Review

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Anti-inflammatory, anti-oxidative stress and novel therapeutic targets for cholestatic liver injury

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Summary **Cholestasis is a pathological process in which bile drainage is poor for a variety of reasons. Many studies have shown that cholestatic liver injury is a neutrophil-mediated inflammatory response, and oxidative stress induced by neutrophils is the main mechanism of liver cell death. The literature summarizes the bile acid signaling pathway, the neutrophil chemotaxis recruitment process during cholestasis, and the oxidative stress damage produced by neutrophil activation, summarizes the latest research progress. Sphingosine-1-phosphate receptor (S1PR) is a potential therapeutic target for cholestasis that reduces neutrophil aggregation without inhibiting systemic immune status. Early growth response factor 1 (Egr-1) may play a central role in the inflammation induced by cholestasis, and it is also a potential therapeutic target to inhibit the inflammation induced by cholestasis. Strengthening the antioxidant system of hepatocytes to cope with oxidative stress of neutrophils is a feasible treatment for cholestatic liver injury.**

Keywords: Cholestatic liver injury, sphingosine-1-phosphate receptor, early growth response factor 1, inflammatory response, oxidative stress

1. Introduction

Cholestasis is a pathophysiological state in which bile duct obstruction or hepatocyte surface bile salt export pump (BSEP) function is inhibited by various reasons. Under normal circumstances, bile acids synthesized in hepatocytes are excreted into the bile duct through the bile duct of hepatocytes, and finally collected into the common bile duct to the duodenum, and only a small amount of bilirubin and bile acid are present in the blood (*1*). Under pathological conditions, bile drainage causes bile acids and their bound bile salts to accumulate in hepatocytes and serum. Higher levels of bile acids, such as glycochenodeoxycholate (GCDC), have larger toxicity, which induces apoptosis by directly interfering with mitochondrial function or directly causes cell death (*2*). If pathological obstructive factors are not removed in time, liver fibrosis and cirrhosis will eventually occur (*3*). Patients with cholestasis for a long time and severe jaundice will reduce the indication and success rate of operation for primary diseases, and increase the incidence of postoperative complications. With the further development of the disease, the damage of liver function is aggravated, and even multiple organ failure such as liver, kidney, brain and lung occurs after operation (*4*). However, how cholestasis causes liver damage has always been the focus of researchers' attention (*5*). At present, there is still a lack of effective treatment for cholestatic liver injury. Therefore, it is of great significance to study the pathogenesis of cholestatic liver disease and find potential therapeutic targets for hepatocyte injury. Many animal models of obstructive cholestasis have confirmed that infiltration of inflammatory cells (mainly neutrophils) at sites of hepatic necrosis is a prominent feature of cholestatic liver injury. Ursodeoxycholic acid (UDCA) has been the first-line treatment for cholestatic hepatitis, but some patients have no response to UDCA treatment or even have increased jaundice after treatment. Inhibiting inflammation, controlling oxidative stress, and searching for specific pathways and therapeutic targets are currently the research hotspots for cholestatic liver injury. At present, the mechanism of cell death caused by cholestasis has

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been studied in depth, summarizing the current research situation and providing a new therapeutic target for the treatment of cholestatic hepatitis.

2. The role of bile acids and bilirubin in cholestasis

2.1. *Most bile acids are non-toxic to hepatocytes*

Some bile acids or bile salts such as GCDC are highly toxic, but *in vivo* tests in rodents have shown that bile acids are non-toxic even when the bile acid accumulated during cholestasis reaches a micromolar concentration. *In vivo* experiments with cholestasis can be observed that when exposure of individual hepatocytes to serum and bile with bile acids, most bile acids are not toxic unless metabolized to toxic hydrophobic bile acids such as GCDC or Lithocholic acid (LCA) (*6*).

2.2. *Antioxidation of unconjugated bilirubin*

Unconjugated bilirubin (UCB) is an antioxidant with physiological antioxidant effects (*7*). Traditional liver freezing-resuscitation process during liver transplantation can cause damage to liver cells. It has been reported that after liver transplantation with UCB, liver function is significantly improved compared with untreated liver. UCB may be a potential clinical A cell protectant for liver transplantation. This shows that the antioxidant effect of UCB has a certain protective effect on hepatocytes during cholestasis (*8*).

2.3. *Interaction of bile acids with bilirubin*

Both bile acids and bilirubin are biomarkers of cholestasis, but their roles are diametrically opposite. Bile acid recruits concentrated granulocytes through the bile acid signaling pathway, causing a large amount of neutrophils to infiltrate in the liver tissue, and damaging the liver tissue through oxidative stress; while UCB is a physiological antioxidant, which can resist bile acid toxicity to a certain extent. In animal models of obstructive cholestasis, hepatic histopathology shows significant oxidative stress damage, which may be related to the toxic effect of bile acids on the liver, which reduces intracellular UCB levels, thereby making hepatocytes susceptible to oxidative stress damage (*9*).

3. Neutrophil-mediated inflammatory response exists in common animal models of cholestasis

At present, the representative model of extrahepatic cholestasis induction is the bile duct ligation (BDL) model. The representative model of intrahepatic cholestasis induction is alpha-naphthylisothiocyanate (ANTI)-induced cholestasis. Depending on the cause of cholestasis, there are differences between different animal models, but neutrophil-mediated inflammatory responses are considered to be a common pathogenesis of multiple cholestasis models (*10,11*).

4. Inflammatory response is the damage mechanism of cholestatic liver injury

4.1. *The role of neutrophils in cholestasis*

For humans and mice, neutrophils are the most important cellular components of the innate immune system. Liver histopathology showed a large amount of neutrophil infiltration in the necrotic area during cholestasis, suggesting that neutrophils play an important role in liver injury caused by cholestasis.

