Review

Traditional Chinese medicine and related active compounds against hepatitis B virus infection

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Summary **Hepatitis B induced by hepatitis B virus (HBV) remains a major public health problem worldwide. Although several antiviral drugs have been approved for hepatitis B, they cause significant dose-dependent side-effects (interferon-α) and drug resistance (lamivudine, *etc.*). Safe and potent new anti-HBV drugs are urgently needed. Traditional Chinese medicine (TCM) is an established segment of the health care system in China and widely used for hepatitis B in China and many parts of the world. Many TCMs and related active compounds have been reported that have promising and potent anti-HBV activities, including *Phyllanthus*, *Salvia miltiorrhiza*, *Rheum palmatum* L., *Radix Astragali*, oxymatrine, artemisinin and artesunate, and wogonin. Thus, TCM is a potential candidate for anti-HBV drugs. More information is needed regarding TCMs, including preparation, standardization, identification of active ingredients, and toxicological evaluation. Therefore, TCM development needs to apply advanced and interdisciplinary methodology and technology and perform further rigorously designed experimental and clinical investigations.**

Keywords: Hepatitis B virus (HBV), traditional Chinese medicine, *Phyllanthus*, *Salvia miltiorrhiza*, oxymatrine

1. Introduction

Hepatitis B is a significant public health concern and it may develop into hepatic fibrosis, liver cirrhosis, and hepatocellular carcinoma, which result in one million deaths annually (*1*). According to the World Health Organization (WHO), there are two billion people worldwide infected by hepatitis B virus (HBV) at some time in their lives (*2*). Of these, more than 350 million people are estimated to chronically infect and become carriers of the virus (*3*). Although several anti-virus drugs have been approved for hepatitis B, they induce significant dose-dependent side-effects and drug resistance. Interferon-α (IFN-α) was the firstly approved therapy for chronic HBV infection around the world. However, its therapeutic effect is not satisfactory and is related with some side-effects such as influenza-like syndrome, and leukocyte and platelet decreases (*4*). Lamivudine (3TC) was the firstly approved nucleotide analog for HBV infection, but its efficacy resembles IFN-α and is associated with drug resistance following prolonged administration (*5*). Thus there exists an urgent need for safe and effective new anti-HBV drugs.

Traditional Chinese medicine (TCM) is an established segment of the health care system in China. There are many TCMs widely used for hepatitis B in China and many parts of the world. In China, Chinese medicine is used as an adjunct or alternative treatment and accounts for 30% to 50% of total medicine consumption, with low costs and low toxicity (*6*). The 2002 National Health Interview Survey (NHIS) of the

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United States suggested that 19% of adults used some form of herbal supplements within the past 12 months (*7*). McCulloch *et al.* (*8*) showed that Chinese medicine may have a potential therapeutic value for treatment of chronic hepatitis B using meta-analysis data. TCM is composed of complex mixtures of compounds. Although the active ingredients of many mixtures have not been completely identified, some ingredients have been isolated and identified as potential therapeutic agents. These natural active compounds offer major opportunities for finding novel active lead structures against a wide range of assay targets because they contain more characteristics of high chemical diversity and biochemical specificity than standard combinatorial chemistry. Moreover, biologically active small molecules derived from natural products have drug-like properties and they can be absorbed and metabolized by the body (*9*). Furthermore, TCM is easily available without the need for laborious pharmaceutical synthesis (*10*). Therefore, TCM may be a good candidate for special antiviral characteristics and it has drawn more attention from researchers making an effort to identify effective antiviral agents (*11*).

2. Virologic features of HBV

HBV is the prototype member of the Hepadnaviridae (hepatotropic DNA virus) family and HBV virions are double-shelled particles (*12*) with an outer lipoprotein envelope containing surface antigens (*13*). The viral nucleocapsid is within the envelope (*14*) and includes the viral genome (relaxed circular, partially double-stranded, 3.2 kb) and a polymerase for the synthesis of viral DNA in infected cells (*15*).

The HBV genome possesses only four long open reading frames: presurface-surface (preS-S) region, precore-core (preC-C) region, P coding region, and X open reading frame. Their translations ultimately yield the viral surface, e, core, and polymerase proteins, as well as the X polypeptides. All the HBV proteins play important roles in HBV transcriptional regulation, viral packaging, reverse-transcription, and viral DNA recycling. Therefore, serum HBV markers are the most important clinical data for epidemic screening and diagnosis of HBV infection (*16*). Among these markers, hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) are most commonly used in experimental and clinical studies.

