Acta Pharmaceutica Sinica B 2024;14(5):1939−1950

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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REVIEW

Bile acids and coronavirus disease 2019

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Received 14 August 2023; received in revised form 8 December 2023; accepted 28 January 2024

KEY WORDS

Bile acids;
COVID-19;
Farnesoid X receptor;
G-protein-coupled bile acid receptor;
SARS-CoV-2;
Angiotensin-converting enzyme 2;
Ursodeoxycholic acid;
Chenodeoxycholic acid

Abstract The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been significantly alleviated. However, long-term health effects and prevention strategy remain unresolved. Thus, it is essential to explore the pathophysiological mechanisms and intervention for SARS-CoV-2 infection. Emerging research indicates a link between COVID-19 and bile acids, traditionally known for facilitating dietary fat absorption. The bile acid urso deoxycholic acid potentially protects against SARS-CoV-2 infection by inhibiting the farnesoid X receptor, a bile acid nuclear receptor. The activation of G-protein-coupled bile acid receptor, another mem brane receptor for bile acids, has also been found to regulate the expression of angiotensin-converting enzyme 2, the receptor through which the virus enters human cells. Here, we review the latest basic and clinical evidence linking bile acids to SARS-CoV-2, and reveal their complicated pathophysiological mechanisms.

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Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2024.02.011

1. Introduction

Bile acids are known for aiding digestion and the absorption of lipids and fat-soluble vitamins. Recent research suggests that they also regulate the expression of angiotensin-converting enzyme 2 (ACE2), the receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. Although the coronavirus disease 2019 (COVID-19) pandemic has been significantly alleviated, its long-term health effects and prevention strategy are still unresolved[2,3]. Thus, it is essential to explore the pathophysiological mechanisms and interventions for SARS-CoV-2 infection. COVID-19 is a highly contagious respiratory disease that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The SARS-CoV-2 virus primarily enters host cells by binding to ACE2. A recent study has demonstrated that ursodeoxycholic acid (UDCA) can reduce ACE2 levels by inhibiting the bile acid nuclear receptor farnesoid X receptor (FXR) in the nasal epithelium, which leads to positive clinical outcomes in COVID-19 patients[4]. The activation of G-protein-coupled bile acid receptor (GPBAR, also known as TGR5), another receptor for bile acids, has also been found to regulate the expression of ACE2[5]. Additionally, bile acids act as cell signaling moleculars that can modulate the immune system and inflammatory response[6,7]. Metabolite reanalysis has shown that tauroursodeoxycholic acid and taurocholic acid are elevated in COVID-19 patients, both of which have been implicated in alleviating inflammation in the intestines and bile ducts[8]. All of the evidence highlights an intriguing association between bile acids and COVID-19. This suggests that drugs like obeticholic acid (OCA), currently approved for treating primary biliary cholangitis, which indirectly decreases bile acid synthesis *via* FXRs, could have both direct and indirect effects on the immune response to SARS-CoV-2 infection and be an effective treatment for COVID-19[9]. Here, we discuss the latest basic and clinical evidence linking bile acids with COVID-19 and summarize the probable underlying mechanisms with a focus on bile acid receptors.

2. Bile acid system

2.1. Bile acids

Bile acids encompass a diverse array of amphipathic steroid acids and serve as the principal organic constituents of bile. They can be classified into primary and secondary. Primary bile acids are synthesized by hepatocytes from cholesterol *via* both the classical pathway and the alternative one, inhibiting interspecies variability[10,11]. For instance, humans produce cholic acid (CA) and chenodeoxycholic acid (CDCA). In mice, CDCA undergoes conversion to α- and β-muricholic acids (MCA), while in pigs, it is transformed into hyocholic acid[12]. Most primary free bile acids subsequently undergo conjugation with taurine (predominantly in mice) or glycine (primarily in humans) *via* bile acyl-CoA synthetase and bile acid-CoA: amino acid *N*-acyltransferase, enhancing their hydrophilicity and facilitating passage through the bile canalicular[13].

After synthesis, primary bile acids are secreted into bile and sequestered in the gallbladder, which contracts postprandially to discharge bile into the duodenum *via* the common bile duct. A minor proportion of primary bile acids is transformed into secondary bile acids through microbial enzyme-mediated reactions in the ileum and proximal colon. In humans, CA is metabolized into deoxycholic acid (DCA) and CDCA into lithocholic acid (LCA) and UDCA. Mice and rats predominantly convert β-MCA to ω-MCA[14]. Over 95% of bile acids are absorbed by either passive diffusion or active transport from the terminal ileum and conveyed to the liver *via* the portal vein, where free bile acids are reconjugated in hepatocytes and reintroduced into bile. This continuous process of bile acid exchange between the liver and intestines, termed "enterohepatic circulation", effectively recycles a finite bile acid pool to fulfill the human physiological demand for bile acids.

2.2. Bile acid receptors

Bile acids mediate downstream signals through a variety of bile acid receptors, including nuclear and membrane receptors. In 1999, three separate laboratories reported that FXR was the primary nuclear receptor most responsive to bile acids[15-17]. Subsequently, specific nuclear receptors pregnane X receptor[18], vitamin D3 receptor[19], constitutive androstane receptor[20], and surface membrane receptor TGR5 were also found to be activated by bile acids[21,22]. Bile acids are amphipathic steroid acids with different degrees of hydrophobicity. The order of hydrophobicity, from most to least, is LCA, DCA, CDCA, CA, and UDCA[23]. The structural differences result in different affinities to different receptors[24]. For nuclear receptors, their activation by bile acids necessitates cellular uptake first, which occurs through simple diffusion for hydrophobic free bile acids or *via* active transport for conjugated and hydrophilic bile acids. Unlike nuclear receptors, bile acids can directly interact with cell surface membrane receptors[25]. Bile acids have recently been recognized as important hormone-like signaling molecules[16]. Among the various signaling pathways linked to bile acids, FXR and TGR5 are the two bile acid receptors receiving the most attention[26,27], suggesting a pivotal role for these receptors.