Experiments showed that after 3 days in the wild-type mouse BDL model, ICAM-1 expression levels increased along the hepatic sinus, portal vein, and hepatocytes, and neutrophils accumulated in large amounts, accompanied by a sharp rise in serum ALT and severe cell necrosis (*12*). The serum ALT level of ICAM-1 deficient rats was 67% lower than that of wild type mice, the degree of cell necrosis was significantly reduced, and the total amount of neutrophil infiltration in liver tissues was significantly reduced, 85% of which was still in hepatic sinusoids (*13*). The anti-inflammatory effects of glucocorticoids have protective effects on liver damage in BDL model mice. This indicates that a large amount of neutrophils infiltrate during obstructive cholestasis, and reducing neutrophil aggregation can help reduce liver damage caused by cholestasis.

Neutrophils express myeloperoxidase, which catalyzes the production of large amounts of hypochlorous acid, which kills liver cells by strong oxidation. Immunohistochemistry confirmed that 3-chlorotyrosine-protein complex staining was positive in hepatic necrotic tissue during cholestasis, indicating that neutrophils in the hepatic necrosis area release highly toxic hypochlorous acid, leading to hepatocyte death (*14*).

Other cells in the innate immune system also play an important role in cholestasis. Kupffer cells are thought to have protective effects against cholestatic liver damage. Experiments showed that the serum transaminase level of Kupffer cells or IL-6 deficient mice was significantly higher than that of normal mice after the BDL model. Histopathology suggested that inflammatory cell infiltration, bile duct proliferation and hepatocyte necrosis were more significant than normal mice. Kupffer cells extracted from the liver of normal BDL model mice were cultured, and IL-6 expression levels were significantly increased (*15*). This indicates that the protective effect of Kupffer cells on liver during cholestasis is achieved by a IL-6-mediated cytokine-dependent protection mechanism.

4.2. *Novel mediators that recruit neutrophil aggregation during cholestasis*

Neutrophil-mediated inflammatory response is the main mechanism of liver damage during cholestasis, so the study of specific signaling pathways for concentrated neutrophil aggregation during cholestasis has brought new therapeutic targets for the treatment of cholestatic liver injury. In recent years, studies have found that receptors recognize damage-associated molecular patterns (DAMPs) and thereby activate immune cells, ultimately stimulating a sterile inflammatory response (*16*).

DAMPs are rapidly released from damaged or necrotic tissue and can also be released by certain activated immune cells, such as mitochondrial DNA, nuclear DNA fragments or ATP (*17*). DAMPs can be considered as biomarkers for clinical detection of injury. Once DAMPs are released into the blood, the damage can be reflected indirectly by detecting markers in the serum. DAMPs activate cells expressing pattern recognition receptors (PRRs) in the innate immune system, elicit an innate immune response, and initiate an adaptive immune response either directly or indirectly. A variety of DAMPs have been discovered, including high mobility group protein box (HB1), heat shock protein (HSP), uric acid crystals, and hepatomaderived growth factor (HDGF).) *etc*. (*18*). Among the most important PRRs are Toll-like receptors (TLRs), which are a class of transmembrane receptors with a wide distribution. TLR4 is an important PRR that mediates signal transduction (*16*).

HMGB1 is a DNA-associated histone deacetylase that is transferred to the cytosol when cells are damaged. Cell necrosis due to cholestasis can cause HMGB1 to be released into serum within 6 hours after liver injury. The acetylated HMGB1 secreted by macrophages acts as a pro-inflammatory signal to promote inflammatory responses and is also an effective chemokine for the recruitment of concentrated neutrophils during cholestasis (*19*). As a receptor for recognizing DAMPs, TLR expression is significantly up-regulated in patients with chronic cholestasis. Activation of TLR by immune cells in the liver of PBC stimulates local NK cells to attack and kill capillary bile ducts. Experiments have confirmed that blocking the TLR4 signal transduction pathway or blocking HMGB1 with neutralizing antibodies in the ischemia-reperfusion model can effectively inhibit the production of aseptic inflammation (*20*).

Osteopontin (OPN) is a recently discovered molecule that has chemotactic effects on neutrophils during cholestasis (*21*). OPN is a pleiotropic protein that can play different roles in different cells (*22*). Secreted OPN binds to the integrin receptor and acts as a chemokine. Under normal conditions, OPN is expressed in biliary epithelial cells, whereas in chronic liver injury, hepatocytes induce the expression of OPN. Animal experiments show that OPN-/- mice can inhibit neutrophil recruitment and anti-BDL-induced liver injury in the early stage of the experiment, but this protection is temporary, and the BDL model can show similar liver injury like wild type after 72 hours, which may be related to the production of other chemokines and induction of neutrophil recruitment (*23*).

Therefore, it is difficult to achieve long-term protection against the liver by inhibiting one of the chemokines. At present, a feasible solution is to inhibit the upstream signaling pathway to activate the immune system or prevent neutrophil-mediated oxidative stress damage, thereby achieving the purpose of alleviating or even eliminating the liver damage caused by the inflammatory reaction.

4.3. *Inflammatory stress mediated by the innate immune system during cholestasis*

Neutrophils exert cytotoxic effects mainly by producing reactive oxygen species (ROS)(24). Under normal conditions, ROS is produced by mitochondrial respiration and detoxification by intracellular antioxidants. Innate immune cells such as neutrophils produce a large amount of highly toxic ROS to kill hepatocytes during aseptic inflammation. Neutrophils are infiltrated in the liver parenchyma after activation, releasing these toxic mediators to directly kill liver cells. According to reports in the literature, cytokines, chemokines, complement, and even HMGB1, and bile acids can activate neutrophils. Activated neutrophils adhere to target cells *via* CD 18-ICAM-1 interaction, triggering persistent adhesion-dependent oxidative stress damage (*25*).

Neutrophils produce hypochlorous acid (HClO) through a series of biochemical reactions. As a strong oxidant, hypochlorous acid can bind to various components in cells and kill cells. It is now known that HClO can bind to DNA and proteins, especially to proteins. The combination of sulfhydryl groups further produces toxic chloramines. Exposure of hepatocytes to ROS can impair mitochondrial function, leading to cell death. Experiments have shown that if a cyclosporin analog, such as NIM-811, is used to ablate mitochondrial permeability transition pores, it can counteract early damage to BDL and produce a protective effect (*26*). In addition, the addition of intracellular antioxidants, such as reduced glutathione, can also produce protection.

