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Review Article

Bile acid receptors and gastrointestinal functions☆

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ABSTRACT

Bile acids modulate several gastrointestinal (GI) functions including electrolyte secretion and absorption, gastric emptying, and small intestinal and colonic motility. High concentrations of bile acids lead to diarrhea and are implicated in the development of esophageal, gastric and colonic cancer. Alterations in bile acid homeostasis are also implicated in the pathophysiology of irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). Our understanding of the mechanisms underlying these effects of bile acids on gut functions has been greatly enhanced by the discovery of bile acid receptors, including the nuclear receptors: farnesoid X receptor (FXR), vitamin D receptor (VDR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR); and G protein-coupled receptors (GPCRs): Takeda G protein-coupled receptor 5 (TGR5), sphingosine-1-phosphate receptor 2 (S1PR2), and muscarinic acetylcholine receptor M3 (M3R). For example, various studies provided evidence demonstrating the anti-inflammatory effects of FXR and TGR5 activation in models of intestinal inflammation. In addition, the activation of TGR5 in enteric neurons was recently shown to increase colonic motility, which may lead to bile acid-induced diarrhea (BAD). Interestingly, TGR5 induces the secretion of glucagon-like peptide-1 (GLP-1) from L-cells to enhance insulin secretion and modulate glucose metabolism. Because of the importance of these receptors, agonists of TGR5 and intestine-specific FXR agonists are currently being tested as an option for the treatment of diabetes mellitus and primary bile acid diarrhea, respectively. This review summarizes current knowledge of the functional roles of bile acid receptors in the GI tract.

1. Introduction

Bile acids are synthesized in the liver from cholesterol, secreted into the intestine, and reabsorbed in the ileum. Reabsorbed bile acids circulate back to the liver and are resecreted to establish their enterohepatic circulation.[1,2] In the intestine, the entry of bile acids into enterocytes of the distal ileum stimulates the expression and secretion of fibroblast growth factor (FGF) 19 (FGF15 in rodents) that functions as a hormone affecting several biological processes in the liver, including the suppression of bile acid synthesis (Fig. 1). Bile acids in the intestine emulsify dietary lipids and cholesterol to facilitate their absorption. However, extensive research during the last two decades has provided interesting evidence showing that bile acids exert multiple important physiological and pathophysiological roles beside their function as detergents. Since the gut is a large organ that represents the longer limb of the enterohepatic circulation of bile acids, the effects of bile acids on physiological functions of the gut have been extensively studied. The intestine is equipped with elaborate bile acid-sensing mechanisms that allow elegant coordination of different intestinal functions and control the crosstalk between the gut and other organs in the body. Seminal work by several investigators addressed the roles of bile acids as signaling molecules that trigger cellular signaling pathways by activating specific receptors to modulate biological processes. Bile acids have been shown to activate both nuclear receptors and plasma membrane associated receptors expressed in different cell types to elicit the physiological effects of bile acids. In light of the complex nature of the gastrointestinal (GI) tract and the intricate network of signaling induced by bile acids, the influence of bile

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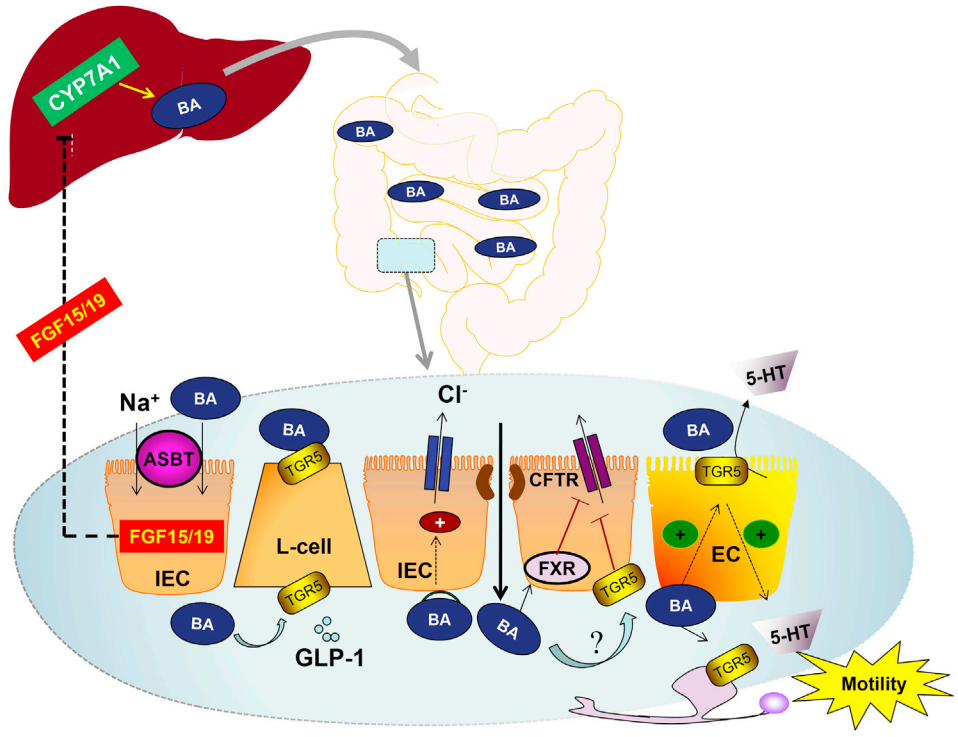