2.2.1. Farnesoid X receptor (FXR)

FXR is a ligand-activated transcription factor first identified as an orphan nuclear receptor activated by farnesol metabolites[28]. It is primarily expressed in hepatocytes, hepatic stellate cells, and intestinal epithelial cells, with lower levels of expression in monocytes and lymphocytes[29,30]. There are two distinct FXR members in mammals: FXRα and FXRβ. In humans, rodents, and rabbits, FXRα has been well-studied and encodes four functional isoforms with different DNA binding affinities and tissue distributions[31]. FXRβ, on the other hand, is considered a pseudogene in humans but has unresolved functions in mice, rats, rabbits, and dogs[32].

The rank order of endogenous bile acids activating FXR (CDCA > DCA > LCA > CA) has been reported from many *in vitro* studies[15,33], but may differ *in vivo*, particularly in mice[34]. Tauro-β-muricholic acid is a natural FXR antagonist in mice, while glyco ursodeoxycholic acid (GUDCA) is a natural FXR antagonist in humans[35]. UDCA has a negligible activation effect on FXR in coactivator association *in vitro* assays but can inhibit FXR signaling as evidenced by decreased short heterodimer partner (SHP) levels[4,36]. OCA, a synthetic derivative of bile acid, is a specific FXR agonist. After ligand binding, FXR regulates the expression of many target genes by binding to the FXR response element, which is an inverted repeat with one bae spacing (*IR1*)[37]. FXR forms a heterodimer with retinoid X receptor to bind to *IR1* element to activate target gene expression. FXR monomer usually acts as a repressor. It is well-established that FXR is a master regulator of bile acid ho meostasis involving bile acid synthesis, conjugation, and enterohepatic circulation in the liver and intestine[38]. FXR also plays an essential role in lipid and glucose metabolism. On one hand, FXR

increases serum low-density lipoprotein cholesterol *via* decreasing bile acid synthesis and decreases serum high-density lipoprotein cholesterol *via* increased high-density lipoprotein clearance. On the other hand, FXR decreases serum triglycerides *via* decreased very low-density lipoprotein production through the FXR-SHP-sterol regulatory element-binding protein 1c pathway and increased very low-density lipoprotein clearance[39]. As for glucose homeostasis, FXR activation or inhibition in different tissues can also improve glucose regulation by enhancing glycogenesis and insulin sensitivity and reducing gluconeogenesis[40-42]. More interestingly, it has recently become clear that FXR has an immune-modulatory prop erty and can suppress intense immune responses through direct ef fects on the immune system and indirect effects on metabolic balance regulation and maintenance of the intestinal barrier.

2.2.2. *G-protein-coupled bile acid receptor (GPBAR/TGR5)*

In 2002, TGR5 was identified as the first transmembrane G protein-coupled receptor responsive to bile acids[43]. It is highly expressed in the gallbladder but exhibits low expression in the liver, intestine, brown adipose tissue, and central nervous system[44,45]. The affinity of natural bile acids to activate TGR5 follows the order LCA > DCA > CDCA > CA > UDCA[21]. Upon bile acid stimulation, TGR5 induces the Gαs−adenylate cyclase−cyclic adenosine monophosphate (cAMP) pathway, triggering pleiotropic downstream signaling effectors[46] and subsequently exerting a crucial impact on immunomodulation, glucose, and energy homeostasis, and liver and gallbladder physiology[22,47]. TGR5 negatively immunomodulates inflammation by suppressing the nuclear factor-kappa B (NF-κB) pathway and nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing 3 (NLRP3) inflammasomes in immune cells, such as monocytes/macrophages, Kupffer cells, and dendritic cells[48,49]. In addition, TGR5 mediates increases in glucagon-like peptide-1 (GLP-1) and insulin secretion, playing a protective role in glucose homeostasis[50,51]. Through the TGR5 signaling pathway, bile acids not only suppress CCAAT/enhancer binding protein β-dependent macrophage migration into white adipose tissue[52] but also induce cAMP-dependent thyroid hormone activation in brown adipose tissue and muscle to promote energy expenditure[53]. In the gallbladder, the deletion of TGR5 protects mice from cholesterol gallstone formation[54]. Moreover, TGR5 activates cAMP-dependent endothelial nitric oxide synthase, inducing nitric oxide production in sinusoidal endothelial cells to protect the liver against lipid peroxidation and bile acid-induced reactive oxygen injury[55].

The extensive interrelationships between bile acid activity and multiple signaling pathways suggest that acute physiological assaults, like COVID-19, could have significant effects involving bile acids.

3. Bile acids and COVID-19

3.1. *Clinical research*

Bile acid levels may be useful in predicting COVID-19 severity and prognosis. Compared to COVID-19-negative patients, CA and GUDCA were upregulated in asymptomatic patients[56]. Moreover, severe COVID-19 patients exhibited higher serum total bile acid serum levels compared to non-severe patients[57]. Metabolomic analysis demonstrated that COVID-19 patients with elevated bile acid derivatives were more likely to progress to severe disease[58]. In a prospective observational cohort study, metagenomic and metabolomic analyses of fecal samples from patients with COVID-19 admitted to intensive care unit revealed that increased concentrations of secondary bile acids (DCA, LCA, and isoDCA) were associated with improved COVID-19 survival[59].