Although Kupffer cells can produce oxygen free radicals in the liver ischemia-reperfusion model, oxidative stress is mainly caused by neutrophils during cholestasis. When the number of Kupffer cells is reduced, the damage is aggravated, because the amount of protective cytokine protein produced by Kupffer cells is also decreased.

Based on the above studies, we can find that bile acid can agglomerate neutrophils by increasing ICAM-1 expression level in cholestasis. Neutrophils produce myeloperoxidase, catalyze the production of a large amount of HClO, and kill hepatocytes by strong oxidation. Inflammation-induced cell damage can

produce DAMPs, such as acetylated HMGB1 and TLR4, and then further recruit centralized granulocytes, which can amplify the inflammatory response and further damage the liver cells. Therefore, strengthening the hepatocyte antioxidant system to cope with neutrophil oxidative stress is also a feasible treatment.

In recent years, many scholars have found new drugs and new targets for the treatment of cholestatic liver injury. We searched the existing literature and summarize the new therapeutic drugs and related molecular pathways and therapeutic targets. As shown in Table 1, many drugs and pathways are involved, but the main ways of action are anti-inflammation, anti-oxidation and anti-fibrosis (*27-51*).

5. Bile acid signaling pathway and its relationship with the innate immune system

5.1. *Genetic changes caused by cholestasis*

Choles deposition induces many genes in liver cells to change (*52*). In the BDL model, hepatocytes rapidly up-regulate exporter transporters, such as the multidrug resistance (Mdr) transporter family and BSEP (*53*), while down-regulating uptake transporters, especially sodium taurocholate cotransporting (sodium taurocholate cotransporting) Polypeptide, Ntcp), aims to reduce the accumulation of bile salts in the liver (*54*). Post-transcriptional regulation of BSEP results in an overall down-regulation of hepatocyte activity. This series of changes may be an intrinsic regulation process to reduce bile acid emissions into bile after obstruction of bile drainage. In the Mdr-deficient mouse model, it was observed that BSEP mRNA levels increased gradually after birth, but BSEP protein expression levels decreased significantly (*55*). This compensatory change is to protect the liver cells from the damage caused by the accumulation of bile and to promote the bile acid into the blood and then filter through the urine.

5.2. *Endogenous Bile Acid Nuclear Receptor - Farnesol Receptor*

The hepatoenteral circulation, bile acid synthesis and metabolic processes of bile acids after cholestasis are automatically regulated by changes in the endogenous bile acid nuclear receptor, Farnesoid X Receptor (FXR). Bile acids act as ligands to activate FXR, up-regulating or down-regulating the expression of these genes by CYP7A1, a rate-limiting enzyme in the classical bile acid synthesis pathway (*56*). FXR is considered to be an important response element for bile acids during cholestasis. FXR-/- mice are unable to up-regulate BSEP expression and therefore cannot increase bile secretion or tolerate more damage caused by cholestasis (*57*).

At present, obeticholic acid is an FXR agonist with great potential in the clinical trial of primary biliary cirrhosis (PBC). Clinically, UDCA is the first-line treatment for patients with PBC, but some patients have poor or no response to UDCA. Alkaline phosphatase, γ-glutaminotransferase and alanine aminotransferase in the serum of these patients were all decreased after treatment with obenzolic acid, indicating that obeticholic acid has a positive effect on protecting liver cells, bile duct epithelial cells and resisting PBC-related liver damage (*58*).

In the BDL model-induced complete biliary obstruction, the first-line use of cholestasis-induced liver injury, UDCA, may result in more severe liver damage, as the biliary drug UDCA increases biliary pressure and aggravates biliary infarction (*59,60*). However, patients often do not show an increase in injury when taking the drug. On the one hand, the therapeutic dose is small, and UDCA has a protective effect on mitochondria (*61*). Therefore, the value of the BDL model in testing the therapeutic effect of cholestatic liver injury remains to be seen.

5.3. *Extracellular receptors − G protein coupled receptors*

In addition to acting on intracellular receptors, bile acids can also act on extracellular receptors, such as tyrosine kinase receptors and G-protein coupled receptors (GPCRs), or by activation of epidermal growth factor. The epidermal growth factor receptor (EGFR) acts on the downstream mitogen-activated protein kinase. Although these signal cascade amplifications are dependent on the generation of reactive oxygen species and ultimately lead to damage to rat hepatocytes (*62*).

5.3.1. *Sphingosine 1-phosphate receptor family*

Sphingosine kinases (SphKs) and its product sphingosine-1-phosphate (S1P) have the effect of regulating hepatocyte apoptosis and survival. The sphingosine-1-phosphate receptor family (S1PR family) is a G protein-coupled receptor with important biological activity (*63*). In rat hepatocyte experiments, bile acid is a sphingosine-1-phosphate receptor 2 (S1PR2) ligand in the mitogen-activated protein kinase (MAPK) pathway (*64*). It has been reported in the literature that sphingosine kinase inhibitors can significantly inhibit apoptosis of hepatocytes induced by GCDC and inhibit hepatocyte necrosis. At this time, S1PR1 and S1PR2 are simultaneously inhibited. Exogenous sphingosine kinase also significantly inhibits hepatocyte apoptosis by inhibiting S1PR2 (*65*). Therefore, S1PR is a potentially valuable therapeutic target for reducing neutrophil aggregation while not inhibiting systemic immune status in cholestasis.