Fig. 1. Bile acid signaling in the intestine. Bile acids are synthesized in the liver by the rate-limiting enzyme CYP7A1 and secreted into the intestine. Bile acids are reabsorbed by IECs in the distal ileum via ASBT where they activate FXR, inducing the expression and secretion of FGF15/19 to inhibit hepatic bile acid synthesis. Luminal and basolateral bile acids activate TGR5 in enteroendocrine L-cells, resulting in the release of the incretin GLP-1, and thus promoting glucose tolerance. Overproduction of bile acids can promote diarrhea by several distinct mechanisms. In the colon, bile acids disrupt barrier integrity allowing bile acids to reach the basolateral membrane of epithelial cells and induce chloride secretion, though the receptor(s) underlying this phenomenon is unknown. Interestingly, colonic FXR and TGR5 activation inhibits the secretion of chloride and other electrolytes, which may be a compensatory mechanism. Excessive bile acids also promote gut motility. Colonic bile acids activate TGR5 on ECs, promoting 5-HT release and motility. Bile acids activate neuronal TGR5, which either stimulates or inhibits motility, depending on the type of neuron and region of the GI tract. Overall, overproduction of bile acids induces colonic secretion and enhances motility, producing a diarrheal phenotype. Abbreviations: 5-HT, 5-hydroxytryptamine; ASBT, apical sodium-dependent bile acid transporter; BA, bile acid; CFTR, cystic fibrosis transmembrane conductance regulator; CYP7A1, cytochrome P450 7A1; EC, enterochromaffin cell; FGF15/19, fibroblast growth factor 15/19; FXR, farnesoid X receptor; GI, gastrointestinal; GLP-1, glucagon-like peptide-1; IEC, intestinal epithelial cell; TGR5, Takeda G protein-coupled receptor 5.

acids on intestinal functions deserves special attention. Many recent advances have been made with respect to our understating of the effects of bile acids on GI tract physiology. In this review, we will focus on summarizing the current knowledge about the roles of bile acid receptors in modulating gut functions.

2. Nuclear receptors

One of the mechanisms by which bile acids regulate intestinal physiology is altering gene expression via binding to and activating nuclear receptors.[3] These are a group of ligand-activated transcription factors that are involved in regulating a variety of biological processes. At least four nuclear receptors are known to be activated by bile acids: farnesoid X receptor (FXR, nuclear receptor subfamily 1, group H, member 4 (NR1H4)), vitamin D receptor (VDR, NR1I1), pregnane X receptor (PXR, NR1I2), and constitutive androstane receptor (CAR, NR1I3).[3] We will summarize in the following sections what is currently known about the roles of bile acid nuclear receptors in influencing gut functions and their relevance to the development of GI diseases.

2.1. Farnesoid X receptor (FXR)

FXR was the first identified receptor for bile acids and its activation meditated the canonical pathways triggered by bile acids.[4–8] FXR is expressed in different types of cells and tissues including the liver and small intestine and has been shown to play crucial roles in the maintenance of bile acid and cholesterol homeostasis as well as glucose and lipid metabolism.[9] Bile acids are the endogenous ligands for FXR with different potency in the following order: chenodeoxycholic acid (CDCA)> deoxycholic acid (DCA)> lithocholic acid (LCA)> cholic acid (CA).[9] There are two genes for FXR: *FXRα* and *FXRβ*. The function of *FXRβ* is not fully understood in mice and it is considered to be a pseudo gene in humans. Thus, the name FXR in this review refers to FXRα. Intestinal FXR was shown to be crucial for controlling bile acid biosynthesis in the liver. Indeed, tissue-specific knock out in mice provided compelling evidence demonstrating that intestinal FXR, via the induction of FGF15/19, is more important than liver FXR in mediating the negative feedback of bile acids on their synthesis in the liver. Beside its importance in controlling hepatic bile acid synthesis, intestinal FXR is relevant to a number of important intestinal functions and is considered as a target for the treatment of a number of GI disorders.[9] We will discuss in the following sections the roles of FXR in modulating intestinal functions and its potential roles in the development of intestinal diseases.

2.1.1. FXR and mucosal immune response

Effects of bile acids on immune cells were known from the early studies of Calmus *et al.*,[10] which showed that the incubation of monocytes with CDCA inhibited lipopolysaccharide (LPS)-stimulated secretion of cytokines including tumor necrosis factor (TNF) α. Since CDCA is a potent activator of FXR, these findings suggested the involvement of FXR in the observed anti-inflammatory effects

of bile acids. In this regard, FXR was found to be expressed in human peripheral blood mononuclear cells (PBMCs) along with other nuclear receptors.[11] These studies showed FXR messenger ribonucleic acid (mRNA) and protein expression in $CD4^+$ T cells, $CD8^+$ T cells, and $CD14^+$ monocytes subpopulations, suggesting a modulatory role for this nuclear receptor in immune responses. Consistently, the synthetic FXR agonist INT-747 (obeticholic acid) decreased TNFα secretion from activated human PBMCs and $CD14^+$ monocytes. Also, INT-747 was able to suppress the secretion of TNFα from mononuclear cells isolated from the lamina propria of inflammatory bowel disease (IBD) patients. It was observed that the FXR agonist not only inhibited the secretion of cytokines from active mononuclear immune cells but also decreased the expression of proinflammatory cytokines in differentiated intestinal epithelial cells.[12] Activation of FXR was also shown to inhibit cellular signaling pathways known to be triggered during inflammation via interactions with activator protein 1 (AP-1) and signal transducers and activators of transcription-3 (STAT3) transcription factors. In addition, FXR inhibits the expression of genes that are known targets for the major modulators of immune responses, namely, nuclear factor-κB (NF-κB) and AP-1 transcription factors.[13–15] Collectively, studies of FXR and the immune response provided strong evidence for the involvement of FXR in the regulation of innate and adaptive immunity, as well as the inhibition of the pro-inflammatory response, suggesting its potential role in the pathophysiology of chronic inflammatory diseases of the intestine.

