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Bile salts increase epithelial cell proliferation through HuR-induced c-Myc expression

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ABSTRACT

Background: Bile salts increase intestinal mucosal proliferation through an increase in c-Myc, a transcription factor that controls the expression of numerous translation regulatory proteins. HuR is an RNA-binding protein that regulates translation of target mRNAs. RNA-binding proteins can control mRNA stability by binding to AU- and U-rich elements located in the 3′-untranslated regions (3′-UTRs) of target mRNAs.

Aim: To determine how bile salt–induced c-Myc stimulates enterocyte proliferation.

Methods: Enterocyte proliferation was measured both *in vivo* using C57Bl6 mice and *in vitro* using IEC-6 cells after taurodeoxycholate (TDCA) supplementation. HuR and c-Myc protein expression was determined by immunoblot. c-Myc mRNA expression was determined by PCR. HuR expression was inhibited using specific small interfering RNA. HuR binding to c-Myc mRNA was determined by immunoprecipitation.

Results: TDCA increased enterocyte proliferation *in vivo* and *in vitro*. TDCA stimulates translocation of HuR from the nucleus to the cytoplasm. Cytoplasmic HuR regulates c-Myc translation by HuR binding to the 3′-UTR of c-Myc mRNA. Increased TDCA-induced c-Myc increases enterocyte proliferation.

Conclusions: Bile salts have beneficial effects on the intestinal epithelial mucosa, which are important in maintaining intestinal mucosal integrity and function. These data further support an important beneficial role of bile salts in regulation of mucosal growth and repair. Decreased enterocyte exposure to luminal bile salts, as occurs during critical illness, liver failure, starvation, and intestinal injury, may have a detrimental effect on mucosal integrity.

1. Introduction

Mucosal integrity is necessary for digestive and secretory functions as well as the formation of a barrier against noxious substances within the intestinal lumen. This integrity is maintained by a strict balance of proliferation, differentiation, and apoptotis [1,2].

Bile salts are normally found within the intestinal lumen, where their primary function is to aid in the absorption of lipids and lipid-soluble vitamins [3]. They also have cellular

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effects. The bile salt deoxycholic acid has been shown to increase proliferation and induce apoptosis in colon cancer cell lines [4,5]. Taurodeoxycholic acid (TDCA) increases growth of esophageal mucosa in a rabbit explant model [6]. The intestinal mucosa requires the presence of luminal contents to maximize the adaptive response [7–11]. After bile duct ligation, oral administration of sodium taurocholate improved anastomotic healing [12]. Animals with biliary diversion after small bowel resection demonstrate an impaired adaptive response [7–9]. Clearly, bile salts have many functions besides facilitating absorption of lipids and lipid-soluble vitamins. The presence of bile salts is important for regulation of these functions; their absence may be detrimental. Mucosal atrophy caused by starvation and critical illness, while multifactorial in etiology, may be related to cholestasis and diminished luminal bile. Further characterization of the beneficial functions of bile salts on the small intestinal mucosa is warranted.

Previous work from our lab has shown that TDCA supplementation into the growth media of intestinal epithelial cells results in increased cellular restitution and proliferation [13–15]. We have also shown that this increase in epithelial cell proliferation is due to a c-Myc-dependent pathway [16]. c-Myc is a proto-oncogene that plays a major role in the control of cell cycle [17,18]. Decreased expression of c-Myc mRNA has been associated with prevention of G_1-S phase transition and inhibition of cell proliferation [19,20]. The c-Myc gene codes for a nuclear phosphoprotein that functions as a transcription factor controlling cell division, differentiation, and apoptosis [17,18]. Constitutive expression of the c-Myc gene prevents exit from the cell cycle as well as differentiation [21,23]. Moreover, c-Myc activity is sufficient to drive resting cells into the cell cycle [22–24], and decreased expression of c-Myc gene prevents the transition from the G_1 to the S phase. c-Myc is a physiologic regulator of normal intestinal epithelial cell proliferation and is implicated in maintenance of mucosal epithelial integrity.