5.3.2. *G protein coupled bile acid receptor*

G-protein coupled bile acid receptor (GPBAR/TGR5) is

Table 1. Drug therapy for cholestatic liver injury

Drugs	Methods	Signaling Pathway	Targets
Celastrol (*27*)	ANIT and TAA rat model	SIRT1-FXR signaling pathway	SIRT1, FXR
Andrographolide (*28*)	ANIT rat model	Anti-inflammatory Anti-oxidative	IL-6, TNF-α, MDA, SOD, GSH, GSH-PX
Rosuvastatin (*29*)	BDL rat model	HMGB1/TLR4 axis miR-21 signaling	HMGB1, TLR4, miR-21
Shenqi Fuzheng Injection (*30*)	BDL rat model		PPAR-gamma, COX-2 NF-kappaBp65
Peroxiredoxin 4 (*31*)	BDL rat model	Anti-inflammatory Anti-oxidative stress Anti-fibrosis	
9-cis-retinoic acid (*32*)	BDL rat model		MRP3, RXRalpha
Schisandrol B (*33*)	LCA rat model	PXR pathway	PXR
Glechoma hederacea (*34*)	BDL rat model	Anti-oxidative Anti-inflammatory Anti-fibrotic HMGB1/TLR4 signaling pathways TGF-beta/Smad signaling	NF-kappaB AP-1 TGF-beta HMGB1 TLR4
Chicken bile powder (*35*)	ANIT rat model	Restoring bile acid homeostasis Anti-inflammation	FXR NF-kappaB
Auraptene (*36*)	LCA rat model and *in vitro*	FXR pathway	FXR
SRT1720 (*37*)	17alpha-ethinylestradiol rat model	HNF1alpha/FXR signalling pathway Anti-inflammatory	HNF1alpha FXR
Sweroside (*38*)	ANIT rat model	Regulating bile acids Anti-inflammatory	beta-MCA, CA, TCA
Chlorogenic acid (*39*)	ANIT rat model	STAT3 and NFkappaB signalling	STAT3, NFkappaB
Vitamin C (*40*)	LCA rat model	Anti-fibrotic	Gulo
Thymoquinone (*41*)	BDL rat model	Anti-oxidative	MDA, SOD, GPx
Cilostazol (*42*)	BDL rat model	Anti-inflammatory Anti-fibrotic	TNF-alpha, TGF-beta PDGF-B
Quercetin (*43*)	BDL rat model	Anti-inflammatory Anti-oxidative Anti-fibrotic NF-kappaB signaling. TGF-beta/Smad signaling TLR signaling	NF-kappaB TGF-beta TLR
Guava Pulp (*44*)	BDL rat model and *in vitro*	Anti-fibrotic Src/MEK/ERK1/2/c-Myc pathway	TGF-beta1, TIMP, COL1alpha1, GP, IL-6 QBC939, p-ERK, c-Myc
Serotonin (*45*)	BDL rat model		Tph1
Docosahexaenoic acid (*46*)	BDL rat model	Hepatoprotective Anti-inflammatory Anti-fibrotic NF-kappaB signaling ERK/TGF-beta/Smad pathway	NF-kappaB ERK TGF-beta1
Cyclopamine (*47*)	BDL rat model	Hedgehog signaling pathway	Sonic Hedgehog, Patched-1 Glioblastoma-1, TNF-alpha IL-1beta, Akt, ERK
Resveratrol (*48*)	BDL rat model	Hepatoprotective Anti-inflammatory Anti-fibrotic	TNF-alpha, IL-6, collagen Ialpha1, TIMP-1
Caffeic acid phenethyl ester (*49*)	BDL rat model	Anti-inflammatory Anti-oxidative	MDA, MPO, IL-1alpha IL-6
Tetrathiomolybdate (*50*)	BDL rat model	Anti-fibrotic	TNF-alpha, TGF-beta1
Aminoguanidine (*51*)	BDL rat model	Anti-inflammatory Anti-oxidative	IL-1alpha, TNF-alpha

ANIT, alpha-naphthyl isothiocyanate; TAA, thioacetamide; BDL, bile duct ligation; LCA, lithocholic acid.

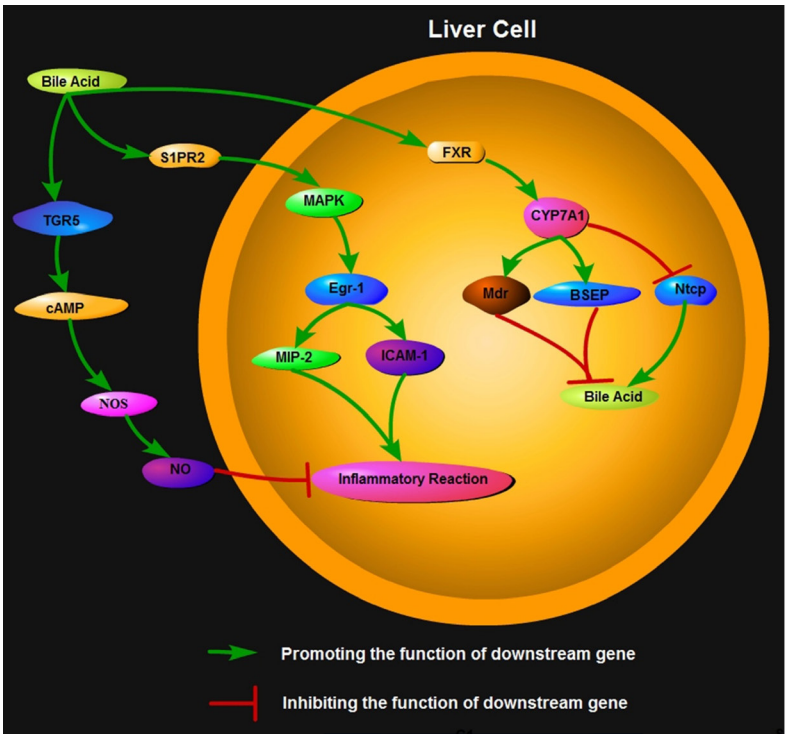

Figure 1. Bile acid signaling pathway and its relationship with the innate immune system. Bile acid acts on intracellular receptors: Bile acid can activate FXR, and then up-regulate Mdr and BSEP genes or down-regulate Ntcp genes through CYP7A1 to reduce intracellular bile acid accumulation. Bile acid acts on extracellular receptors: Bile acid can activate S1PR2 as ligand, up-regulate the level of Egr-1 through the MAPK pathway, and then up-regulate the levels of MIP-2 and ICAM-1 to promote inflammation. In addition, bile acid can also activate TGR5. Activated TGR5 induces NOS synthesis through the cAMP pathway, produces NO and inhibits inflammatory response.