2.1.2. FXR and intestinal inflammation

IBDs, which consist of Crohn's disease and ulcerative colitis (UC), are chronic inflammatory conditions of the gut. While the development of intestinal inflammation is multifactorial, there is recent experimental evidence suggest that increased levels of luminal bile acids may play a role in the progression of these disorders.[16,17] FXR expression was shown to be reduced in patients with Crohn's disease and in mouse models of colitis.[18] Since FXR was shown to influence immune function, recent studies have focused on its involvement in IBD. Furthermore, the studies of Gadaleta *et al.*[12] showed that administration of INT-747 protected mice from dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis. On the other hand, induction of inflammation by DSS and TNBS in *FXR* knockout mice resulted in more severe inflammation and increased levels of proinflammatory cytokines, which was accompanied by a greater loss in body weight as compared to wildtype mice.[18] Also, administration of an FXR agonist failed to attenuate DSS- or TNBS-induced inflammation in *FXR* knockout mice.[18] These findings strongly suggest that a decrease in FXR expression contributes to the development of intestinal inflammation and indicate that exploitation of the anti-inflammatory effects of FXR should be considered for the treatment of IBD.

2.1.3. FXR, gut bacteria, and intestinal permeability

In addition to the role of bile acids in modulating immune response, it has become evident that blocking the secretion of bile acids and decreasing bile acid concentrations in the colonic lumen result in bacterial overgrowth and compromised epithelial barrier function, leading to enhanced bacterial translocation across colonic epithelia.[19,20] Studies showed that the administration of CDCA to rats with obstructive jaundice decreased bacterial overgrowth and translocation, providing supportive evidence for the protective roles of bile acids and suggesting the involvement of FXR in this process.[19,21] The first evidence directly supporting the antibacterial effects for FXR was established by the studies of Inagaki *et al.*[20] The authors showed that in mice subjected to bile duct ligation, administration of the FXR agonist GW4064 attenuated bacterial overgrowth and translocation. Further, they showed that FXR deficient mice exhibited enhanced bacterial overgrowth accompanied by increased translocation of bacteria to mesenteric lymph nodes. Activating FXR by GW4064 resulted in a significant increase in the expression of genes in epithelial cells that are known to have antibacterial effects, including angiogenin, carbonic anhydrase 12, inducible nitric oxide, and interleukin (IL)-18.[19,20] Similar results were shown in recent studies using the INT-747, which further demonstrated that experimentally-induced obstructive jaundice in rats is associated with increased epithelial permeability along with elevated expression of claudin-2.[22] The authors showed that obeticholic acid normalized intestinal permeability and increased the expression of tight junction proteins claudin-1 and occludin. The conclusion from these studies and many others has established pivotal roles of FXR in the protection against mucosal damage by inducing antibacterial effects and maintaining epithelial integrity.

2.1.4. FXR and bile acid-induced diarrhea (BAD)

Increased levels of luminal bile acids in the colon induces chloride secretion and impairs fluid absorption, leading to BAD.[23] BAD often presents secondary to ileal resection or ileal disease such as Crohn's disease.[23] However, there is also a prevalent type of primary (idiopathic) BAD that is common in patients with functional bowel disorders. Recent studies suggested that primary BAD is associated with overproduction of bile acids from the liver due to a decrease in ileal FGF19/15 secretion and attenuated feedback inhibition.[24] Since activation of intestinal FXR stimulates the secretion of FGF19, it is reasonable to propose that FXR agonists are useful for the treatment of bile acid diarrhea. Recent studies indeed have shown promising results demonstrating that treatment with obeticholic acid was well-tolerated in patients with BAD and caused a decrease in hepatic synthesis of bile acids along with improved symptoms of diarrhea.[25] It should also be noted that the studies of Mroz *et al.*[26] showed that FXR agonists exhibited anti-secretory effects by reducing the expression of the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR) and inhibiting the activity of Na^+/K^+-ATPase transporter on the basolateral membrane of epithelial cells. Thus, the influence of FXR activation on epithelial transport processes may also contribute to the antidiarrheal effects of FXR agonists (Fig. 1).

2.1.5. FXR and colorectal cancer

Multiple lines of evidence suggest a role for FXR in the development of colorectal cancer. The studies of Bailey *et al.*[27] showed that FXR expression is decreased in precancerous lesions and absent in the majority of samples from stages I to IV colonic adenocarcinoma patients. The authors showed that this silencing of FXR was the result of FXR promoter hypermethylation and V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) signaling. The findings implicate FXR in the suppression of genes involved in the epithelial-to-mesenchymal transition and other oncogenic signaling pathways.[27] Similar findings were also reported showing a decrease in FXR in colonic polyps and a remarkable reduction in its expression in colon adenocarcinoma.[28] In other studies, the loss of FXR was associated with an increase in the number and the size of intestinal epithelial tumors in mice treated with azoxymethane (AOM).[29] Also, the lack of FXR was shown to be associated with an increased tumor progression and early mortality in the $Apc^{Min/+}$ (a genetically predisposed model of human familial adenomatous polyposis) and in chronic colitis mouse models.[29] The authors demonstrated an activation of Wnt signaling by infiltrating neutrophils and elevation in the levels of TNFα secreted from macrophages when FXR was absent. Importantly, the administration of cholestyramine, a bile acid-binding resin, did not affect the

promotion of tumorigenicity in *FXR*$^{-/-}$ mice, clearly demonstrating that the loss of FXR and not the increase in bile acids is responsible for the high susceptibility to tumorigenesis. These findings provided novel insights into the potential role of FXR as a target for the treatment of colorectal cancer.