RNA-binding proteins like HuR control mRNA stability by binding to AU- and U-rich elements located in the 3′-untranslated regions (3′-UTRs) of target mRNAs [25,26]. HuR is a key regulator of genes that are central to cell proliferation, stress response, immune cell activation, carcinogenesis, and replicative senescence. HuR is predominantly nuclear in unstimulated cells, but when stimulated, HuR rapidly translocates to the cytoplasm, where it stabilizes specific mRNAs and affects the translation of target mRNAs [27,28]. We hypothesized that TDCA, which stimulates intestinal epithelial proliferation through a c-Myc-dependent pathway, increases c-Myc expression through an increase in HuR-regulated c-Myc expression. We studied the effect of TDCA on HuR binding to c-Myc mRNA and its effect on c-Myc expression.

2. Materials and methods

2.1. Animals

Six- to 8-wk-old C57Bl/6J male mice were used and were obtained from Jackson Laboratory (Bar Harbor, ME). Animals were housed in a standard facility, were kept on a 12-h light-dark cycle, and received water and mouse chow *ad libitum* until time for experimentation. All studies were approved by the University of Maryland School of Medicine Animal Studies Committee (IACUC protocol #0807008 and #1108007). Mice were treated in accordance with the National Institutes of Health laboratory animals use guidelines.

Mice underwent a sham laparotomy after isoflurane anesthesia. Postoperatively, the mice were placed on a liquid rodent diet (Micro-Stabilized Rodent Liquid Diet LD101; Purina Mills, St. Louis, MO) with or without the addition of TDCA (50 mg/kg/d TDCA, T0557; Sigma-Aldrich, St. Louis, MO). Mice were sacrificed after 7 d of this treatment in order to evaluate intestinal proliferation.

2.2. In vivo *quantification of intestinal proliferation*

Quantification of intestinal proliferation was done in 100 well-oriented jejunal crypt-villus units in a masked manner. Mice were injected 90 min prior to sacrifice with 5-bromo-2′deoxyuridine (BrdU) (200 μL intraperitoneal injection per animal; Invitrogen Corporation, Carlsbad, CA) to label S-phase cells. BrdU was later detected via immunohistochemistry as previously described [29]. Briefly, paraffin-embedded slides were deparaffinized and rehydrated, and then underwent blocking of endogenous peroxidase activity. Slides were immersed in citric acid–based antigen unmasking solution (Vector, Burlingame, CA) and heated in a microwave for 15 min. After cooling to 42°C, sections underwent protein block (Dako, Carpinteria, CA) and were then incubated with rat monoclonal anti-BrdU (1:500; Accurate Chemical & Scientific) overnight at 4°C. On the subsequent day, slides were incubated with goat anti-rat secondary antibody (1:500; Accurate Chemical & Scientific) followed by incubation with streptavidin/horseradish peroxidase (Dako). Finally, sections were developed with diaminobenzidine and counterstained with hematoxylin.

2.3. *Cell culture*

The IEC-6 small intestinal cell line was purchased from the American Type Culture Collection at passage 13, and passages 15–20 were used for experimentation as previously described [16]. The cell line was derived from normal rat jejunal crypt cells and was developed and characterized by Quaroni *et al.* [30]. Dulbecco's modified Eagle medium supplemented with 5% heat-inactivated dialyzed fetal bovine serum, 1% gentamicin, and 0.1 U/mL insulin was used as control media. TDCA was supplemented to control media at 0, 0.05, 0.5, or 1.0 mmol/L when specified.

2.4. In vitro *quantification of intestinal proliferation*

IEC-6 cells were plated at 2×10^4 cells/cm^2 in Dulbecco's modified Eagle medium. After 24 h of growth, media were changed to contain 0 to 1 mmol/L TDCA ($n = 3$–4 per group) as previously described [16]. Media were changed every 2 d thereafter and cells were harvested with 0.4% trypsin after 2, 4, or 6 d of growth. Total cell numbers were quantified using a hemocytometer and light microscopy and are reported as total cells per plate.