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REVIEW ARTICLE

Research progress on the protective effects of licorice-derived 18β-glycyrrhetinic acid against liver injury

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The first description of the medical use of licorice appeared in "Shennong Bencao Jing", one of the well-known Chinese herbal medicine classic books dated back to 220–280 AD. As one of the most commonly prescribed Chinese herbal medicine, licorice is known as "Guo Lao", meaning "a national treasure" in China. Modern pharmacological investigations have confirmed that licorice possesses a number of biological activities, such as antioxidation, anti-inflammatory, antiviral, immune regulation, and liver protection. 18β-glycyrrhetinic acid is one of the most extensively studied active integrants of licorice. Here, we provide an overview of the protective effects of 18β-glycyrrhetinic acid against various acute and chronic liver diseases observed in experimental models, and summarize its pharmacological effects and potential toxic/side effects at higher doses. We also make additional comments on the important areas that may warrant further research to support appropriate clinical applications of 18β-glycyrrhetinic acid and avoid potential risks.

Keywords: 18β-glycyrrhetic acid; licorice preparation; liver injury; glycyrrhetinic acid target

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INTRODUCTION

18β-Glycyrrhetinic acid (18β-GA) is an in vivo metabolic component of glycyrrhizic acid (GL) formed after two sugar moieties are removed by the intestinal flora. 18β-GA is widely considered one of the main active substances of licorice [1]. The current research on the biological activities of 18β-GA mainly focuses on its adrenal cortical hormone-like properties and anti-inflammatory [2, 3], immunoregulatory [4], anti-tumor [5, 6], anti-injury [7], and antioxidative activities [8, 9]. Based on the liver protective properties of licorice, some scholars have suggested that 18β-GA may also possess a strong liver protective effect [10–12]. In this review, we will summarize the liver protective effects of 18β-GA and its mechanisms in different models of liver injury.

BIOLOGICAL ACTIVITY OF 18B-GA

Licorice was initially described in "Shennong Bencao Jing", an ancient Chinese book on agriculture and medicinal plants. Licorice remains the most commonly used traditional Chinese medicine today. It is also a commonly used ingredient in food, beverages, tobacco, and cosmetics. One of the most important known naturally occurring components of licorice is GL. This component has been explicitly recommended as a hepatoprotective drug by several guides [13, 14]. There are two epimers of GL, namely, 18α-GL and 18β-GL (Fig. 1) [9]. After oral administration, 18α-GL accumulates mainly in the liver [9] and has potent antifibrotic effects, which are significantly higher than those of 18β-GL [15]. 18α-GL is the main active ingredient of TianqingGanmei and Ganlixin, two anti-inflammatory and liver enzyme-lowering hepatoprotective drugs developed in China. Additionally, pharmacokinetic studies have shown that 18α- and 18β-GL can be converted to their respective metabolites, 18α- and 18β-GA, at a significantly higher rate. A preliminary study suggested that 5 min after mice were intraperitoneally administered 18α-GL and 18β-GL, the substances were distributed in all organs. Except for those in blood, the cumulative levels of these substances were the highest in the liver, and the blood concentration of 18α-GL was significantly higher than that of 18β-GL. As time passed, 18α-GL decreased rapidly, and by 3 h, the 18α-GL content was only one tenth of the 18β-GL content. This indicates that the metabolism rate of 18α-GL in mice is significantly faster than that of 18β-GL [16]. Furthermore, pharmacokinetics studies have indicated that the rate of conversion of 18α-GL to GA (including 18α-GA and 18β-GA) is significantly higher than that of 18β-GL. This may be the main reason why 18α-GL is more potent than 18β-GL. When GL is hydrolyzed, the content of 18β-GA is above 97% [17]. In addition, almost 100% of GL is irreversibly transformed into 18β-GA in the gastrointestinal tract by bacterial β-*D*-glucuronidase after it is orally administered [12, 18, 19]. Therefore, 18β-GA is the main metabolite of GL and the main active component of licorice root [20]. GA is a pentacyclic triterpenoid derivative of beta-amyrin and is the aglycone of GL [12], which has two major optical isomers, as shown in Fig. 2.

18B-GA PROTECTS AGAINST DIFFERENT TYPES OF LIVER DISEASES

The liver is the main metabolic and defensive organ of the human body, and it performs multiple functions, such as biotransformation

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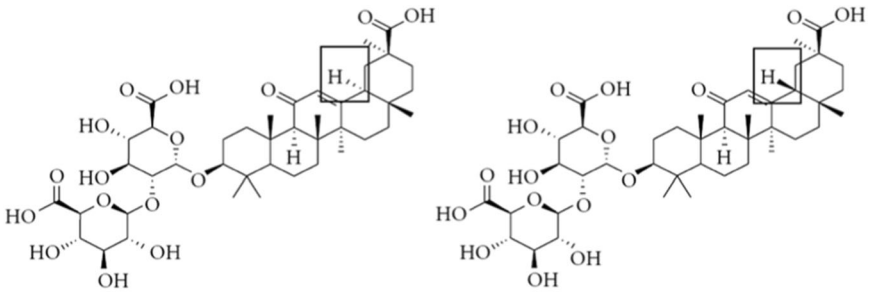