2.2. VDR, PXR, and CAR

In addition to FXR, it has also been shown that bile acids bind to VDR, PXR, and CAR. In contrast to FXR, bile acids are not the main ligands for these receptors. VDR is activated by calcitriol (1,25-dihydroxyvitamin D3) and by the oncogenic secondary bile acid LCA, but not by other bile acids.[30,31] The activation of VDR resulted in the induction of cytochrome P450 3A4 (CYP3A4) that is responsible for detoxifying LCA.[30] Also, the activation of VDR resulted in a suppression of CYP7A1 expression in the liver, decreasing hepatic bile acid synthesis.[32] Interestingly, intraperitoneal (IP) injection of vitamin D3 resulted in induced expression of ileal FGF15 in mice, suggesting that FGF15 is also a target of VDR.[33] Furthermore, VDR is also known to increase the expression of the ileal bile acid transporter apical sodium-dependent bile acid transporter (ASBT).[34] Collectively, activation of VDR may be involved in increasing bile acid absorption, suppressing hepatic bile acid synthesis, and promoting the expression of enzymes involved in the detoxification of LCA.

PXR is primarily activated by xenobiotics as well as drugs such as rifampicin and is responsible for the induction of phase I and phase II metabolism of many compounds including bile acids. LCA is the main bile acid ligand for PXR, and PXR activation induces the expression of detoxifying enzymes (including CYP3A family enzymes), as well as enzymes involved in sulphation and conjugation of bile acids.[3,9] Indeed, activation of PXR has been suggested as a useful approach to attenuate the cytotoxic effects of bile acids during cholestasis.[3,9] Furthermore, activating PXR in colonic cell line was shown to induce the expression of the FGF19, suggesting that PXR also contributes to the suppression of bile acid synthesis in the liver.[35] CAR has also been shown to bind LCA and cooperate with PXR in stimulating the pathways involved in LCA detoxification in the liver.[36]

3. Membrane receptors: G protein-coupled receptors (GPCRs)

Besides their roles as ligands for the nuclear receptors, a broad spectrum of bile acid effects throughout the body are now known to be mediated by direct activation of GPCRs at the plasma membrane. Three major GPCRs are known to interact with bile acids to modulate GI functions: the Takeda G protein-coupled receptor 5 (TGR5; also known as the G protein-coupled bile acid receptor 1 (GPBAR1)),[37] the muscarinic acetylcholine receptor M3 (M3R),[38] and the sphingosine-1-phosphate receptor 2 (S1PR2).[39] Although little is known about the roles of M3R and SIPR2 in modulating GI functions, extensive work has demonstrated the importance of TGR5 in controlling a wide range of GI physiology.[40] We will focus in this section on reviewing the current knowledge regarding the roles of these receptors in modulating gut function, and with a focus on TGR5.

3.1. TGR5

A major breakthrough came with the discovery of a plasma membrane-associated GPCR mediating non-genomic effects of bile acids through $G_{\alpha s}$.[40] This receptor is widely referred to as the TGR5 and has been implicated in a wide range physiological functions in the body such as maintaining energy homeostasis and stimulating insulin secretion.[40] Bile acids are the endogenous ligands for TGR5 and the potency of different types of bile acids are in the order of LCA > DCA > CDCA > CA.[9] In the following sections, we will attempt to summarize the results of a large body of literature related to the involvement of TGR5 in modulating GI functions.

3.1.1. Role of TGR5 in modulating intestinal electrolyte transport and gut motility

Excessive bile acids in the lumen of the small intestine and colon has long been known to cause diarrhea.[41–43] Earlier studies in humans showed that perfusion of the colon with bile acids stimulated water and electrolyte secretion and inhibited absorption.[41,44] In particular, bile acid-dependent induction of chloride secretion across colonic epithelia was shown using different experimental models.[45,46] One noteworthy aspect of this phenomenon is the sidedness of bile acid effects on chloride secretion. Interestingly, chloride secretion from colonic epithelial monolayers was shown to be significantly induced when cells were exposed to bile acids from the basolateral membrane, while higher concentrations of bile acids at the luminal membrane of colonic epithelial monolayers were needed to stimulate chloride secretion.[47] The observed effect on chloride secretion from the luminal membrane has been attributed to compromised epithelial tight junctions that allow bile acids to reach the basolateral membrane, where they subsequently stimulate chloride secretion (Fig. 1).[47] The sidedness of bile acid effects on chloride secretion indicated a mechanism by which bile acids may activate a membrane receptor exclusively expressed on the basolateral membrane of epithelial cells. In this regard, the expression of TGR5 was recently shown in T84 cells and in epithelial cells of the rat colon.[47,48] However, potent activators of TGR5 including LCA and ciprofloxacin failed to induce chloride secretion in T84 cells.[49] Also, the activation of TGR5 by the synthetic agonist INT-777 rather decreased basal secretion in rat colon and significantly attenuated Ca^{2+}-dependent chloride secretion.[48] These observations strongly indicated that TGR5 is not involved in bile acid-induced chloride secretion.