The therapeutic and preventive effects of bile acids on COVID-19 are contradictory. Some clinical studies have shown that UDCA can reduce susceptibility to SARS-CoV-2 and improve the symptoms of COVID-19[4,60,61]. For example, a retrospective study showed that UDCA decreased the infection rate of SARS-CoV-2 and the severity of COVID-19 in patients with cirrhosis[62]. However, several other retrospective investigations have observed that UDCA treatment did not improve mortality and overall survival among COVID-19 patients in the context of cholelithiasis, chronic liver disease, liver cirrhosis, or bone marrow diseases[63,64]. In addition, in the pediatric population with liver disease, UDCA did not reduce susceptibility to SARS-CoV-2 infection[65]. The conflicting conclusion between clinical trials may be caused by the following reasons. Firstly, there are significant disparity in sample sizes between the UDCA-treated and control groups. Secondly, different vaccination rates between the two groups may influence the results. Thirdly, comorbidity diversity between the two groups complicates the interpretation of results[60,63]. Moreover, most of these clinical studies are retrospective, thus large-scale prospective cohort studies or randomized clinical trials are necessary to validate their relationship.

3.2. *Basic research*

Many basic studies have explored the relationship between bile acids and COVID-19 and its potential mechanism[66]. In a non-human primate model, researchers conducted a longitudinal study of fecal bile acids throughout SARS-CoV-2 infection. They observed an increase in the overall quantity of primary bile acids and a corresponding increase in the primary-to-secondary bile acid ratio[67], which is consistent with clinical research findings. Liver and gut damage induced by SARS-CoV-2 infection suppressed hepatic detoxification and gut barrier and reabsorption functions, leading to elevated serum bile acid levels[58,68,69]. Furthermore, the impact of SARS-CoV-2 infection on the composition and interaction network of the gut microbiota played a crucial role in the alteration of bile acid metabolites[21,70]. However, the molecular mechanism behind this remains unclear[71].

Nevertheless, most basic studies suggest that bile acids may have the potential to improve COVID-19 outcomes. The potential mechanisms underlying the influence of bile acids on COVID-19 are encapsulated in Fig. 1. The results for several bile acids that have been more extensively studied are summarized below.

3.2.1. *Chenodeoxycholic acid (CDCA)*

CDCA, the most potent natural agonist of FXR, is a key regulator of ACE2 levels. CDCA can upregulate the expression of ACE2 through activating FXR, which induces SARS-CoV-2 infection. The absence of CDCA resulted in the loss of ACE2 expression[4]. Furthermore, in T84 colonic epithelial cells, treatment with CDCA increased ACE2 mRNA expression[72] (Fig. 1A).

Nonstructural protein 15 (Nsp15), a specific marker for vertebrate nidoviruses, plays a crucial role in SARS-CoV-2 mRNA production and transcription. Antibiotic-induced primary bile acid accumulation (taurocholic acid, CDCA) significantly

