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short communication

Effect of Conjugated Bile Salt Taurodeoxycholic Acid (TDCA) on Mice Colonic Motor Activity

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Abstract

Background & Aims: *There is an ongoing discussion concerning the role of bile salts on the gastrointestinal motility. Therefore, Taurodeoxycholic acid (TDCA) was studied to investigate the effect of TDCA on mice colonic motility and to examine this mechanism of action in presences histamine antagonist (pyrilamine maleate salt, H1) and serotonin agonist (5-hydroxytryptamine hydrochloride, 5-HT).*

Methods: *Dose response curve of TDCA was performed using different doses of TDCA (0.3, 30, 50, 100, 200, 300 and 500 μM). Peristaltic motor complexes (PMCs) amplitudes and intervals of contractions were recorded. TDCA inhibitory effect (300 μM) on PMCs was studied with presence with H1 (10 μM) and 5-HT (25 μM).*

Results: *TDCA inhibits intestine motility through increasing PMC intervals and decreasing PMC amplitudes with doses of 100, 200, 300 and 500 μM compared to the control values (P< 0.03, 0.03, 0.01, 0.01 respectively). H1 antagonist (10 μM) induced significant reducing in the PMC intervals (137.2 ± 13 S) compared to the control values (255.8 ± 31 S, P < 0.04) while there was no response of 5-HT agonist (25 μM) on interval or amplitude of PMCs regardless applying of TDCA (300 μM).*

Conclusion: *TDCA directly inhibited colon motility may be due to slowing intestinal transit time. These finding is not revealed with presences of histamine antagonist and serotonin agonist.*

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INTRODUCTION

Bile salts are surface active compounds; they have physiological functions correlated to their amphipathic molecular structures as digestion and absorption of lipophilic compounds and their detergent nature on protein denaturation (1). Bile salts are also considered as effective signaling molecules in liver and intestine (2).

Bile salts can be synthesized from cholesterol or extracted from the bloodstream by the liver (3). In the liver, bile salts directly modulate their hepatocellular uptake, synthesis and biliary secretion *via* activation of nuclear receptors and *via* modulation of cytosolic signaling cascades. They pass from the liver into the small intestine where they reabsorbed from the intestinal lumen to undergo enterohepatic circulation (2).

Bile salts are composed of the salts of bile acids combined with taurine or glycine during many conversions include deconjugation, oxidation and epimerization of hydroxyl groups at C_3, C_7 and C_{12}, 7-dehydroxylation, esterification and desulfatation (4). Taurodeoxycholic acid

(TDCA) is one of bile salts formed by conjugation of deoxycholate with taurine (5).

TDCA inhibits motility, delays gastric emptying and inhibits small bowel transit time (6). This inhibition may due to molecular mechanisms induce gene expression (7) or prostaglandin dependent due to bile acid action on intestinal epithelial cells by elicitation chloride ion secretion (8). Kalia *et al.* (9) supported the bile-induced secretion resulting from increasing concentrations in the colon.

Gastrointestinal (GI) motility could be influenced by muscarinic and histaminergic modulation (10). Bile acids induce prostaglandin and histamine secretion (8). Histamine is an important cellular messenger of the GI tract while histamine antagonist (pyrilamine maleate salt, H1) can stimulate various smooth muscles through activation of H1 receptors (10).

The secretory actions of TDCA may be partly mediated *via* a neural pathway through interactions between the intestinal nervous and immune systems (9). Serotonin (5-hydroxytryptamine; 5-HT) has become one of the most investigated and complex biogenic amines (11). Serotonin is found in the enteric nervous system where it is implicated in controlling gastrointestinal motor function (12) through the receptors of serotonin that mediate reflex control of GI motility and secretion (13).

The aim of this study is to investigate the effect of TDCA as a bile salt on mice colonic motility and to examine this mechanism of action in presences of histamine antagonist and serotonin agonist.

MATERIALS AND METHODS

Experimental Animals

Experiments were performed on colon segments from Swiss male mice (25– 30 g, 4-wk-old). Animals were kept and fed *ad libitumin* in Animal House of King Fahd Medical Research Centre (KFMRC), Jeddah, Saudi Arabia. The study was approved by the Ethics Committee of KFMRC, King Abdulaziz University.