Fig. 1 Chemical structure of glycyrrhizic acid (GL)

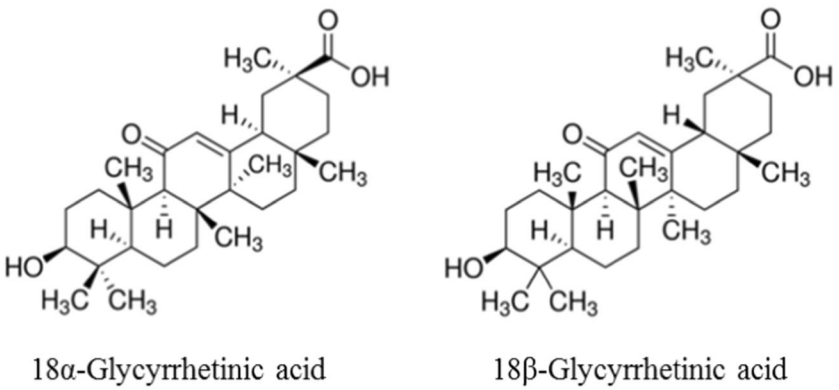

Fig. 2 Chemical structure of glycyrrhetinic acid (GA)

and detoxification [21]. When the liver is overloaded, liver dysfunction, massive hepatocyte necrosis, abnormal liver histopathology, and liver failure develop, resulting in various complications, such as jaundice, hepatic encephalopathy, hepatic nephropathy, and even death. According to statistics, the number of liver disease cases worldwide is as high as 1.3 billion, and approximately one-third of these cases occur in China [22]. Liver disease can be divided into chronic, subacute, and acute liver diseases based on the onset of liver injury [23]. Subacute liver injury refers to acute liver dysfunction that occurs in chronic liver disease. However, due to regional differences in the definition and diagnostic criteria of subacute liver injury both domestically and internationally and because there is no unified prognostic evaluation system, relatively few studies on subacute liver injury have been performed. Due to the different pathogeneses and clinical manifestations of various liver diseases and the mutual influence of pathogenic factors, the treatment of liver injuries remains a difficult challenge to healthcare providers.

Acute liver injury

Recent wide-ranging studies on acute liver injury have focused on massive hepatocyte death or the loss of liver function caused by various etiologies or pathogenic factors in the absence of chronic liver disease. The causes of acute liver injury are numerous and complex and include viruses, drugs, excessive alcohol intake, ischemia, and food poisoning. The incidence of liver damage remains high in China and around the world.

Drug-induced liver injury (DILI). Toxic damage to the liver caused by various prescription or nonprescription drugs or their metabolites is called drug-induced liver injury (DILI) and is common. DILI is one of the most common and serious adverse drug reactions and can cause acute liver failure or even death [24]. The main reason for the susceptibility of the liver to adverse drug reactions is probably its central role in biotransformation, which involves cytochrome P450 (CYP; phase 1), conjugation (phase 2), and transportation (phase 3) [20, 25, 26]. Recent studies have shown that 18β-GA pretreatment can minimize cyclophosphamide-induced hepatotoxicity [27] and reduce methotrexate-induced oxidative stress and liver injury [28], both of which are closely related to the activation of the Nrf2 [29] and PPARγ pathways, which may be related to ERK [30] or Sirt1 [7]. A variety of Chinese herbal medicines contain hepatotoxic pyrrolizidine alkaloids (PAs), which may cause acute and chronic liver damage in humans [31]. 18β-GA pretreatment for three days significantly reverses PA-induced liver injury in SD rats, as indicated by significant reductions in serum GOT and GPT levels and various transaminase levels [32]. It was reported that 18β-GA significantly reduces APAP-induced hepatic inflammatory cell infiltration (which involves the JNK and NF-κB pathways [33]), decreases HMGB1-TLR4 signaling activation [29], downregulates CYP2E1 expression and significantly reduces ROS production [34]. In addition, 18β-GA can increase the expression level of Sirt 6, inhibit the translocation of the HMGB1 protein from the nucleus and reverse the lipopolysaccharide-induced extracellular accumulation of HMGB1 through the same mechanism [35]. 18β-GA pretreatment can significantly reduce liver ALT and AST levels and serum fatty acid and carnitine level and improve APAP-induced hepatotoxicity by reversing the metabolism of fatty acids [11]. Conversely, it has also been reported that GL can rapidly improve APAP-induced liver damage by directly inhibiting TNFα-induced hepatocyte apoptosis. However, 18β-GA does not have the potential to treat the effects of excess APAP [36]. 18β-GA promotes the production and accumulation of GSH, improves the viability of hepatocytes, and alleviates hepatotoxicity induced by isoniazid (INH) [37]. 18β-GA pretreatment

significantly improves 2-AAF-induced lipid peroxidation and decreases ALT and AST levels, xanthine oxidase activity, and 2-phase detoxification enzyme activity. 18β-GA has a potential hepatoprotective effect by attenuating oxidative stress, inflammation, and hyperproliferation [38].