Bile acids are also well known to modulate intestinal and colonic motility, but until recently the underlying mechanisms were poorly understood. Bile acids inhibit gastric emptying and reduce small intestinal transit time but stimulate colonic peristalsis and increase colonic transit time.[50,51] With respect to gastric emptying, TGR5 was found to be expressed in gastric smooth muscle, where its activation triggers a signaling via exchanged protein directly activated by 3′,5′-cyclic adenosine monophosphate (cAMP) (Epac) and protein kinase A (PKA)-mediated pathway to inhibit RhoA and cause gastric muscle relaxation.[52] Besides gastric smooth muscle, TGR5 was also shown to be expressed in nitrergic inhibitory neurons of the myenteric plexuses in the small intestine, colon, and stomach, as well as in the cholinergic neurons of the submucosal plexuses in the small intestinal and colon.[53] TGR5 expression was also detected in the intrinsic primary afferent neurons (IPAN) that represent the sensory limb of the enteric neurogenic reflexes of the gut.[53] Studies in transgenic mice provided important insights into the roles of TGR5 in modulating gut motility. In this regard, the lack of TGR5 in *TGR5* knockout mice resulted in a constipated phenotype with a decline in colonic transit time, a reduction in the frequency of defecation, and a decrease in water content in the stool.[54] On the other hand, TGR5 overexpression in transgenic mice caused a faster colonic transit time as well as an increased frequency of defecation. Alemi *et al.*[54] further showed that the induction of colonic peristalsis by bile acids was inhibited by antagonists to 5-hydroxytroptamine (5-HT) and calcitonin gene-related peptide (CGRP), suggesting the involvement of 5-HT and CGRP in mediating the effects of TGR5 receptor on motility (Fig. 1). It is interesting to note that TGR5 was recently shown to be expressed in enterochromaffin cells (ECs) of the colon but not in the small intestine, suggesting that bile acids may directly induce 5-HT secretion only

in the colon.[55] The secreted 5-HT from EC may also contribute to bile acid effects on motility.

Overall, TGR5 appears to play a key role in coordinating the responses of different intestinal cell types to bile acids. For instance, activation of TGR5 on L-cells may be involved in mediating the ileal brake phenomenon, resulting in a decrease in small intestinal motility and slowing of gastric emptying. Also, TGR5 on ECs stimulates the secretion of 5-HT to enhance peristalsis in the colon. The presence of TGR5 in IPAN sensory neurons triggers the enteric reflex, promoting peristalsis mediated by CGRP. Peristalsis occurs by orchestrated contraction and relaxation of circular and longitudinal smooth muscles of the GI tract. The direct activation of TGR5 on inhibitory neurons leads to inhibition of longitudinal muscle contractility, consistent with the induction of peristalsis. It is intriguing to note that the activation of TGR5 seems to mediate the laxative effects of bile acids mainly by altering gut motility patterns and not via induced secretion. Indeed, the activation of TGR5 on cholinergic secretomotor neurons in the submucosal plexuses was shown to inhibit basal as well as agonist-stimulated electrolyte secretion from the colonic epithelia.[56]

With regard to diarrhea, it appears that the roles of bile acid receptors are complex and may be summarized as follows: diarrhea is multifactorial and may result from increased colonic motility, increased electrolyte secretion, decreased electrolyte absorption, or a combination of these factors.[43] With respect to TGR5, the activation of this receptor on enteric neurons causes an increase in colonic motility. However, TGR5 on cholinergic neurons in submucosal plexuses inhibits electrolyte secretion. Also, TGR5 activation results in a decrease in agonist-induced chloride secretion in epithelial cells. The inhibition of secretion by TGR5 could be construed as compensatory to stimulated colonic motility. Therefore, TGR5 expressed on enteric neurons and ECs, but not the TGR5 expressed on epithelial cells, may explain BAD. However, bile acids directly induce chloride secretion when added to the basolateral membrane of epithelial cell and the receptor(s) involved remain to be identified (Fig. 1). Finally, it should be noted that the effects of TGR5 on electrolyte absorption have not been fully delineated. We have previously shown that bile acids decrease chloride uptake in epithelial cells.[44] However, the role of TGR5 or other receptors in mediating bile acid-induced inhibition of chloride uptake has not been thoroughly investigated.

Not only has TGR5 been shown to modulate GI motility, it has also been demonstrated that alterations in TGR5 expression may also be involved in the pathophysiology of several disease states. In this regard, an increase in TGR5 expression in the gastric myenteric plexus was implicated in the pathogenesis of delayed gastric emptying in mice.[57] These studies showed that depleting the bile acid pool using cholestyramine reversed the effects of high fat diet on TGR5 expression and gastric emptying. These data suggest that the high levels of serum bile associated with high fat diets may explain the increase in TGR5 expression. Along with studies in mice, recent investigations in humans showed that a functionally relevant single nucleotide polymorphism (SNP) in the *TGR5* gene (rs11554825) increases susceptibility to fast colonic transit time and increased levels of fecal bile acids in carriers of this allele.[58] This evidence reinforces the essential role of TGR5 in gut motility and supports the notion that alterations in TGR5 expression may be involved in the development of disorders such as delayed gastric emptying and irritable bowel syndrome (IBS). It is clear from these studies that TGR5 is an attractive therapeutic target for the management of functional GI and motility disorders.