Chemicals

Sodium taurodeoxycholate hydrate (Taurodeoxycholic acid, TDCA) and Pyrilamine maleate salt (Histamine antagonist, H1) were purchased from Sigma-Aldrich Corporation (St. Louis, MO USA). 5-Hydroxytryptamine Hydrochloride (Serotonin agonist, 5-HT) was purchased from Spectrum Chemical Manufacturing Corporation (New Brunswick, Gardena, CA).

TDCA was dissolved in dimethylsulphoxide (DMSO, 0.1%), while H1 antagonist and 5-HT agonist were dissolved in distilled water; Solutions were stored at -20°C and then they were freshly diluted and added in microliter volumes to the segment bath during the experiments.

Tissue Preparation

The experiment was performed according to Abdu *et al.* (14, 15); mice were sacrificed by cervical dislocation, colon segment was rapidly excised and placed in gassed (95% O_2 and 5% CO_2) Krebs bicarbonate buffer solution (composition in mM: 117 NaC1, 4.7 KC1, 25 $NaHCO_3$, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 2 NaH_2PO_4, 1.2 H_2O, and 11 D-glucose), cleared of mesenteric connective tissue, and the lumen was flushed with Krebs solution. Colon segment was prepared and mounted horizontally in separate 20-ml perfusion chambers. The oral and aboral ends of colon segment were secured to two metal catheters fixed at either end of the chamber; the oral end was connected to a perfusion pump for intra-infusion of Krebs solution at a rate of 0.16 ml/min, and the aboral end was attached to a pressure transducer (NeuroLog™ System, NL900D, Digitimer Ltd., Hertfordshire, UK) to record contractile activity as changes in intraluminal pressure under isovolumetric conditions. Tissues were maintained at 37°C, perfused with Krebs solution and allowed to equilibrate for at least 10 min before starting of the experiments. The productivity from the pressure transducers was transmitted to a data-acquisition system (Power1401, Cambridge Electronic Design Ltd., Cambridge, UK) and from there to a computer running Spike 2 version 4 software (Cambridge Electronic Design Ltd., Cambridge, UK), which stored the records for subsequent offline analysis.

Colon Motility

Peristaltic motor complexes (PMCs) were measured in conditions of their peak amplitude above baseline and were expressed as cm/H_2O, while period and interval between them were expressed in seconds (S). PMC amplitudes and intervals of contractions were determined 15 min before and after appropriate vehicle.

Dose response curve (DRC) was done for TDCA for the following doses (0.3, 30, 50, 100, 200, 300 and 500 µM, n = 28, 4 mice for each dose). H1 antagonist and 5-HT agonist were added for 5 min after stopping perfusion and prior to challenge with TDCA (300 µM, n = 8, 4 mice for each chemical), the recording continued for a further 15 min.

TDCA dose (300 µM) was selected according to studied DRC. Dose of H1 antagonist (10 µM) was chosen according to Abdu *et al*. study done in (15) while 5-HT agonist (25 µM) dose was obtained according to Michel and Kendall study (16).

Statistical analysis

Responses are expressed as absolute values ± SE. Data compared using „student's" t-test paired or unequal variances as appropriate. Probability of $P< 0.05$ was considered as significant.

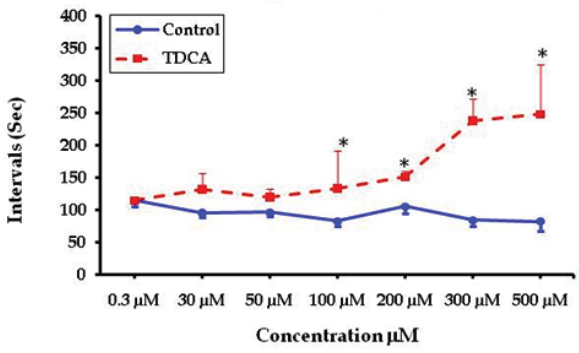

Figure 1. *Effect of TDCA on PMC intervals*
*Effect of different doses (0.3, 30, 50, 100, 200, 300 and 500 mM) of TDCA on the PMC intervals. Values represents mean ± SE of 4 colon segments from different animals. (*P < 0.05) Significantly different compared to the control group.*