Cholestatic liver injury. Cholestasis is a complication of a variety of hepatobiliary diseases. The main manifestation of cholestasis is abnormal bile secretion or excretion. α-Naphthyl isothiocyanate (ANIT) is a toxin widely used to mimic human clinical cholestatic liver damage. It was found that 18β-GA can promote cholestasis by promoting bile flow and has significant effects against ANIT-induced liver injury in rats [39, 40]. Data have also demonstrated that 18β-GA can prevent ANIT-induced liver damage by regulating the expression of bile acid transporters and reversing bile acid metabolites [39]. Our laboratory also demonstrated that 18β-GA can promote bile acid efflux transporter expression, regulate bile acid balance, and improve ANIT-induced cholestatic liver injury by activating the Sirt1/FXR signaling pathway [7]. The 18β-GA derivative TY501 also reduces LCA-induced cholestatic liver injury by activating the FXR-mediated upregulation of efflux transporters such as Bsep, Mrp2, and Ntcp [41].

Fulminant liver failure. Fulminant hepatic failure (FHF) is caused by many factors, such as viral infection and APAP overdose. FHF is characterized by rapid hepatocyte death and a loss of liver function with temporal and regional differences as well as poor prognosis that may be related to high mortality [42]. Liver injury induced by *D*-galactosamine/lipopolysaccharide (*D*-GalN/LPS) is the most commonly used experimental model of fulminant hepatic failure. A mouse model of acute inflammation induced by *Propionibacterium acnes*/LPS has been used to study the potential mechanism of 18β-GA. 18β-GA can improve acute *P. acnes*-induced liver injury by downregulating MyD88 expression, inhibiting NF-κB activation, and decreasing MIP-1α expression in Kupffer cells [43]. 18β-GA pretreatment can inhibit IRAK-1, subsequently hinder the MAPK and NF-κB signaling pathways, inhibit TNF-α production, and reduce LPS/*D*-GalN-induced liver inflammation and liver failure by upregulating IRAK-M [44]. 18β-GA has a strong anti-inflammatory effect by inhibiting the expression of NO, PGE_2 and ROS induced by LPS and the expression of proinflammatory genes by inhibiting NF-κB and PI3K activities [45–47]. 18β-GA significantly inhibits the expression of the dendritic cell (DC) surface molecules CD80, CD86, and major histocompatibility complex (MHC) class I and class II and the production of interleukin-12 in LPS-stimulated DCs and thus has the potential to treat DC-related acute and chronic liver diseases [48].

Chronic liver disease

Chronic liver injury is a long-term progressive disease caused by a variety of factors that can progress to liver fibrosis and cirrhosis [49]. Common experimental chronic liver injury models include alcoholic liver injury, nonalcoholic fatty liver disease, and CCl_4-induced liver fibrosis.

Fatty liver disease. Fatty liver disease is the excessive deposition of lipids in the liver caused by excessive fat intake and decreased triglyceride secretion [50]. The incidence of fatty liver disease in China is reported to be as high as 10% [51] and is increasing each year. Thus, fatty liver disease is one of the most serious public health threats in China. It was demonstrated that 18β-GA inhibits fat production by inhibiting MAPK activation and significantly reduces the obesity index, lipid deposition and plasma lipid levels in HFD-induced animals [52]. 18β-GA can also inhibit the expression of cathepsin B and enzyme activity by stabilizing the integrity of lysosomes and mitochondria, significantly reducing FFA/HFD-induced hepatic lipotoxicity and protecting against fatty liver disease [53]. 18β-GA can significantly improve hepatic steatosis, inflammation and fibrosis induced by methionine- and choline-deficient diets by regulating the bile acid balance and inhibiting inflammatory damage [54].

Licorice has been shown to have a significant protective effect against alcohol-induced fatty liver disease due to its potent anti-inflammatory and antioxidant activities [55]. Glycyrrhizic acid, as one of the major active compounds in licorice, also has significant effects against alcoholic liver disease (ALD) by regulating oxidative stress and lipid metabolism [56]. Glycyrrhizic acid may also prevent alcoholic hepatitis (AH) through its anti-inflammatory effect [57]. It is well known that excessive drinking causes ALD. Its main features are fat accumulation, inflammation and scarring. The current standard drug treatments for alcoholic liver disease, such as corticosteroids and pentoxifylline, which are mainly used to reduce inflammation in patients with acute alcoholic hepatitis, target the inflammatory pathway. In addition, 18β-GA is an active in vivo metabolite of glycyrrhizic acid (GL), which has also been shown to have significant anti-inflammatory and lipid metabolism regulatory effects. To conclude, the aforementioned scientific data suggest that 18β-GA has a potent protective effect against alcohol-induced liver injury.