Table 2. The treatment of cholestatic liver injury

Therapeutic method	Bile acid signaling pathway	Therapeutic targets
Anti-inflammatory		OPN, HMGB1, HSP, Uric acid crystal, HDGF
Anti-oxidative stress		GSH, ROS, HClO, Chloramines
Novel therapeutic targets	Intracellular signaling pathway	FXR, CYP7A1, Mdr, BSEP, Ntcp
	Extracellular signaling pathway I	S1PR2, MAPK, Egr-1, MIP-2, ICAM-1
	Extracellular signaling pathway II	TGR5, cAMP, NOS, NO

a novel membrane-bound bile acid receptor expressed in many types of cells, which plays an important role in regulating energy homeostasis and glucose metabolism. *In vitro* experiments showed that TGR5 significantly inhibited macrophage function (*66*). It is worth noting that the negative regulation of TGR5 on inflammatory responses makes it a potential therapeutic target for immunological liver disease and inflammatory liver disease. It is known that TGR5 is expressed in monocytes, sinusoidal endothelial cells, Kupffer cells, and bile duct epithelial cells, and is also expressed in small amounts in hepatocytes (*67*).

When cholestasis occurs, Kupffer cells inhibit cytokine synthesis through the TGR5-cAMP pathway, thereby inhibiting inflammatory immune responses (*68*). The activation of TGR5 on the surface of sinusoidal endothelial cells induces the synthesis of nitric oxide synthase (NOS) by endothelial cells through the cAMP pathway, thereby producing NO. NO can cause vasodilation to relieve portal hypertension and also play an important role in protecting the liver. In addition, TGR5 stimulates gallbladder filling. Under the action of agonists such as TGR5 agonist and lithocholic acid, the degree of gallbladder filling in TGR5-/- mice is significantly less than that in wild-type mice (*69*). TGR5 controls bile load during cholestasis and promotes liver regeneration. Animal experiments show that in the BDL model, TGR5 knockout mice have more severe liver damage than normal mice, and there is more inflammatory cell infiltration in the necrotic area (*70*). This evidence suggests that TGR5 has an important protective effect on the liver during cholestasis, protecting the liver from inflammatory reactions and promoting bile excretion to fill the gallbladder.

INT-767 is a TGR5/FXR dual agonist. Experiments suggest that in the Mdr2-/-(Abcb4-/-) mouse model, INT-767 can significantly induce bile drainage,cause high gene expression of carbonic anhydrase-14 and increase HCO_3^- release. Carbonic anhydrase-14 increases HCO_3^- transportation, which has a significant effect on improving serum liver enzyme levels, reducing inflammatory infiltration and gallbladder fibrosis (*71*).

5.4. *Early growth response factor 1 (early growth response factor 1, Egr-1)*

After exposure of primary mouse hepatocytes to chenocholalic acid (DCA), chenodeoxycholic acid (CDCA), or taurocholic acid (TCA), the level of intracellular adhesion molecule-1 and macrophage inflammatory protein 2 (MIP-2) increased significantly. Experiments have shown that the upregulation of ICM-1 and MIP-2 levels is dependent on the transcriptional expression of the Egr-1 gene (*72*). Egr-1 is an important regulator of the expression of many target genes involved in the coupling of external signals to target gene expression. It was reported that normal mouse hepatocytes and FXR knockout mouse liver cells were simultaneously exposed to DCA and CDCA, and there was no difference in Egr-1 expression level, indicating that Egr-1 expression was not associated with FXR (*73*). The expression level of Egr-1 in hepatocytes using highly-selective MEK inhibitors such as U0126 was significantly decreased, and pretreatment with U0126 prevented Egl-1 levels in BDL model mice from increasing, indicating the level of Egr-1 upregulation is dependent on the MAPK pathway during cholestasis (*74*). Egr-1 may be central to the inflammatory response caused by cholestasis and a potential therapeutic target for inhibition of inflammatory response during cholestasis (Figure 1).

6. Conclusion

Cholestasis is a complex pathophysiological process with multiple causes, multi-cell involvement. Cholestasis-induced liver injury is mainly a neutrophil-mediated inflammatory response. Therefore, the treatment of cholestatic liver injury is mainly reflected in the inhibition of neutrophil chemotaxis and inhibition of neutrophil-induced oxidative stress. The specific conduction pathway or target can provide new, more specific treatment options to treat cholestatic liver injury (Table 2).

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References

1. Woolbright BL, Jaeschke H. Therapeutic targets for cholestatic liver injury. Expert Opin Ther Targets. 2016; 20:463-475.
2. Li Y, Tang R, Leung PSC, Gershwin ME, Ma X. Bile acids and intestinal microbiota in autoimmune cholestatic liver diseases. Autoimmun Rev. 2017; 16:885-896.
3. Yang T, Khan GJ, Wu Z, Wang X, Zhang L, Jiang Z. Bile acid homeostasis paradigm and its connotation with cholestatic liver diseases. Drug Discov Today. 2019; 24:112-128.
4. Pena Polanco NA, Levy C, Martin EF. Cholestatic Liver Diseases After Liver Transplant. Clin Liver Dis. 2017; 21:403-420.
5. Santiago P, Scheinberg AR, Levy C. Cholestatic liver diseases: new targets, new therapies. Therap Adv Gastroenterol. 2018; 11:1756284818787400.
6. Woolbright BL, Li F, Xie Y, Farhood A, Fickert P, Trauner M, Jaeschke H. Lithocholic acid feeding results in direct hepato-toxicity independent of neutrophil function in mice. Toxicol Lett. 2014; 228:56-66.
7. Galbiati G, Muraca M, Mitry RR, Hughes RD, Lehec SC, Puppi J, Sagias FG, Caruso M, Mieli-Vergani G, Dhawan A. Bilirubin, a physiological antioxidant, can improve cryopreservation of human hepatocytes. J Pediatr Gastroenterol Nutr. 2010; 50:691-693.
8. Qaisiya M, Coda Zabetta CD, Bellarosa C, Tiribelli C. Bilirubin mediated oxidative stress involves antioxidant response activation *via* Nrf2 pathway. Cell Signal. 2014; 26:512-520.
9. Masubuchi N, Sugihara M, Sugita T, Amano K, Nakano M, Matsuura T. Oxidative stress markers, secondary bile acids and sulfated bile acids classify the clinical liver injury type: Promising diagnostic biomarkers for cholestasis. Chem Biol Interact. 2016; 255:83-91.
10. Zhang Y, Hong JY, Rockwell CE, Copple BL, Jaeschke H, Klaassen CD. Effect of bile duct ligation on bile acid composition in mouse serum and liver. Liver Int. 2012; 32:58-69.
11. Kodali P, Wu P, Lahiji PA, Brown EJ, Maher JJ. ANIT toxicity toward mouse hepatocytes *in vivo* is mediated primarily by neutrophils *via* CD18. Am J Physiol Gastrointest Liver Physiol. 2006; 291:G355-G363.
12. Saito JM, Maher JJ. Bile duct ligation in rats induces biliary expression of cytokine-induced neutrophil chemoattractant. Gastroenterology. 2000; 118:1157-1168.
13. Gujral JS, Liu J, Farhood A, Hinson JA, Jaeschke H. Functional importance of ICAM-1 in the mechanism of neutrophil-induced liver injury in bile duct-ligated mice. Am J Physiol Gastrointest Liver Physiol. 2004; 286:G499-507.
14. Li M, Cai SY, Boyer JL. Mechanisms of bile acid mediated inflammation in the liver. Mol Aspects Med. 2017; 56:45-53.
15. Jones H, Alpini G, Francis H. Bile acid signaling and biliary functions. Acta Pharm Sin B. 2015; 5:123-128.
16. Chu M, Zhou M, Jiang C, Chen X, Guo L, Zhang M, Chu Z, Wang Y. Staphylococcus aureus Phenol-Soluble Modulins alpha1-alpha3 Act as Novel Toll-Like Receptor (TLR) 4 Antagonists to Inhibit HMGB1/TLR4/NF-kappaB Signaling Pathway. Front Immunol. 2018; 9:862.
17. McGill MR, Staggs VS, Sharpe MR, Lee WM, Jaeschke H, Acute Liver Failure Study G. Serum mitochondrial