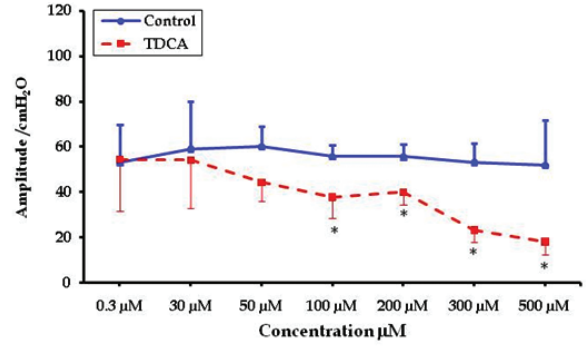

Figure 2. *Effect of TDCA on PMC amplitudes*
*Effect of different doses (0.3, 30, 50, 100, 200, 300 and 500 mM) of TDCA on the PMC amplitudes. Values represents mean ± SE of 4 colon segments from different animals. (*P < 0.05) Significantly different compared to the control group.*

RESULTS

Dose response curve of TDCA

The contractile activity of luminal distension of isolated colon segment expressed physiologically by continuous peaks (amplitudes) separated by relaxation (intervals). The dose response effect of TDCA, a bile salt, on the colonic PMCs was done using different doses (0.3, 30, 50, 100, 200, 300 and 500 µM, n = 28, 4 mice for each dose).

TDCA significantly amplified the values of the PMC intervals (Fig. 1) in a dose response manner after treatment of the colon with the 100, 200, 300 and 500 µM doses compared to the control values (P< 0.03, 0.03, 0.01, 0.01 respectively).

In the meantime, treatment of the mice colon segments with TDCA doses caused significant dose response drop in the amplitudes of the PMCs at the 100, 200,300 and 500 µM doses in comparison with the untreated colon segments (P< 0.02, 0.03, 0.01, 0.05 respectively, Fig. 2).

Effect of TDCA in the presence of Histamine antagonists on PMCs

There was no effect for TDCA treatment (300 µM) after incubation of histamine antagonist (H1, 10 µM) on interval or amplitude of PMCs of mice colon, Fig. 3. Moreover, H1 antagonist (10 µM) induced significant reducing in the PMC intervals (137.2 ± 13 S) compared to the control values (255.8 ± 31 S, P < 0.04), Fig. 5.

Effect of TDCA in the presence of Serotonin agonists on PMCs

The response of serotonin (5-HT, 25 µM) as agonist for bile salt TDCA on colonic motility is illustrated in Fig. 4 and 6. There was no response of 5-HT agonist (25 µM) on interval or amplitude of PMCs regardless applying of TDCA (300 µM).

Discussion

The study findings indicate that TDCA inhibits intestine motility through increasing PMC intervals and decreasing PMC amplitudes. This finding is demonstrated

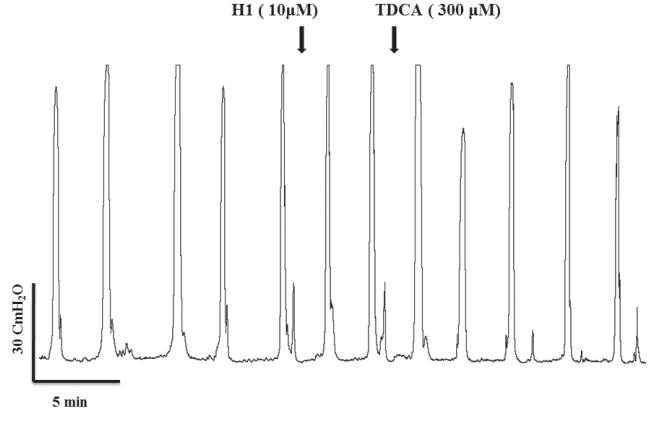

Figure 3. *Effect of TDCA in the presence of histamine antagonist on colon motility*
Representative recording traces showing the effect of TDCA (300 µM) in the presence of histamine antagonist (H1, 10 mM) on colon motility.