Hepatic fibrosis. Hepatic fibrosis is the abnormal proliferation of connective tissue in the liver caused by a variety of factors, including viral hepatitis, alcohol, autoimmune diseases, drug induction and biliary obstruction [58]. Further development of liver cirrhosis leads to hepatic fibrosis. Long-term subcutaneous injection of CCl_4 is widely used to model liver fibrosis in animals, as CCl_4 can cause lipid peroxidation and destroy the membrane structure of hepatocytes [59, 60]. 18β-GA can exert strong effects against chronic liver fibrosis by activating the nuclear translocation of Nrf2 to reverse CCl_4-induced liver oxidative stress in mice [61]. 18β-GA inhibits CCl_4-induced hepatocyte apoptosis via the p53-dependent mitochondrial pathway, thereby delaying the progression of hepatic fibrosis in rats [62]. 18β-GA can prevent alcohol- and CCl_4-induced liver fibrosis in rats by inhibiting the proliferation and activation of HSCs to reduce the production of collagen [63]. 18β-GA significantly inhibits liver fibrosis in mice by inhibiting the nuclear accumulation of Smad3 and Smad3-dependent type 1 collagen synthesis and the transdifferentiation of resting HSCs into activated HSCs [64].

Bile duct ligation (BDL) is another animal model that is widely used to simulate liver fibrosis and cirrhosis caused by long-term cholestasis. 18β-GA treatment significantly reduces collagen deposition in the liver of BDL rats and the gene transcription of α-SMA and TGF-β1 in HSCs [65]. In addition, 18β-GA significantly inhibits hepatitis B surface antigen (HBsAg) and significantly improves HBV-induced liver dysfunction in humans and animals [66].

Cirrhosis. Cirrhosis is a common intrahepatic diffuse fibrosis caused by one or more of the following factors: hepatocyte necrosis, interstitial inflammatory reactions, or liver deformation and hardening. Cirrhosis eventually causes a variety of complications, including liver cancer. It has been reported that the long-term administration of 18β-GA can significantly inhibit liver fibrosis in SD rats by reducing interstitial inflammation and the occurrence of cirrhosis [67], although the detailed anticirrhotic mechanism of 18β-GA has not been further studied.

Hepatocellular carcinoma (HCC). Hepatocellular carcinoma (HCC) is one of the most serious forms of liver diseases worldwide, and in the absence of proper care and treatment, HCC is one of the leading causes of death [22]. Although anticancer drugs and surgery are widely used for the treatment of liver cancer in the clinic, side effects and other adverse reactions influence the treatment outcomes of liver cancer. Recently, a traditional Chinese medicine product, which may reduce the side effects of standard chemotherapy, prolong survival and improve quality of life, has been used as an alternative therapy for patients with

liver cancer [68]. A variety of Chinese medicines have been used alone to treat liver cancer with perceived benefits; however, their treatment effects are often difficult to evaluate due to various compounding factors and the complexity of the disease. On the other hand, the benefits of immunomodulatory and/or anti-inflammatory activities for cancer patients are widely accepted. One Chinese medicine commonly used is Bu-Zhong-Yi-Qi-Tang (BZYQT), which can strengthen immune functions, improve the defense system, and fight against liver cancer in HCC patients [69]. Danshen (*Salvia miltiorrhiza*) is a typical Chinese medicine that can exert anti-inflammatory, antifibrotic and anticancer effects by inhibiting p38 and NF-κB signaling [70]. Glycyrrhizic acid and glycyrrhetinic acid have been used as liver protective drugs for treating chronic hepatitis in China for over a decade. It was reported that 18β-GA can inhibit the proliferation of HepG2 cells by increasing reactive oxygen species, increasing the loss of mitochondrial membrane potential, and relieving the effects of hepatocellular carcinoma [71]. A recent study suggested that 18β-GA induces apoptosis in HCC cells by inducing cell cycle arrest, activating caspase-dependent pathways, and activating the PPARγ pathway [72]. 18β-GA reverses HSC-mediated immunosuppression in HCC and eventually abolishes HCC cell invasion and metastasis [4].

In addition, new formulations of 18β-GA using functionalized nanomaterials, including liposomes, micelles, and nanospheres, and other formulations have been used to treat hepatocellular carcinoma [73–76]. These modified formulations of 18β-GA using nanomaterials induce higher uptake of 18β-GA by HCC cells than unmodified formulations; thus, they are expected to have a stronger inhibitory effect on HCC. The potential mechanisms of the anticancer activity of 18β-GA involve antiproliferative, pro-apoptotic and anti-invasive/antimetastatic activities [77].