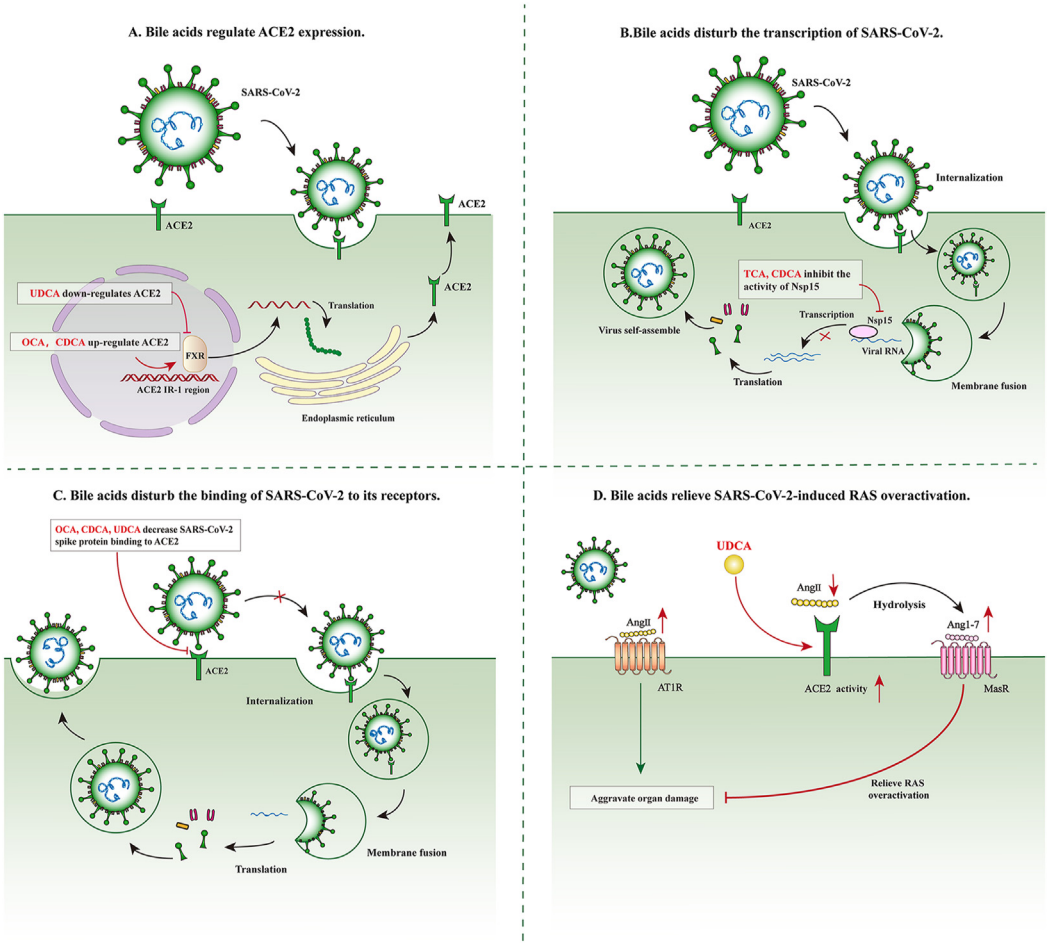

Figure 1 Potential mechanisms underlying the influence of bile acids on COVID-19. Bile acids play a significant role in influencing COVID-19 through four major pathways. (A) CDCA and UDCA control ACE2 expression through FXR. (B) TCA and CDCA inhibit the activity of Nsp15 to disturb the transcription of SARS-CoV-2. (C) OCA, CDCA, and UDCA inhibit SARS-CoV-2 spike protein binding to its receptors mediating viral entry to host cells. (D) UDCA increases ACE2 activity and thus relieves SARS-CoV-2-induced RAS activation. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; ACE2, angiotensin-converting enzyme 2; TCA, taurocholic acid; CDC, chenodeoxycholate; CDCA, chenodeoxycholic acid; UDC, ursodeoxycholate; UDCA, ursodeoxycholic acid; OCA, obeticholic acid; Nsp15, nonstructural protein 15; FXR, farnesoid X receptor; Ang II, angiotensin II; AT1R, angiotensin II type 1 receptor; RAS, renin-angiotensin system; MasR, Mas receptor.

inhibited the activity of SARS-CoV-2 Nsp15 in the mouse gut[73] (Fig. 1B). Moreover, CDCA and its metabolite glycochenodeoxycholic acid suppressed the binding of the SARS-CoV-2 spike protein receptor binding domain (RBD) to ACE2 in a concentration-dependent manner *in vitro*[74] (Fig. 1C).

3.2.2. *Obeticholic acid (OCA)*

OCA, a semisynthetic FXR agonist, has been approved by the Food and Drug Administration for treating primary biliary cholangitis and is being investigated as a potential treatment for nonalcoholic steatohepatitis[75,76]. Based on *in vitro* screening with an assay kit, OCA could reduce the SARS-CoV-2 spike protein RBD/ACE2 binding[74] (Fig. 1C). Interestingly, while OCA upregulated ACE2 expression in undifferentiated colonic and ileal enteroids (Fig. 1A), it did not affect differentiated enteroids[72]. It is noteworthy that the primary route of viral infection in the gastrointestinal system is through differentiated enterocytes of the ileal villus or colonic surface cells[77]. The ACE2/Angiotensin (Ang)-(1−7)/Mas receptor (MasR) axis plays an important role in preventing proinflammatory effects through angiotensin II type 1 receptor signaling[78]. In SARS-CoV-2 infected patients, the weakened ACE2-Ang-(1−7)-MasR axis leads to increased Ang II and decreased vasodilator Ang-(1−7) levels. This dysregulation correlates with viral titer and organ damage[1]. Thus, OCA through its effects on ACE2 may not increase viral entry but rather mitigate the viral impact due to the increase of anti-inflammatory Ang-(1−7) induced by ACE2[72].

3.2.3. *Ursodeoxycholic acid (UDCA)*

UDCA was originally synthesized as a drug for the treatment of liver diseases such as PBC[79]. The interactions between ACE2 and the SARS-CoV-2 spike protein RBD can be slightly and dose-dependently inhibited by UDCA and its taurine conjugate taurursodeoxycholic acid *in vitro*[5,74]. In Beas-2B human bronchial epithelial cells, SARS-CoV-2 spike protein reduced cell migration by interacting with ACE2. Pretreatment with UDCA inhibited the spike protein-ACE2 interaction and ameliorated Beas-2B cell migration[80] (Fig. 1C).

Interestingly, although UCDA inhibits FXR signaling, it was found not to be a direct FXR antagonist but a direct TGR5 ligand in transactivation assays[81]. Nevertheless, by inhibiting FXR signaling, UDCA prevented FXR from attaching to *IR1* in the *ACE2* promoter region, which lowered the expression of ACE2 at the mRNA and protein levels[4]. This inhibitory effect reduced SARS-CoV-2 infection (Fig. 1A).

Aside from affecting the expression of ACE2, UDCA also regulated ACE2 activity in the lung[82]. *In vitro,* pharmacological assays showed that UDCA and its derivatives increased the exopeptidase activity of ACE2. Therefore, UDCA has therapeutic potential against COVID-19-associated inflammation *via* activation of ACE2/Ang (1−7)/MasR axis[83] (Fig. 1D).

4. Bile acid receptor and COVID-19

FXR and TGR5 are two primary receptors for bile acids. Both receptors are involved in regulating the expression of ACE2, which directly controls the entry of SARS-CoV-2 into host cells. Additionally, they have been shown to modulate the immune system and attenuate inflammation, potentially contributing to the preventive and therapeutic effects of bile acid receptors on COVID-19. The next section provides a summary of the relationship between bile acid receptors and COVID-19, as depicted in Fig. 2.