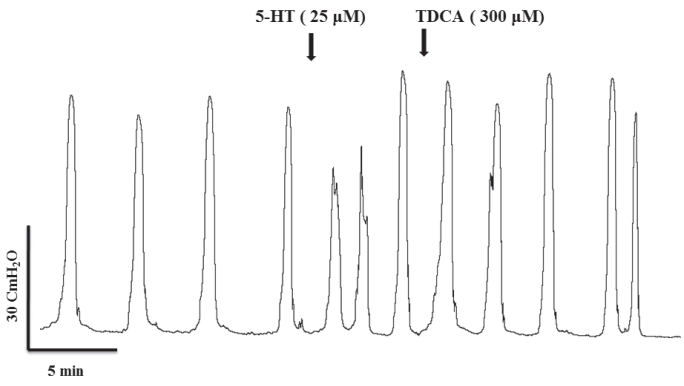

Figure 4. *Effect of TDCA in the presence of serotonin agonist on colon motility*

Representative recording traces showing the effect of TDCA (300 µM) in the presence of serotonin agonist (5-HT, 25 mM) on colon motility

with previous studies (6, 8) while it is inconsistent with (9, 17). Kalia *et al.* (9) reported that TDCA increases the motility through amplification of intestinal secretion, activation of mast cells and other inflammatory cells, while Poole *et al.* (17) suggested the inability of TDCA to inhibit contractile activity of the isolated ileum due to the hydrophilic property of TDCA which does not effectively access target neurons within the small intestine.

The ability of TDCA to inhibit motility can be explained by Mühlbauer *et al.* (7) who suggested molecular mechanisms underlying the bile acid-induced gene expression; TDCA induced IL-8 expression in a dose and time-dependent manner. Martínez-Augustin and his colleague (8) proposed that TDCA can exert direct actions on intestinal epithelial cells by a Ca^{2+} dependent mechanism which is related by to the actions of bile acids in the colon which is claimed to be prostaglandin dependent. TDCA can stimulate induction of cyclooxygenase-2 and release of prostaglandins (18), moreover, mast cells also involve in response to prostaglandins (8).

It is worth to mention that high level of dietary-fat intake increases the flow of bile into the intestine, TDCA was significantly increased in the high fat-intake (19). This is in agreement with this study that TDCA, which is synthesized from cholesterol, produced an inhibition of intestinal motility.

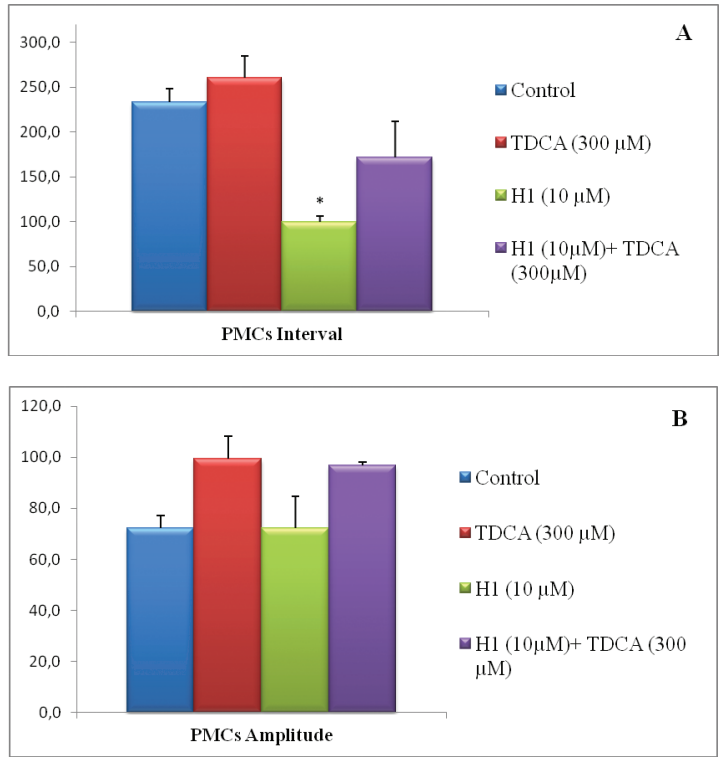

Figure 5. *Effect of TDCA in the presence of histamine antagonist on interval (A) and amplitude (B) of PMCs*