Autoimmune liver disease. Autoimmune liver disease (AILD) is attributed to autoimmune abnormalities characterized by the presence of autoantibodies in the circulation. AILD is mainly divided into the following three categories: autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Among those AILDs, AIH is mainly characterized by hepatitis, and PBC and PSC are mainly characterized by cholestasis [78]. Clinical or laboratory diagnosis of AILD, as a chronic liver injury, is usually based on partial symptoms. Interventions for AILD include but are not limited to the use of prednisolone to improve inflammation, the administration of ursodeoxycholic acid (UDCA) to improve cholestasis, and the use of rifampicin to improve pruritus. 18β-GA inhibits the development of autoimmune diseases in autoimmune *lpr* mice. 18β-GA also inhibits urinary protein excretion and serum IgG levels and improves autoimmune diseases in mice [79].

GL preparations have been used in combination with UDCA to treat AILD, and such combinations have been reported to have significant therapeutic effects. One such preparation, Tianqing-Ganping combined with UDCA, was used to treat PSC patients and showed a significant effect in the early stage; initially, the effect was remarkable. However, after 1 year of treatment, the bile acid, total bilirubin, glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) levels of patients increased to varying degrees and fluctuated repeatedly, and the treatment effect decreased with the progression of the disease [80, 81]. It has been found that the combination of magnesium isoglycyrrhizinate and UDCA is more effective than the individual agents alone in patients with PBC [82, 83]. Diammonium glycyrrhizinate combined with UDCA can significantly improve the symptoms of autoimmune hepatitis patients and improve autoimmune function and liver function [84, 85]. 18β-GA has not been tested in patients with autoimmune liver disease in clinical trials. The exact reasons are unknown but might be related to the poor solubility of 18β-GA and insufficient experimental evidence.

PRACTICAL ISSUES OF 18B-GA AS A HEPATOPROTECTIVE AGENT

The use of licorice as a detoxifying herb was reported in "Shennong Bencao Jing", a classic Chinese herbology book published more than 2500 years ago. Later, many licorice-containing prescriptions were also recorded in traditional Chinese medicine masterpieces, such as Shanghan Lun (Han Dynasty), JinGui Yaolue (Han Dynasty) and Bencao Gangmu (Ming Dynasty). The successful extraction and purification of GL from licorice and semisynthetic preparations of glycyrrhizin by Japanese pharmaceutical companies were reported in the 1940s. Japanese scientists also combined glycyrrhizin (GL) with glycine and cysteine (methionine) to form a compound glycyrrhizin product, commonly known as SNMC, Meineng, and Qianglixin, which has been used for the treatment of chronic hepatitis since 1958 with apparent success. Two SNMC-like GL preparations, Ganlixin and TianqingGanmei, were developed in China as first- and second-generation liver protective medicines. The main component of Ganlixin is a mixture of α- and β-glycyrrhizic acid diammonium salts (also known as diammonium glycyrrhizinate). β-Glycyrrhizic acid diammonium can cause hypertension and edema in a small number of patients. TianqingGanmei, prepared from a pure α-GL derivative (magnesium isoglycyrrhizinate), is considered safer than Ganlixin and is thus widely regarded as a second-generation licorice-derived liver protective drug in China. 18β-GA is one of the major active metabolites of α-GL in vivo. Experiments conducted in our lab also demonstrated that the liver protective effect of 18β-GA against ANIT-induced liver damage is significantly stronger than that of diammonium glycyrrhizinate, a result consistent with findings reported in the literature (data not shown here). The strong hepatoprotective effects of 18β-GA in rodents lead us to speculate that 18β-GA may also be a liver protective metabolite of diammonium glycyrrhizinate and magnesium isoglycyrrhizinate.

The chemical structure of 18β-GA has similarities with steroid hormones, indicating that 18β-GA may play a hormone-like role in combination with steroid hormone receptors. The data indeed indicate that 18β-GA increases glucocorticoid activity and alleviates adverse reactions such as drug withdrawal and drug dependence. Data from the literature also indicate that 18β-GA can reduce the inactivation of glucocorticoids by inhibiting 11β-HSD2 to exert anti-inflammatory activity [86]. Further studies have confirmed that 18β-GA promotes the dissociation of the glucocorticoid receptor (GR)-HSP90 complex, which activates GR and negatively interacts with NF-κB and AP-1 to block inflammation [46, 87]. It has also been confirmed that 18β-GA can inhibit ROS production by activating the PI3K/AP-1/HO-1 signaling pathway and the GR/DUSP1 signaling pathway, thereby restoring glucocorticoid sensitivity and reversing glucocorticoid resistance [86].