3. TCM and related active compounds with potential anti-HBV activity

The progress of the major TCM and related active compounds for treatment of HBV infection, including *Phyllanthus*, *Salvia miltiorrhiza*, *Rheum palmatum* L., *Radix Astragali*, oxymatrine, artemisinin and artesunate, and wogonin, are summarized in this section. The main focus is on cell experiments, animal studies, clinical trials, and lack of cytotoxicity. Anti-HBV activities of these TCMs and related active compounds in cell experiments and in clinical trials are shown in Table 1 and Table 2, respectively. Therapeutic index (TI) is defined as the ratio between CC_{50} (drug concentration inducing 50% reduction in host cell viability) and IC_{50} (drug concentration inducing 50% inhibition in HBsAg or HBeAg or HBV DNA release) for the most sensitive parameters to detect reduction in HBV production in each case.

3.1. *Phyllanthus*

The plant genus *Phyllanthus* (Yexiazhu) is widely distributed in most tropical and subtropical countries and consists of approximately 550-750 species throughout the world. It has long been used in traditional medicine to treat chronic liver disease in China and India (*17*). In China, it is estimated that 33 species exist in more than 10 provinces, which is approximately the same number of species as are in India. The most widely studied species have been *P. amarus* (Kuweiyexiazhu), *P. nanus*

Table 1. Anti-HBV activities of TCM and related active compounds in cell experiments

Treatments	Duration (days)	CC_{50}[a]	IC_{50}[b]			TI[c]	*Ref.*
			HBsAg	HBeAg	HBV DNA		
Phyllanthus nanus ethanolic extract	7	100	> 200	-	> 50	< 2	*20*
PA from *Salvia miltiorrhiza*	9	> 96	3.94	2.46	4.17	> 39.02	*38*
Astragaloside IV from *Radix Astragali*	9	388	> 200	> 200	-	< 1.94	*41*
Rheum palmatum L. ethanol extract	8	1,628	292.42	1,435	212.36	7.67	*51*
Chrysophanol 8-*O*-β-D-glucoside from *Rheum palmatum* L.	8	> 10,000	237.4	183.41	36.98	> 270.42	*51*
Oxymatrine	3	> 2,000	-	-	< 1,000	> 2	*53*
Artesunate	21	7.69	0.88	-	0.19	40.47	*58*
Wogonin	9	> 200	4	4	> 20	> 50	*62*

[a] CC_{50}: drug concentration (μg/mL) inducing 50% reduction in host cell viability.
[b] IC_{50}: drug concentration (μg/mL) inducing 50% inhibition in HBsAg, HBeAg, and HBV DNA release.
[c] Therapeutic index (TI) was the ratio between CC_{50} and IC_{50} for the most sensitive parameters to detect reduction in HBV production (HBsAg or HBeAg or HBV DNA) in each case.
PA: protocatechuic aldehyde.

(Aiyexiazhu), and *P. urinaria* (Yexiazhu) (*18*). Many kinds of compounds, including alkaloids, flavonoids, lactones, steroids, triterpenes, lignans, and tannins, were isolated from *Phyllanthus*. These compounds were reported to be responsible for the pharmacologic actions of the plant (*19*).

Phyllanthus is currently used in preclinical and clinical evaluations. Its promising biological activities were shown by *in vitro* and *in vivo* assays. Lam *et al.* (*20*) showed that the ethanolic extract of *P. nanus* produced a suppressive effect on HBsAg secretion, HBsAg mRNA expression, and HBV replication *in vitro*. The TI of the ethanolic extract of *P. nanus* was less than 2 (Table 1). Moreover, Lee *et al.* (*21*) demonstrated that *P. amarus* inhibited HBV production in cell culture and HBV transgenic mice by affecting HBV polymerase and decreasing HBV mRNA accumulation. In the clinic, *P. amarus* was reported to significantly increase the negative conversion rate of serum HBeAg compared with control (*22*) (Table 2). Liu *et al.* (*23*) published a meta-analysis of the efficacy and safety of *Phyllanthus* for chronic HBV infection. Twenty-two randomized clinical trials (n = 1,947) were included. The combined results revealed that *Phyllanthus* had a positive effect on clearance of serum HBsAg compared with placebo or no intervention. There was no significant difference between *Phyllanthus* and interferon in clearance of HBsAg, HBeAg, and HBV DNA (*24*,*25*). *Phyllanthus* plus interferon was better than interferon alone (*26*,*27*) and *Phyllanthus* was better than nonspecific treatment or other herbal medicines for the negative conversion of HBsAg, HBeAg, and HBV DNA (*28*-*35*). No serious adverse reactions were reported. This meta-analysis showed that *Phyllanthus* might have an antiviral effect.