3.1.2. Emerging role of TGR5 in intestinal inflammation

The expression of TGR5 has been established in immune cells including monocytes and macrophages.[37,59] In macrophages activated by LPS, TGR5 agonists suppressed the production of TNFα through the mammalian target of rapamycin (mTOR) pathway.[60] These studies also showed that TGR5 deficiency in macrophages was associated with an increase in cytokine secretion and macrophage migration.[60] Such findings represent strong evidence for the important role of TGR5 in chronic inflammatory conditions. Incubation of human peripheral blood monocytes with a specific TGR5 agonist (benzyl 2-keto-6-methyl-4-(2-thienyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate) but not an FXR agonist (fexaramine) induced differentiation into dendritic cells with an IL-12 hypo-producing phenotype.[61] IL-12 produced by dendritic cells promotes the immune response mediated by type 1 helper T cells, contributing to the pathogenesis of chronic inflammatory diseases such as IBD.

In this regard, Cipriani *et al.*[62] showed TGR5 agonists (ciprofloxacin and oleanolic acid) mitigated intestinal inflammation induced by TNBS in mice. Consistent with the anti-inflammatory effects of TGR5 agonists, the authors showed that *TGR5* knockout mice are more susceptible to TNBS-induced colitis. Similarly, TGR5 agonists were also shown to reduce the inflammatory index in the DSS model of induced colitis and other models of intestinal and gastric epithelial injury, such as that induced by acetylsalicylic acid.[63] It is also notable that the lack of TGR5 resulted in altered epithelial tight junctions associated with increased intestinal permeability, which may contribute to the increased susceptibility to experimentally-induced intestinal inflammation.[62]

One important observation is that TGR5 expression was increased in the colon of mice with TNBS-induced colitis and in patients with Crohn's disease.[62] Furthermore, it was shown that the majority of $CD14^+$ cells isolated from intestinal lamina propria of inflamed intestine in mice treated with TNBS and from patients with Crohn's disease are also positive for TGR5. The fact that TGR5 exhibits anti-inflammatory effects highlights the potential for targeting TGR5 in the treatment of intestinal inflammation. Indeed, a recent study by Biagioli *et al.*[64] demonstrated that a putative small molecule activator of TGR5, BAR501, was able to significantly decrease the disease activity index in TNBS-induced colitis by about 70%. This decrease in the inflammatory response was associated with a shift in the macrophages of the lamina propria from the classically activated macrophages ($CD11b^+$, $CCR7^+$, $F4/F80^-$) to alternatively activated phenotype ($CD11b^+$, $CCR7^-$, $F4/F80^+$). The treatment with TGR5 agonist resulted in a decrease in the levels of pro-inflammatory cytokines (TNFα, IL-1b, IL-6 and C-C motif chemokine ligand 2), along with an increase in the levels of anti-inflammatory cytokines (IL-10 and transforming growth factor β). Interestingly, TGR5-mediated shift in M1/M2 phenotype of intestinal macrophages was dependent on IL-10, as the anti-inflammatory effects of 6β-ethyl-3a, 7b-dihydroxy-5b-cholan-24-ol (BAR501) were lost in *IL-10* knockout mice.[64]

3.1.3. TGR5 and gut hormone secretion: beneficial role in metabolic disorders

The link between bile acids and glucagon-like peptide-1 (GLP-1) secretion was initially established by Katsuma *et al.*,[65] who demonstrated an increase in the secretion of this incretin from the mouse STC-1 enteroendocrine cell line in response to the incubation with bile acids (Fig. 1). The increase was blocked by inhibitors of adenylate cyclase (MDL12330A),[65] strongly suggesting the involvement of TGR5 receptor. These results were later confirmed using the specific TGR5 synthetic agonist INT-777 and were reproduced in various experimental models.[40,66] Similar observations were also made in humans. Rectal administration of taurocholic acid in male human subjects showed an increase in both GLP-1 and peptide YY (PYY).[67] Indeed, activation of TGR5 receptor on L-cells also triggers the secretion of PYY along with GLP-1 via

a cAMP-dependent mechanism and the involvement of Epac/phospholipase C (PLC)-mu/Ca^{2+} pathway.[68] These studies also showed that H_2S, which may be generated by gut bacteria, blocks TGR5-mediated secretion of GLP-1 and PYY via the inhibition of PLCε/Ca^{2+} pathway.[68] These findings have significant implications showing that bacterial products may influence the induction of GLP-1 secretion by bile acids.