One of the safety concerns of the long-term use of GL preparations, especially at high doses, is the development of pseudoaldosteronism [88–90]. The clinical manifestations of pseudoaldosteronism include elevated blood pressure, sodium retention, decreased urine potassium, and decreased plasma aldosterone concentrations. Studies have demonstrated that 18β-GA inhibits 11β-HSD2 in the kidney and leads to the corticosteroid-induced excessive accumulation of cortisol in renal tubular epithelial cells and pseudohyperaldosteronism [90–92].

The in vivo metabolic processes of GL and 18β-GA have not been extensively studied. After the oral administration of GL to humans and rats, the concentrations of 18β-GA in the urine are extremely low (below the detection limit (1 ng/100 g body weight)) [18, 93]. In contrast, the concentrations of 3-monoglucuronyl-glycyrrhetinic acid (3MGA, another important metabolite of GL) in the plasma and urine are reported to be much higher [93]. Studies in normal and Eisai hyperbilirubinemia rats (Mrp2-deficient) have confirmed that 3MGA (but not 18β-GA) accumulates in the renal tubule after the oral administration of 18β-GA. The accumulation of

Table 1. Summary of in vivo studies on the effect of 18β-GA in liver injury

Model	18β-GA treated dose	Pharmacological activities	Mechanisms	References
CP-induced wistar rats liver injury	18β-GA, ip 25, 50 mg/kg single dose	Antioxidant defenses and reducing inflammation	Inhibited activating Nrf2 and PPARc	[27]
HFD-induced SD rat NAFLD models	18β-GA, ig 25, 50 mg/kg for 8 weeks	Reduced hepatic lipotoxicity and cell appotosis	Lysosomal and mitochondrial pathways	[53]
Carbon tetrachloride-induced mice liver fibrosis	18β-GA, ip 15 mg/kg three times a week for 4 weeks	Inhibition of hepatic fibrosis	Inhibited nuclear accumulation of Smad3 in activated HSCs	[64]
LPS/*D*-GalN-induced Balb/c mice liver injury	18β-GA, ip 10, 30 or 100 mg/kg single dose	Alleviation of mortality; anti-inflammation	Upregulating IRKM	[44]
Ischemia/reperfusion-induced C57BL/6 mice liver injury	18β-GA, ip 100 mg/kg single dose	Anti-inflammation	Inhibiting TLR4 signaling cascade	[99]
Acetaminophen-induced Balb/c mice acute liver injury	18β-GA, ip 100 mg/kg single dose	Alleviated hepatic damages with hepatocellular apoptosis	Inhibition of CYP2E1 expression and HMGB1-TLR4 pathway	[34]
CCl_4-induced SD rat cirrhosis	18β-GA, ig 72 mg/kg for 7 days)	Inhibition of hepatic fibrosis	Restoring the composition of Lactobacillus	[100]
ANIT-induced 129/Sv mice liver injury	18β-GA, ip 50 mg/kg for 7 days	Inhibited bile acid cycle disruption	Increase transporter expression	[39]
ANIT-induced SD rat liver injury	18β-GA, ip 60 mg/kg for 7 days		Increase transporter expression through Sirt1/FXR	[7]
P. acnes-induced C57BL/6 mice liver injury	18β-GA, ip 75 mg/kg for 1 day	Suppressed mortality and improving the liver dysfunction	Inhibition of macrophage inflammatory protein-1	[43]
Methotrexate-induced wistar rats hepatotoxicity	18β-GA, ig 50, 100 mg/kg 7 days	Attenuation of inflammation, oxidative stress and apoptosis	Inhibited activating Nrf2 and PPARγ	[28]
Realgar-induced ICR mice subchronic hepatotoxicity	18β-GA, ig 16, 48 mg/kg for 8 weeks	Regulating changes in the lipid and energy metabolism	None	[101]
CCl_4-induced Kunming mice chronic liver fibrosis	18β-GA, ig 25, 50, 100 mg/kg for 30 days	Antioxidant defenses	Decreased nuclear Nrf2 expression	[61]
Retrorsine-induced SD rat hepatotoxicity	18β-GA, ip 10 mg/kg for 30 min	Improving the liver dysfunction	None	[32]
BDL-induced SD rat chronic liver fibrosis	18β-GA, iv 2 mg/kg, M6P26-HAS-GA, iv 10 mg/kg, three times a week for 4 weeks	Inhibition of hepatic fibrosis	Target hepatic stellate cell	[65]
CCl_4-plus ethanol-induced SD rat liver cirrhosis	–	Inhibition of hepatic fibrosis	Target hepatic stellate cell	[63]
Dimethyl nitrosamin-induced SD rat liver injury	18α-GL,18β-GL ip,150 mg/kg, three times a week for 4 weeks	Improving the liver dysfunction	None	[102]
Con-A-induced ICR mice immunological liver injury	18α-GL, 18β-GL, ip 15, 30, 60 mg/kg for 5 days	Improving the liver dysfunction	None	[103]
CCl_4-induced ICR mice chronic liver injury	18β-GA, s.c. 10, 50, 100 mg/kg for 3 days	Attenuation of CCl_4-induced hepatotoxicity	Inhibited cytochrome P450 2E1 expression	[10]
CCl_4-induced wistar rat acute liver injury	18β-GA, ip 50, 100 mg/kg for 30 min	Attenuation of acute liver injury	Inhibited β-glucuronidase	[104]
Acetylaminofluorene-induced Wistar rats hepatotoxicity	18β-GA, ig 45, 75 mg/kg for 15 days	Attenuation of oxidative stress, inflammation and hyperproliferation	Restored the expressions of PCNA, COX- 2, iNOS and NF-κB	[38]