However, some papers reported that *Phyllanthus* had no demonstrable antiviral effect in chronic hepatitis B. A double-blind placebo-controlled study was conducted for treatment of chronic hepatitis B (*36*). After 6 months treatment, there was no difference between *P. urinaris* and placebo in HBV DNA reduction, HBeAg seroconversion, and alanine aminotransferase (ALT) normalization. The discrepancy in the clinical effect in these studies could be attributed to different species, different growing conditions and harvest seasons, and different processing methods. Therefore, standardization of the genus *Phyllanthus* and large-scale prospective, multicenter, randomized, controlled trials are needed.

3.2. *Salvia miltiorrhiza*

Salvia miltiorrhiza (SM, Danshen), a herb, is traditionally used to treat liver disease in China. SM is believed to be one of the most highly recommended and widely accepted medicines for the treatment of hepatitis B in China (*18*).

Like most herbal medicines, SM is not a single entity but comprises different ingredients. Both its lipophilic and hydrophilic fractions have biological activities (*37*). Zhou *et al.* (*38*) isolated and characterized a functionally unique anti-HBV water-soluble substance, protocatechuic aldehyde (PA), from SM. They found that in HepG2.2.15 cells PA (Figure 1A) significantly inhibited the production of HBV DNA with an IC_{50} of 4.17 μg/mL and suppressed the expression of HBsAg and HBeAg with an IC_{50} of 3.94 and 2.46 μg/mL, respectively. The TI of PA was more than 39.02 (Table 1). Moreover, their results showed that PA inhibited duck hepatitis B virus (DHBV) DNA replication in ducks.

In a clinical evaluation, 30 patients with chronic hepatitis B were treated with SM (*39*). After 3 months of treatment, the negative conversion rate of HBeAg was 16.7%. A follow up of 3 and 9 months after the end of treatment showed negative conversion rates of HBeAg were 22.7% and 25.0%, respectively. In another clinical trial (*40*), 123 cases were randomly divided into a treatment group (n = 63) and a control group (n = 60). The treatment group was treated with SM injections and *Radix Astragali* injections and the control group was treated with oral administration of Gankangning tablets and fufang yiganling tablets. The treatment group was significantly better than the control group in the negative conversion of HBeAg and HBV DNA (Table 2).

In summary, the active compound of SM, PA, has been isolated and characterized and clinical assays showed that SM possessed anti-HBV activity.

3.3. *Radix Astragali*

Radix Astragali (Huangqi) derives from the dried

Table 2. Anti-HBV response of TCM and related active compounds in clinical trials

Treatments	n	Control	Duration (days)	Negative conversion of serum HBV markers			*Ref.*
				HBsAg	HBeAg	HBV DNA	
Phyllanthus amarus	122	Vitamins, Hypoxanthosine	30	6/62 (9.7%)	21/48 (43.8%)**	-	*22*
Salvia miltiorrhiza	123	Gankangning, fufang yiganling	90	-	41/57 (71.9%)**	19/26 (73.1%)**	*40*
Astragali compound	208	other regular drugs	60	2/94 (2.1%)	13/47 (27.7%)**	14/50 (28.0%)*	*43*
Oxymatrine	100	Vitamins	182	-	21/50 (42.0%)**	22/50 (44.0%)**	*56*

*$p < 0.05$; **$p < 0.01$ compared with control group.