4.1. FXR and COVID-19

4.1.1. FXR-mediated signaling pathways in COVID-19

FXR plays a crucial role in controlling ACE2 expression and modulating the immune system, making it a potential therapeutic target for reducing SARS-CoV-2 infection and improving COVID-19 outcomes. As mentioned above, FXR directly controls ACE2 expression by binding *IR1* of the ACE2 promoter region. Regulation of ACE2 by FXR is associated with the organs' vulnerability to SARS-CoV-2. FXR inhibition reduced ACE2 expression and SARS-CoV-2 infection rate in gallbladder cholangiocyte, airway, and intestinal organoids[4]. Smyth et al.[72] extended

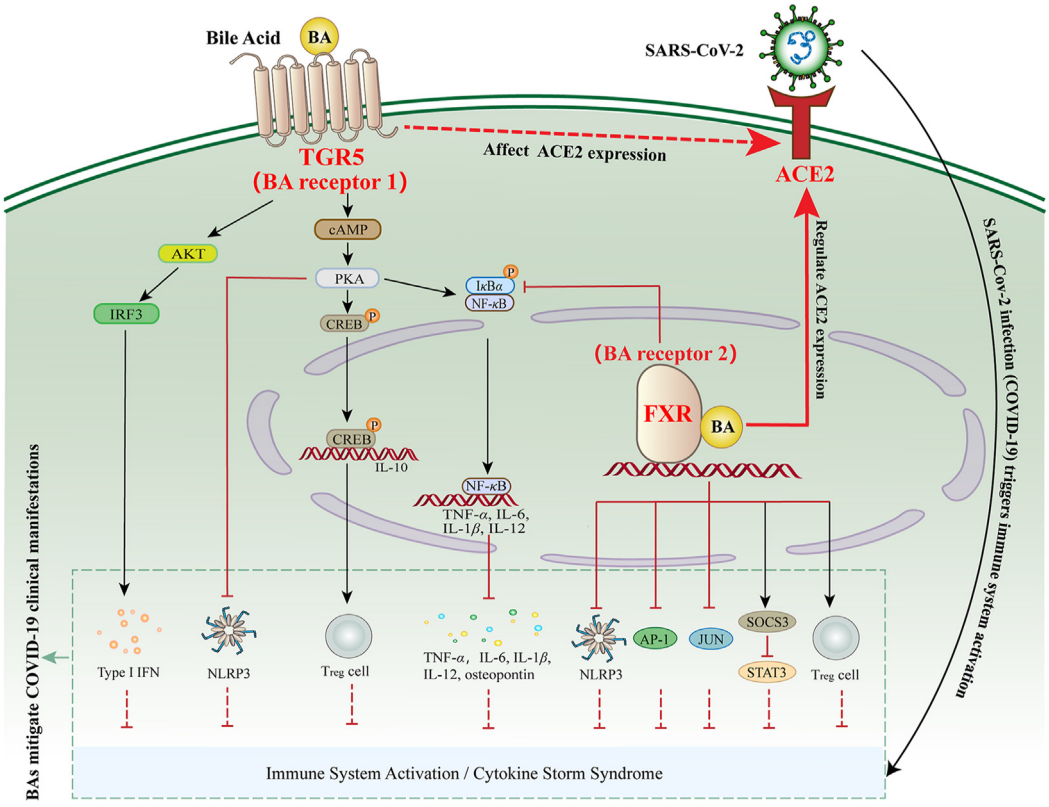

Figure 2 The relationship between bile acid receptor and COVID-19. Both TGR5 (bile acid receptor 1), a membrane receptor, and FXR (bile acid receptor 2), a nuclear receptor, serve as the primary receptors for bile acids. Both receptors participate in affecting the expression of ACE2, which directly controls the entry of SARS-CoV-2 into host cells. SARS-CoV-2 infection/COVID-19 triggers immune system activation, which in some cases can lead to an excessive release of cytokines, causing cytokine storm syndrome and potentially severe complications. Bile acids regulate crucial pathways involved in immune system activation and the development of cytokine storm syndrome triggered by SARS-CoV-2, potentially contributing to the mitigation of COVID-19's clinical manifestations. BA, bile acid; FXR, farnesoid X receptor; TGR5, G-protein-coupled bile acid receptor; ACE2, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; AKT, protein kinase B; IRF3, interferon regulatory factor 3; PKA, protein kinase A; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element-binding protein; IL, interleukin; TNF-α, tumor necrosis factor-alpha; NF-κB, nuclear facto-kappa B; IκBα, NF-κB inhibitor alpha; IFN, interferon; NLRP3, nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing 3; T_{reg} cell, regulatory T cell; AP-1, activator protein 1; SOCS3, suppressor of cytokine signaling 3; STAT3, signal transducer and activator of transcription 3.

those findings to the colon. However, elevated ACE2 expression is not definitively linked to an increased susceptibility to SARS-CoV-2, and reducing it may not reduce susceptibility. For example, although the ACE2 expression is the highest in the ileum, the ileum is not the most susceptible organ in humans. These indicate that more complicated processes may contribute to both organ-specific and general susceptibility to SARS-CoV-2 infection[1].

FXR has an immunomodulatory role through both its direct effects on the immune system and its indirect effects on metabolic balance regulation and maintenance of the intestinal barrier[84]. FXR can directly control the activation and differentiation of immune cells. It decreases macrophage proinflammatory (TNF-α, interleukin-6 (IL-6), IL-1β, IL-12) activity through negatively regulating NLRP3 inflammasome and NF-κB activation by SHP[85]. In dendritic cells, the secondary bile acid 3β-hydroxydeoxycholic acid/isoDCA limits FXR activity, potentiating peripheral anti-inflammatory regulatory T cell generation[86,87]. In hepatic natural killer T cells, FXR activation inhibits proinflammatory mediators osteopontin production *via* the SHP−JUN axis[88]. Furthermore, FXR agonism decreased hepatic inflammation by preventing activator protein 1 binding to inflammatory genes through SHP[6]. In liver injury, the activation of FXR increased the expression of suppressor of cytokine signaling 3, negatively regulating signal transducer and activator of transcription 3 signaling[89]. FXR also has essential roles in responding to inflammation and regulating immunity within the gastrointestinal tract, linked to the intestinal peroxisome proliferator-activated receptor α-uridine diphosphate−glucuronosyltransferases axis, and reduction of pro-inflammatory including IL-6, IL-1β, and TNF-α[84,90]. Research has shown that the activation of FXR decreases the release of IL-6 in both SARS-CoV-2-infected Caco-2 cells and T84 cells treated with polyinosinic: polycytidylic acid, a viral mimic[72].