biomarkers and damage-associated molecular patterns are higher in acetaminophen overdose patients with poor outcome. Hepatology. 2014; 60:1336-1345.
18. McGill MR, Jaeschke H. Mechanistic biomarkers in acetaminophen-induced hepatotoxicity and acute liver failure: from preclinical models to patients. Expert Opin Drug Metab Toxicol. 2014; 10:1005-1017.
19. Toki Y, Takenouchi T, Harada H, Tanuma S, Kitani H, Kojima S, Tsukimoto M. Extracellular ATP induces P2X7 receptor activation in mouse Kupffer cells, leading to release of IL-1beta, HMGB1, and PGE2, decreased MHC class I expression and necrotic cell death. Biochem Biophys Res Commun. 2015; 458:771-776.
20. Yin H, Huang L, Ouyang T, Chen L. Baicalein improves liver inflammation in diabetic db/db mice by regulating HMGB1/TLR4/NF-kappaB signaling pathway. Int Immunopharmacol. 2018; 55:55-62.
21. Singh R, Hui T, Matsui A, Allahem Z, Johnston CD, Ruiz-Torruella M, Rittling SR. Modulation of infection-mediated migration of neutrophils and CXCR2 trafficking by osteopontin. Immunology. 2017; 150:74-86.
22. Castello LM, Raineri D, Salmi L, Clemente N, Vaschetto R, Quaglia M, Garzaro M, Gentilli S, Navalesi P, Cantaluppi V, Dianzani U, Aspesi A, Chiocchetti A. Osteopontin at the Crossroads of Inflammation and Tumor Progression. Mediators Inflamm. 2017; 2017:4049098.
23. Apte UM, Banerjee A, McRee R, Wellberg E, Ramaiah SK. Role of osteopontin in hepatic neutrophil infiltration during alcoholic steatohepatitis. Toxicol Appl Pharmacol. 2005; 207:25-38.
24. Winterbourn CC, Kettle AJ, Hampton MB. Reactive Oxygen Species and Neutrophil Function. Annu Rev Biochem. 2016; 85:765-792.
25. Yang M, Ramachandran A, Yan HM, Woolbright BL, Copple BL, Fickert P, Trauner M, Jaeschke H. Osteopontin is an initial mediator of inflammation and liver injury during obstructive cholestasis after bile duct ligation in mice. Toxicol Lett. 2014; 224:186-195.
26. Okada K, Shoda J, Taguchi K, Maher JM, Ishizaki K, Inoue Y, Ohtsuki M, Goto N, Sugimoto H, Utsunomiya H, Oda K, Warabi E, Ishii T, Yamamoto M. Nrf2 counteracts cholestatic liver injury *via* stimulation of hepatic defense systems. Biochem Biophys Res Commun. 2009; 389:431-436.
27. Zhao Q, Liu F, Cheng Y, Xiao XR, Hu DD, Tang YM, Bao WM, Yang JH, Jiang T, Hu JP, Gonzalez F, Li F. Celastrol protects from cholestatic liver injury though modulation of SIRT1-FXR signaling. Mol Cell Proteomics. 2019; pii: mcp.RA118.000817.
28. Wang L, Cao F, Zhu LL, Liu P, Shang YR, Liu WH, Dong X, Bao HD, Gong P, Wang ZY. Andrographolide impairs alpha-naphthylisothiocyanate-induced cholestatic liver injury *in vivo*. J Nat Med. 2019; 73:388-396.
29. Nabih ES, El-Kharashi OA. Targeting HMGB1/TLR4 axis and miR-21 by rosuvastatin: role in alleviating cholestatic liver injury in a rat model of bile duct ligation. Naunyn Schmiedebergs Arch Pharmacol. 2019; 392:37-43.
30. Cao F, Liu P, Zhang X, Hu Y, Dong X, Bao H, Kong L, Wang L, Gong P. Shenqi Fuzheng Injection impairs bile duct ligation-induced cholestatic liver injury *in vivo*. Biosci Rep. 2019; 39. pii: BSR20180787
31. Zhang J, Guo X, Hamada T, Yokoyama S, Nakamura Y, Zheng J, Kurose N, Ishigaki Y, Uramoto H, Tanimoto A, Yamada S. Protective effects of peroxiredoxin 4 (PRDX4) on cholestatic liver injury. Int J Mol Sci. 2018; 19.