Because TGR5 activation induces the secretion of the incretin hormone GLP-1, it is reasonable to expect that TGR5 may be intimately involved in the control of glucose homeostasis. Indeed, the beneficial effects of TGR5 activation in the control of hyperglycemia and hyperinsulinemia have been shown in mouse models of diet-induced obesity as well as in human patients. These studies showed that treatment of obese mice with the bile acid sequestrant colesevelam reduced hepatic glucose production by blocking hepatic glycogenolysis.[69] Using both *TGR5* knockout mice and GLP-1 antagonists, these studies further showed that the effects of colesevelam on hepatic glucose production were mediated by the TGR5-GLP-1 axis. Bile acid sequestrants such as colesevelam are resins that block bile acid absorption, retaining them in the intestinal and colonic lumen. The authors of this study posited that luminal colesevelam-bile acid complexes remain capable of activating TGR5. This hypothesis was proven using non-polarized HEK 293 cells as an *in vitro* model. Also, injecting colesevelam-bile acid complexes via the rectum was able to stimulate GLP-1 secretion. However, the idea that bile acid-sequestrant complexes activate TGR5 was later challenged by at least two studies. In the first study, Brighton *et al.*[70] showed that adding bile acids to the basolateral side of the intestinal epithelia was more effective in inducing GLP-1 secretion from L-cells than the luminally added bile acids. They also showed that the luminal effects were abolished in the presence of an ASBT inhibitor. In the second study, GLP-1 secretion was assessed in metformin-treated patients after cholecystokinin (CCK) infusion to induce the release of bile acids. The results showed that the secretion of bile acids into the gut lumen by CCK-mediated gallbladder contraction caused an increase in GLP-1 secretion. Importantly, this effect was blocked by oral supplementation of bile acid sequestrant sevelamer.[71] Furthermore, administration of CDCA to diabetic patients was able to increase GLP-1 level when given alone but not when it was given with colesevalam.[72] Taken together, these findings strongly suggest that the luminal uptake of bile acids via ASBT is essential for the delivery of bile acids to the basolateral side of L-cells to activate TGR5 and secrete GLP-1 (Fig. 1). However, this conclusion is in conflict with the earlier proposition of luminal bile acids activating TGR5-GLP1, even if they are complexed with sequestrants. The discrepancy between these findings requires further critical investigation.

In relation to obesity, strong evidence suggests that alterations in bile acid homeostasis and TGR5-mediated increase in GLP-1 levels drive the beneficial effects of Roux-en-Y gastric bypass surgery on weight loss and improved insulin sensitivity, independent of restricted caloric intake.[73,74] There is also convincing evidence for the role of TGR5 in the beneficial response to other types of bariatric surgeries such as vertical sleeve gastrectomy.[75]

In light of these numerous studies, TGR5 receptor agonists (synthetic or naturally produced by gut bacteria) are now considered as therapeutics for the treatment of diabetes mellitus due to their ability to induce GLP-1 secretion.[74,76] However, TGR5 agonists my cause systemic side effects such as increased gallbladder filling, as TGR5 is also expressed in the gallbladder.[77] Thus, recent studies have focused on the development of poorly absorbed agonists to avoid systemic effects.[77] Moreover, the combination of TGR5 agonists with inhibitors of the dipeptidyl peptidase-4 (DPP-4) potentiated the glucose lowering effects, galvanizing the research in this area for developing advanced and more effective interventions for the management of hyperglycemia in diabetic patients.[77] For instance, recent studies by Pathak *et al.*[78] using the dual FXR and TGR5 agonist (INT-767) have unraveled novel pathways via which FXR increases TGR5 expression to enhance GLP-1 secretion. The same group further showed the important role of altered gut microbial composition in mediating the effects of the intestine-specific FXR agonist (fexaramine) on TGR5 signaling. They demonstrated that antibiotic treatment blunted the effects of fexaramine on GLP-1 secretion, impairing the subsequent improvement in insulin sensitivity and metabolic conditions.[79] These studies highlight the increased efficacy of treating obesity and diabetes mellitus by simultaneously activating both FXR and TGR5.

3.1.4. TGR5 and GI cancer

The link between TGR5 signaling and tumorigenesis was first shown in human gastric carcinoma AGS cells.[80] These studies of Yasuda *et al.*[80] demonstrated an induction of epidermal growth factor receptor (EGFR) phosphorylation in response to incubation with bile acids, an event that was blocked by small interfering RNA (siRNA)-mediated attenuation of TGR5 expression. Further, studies by Cao *et al.*[81] showed an increase in TGR5 in the majority of cases of gastric intestinal-type adenocarcinoma. Interestingly, the level of TGR5 expression in these studies was also shown to negatively correlate with patient survival. The authors further showed that the activation of TGR5 and coupling to $G_{\alpha q}$ and $G_{\alpha i\text{-}3}$ proteins enhances the proliferation of gastric adenocarcinoma cells. Studies by Carino *et al.*[82] provided additional evidence for the increased expression of TGR5 in advanced stages of gastric cancer and showed that TGR5 activation increases the expression of markers for epithelial-mesenchymal transition (EMT) in gastric cancer cells. These studies suggest the pivotal role for TGR5 in the development of gastric cancer.

The reflux of bile acids back into the stomach and esophagus is also implicated in the development of Barrett's esophagus and esophageal adenocarcinoma (EAC).[83] Strong evidence showed higher levels of TGR5 expression in human tissues of EAC as compared to normal esophageal mucosa and to mucosa from Barrett's esophagus.[84] It was shown that TGR5 expression was associated with worse survival in EAC patients.[84] The activation of TGR5 receptor in EAC cells was linked to the increase in inducible nitric oxide synthase, nicotinamide adenine dinucleotide phosphate (reduced) (NADPH) oxidase NOX5-S, and the production of H_2O_2.[83] The pathway was further delineated, and the evidence suggested that in EAC cells, bile acid-dependent activation of TGR5 caused DNA damage via NOX5-S and cAMP-response element binding (CREB) transcription factor.[85] Interestingly, there was an association between TGR5, VDR, and claudin-2 expression, suggesting a potential mechanism that may contribute to the development of EAC.[86] It is also interesting to note that increased expression of TGR5 in EAC patients was more prominent in males as compared to females, which may help to explain the increased incidence of EAC in males.[84]