However, given the complexity of FXR functions, the activation and inhibition of FXR produced paradoxical or even opposite effects in some experimental disease models, implying that the signaling pathways regulated by FXR are intricate and tissue/cell-specific[26]. Nevertheless, FXR has the potential to offer protection against COVID-19 and other inflammation-heavy conditions by modulating the immune system directly and reducing inflammation in various organs.

4.1.2. *Non-bile acid compounds targeting FXR for COVID-19*

In addition to the bile acids mentioned above, non-bile acid compounds targeting FXR have been shown to influence COVID-19 (Table 1). A comprehensive analysis of the impact of FXR agonists and antagonists on COVID-19 may offer insights into the underlying relationship between FXR and COVID-19.

4.1.2.1. *FXR agonists*

4.1.2.1.1. Berberine. Berberine can activate intestinal FXR[91]. Therefore, it has the potential to improve COVID-19 by targeting SARS-CoV-2[92]. In Vero E6 cells, berberine exhibited dose-dependent antiviral effects against SARS-CoV-2[93]. This antiviral efficacy was also replicated in differentiated human nasal epithelial cells, where berberine effectively suppressed SARS-CoV-2 RNA levels[94]. Furthermore, berberine significantly lowered viral replication, repressed viral entry host receptor ACE2 and transmembrane serine protease 2, and decreased inflammatory markers in SARS-CoV-2-infected human lung epithelial cell lines[95]. In clinical research, a prospective study showed that berberine significantly reduced circulating inflammatory mediators (IL-6, TNF-α, and C-reactive protein) in patients with severe COVID-19[96]. A phase IV clinical trial of berberine in patients with severe COVID-19 has been completed in China (NCT04479202)[97]. The results of this clinical trial have not yet been made public.

4.1.2.1.2. Epigallocatechin-3-gallate. Epigallocatechin-3-gallate (EGCG), a major antioxidative green tea catechin, specifically and dose-dependently activates FXR[98]. EGCG reduced SARS-CoV-2 entry and replication in Vero cells by inhibiting the interaction between the virus spike protein RBD and ACE2[99-102]. Many *in vitro* studies also revealed that EGCG inhibited the activity of SARS-CoV-2 main protease, a vital protease for viral reproduction[103-105]. EGCG can activate nuclear factor erythroid2-related factor-2 to inhibit ACE2 and TMPRSS2 expression that should suppress oxidative stress, endoplasmic reticulum stress, cytokine storm, thrombosis, sepsis, and lung fibrosis to impede COVID-19, but it is important to note that all these mechanisms remain speculative for now[106].

4.1.2.1.3. Others. Papain-like protease plays a role in the reproduction of SARS-CoV-2 and the regulation of host innate immunity[107,108]. Tropifexor, an FXR agonist, was identified as a SARS-CoV-2 papain-like protease inhibitor by drug-repurposing screening and showed antiviral activity against SARS-CoV-2 in Calu-3 cells[109]. Dihydroartemisinin, a derivative of the antimalarial drug artemisinin, can also activate FXR signaling[110]. It showed high anti-SARS-CoV-2 potential *in vitro*, within the plasma concentration range, after clinical intravenous administration[111].

4.1.2.2. *FXR antagonists*

4.1.2.2.1. Guggulsterone. Guggulsterone, an extract of the resin of the guggul tree (*Commiphora mukul*), is a highly efficacious antagonist of FXR[112]. Recent research has revealed that *Z*-guggulsterone, one of its two stereoisomers, effectively decreased the presence of FXR on the ACE2 promoter, leading to a downregulation of ACE2 expression in various organoids, including those from the airway, gall bladder, and intestine, and demonstrated a notable decrease in the susceptibility of these organoids to SARS-CoV-2 infection[4]. This finding highlights the potential therapeutic impact of *Z*-guggulsterone in mitigating the risk of SARS-CoV-2 infection.

4.1.2.2.2. Tempol. Tempol was found to indirectly inhibit FXR by elevating tauro-β-muricholic acid levels as a result of decreases in *Lactobacillus* and bile salt hydrolase activity[113]. Tempol caused the oxidation of RNA-dependent RNA polymerase and Fe−S cofactor clusters, resulting in their disassembly. This process effectively inhibited the activity of RNA-dependent RNA polymerase, halting the replication of SARS-CoV-2 in Vero E6 cells[114]. The inhibitory effect of tempol on SARS-CoV-2 replication was also observed in the Syrian hamster model, a preclinical model of mild infections[115]. A notably lower level of pathologic alterations in the lungs was linked to this inhibition. Additionally, T cells and activated antigen-presenting cells from COVID-19-positive individuals preincubated with tempol had substantially reduced production of multiple cytokines[116]. These findings demonstrate that Tempol has anti-cytokine action *in vitro*.