32. Yuan Z, Wang G, Qu J, Wang X, Li K. 9-cis-retinoic acid elevates MRP3 expression by inhibiting sumoylation of RXRalpha to alleviate cholestatic liver injury. Biochem Biophys Res Commun. 2018; 503:188-194.
33. Zeng H, Jiang Y, Chen P, Fan X, Li D, Liu A, Ma X, Xie W, Liu P, Gonzalez FJ, Huang M, Bi H. Schisandrol B protects against cholestatic liver injury through pregnane X receptors. Br J Pharmacol. 2017; 174:672-688.
34. Wang YY, Lin SY, Chen WY, Liao SL, Wu CC, Pan PH, Chou ST, Chen CJ. Glechoma hederacea extracts attenuate cholestatic liver injury in a bile duct-ligated rat model. J Ethnopharmacol. 2017; 204:58-66.
35. Li YF, Wu JS, Li YY, Dai Y, Zheng M, Zeng JK, Wang GF, Wang TM, Li WK, Zhang XY, Gu M, Huang C, Yang L, Wang ZT, Ma YM. Chicken bile powder protects against alpha-naphthylisothiocyanate-induced cholestatic liver injury in mice. Oncotarget. 2017; 8:97137-97152.
36. Gao X, Fu T, Wang C, Ning C, Kong Y, Liu Z, Sun H, Ma X, Liu K, Meng Q. Computational discovery and experimental verification of farnesoid X receptor agonist auraptene to protect against cholestatic liver injury. Biochem Pharmacol. 2017; 146:127-138.
37. Yu L, Liu X, Li X, Yuan Z, Yang H, Zhang L, Jiang Z. Protective effects of SRT1720 *via* the HNF1alpha/FXR signalling pathway and anti-inflammatory mechanisms in mice with estrogen-induced cholestatic liver injury. Toxicol Lett. 2016; 264:1-11.
38. Yang QL, Yang F, Gong JT, Tang XW, Wang GY, Wang ZT, Yang L. Sweroside ameliorates alpha-naphthylisothiocyanate-induced cholestatic liver injury in mice by regulating bile acids and suppressing pro-inflammatory responses. Acta Pharmacol Sin. 2016; 37:1218-1228.
39. Tan Z, Luo M, Yang J, Cheng Y, Huang J, Lu C, Song D, Ye M, Dai M, Gonzalez FJ, Liu A, Guo B. Chlorogenic acid inhibits cholestatic liver injury induced by alpha-naphthylisothiocyanate: involvement of STAT3 and NFkappaB signalling regulation. J Pharm Pharmacol. 2016; 68:1203-1213.
40. Yu SJ, Bae S, Kang JS, Yoon JH, Cho EJ, Lee JH, Kim YJ, Lee WJ, Kim CY, Lee HS. Hepatoprotective effect of vitamin C on lithocholic acid-induced cholestatic liver injury in Gulo$^{-/-}$ mice. Eur J Pharmacol. 2015; 762:247-255.
41. Kong LY, Li GP, Yang P, Xi Z. Protective effect of thymoquinone on cholestatic rats with liver injury. Genet Mol Res. 2015; 14:12247-12253.
42. Abdel Kawy HS. Cilostazol attenuates cholestatic liver injury and its complications in common bile duct ligated rats. Eur J Pharmacol. 2015; 752:8-17.
43. Lin SY, Wang YY, Chen WY, Chuang YH, Pan PH, Chen CJ. Beneficial effect of quercetin on cholestatic liver injury. J Nutr Biochem. 2014; 25:1183-1195.
44. Peng J, Yue C, Qiu K, Chen J, Aller MA, Ko KS, Yang H. Protective effects of guava pulp on cholestatic liver injury. ISRN Hepatol. 2013; 2013:601071.
45. Jang JH, Rickenbacher A, Humar B, Weber A, Raptis DA, Lehmann K, Stieger B, Moritz W, Soll C, Georgiev P, Fischer D, Laczko E, Graf R, Clavien PA. Serotonin protects mouse liver from cholestatic injury by decreasing bile salt pool after bile duct ligation. Hepatology. 2012; 56:209-218.
46. Chen WY, Lin SY, Pan HC, Liao SL, Chuang YH, Yen YJ, Lin SY, Chen CJ. Beneficial effect of