3.2. Muscarinic receptors

Earlier studies demonstrated the selective interactions and activation of muscarinic receptors by bile acids in gastric epithelial cells.[87] Studies further showed that the activation of muscarinic receptor M3R in colonic epithelial cells caused a transactivation of EGFR and an increase in cell proliferation.[88] Interestingly, mice lacking ASBT, in which the colonic levels of bile acids are significantly higher due to the malabsorption, exhibited a significant increase in the number and size of tumors when challenged with the carcinogen AOM.[89] These findings could be explained by the involvement of M3R muscarinic receptor in the induction of proliferation of intestinal epithelial cells and the promotion of tumor progression.

Table 1
Functional roles of bile acid receptors in the gut.

Name	Major functions in the intestine	Consequences of loss of function	References
Nuclear receptors			
FXR	(i) Bile acid and cholesterol homeostasis via ileal FGF15/19 (ii) Suppression of mucosal immune response (iii) Maintenance of intestinal barrier	(i) Increased susceptibility to intestinal inflammation (ii) Bacterial overgrowth (iii) Increased bile acid production, bile acid diarrhea (iv) Promoting colorectal tumorigenesis	3,6,7
VDR	(i) Detoxification (ii) Modulation of bile acid biosynthesis via FGF15/19		3,30
PXR	Detoxification		3,36
CAR	Detoxification		3,36
Membrane receptors			
TGR5 (GPBAR1)	(i) Delayed gastric emptying (ii) Increased colonic motility (iii) Anti-inflammatory response (iv) Induction of incretin secretion and modulation of glucose homeostasis via GLP-1	(i) Disturbed motility that varies between intestinal regions (ii) Reduced frequency of defecation (iii) Low stool water content (iv) Disturbed glucose homeostasis	37,54,77
M3R	Cell proliferation		87
S1PR2	(i) Hepatic lipid homeostasis (ii) Cell proliferation		90,94

Abbreviations: FXR, farnesoid X receptor; VDR, vitamin D receptor; PXR, pregnane X receptor; CAR, constitutive androstane receptor; TGR5, Takeda G protein-coupled receptor; GPBAR1, G protein-coupled bile acid receptor 1; M3R, muscarinic acetylcholine receptor M3; S1PR2, sphingosine-1-phosphate receptor 2; FGF15/19, fibroblast growth factor 15/19; GLP-1, glucagon-like peptide-1.

3.3. *Sphingosine-1-phosphate receptors*

S1PR2 was recently shown to be activated by bile acids. Several studies showed that conjugated bile acids stimulate extracellular signal-regulated kinase (ERK) 1/2 as well as protein kinase B (AKT)-dependent pathways in hepatocytes in a manner dependent on $G_{\alpha i}$ to induce the activity of nuclear sphingosine kinase 2 (Sphk2), leading to subsequent increase in nuclear sphingosine-1-phosphate (S1P).[90] Such an increase in S1P inhibits specific histone deacetylases (HDACs), resulting in altered gene expression. This novel bile acid-S1PR2-Sphk2 axis has been implicated in the modulation of processes involved in lipid metabolism. Mice deficient in S1PR2 were more susceptible to the development of fatty liver when fed with a high fat diet.[91] Also, activation of S1PR2 by bile acids regulates hepatic glucose metabolism in a manner similar to that of insulin.[92] S1PR2 activation by bile acids was also implicated in the development of cholangiocarcinoma.[93]

Although much of the research on bile acids and S1PR2 focus on the liver, a recent study showed that S1PR2 is expressed in intestinal epithelial cells and suggested that its activation by conjugated bile acids promotes cell proliferation.[94] Nevertheless, the role of S1PR2 in intestinal physiology requires further investigation.

4. Conclusions

Bile acid effects on the GI tract have shown regional differences between the different segments of the tract. The advances in our knowledge about the receptors mediating the effects of bile acids has provided clues that have helped to explain the differential effects of bile acids in the segments of the gut (Table 1). For example, TGR5 mediates the effects of bile acids in the progression of tumors in the upper GI tract (gastric and esophageal), whereas the loss of FXR expression may be involved in the development of colorectal cancer. Also, activation of TGR5 on gastric smooth muscle results in delayed gastric emptying associated with feeding high fat diet, whereas activation of enteric TGR5 in myenteric pluses in the colon may explain the increase in colonic motility and diarrhea associated with increased levels of bile acids in the colon. Despite the extensive work that has been done, additional investigations are still needed to fully understand the complex network of events that underlie the many interactions between bile acids and the GI system. The receptors involved in the induction of chloride secretion in intestinal epithelial cells and the mechanisms underlying their activation remain elusive. Also, the exact roles of bile acid receptors in shaping the composition of gut microbiota are in interesting area of investigation. Expanding our knowledge about the functional roles of bile acid receptors in the gut will enhance our understanding of gut related disorders and will unravel potential targets for improved therapeutic interventions to treat diseases such as IBD and gut functional disorders.

Authors' contributions

A. L. Ticho, R. K. Gill, and W. A. Alrefai drafted the manuscript. A. L. Ticho, P. Malhotra, P. K. Dudeja, R. K. Gill, and W. A. Alrefai edited the manuscript. A. L. Ticho and W. A. Alrefai approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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