4.1.2.2.3. Metformin. Similar to tempol, metformin was found to indirectly inhibit FXR by suppressing the growth of *Bacteroides fragilis* and increasing levels of GUDCA[35]. Studies have demonstrated that metformin can target proteins involved in SARS-CoV-2 translation[117]. Furthermore, metformin has been found to exhibit antiviral effects against SARS-CoV-2 *in vitro*[118,119]. This protective effect is attributed to its anti-inflammatory properties. Metformin inhibited NLRP3 inflammasome activation, IL-1β production, and inflammasome-independent IL-6 secretion, thereby

Table 1 The effect of non-bile acid compounds targeting FXR on SARS-CoV-2 and the underlying mechanism.

Non-bile acid Compd.	Effect on FXR	Effect on SARS-cov-2 and mechanism	Subject of the study	Ref.
Berberine	Activating intestinal FXR	· Reduced viral replication, suppressed ACE2 and TMPSS2, and decreased inflammatory markers	*In vitro* cell line	91-95
		· Reducing circulating inflammatory mediators (IL-6, TNF-α, and CRP) in patients with severe COVID-19	Severe COVID-19 patients	96,97
Epigallocatechin-3-gallate	FXR agonist	· Inhibiting the entry and replication of SARS-CoV-2	*In vitro* cell line	98-100
		· Inhibiting the binding of the SARS-CoV-2 spike protein receptor-binding domain to the ACE2		101,102
		· Blocking SARS-CoV-2 M^{pro}		103-105
Tropifexor	FXR agonist	· Inhibiting SARS-CoV-2 PL^{pro}	*In vitro* cell line	109
		· Inhibiting SARS-CoV-2 replication		
Dihydroarte-misinin	FXR agonist	· Inhibiting SARS-CoV-2 replication in Vero E6 cells	*In vitro* cell line	110,111
Guggulsterone	FXR antagonist	· Reducing SARS-CoV-2 infection in the airway, gall bladder, and intestinal organoids through the FXR−ACE2 pathway	*In vitro* cell line	4
Tempol	Indirect FXR antagonist	· Suppressing the activity of the RDRP and decreasing the replication of SARS-CoV-2	*In vitro* cell line	114
		· Decreasing the replication of SARS-CoV-2 and pathologic alterations of the lung	*In vivo* Syrian hamster model	115
		· Inhibiting multiple cytokines of acti vated T cells and antigen-presenting cells	*In vitro* cells derived from COVID-19 patients	116
Metformin	Indirect FXR antagonist	· Inhibiting the entry and replication of SARS-CoV-2	*In vitro* cell line	118,119
		· Alleviating SARS-CoV-2-induced acute respiratory distress syndrome through an anti-inflammatory effect	*In vivo hace2* Tg mice	120
		· Lowering COVID-19-related mortality	COVID-19 patients with T2DM	121-123
		· Decreasing the severity of COVID-19	COVID-19 patients with overweight or obese	124
		· Reducing indicators of cardiac injury and heart failure and inflammatory factors	COVID-19 patients with T2DM	125

FXR, farnesoid X receptor; ACE2, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; TMPSS2, transmembrane serine protease 2; TNF-α, tumor necrosis factor-alpha; IL, interleukin; CRP, C-reactive protein; RdRp, RNA-dependent RNA polymerase; $3CL^{pro}$, three chymotrypsin protease; M^{pro}, main protease; PL^{pro}, papain-like protease protein; T2DM, type 2 diabetes; *hACE2* Tg, human *ACE2* transgenic mice.

alleviating SARS-CoV-2-induced acute respiratory distress syndrome in human *ACE2* transgenic mice[120]. In clinical research, some observational studies have indicated that metformin signif icantly lowered COVID-19-related mortality in individuals with type 2 diabetes[121-123], decreased the severity of COVID-19 in overweight or obese adults[124], and was strongly linked to a reduction in heart failure and inflammation in people with both COVID-19 and pre-existing type 2 diabetes[125]. However, a randomized controlled trial showed that metformin was not effective in preventing hypoxemia, emergency department visits, hospitalization, or death related to COVID-19 in either overweight or obese pa tients[126]. As a result, the antiviral and anti-inflammatory properties of metformin require further study to determine if they have any significant impact on the treatment of COVID-19.

Both agonists and antagonists of FXR have the potential to improve COVID-19. However, the supporting evidence for each varies significantly. The majority of evidence supporting FXR agonists is derived from cellular studies, whereas the credibility of FXR antagonists is reinforced by both cellular research and more robust clinical results. Moreover, these agents might have unidentified non-FXR-related activities that contribute to their effects given the complexity of immunomodulation and COVID-19 infectivity. Consequently, the underlying mechanisms are presently intricate and require further investigation.

4.2. TGR5 and COVID-19

4.2.1. TGR5-mediated signaling pathways in COVID-19

TGR5, like FXR, exhibits bile acid-dependent effects on the immune system and inflammation response, which may regulate SARS-CoV-2-induced immune disorders and excessive inflammation. TGR5 promotes type I interferon (IFN) production and antiviral innate immunity through the protein kinase B/interferon regulatory factor 3 signaling pathway[127]. In the immune system, type I IFNs play an important role in innate antiviral immunity[128]. During the early stage of SARS-CoV-2 infection, type I IFNs have insufficient antiviral immunity to protect against the virus, while they can cause long-term damage to patients in the post-inflammatory stage.