docosahexaenoic acid on cholestatic liver injury in rats. J Nutr Biochem. 2012; 23:252-264.
47. Pratap A, Panakanti R, Yang N, Lakshmi R, Modanlou KA, Eason JD, Mahato RI. Cyclopamine attenuates acute warm ischemia reperfusion injury in cholestatic rat liver: hope for marginal livers. Mol Pharm. 2011; 8:958-968.
48. Chan CC, Cheng LY, Lin CL, Huang YH, Lin HC, Lee FY. The protective role of natural phytoalexin resveratrol on inflammation, fibrosis and regeneration in cholestatic liver injury. Mol Nutr Food Res. 2011; 55:1841-1849.
49. Coban S, Yildiz F, Terzi A, Al B, Ozgor D, Ara C, Polat A, Esrefoglu M. The effect of caffeic acid phenethyl ester (CAPE) against cholestatic liver injury in rats. The J Surg Res. 2010; 159:674-679.
50. Song M, Song Z, Barve S, Zhang J, Chen T, Liu M, Arteel GE, Brewer GJ, McClain CJ. Tetrathiomolybdate protects against bile duct ligation-induced cholestatic liver injury and fibrosis. J Pharmacol Exp Ther. 2008; 325:409-416.
51. Yilmaz M, Ara C, Isik B, Karadag N, Yilmaz S, Polat A, Coban S, Duzova H. The effect of aminoguanidine against cholestatic liver injury in rats. Cell Biochem Funct. 2007; 25:625-632.
52. Fani B, Bertani L, Paglianiti I, Fantechi L, De Bortoli N, Costa F, Volterrani D, Marchi S, Bellini M. Pros and Cons of the SeHCAT Test in Bile Acid Diarrhea: A More Appropriate Use of an Old Nuclear Medicine Technique. Gastroenterol Res Pract.. 2018; 2018:2097359.
53. Droge C, Bonus M, Baumann U, *et al*. Sequencing of FIC1, BSEP and MDR3 in a large cohort of patients with cholestasis revealed a high number of different genetic variants. J Hepatol. 2017; 67:1253-1264.
54. Jansen PLM. New therapies target the toxic consequences of cholestatic liver disease. Expert Rev Gastroenterol Hepatol. 2018; 12:277-285.
55. Fickert P, Krones E, Pollheimer MJ, *et al*. Bile acids trigger cholemic nephropathy in common bile-duct-ligated mice. Hepatology. 2013; 58:2056-2069.
56. Dempsey JL, Wang D, Siginir G, Fei Q, Raftery D, Gu H, Cui JY. Pharmacological Activation of PXR and CAR Down-regulates Distinct Bile Acid-metabolizing Intestinal Bacteria and Alters Bile Acid Homeostasis. Toxicol Sci. 2018. doi: 10.1093/toxsci/kfy271
57. Hirschfield GM, Mason A, Luketic V, *et al*. Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid. Gastroenterology. 2015; 148:751-761 e758.
58. Fickert P, Zollner G, Fuchsbichler A, Stumptner C, Weiglein AH, Lammert F, Marschall HU, Tsybrovskyy O, Zatloukal K, Denk H, Trauner M. Ursodeoxycholic acid aggravates bile infarcts in bile duct-ligated and Mdr2 knockout mice *via* disruption of cholangioles. Gastroenterology. 2002; 123:1238-1251.
59. Weerachayaphorn J, Luo Y, Mennone A, Soroka CJ, Harry K, Boyer JL. Deleterious effect of oltipraz on extrahepatic cholestasis in bile duct-ligated mice. J Hepatol. 2014; 60:160-166.
60. Fang YW, Han SI, Mitchell C, Gupta S, Studer E, Grant S, Hylemon PB, Dent P. Bile acids induce mitochondrial ROS, which promote activation of receptor tyrosine kinases and signaling pathways in rat hepatocytes. Hepatology. 2004; 40:961-971.
61. Hsiang CY, Lin LJ, Kao ST, Lo HY, Chou ST, Ho TY. Glycyrrhizin, silymarin, and ursodeoxycholic acid regulate a common hepatoprotective pathway in HepG2 cells. Phytomedicine. 2015; 22:768-777.
62. Li S, Qiu M, Kong Y, *et al*. Bile Acid G Protein-Coupled Membrane Receptor TGR5 Modulates Aquaporin 2-Mediated Water Homeostasis. J Am Soc Nephrol. 2018; 29:2658-2670.
63. McMillin M, Frampton G, Grant S, Khan S, Diocares J, Petrescu A, Wyatt A, Kain J, Jefferson B, DeMorrow S. Bile Acid-Mediated Sphingosine-1-Phosphate Receptor 2 Signaling Promotes Neuroinflammation during Hepatic Encephalopathy in Mice. Front Cell Neurosci. 2017; 11:191.
64. Yang Z, Li J, Xiong F, Huang J, Chen C, Liu P, Huang H. Berberine attenuates high glucose-induced fibrosis by activating the G protein-coupled bile acid receptor TGR5 and repressing the S1P2/MAPK signaling pathway in glomerular mesangial cells. Exp Cell Res. 2016; 346:241-247.
65. Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, Fukusumi S, Habata Y, Itoh T, Shintani Y, Hinuma S, Fujisawa Y, Fujino M. A G protein-coupled receptor responsive to bile acids. J Biol Chem. 2003; 278:9435-9440.
66. Keitel V, Donner M, Winandy S, Kubitz R, Haussinger D. Expression and function of the bile acid receptor TGR5 in Kupffer cells. Biochem Biophys Res Commun. 2008; 372:78-84.
67. Li Z, Huang J, Wang F, *et al*. Dual Targeting of Bile Acid Receptor-1 (TGR5) and Farnesoid X Receptor (FXR) Prevents Estrogen-Dependent Bone Loss in Mice. J Bone Miner Res. 2018. doi: 10.1002/jbmr.3652.
68. Lou G, Ma X, Fu X, Meng Z, Zhang W, Wang YD, Van Ness C, Yu D, Xu R, Huang W. GPBAR1/TGR5 mediates bile acid-induced cytokine expression in murine Kupffer cells. PloS One. 2014; 9:e93567.
69. Wang YD, Chen WD, Yu D, Forman BM, Huang W. The G-protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor kappa light-chain enhancer of activated B cells (NF-kappaB) in mice. Hepatology. 2011; 54:1421-1432.
70. Pean N, Doignon I, Garcin I, Besnard A, Julien B, Liu B, Branchereau S, Spraul A, Guettier C, Humbert L, Schoonjans K, Rainteau D, Tordjmann T. The receptor TGR5 protects the liver from bile acid overload during liver regeneration in mice. Hepatology. 2013; 58:1451-1460.
71. Jadhav K, Xu Y, Xu Y, Li Y, Xu J, Zhu Y, Adorini L, Lee YK, Kasumov T, Yin L, Zhang Y. Reversal of metabolic disorders by pharmacological activation of bile acid receptors TGR5 and FXR. Mol Metab. 2018; 9:131-140.
72. Allen K, Kim ND, Moon JO, Copple BL. Upregulation of early growth response factor-1 by bile acids requires mitogen-activated protein kinase signaling. Toxicol Appl Pharmacol. 2010; 243:63-67.
73. Zhang Y, Xu N, Xu J, Kong B, Copple B, Guo GL, Wang L. E2F1 is a novel fibrogenic gene that regulates cholestatic liver fibrosis through the Egr-1/SHP/EID1 network. Hepatology. 2014; 60:919-930.
74. Koeppel TA, Trauner M, Baas JC, Thies JC, Schlosser SF, Post S, Gebhard MM, Herfarth C, Boyer JL, Otto G. Extrahepatic biliary obstruction impairs microvascular perfusion and increases leukocyte adhesion in rat liver. Hepatology. 1997; 26:1085-1091.

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