CP cyclophosphamide, *CCl_4* carbon tetrachloride, *Con-A* concanavalin A, *BDL* bile duct ligation, *LPS/D-GalN* lipopolysaccharide, LPS/*D*-galactosamine, *P. acnes Propionibacterium acnes*, *ANIT* alpha-naphthylisothi, *IRK-M* interleukin-1 receptor-associated kinase-M, *Nrf2* NF-E2-related factor 2, *PPARg* peroxisome proliferator-activated receptor-gamma, *Sirt1* Sirtuin-1, *FXR* farnesoid X receptor, *TLR4* Toll-like receptor 4, *HFD* high fat diet, *NAFLD* nonalcoholic fatty liver disease, *HSC* hepatic stellate cells, *FAA* fatty acid

Table 2. Summary of in vitro studies on the effect of 18β-GA in liver injury

Cell line	Dose and time	Pharmacological activities	Mechanisms	References
HL-7702 and HepG2	18β-GA and GA derivatives, 5, 10, 20 μM; 24 h	Induced cell cycle arrest at the G_2/M phase, and inhibited cancer cells proliferation and migration	Triggered apoptosis through the mitochondrial pathway	[105]
HepaRG	18β-GA and nanoparticle co-loading entecavir and glycyrrhetinic acid, 2.5, 5, 10, 20 μg/mL, 24 h	Glycyrrhetinic acid can enhance the accumulation of entecavir in HepaRG cell and liver for treating hepatitis B	Improved liver accumulation of entecavir and investigated its ability to deliver both drugs to liver	[106]
HOS and HT1080 cells	20, 40 μM; 24, 48, or 72 h	18β-GA inhibits sarcoma cell proliferation by inducing G_0/G_1-phase arrest	GA induced apoptosis through both extrinsic and intrinsic pathways and GA-induced autophagy, represents a promising therapeutic approach for the treatment of sarcoma	[107]
Hep3B	Serial of concentrations (0.78–25 μM); 24 h	Inhibited tumor growth	GA and poly(*L*-Histidine) mediated polymeric drug delivery system was targeted HCC, and released its encapsulated anticancer drug in the acidic microenvironment of HCC	[74]
HepG2	GA modified curcuminsupramolecular pro-gelator (GA-Cur), serial of concentrations (for IC_{50}); 48 h	Enhanced cellular uptake and better inhibition capacity	–	[108]
HepG2, HLE, LM3, and Hep3B	18β-GA 0, 100, 150, 200 μM; 24 or 48 h	GA suppressed proliferation of various HCC cell lines	GA increased ATF4/CHOP-induced autophagy and IRE1α/XBP1s UPR pathways-induced apoptosis	[6]

HCC hepatocellular carcinoma, *ATF4* activating transcription factor 4, *CHOP* C/EBP homologous protein, *IRE1α* inositol-requiring enzyme 1α, *XBP1* IRE1 activation-dependent X-box-binding protein-1, *UPR* unfolded protein response, *HOS cell* human osteosarcoma cell line, *HT1080 cells* human fibrosarcoma cell line, *HLE* HCC cell lines, *LM3* human hepatocellular carcinoma cell lines

3MGA in the kidney inhibits 11β-HSD2 and in turn leads to pseudohyperaldosteronism [94, 95].

SUMMARY AND PROSPECT

In summary, 18β-GA has significant biological activities against liver injury. Recent studies have shown that 18β-GA has potent liver protective effects in animals (Table 1) and cells (Table 2), which may involve antioxidative (Fig. 3), anti-inflammatory (Fig. 4) and other functions (Fig. 5). However, due to the poor water solubility of 18β-GA, its liver protective effects are still limited to laboratory research. In recent years, many studies have found that 18β-GA is biomodified and targeted to the liver, with the data suggesting that it has significant therapeutic value in different liver disease models and is well tolerated [96, 97]. Preliminary studies have shown that there is a specific 18β-GA-binding protein on the surface of hepatocytes. The binding of GA to this protein is the highest in the liver followed by the kidney. A study of a fluorescent tag (FITC)-labeled 18β-GA analog (FITC-GA) found that