*Effect of TDCA (300 µM) in the presence of H1 antagonist (10 mM) on PMCs intervals (A) and amplitudes (B)of mice colon. Values represents mean ± SE of 4 colon segments from different animals. *Significantly different compared to the control group, (^P < 0.05).*

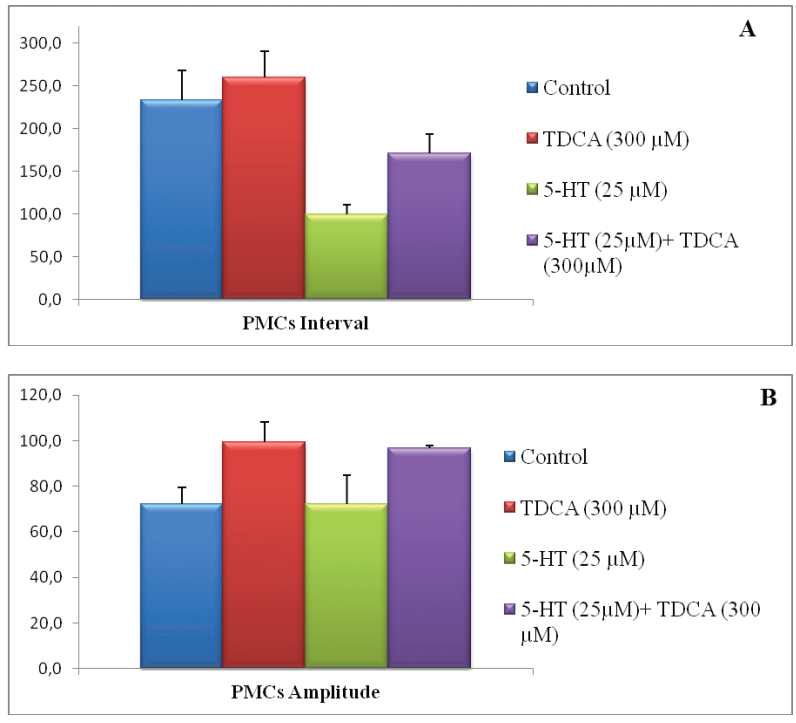

Figure 6. *Effect of TDCA in the presence of serotonin agonist on interval (A) and amplitude (B) of PMCs*
Effect of TDCA (300 µM) in the presence of 5-HT agonist (25 mM) on PMCs intervals (A) and amplitudes (B) of mice colon. Values represents mean ± SE of 4 colon segments from different animals.

Psychological stress plays a major role in functional gastrointestinal disorders, it induced activation or sensitization of mucosal mast cells in the GI tract seem to be involved in stress-associated alterations of visceral sensitivity (20). Psychological stress accelerated reverse cholesterol transport (RCT) by compromising intestinal cholesterol absorption (21) confirming the increase in releasing of TDCA to inhibit the intestinal motility.

Results from this study confirmed that histamine antagonist (10 µM) induced significant reducing in the PMC intervals compared to the control. Earlier studies suggested that antagonists mediate by inhibition of cholinergic neurotransmission through a pertussis toxin sensitive mechanism (6); Histamine antagonist, can block the uptake of monoamines in the central nervous system (22), block central muscarinic receptors (23) and block H1 receptors-mediated channels (10). On other side, pyrilamine enhances dopaminergic activity through H1 receptor blockade at the level of dopamine (22). Cholinomimetic mechanisms are also involved in the regulation of excitatory action of GI smooth muscles (10).

Although autonomic modulation of GI function occurs *via* the actions of neurotransmitters and neuromodulators such as serotonin (24) through mediating the receptors of serotonin of motility and secretion, our results showed no response of serotonin agonist (25 µM) on motor activity with or without TDCA applying (300 µM) which is disagreed with previous study (12, 13, 25). This discrepancy is explained by small dose of applied serotonin (25 µM) since Sasaki-Adams and Kelley (26) suggested that chronically increased levels of 5-HT may facilitate modulatory mechanisms.

In conclusion, this study has shown that TDCA directly inhibited terminal colon motility. It is possible that this effect on colon motor activity exposed by slowing intestinal transit time. These finding is not revealed with low concentration of histamine antagonist and serotonin agonist

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