in Barrett’s mucosa via activation of NFκB (26) and these results have been confirmed by our present experiments with use of NFκB-p65 siRNA. Inhibition of biological of NFκB activity by siRNA caused a significant decrease in CDX-2 expression. Previous reports described that CDX-2 gene upstream sequence contains two putative NFκB binding sites (26, 27). Mutants from each of the NFκB binding sites in bile acid environment inhibited partially CDX-2 promoter activity and were completely inhibited by mutations induced of both binding sites. This finding may suggest a major role of NFκB not only as inflammatory factor but also as transcription factor implicated in the development of metaplastic changes in lower part of the esophagus.

Bile acids show many important biological properties. We have demonstrated that they influence the expression of number crucial genes involved in Barrett’s esophagus carcinogenesis including genes regulating cellular proliferation and apoptosis. Additionally, bile acids and salts can directly or indirectly stimulate the ROS production (27). All these features indicate a double role of bile acids in pathogenesis of Barrett’s adenocarcinoma. On one side, bile acids act like carcinogens and induce damage of DNA, while on the other side, bile acids play a role as the selection factor via induction of cytotoxicity, apoptosis and formation metaplastic phenotype. Trough the above mechanisms they favour the selection of cells clones, which are more resistant to gastric acids and bile action. Furthermore, selected clones cells may escape from cell cycle control and become resistant to apoptosis due to mutations and chromosomal aberrations.

The present study showed a significant difference in biological action between DCA and UDCA. Ursodeoxycholic acid, which has been shown to reduce the rate of malignant progression in patients with ulcerative colitis (28), fail to stimulate the CDX-2 mRNA expression in BE cells. This observation suggests that the treatment of BE patients with UDCA might reduce the malignant progression in Barrett’s esophagus. To clarify this problem, further clinical studies with the use of UDCA as chemopreventive agent are necessary.

The present study demonstrated that some bile acids, like DCA, might cause deleterious biological effects also in neutral environment without the presence of gastric acid. This finding indicates that the blockade of gastric acid secretion e.g. with proton pump inhibitors might not be sufficient to prevent carcinogenesis in patients with Barrett’s esophagus as also suggested by some clinical studies (29). A new approach consisting of gastric acid inhibition, blockade of bile acids and perhaps activation of endogenous mucosal protectors such as melanotin, antioxidants or afferent sensory nerves might be useful in prevention of malignant progression in Barrett’s esophagus (30-34).

We conclude that bile acids are involved in the carcinogenesis of Barrett’s adenocarcinoma via inhibition of DNA repair enzymes and induction of COX-2 and this last effect is, at least partly, mediated by NFκB. Additionally, DCA promotes upregulation of CDX-2 via NFκB, thus, modulating development of Barrett’s metaplasia. Therapy against NFκB in patients with Barrett’s metaplasia may give promising results.

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