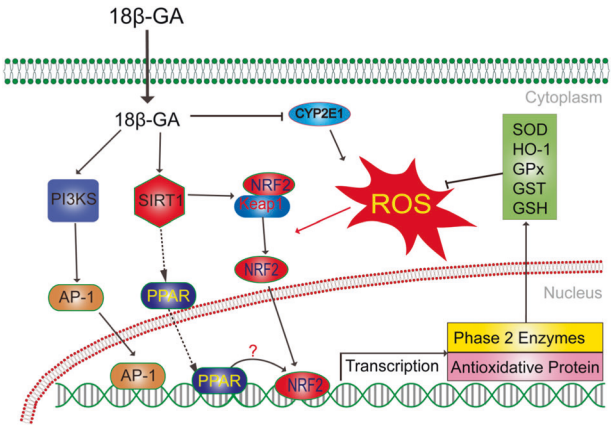

Fig. 3 Networks of molecular signaling underlying antioxidative stress effects of 18β-glycyrrhetinic acid. PI3K: phosphoinositide 3-kinase; AP-1: activator protein-1; ROS: reactive oxygen species; GSH: glutathione; NRF2: NF-E2-related factor 2; PPAR: peroxisome proliferator-activated receptor-gamma; SIRT1: Sirtuin-1; Keap-1: Keleh-like ECH-associated protein-1; CYP2E1: cytochrome P450, family 2, subfamily E, polypeptide 1

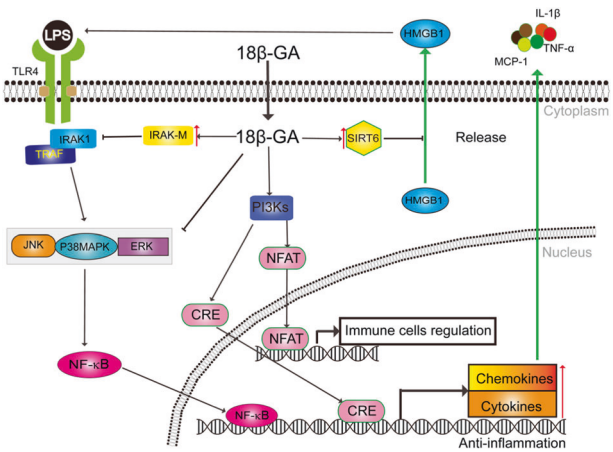

Fig. 4 Networks of molecular signaling underlying anti-inflammation effects of 18β-glycyrrhetinic acid. NFAT: nuclear factor of activated T cells; NF-κB: Nuclear factor κB; CRE: cAMP Responsive Element; JNK: c-Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; ERK: extracellular regulated protein kinases; IRAK: interleukin-1 receptor-associated kinase; TRAF: TNF receptor-associated factor; HMGB1: high mobility group boxe chromosomal protein; SIRT6: Sirtuin-6

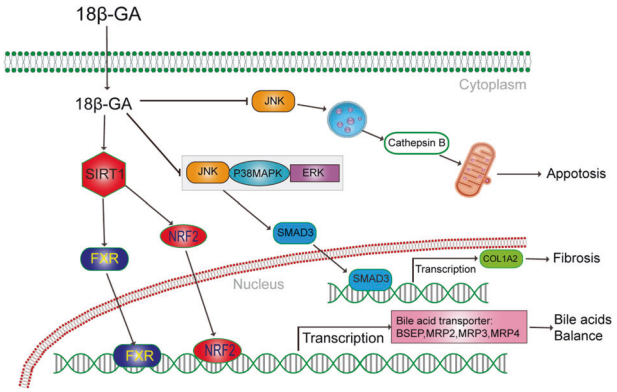

Fig. 5 Networks of molecular signaling underlying other functions of 18β-glycyrrhetinic acid. SMAD3: sekelsky mothers against dpp3; BSEP: bile salt export pump; MRP: multidrug resistance associated protein; COL1A2: collagen, type I, alpha 2

there was competition between FITC-GA and the GA receptor (GA-R) and that 18β-GA and FITC-GA induced similar cytotoxicity in vitro. 18β-GA competitively binds to GA-R with an equilibrium dissociation constant (K_d) of 7.457 ± 2.122 pmol/L and a maximum binding count (B_{max}) of 2.385 ± 0.175 pmol/(2.5×10^6) cells [98]. Studies have also reported that 18β-GA exerts pharmacological effects through intracellular endocytosis and specifically binds GA-R on the cell membrane. However, the specific protein structure of GA-R is currently unknown. We believe that the determination of GA-R, that is, the confirmation of that targets of 18β-GA, warrants further research. First, identifying these targets provides clues for the study of the hepatoprotective mechanism of 18β-GA and serves as a reference biomarker for studying the clinical effects of 18β-GA in the treatment of liver and other diseases. Second, based on these targets, novel liver protective agents could be developed and tested to potentially offer new options for the treatment of relevant human diseases.

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ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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