After SARS-CoV-2 enters the human body, its core component N protein can promote the activation of NLRP3 inflammasomes and induce excessive inflammation[129]. Pyroptosis of infected monocytes can be induced through NLRP3 inflammasome activation and causes systemic inflammation[130]. TGR5 can inhibit NLRP3 activation through the TGR5−cAMP−protein kinase A (PKA) axis. When activated by bile acids, TGR5 activates PKA kinase, thereby ubiquitinating and phosphorylating NLRP3, acting as a brake and inhibiting inflammation[131]. TGR5 also acts as a suppressor modulator of NF-κB-mediated inflammation. TGR5 signaling involves cAMP−PKA-mediated inhibition of NF-κB, which suppresses the phosphorylation of NF-κB inhibitor alpha, the translocation of p65, and NF-κB DNA-binding and tran scriptional activity[132,133]. TGR5 prompts the macrophage polarization toward an anti-inflammatory phenotype M2 and directly increases IL-10 expression *via* the cAMP−PKA−cAMP response element-binding protein pathway, enhancing the production of regulatory T cells[134].

SARS-CoV-2 can not only cause systemic inflammation but also cause damage to the biliary system. Cholestasis is common in abdominal images of infected patients[129]. SARS-CoV-2 can directly damage bile duct cells and destroy the bile transport function, leading to bile accumulation[135]. Bile acids have been shown to induce bile duct cell proliferation by activating TGR5 to protect cells from bile acid toxicity during primary sclerosing cholangitis development[136,137]. As a bile acid receptor, TGR5 alters the composition of bile acids by regulating gallbladder dilation. Its activation induces gallbladder dilation, reduces secondary bile acid concentrations, and increases the primary/secondary bile acid ratio to protect liver cells from cholestasis damage[138].

4.2.2. Non-bile acid compounds targeting TGR5 for COVID-19

BAR501, a selective agonist of TGR5, promotes the synthesis and release of GLP-1 through the TGR5−GLP-1 axis and regulates the expression of ACE2 in the colon. This phenomenon can be reversed by GLP-1 antagonists[5]. Therefore, TGR5 can indirectly regulate colon ACE2 expression. Whether TGR5 has a regulatory effect on ACE2 in other tissues and organs requires further investigation. Theoretically, an agonist of TGR5 like UDAC could increase the level of colon ACE2, but whether UDAC can increase the level of colon ACE2 by activating TGR5 and thereby exacerbate colon susceptibility and aggravate intestinal symptoms of SARS-CoV-2 infection remains to be studied.

5. Summary and future perspectives

Bile acids, traditionally recognized for their role in digestion and lipid metabolism, have recently gained attention in the context of COVID-19. Emerging research indicates a link between bile acids and COVID-19 infection. Here, we review the basic and clinical evidence linking bile acids to COVID-19 and its potential mechanism. Functionally, both clinical and animal studies show that bile acids protect against COVID-19 by various mechanisms. Mechanistically, some studies have suggested that bile acids may play a role in modulating the expression of ACE2, the receptor through which the virus enters human cells. In addition, bile acids may participate in a variety of mechanisms, including immunomodulatory effects, inflammation and metabolism, to regulate COVID-19, which is characterized by an intense inflammatory response. Bile acids have been shown to influence the function of immune cells, such as macrophages and T cells. They may act as modulators of inflammatory responses. Understanding these potential immunomodulatory effects could open new avenues for therapeutic interventions, not only for COVID-19 but for other inflammation-based conditions as well. Bile acids are also involved in metabolic processes, which are closely linked to the immune system. Moreover, metabolic disorders are associated with an increased risk of severe outcomes from viral infections. Exploring the connection between bile acids, metabolism, and COVID-19 outcomes could be a potentially fruitful avenue for research. In addition, bile acids also act as signaling molecules, binding to various receptors such as FXR and TGR5, which in turn have significant physiological effects.

Future research in this field should include both clinical and basic studies. In clinical research, randomized clinical trials or large-scale prospective cohort studies are essential to confirm the preventive or therapeutic effect of bile acids on COVID-19. Importantly, sample size, comorbidity rates, and vaccination rates between treatment and control groups need to be consistently considered to address the limitations of current clinical studies.

In basic research, the nuclear receptor FXR and membrane receptor TGR5, two different types of bile acid receptors, hold promise as potential therapeutic targets for SARS-CoV-2. Whether these two different receptors accept different or multiple bile acid signals and how they subsequently and selectively mediate downstream signals should be the focus of future research into their mechanism of action. Additionally, since the nuclear receptor FXR can regulate the expression of ACE2 as a transcription factor, the fact that ACE2 levels in the intestine are significantly higher than in the colon suggests that FXR could differentially regulate ACE2 expression in different organs, which needs further experimental exploration. For TGR5, a G-protein-coupled receptor, its complex behaviors may be attributed to multiple activation modes[139]. Aside from the classical G-protein activation pathway, bile acid-biased activation of the β-arrestin signaling pathway has been found to exert antiviral activity[140]. There are also other novel modes for GPCR activation, including dimerization activation, translocation activation, and endocytosis activation[139]. Whether different bile acids induce different activation modes and lead to complex biological effects remains an unknown frontier in this research area.

Nevertheless, future research on bile acid-regulated signaling pathways will undoubtedly yield far-reaching results for therapeutic interventions for COVID-19 and other diseases.

Acknowledgments

This study was supported by (1) National Natural Science Foundation of China (NSFC Grant Nos. U21A20336, 81820108031, and 91939301); (2) National Key Research and Development Program of China (Grant No. 2022YFC3602400); (3) Beijing

Municipal Natural Science Foundation (Grant No. 7222218, China); (4) Medical research management/basic and clinical research unit of metabolic cardiovascular diseases, Chinese Academy of Medical Sciences (Grant No. 2021RU003, China).

Author contributions

Xiaoru Huang and Xuening Liu contributed equally to this work. Xiaoru Huang and Xuening Liu drafted the manuscript; Xuening Liu prepared figures; Xiaoru Huang, Xuening Liu, and Zijian Li edited and revised the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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