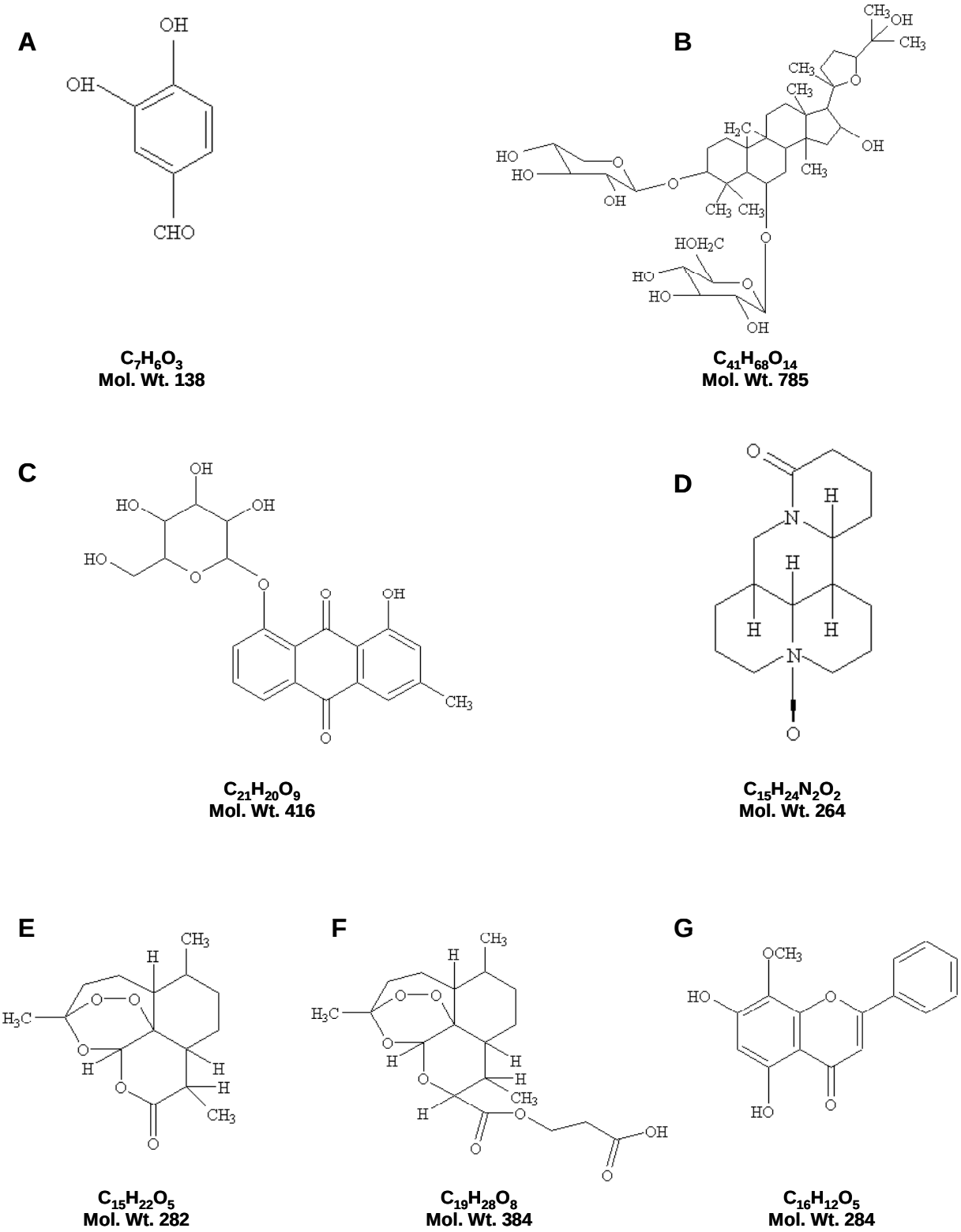

Figure 1. Chemical structures of various anti-HBV compounds in TCM. (**A**) protocatechuic aldehyde from *Salvia miltiorrhiza*, (**B**) astragaloside IV from *Radix Astragali*, (**C**) chrysophanol 8-*O*-β-D-glucoside from *Rheum palmatum* L., (**D**) oxymatrine, (**E**)artemisinin, (**F**) artesunate, and (**G**) wogonin. Mol. Wt. denotes molecular weight.

root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao (Mengguhuangqi) or *A. membranaceus* (Fisch.) Bge. (Mojiahuangqi). It has been widely used in Chinese medicine from ancient times and is one of the most widely prescribed Chinese herbs in many formulas. It has exhibited efficacy in treatment of immune disorders and liver diseases with an excellent safety record (*41*).

The major active constituents of *Radix Astragali* are believed to be the total saponins and the total flavonoids

(*42*). Wang *et al.* (*41*) showed that a major saponin of this herb, astragaloside IV (Figure 1B), suppressed both HBsAg and HBeAg secretion with inhibition rates of 23.6% and 22.9% at 100 μg/mL and possessed a more potent inhibitory activity than 3TC without significant cytotoxicity in HepG2.2.15 cells. Moreover, they found that astragaloside IV inhibited serum DHBV HBsAg by 64.0% at 120 mg/kg and reduced liver DHBV DNA levels in DHBV-infected ducklings. Their results suggested that astragaloside IV from *Radix Astragali* possessed anti-HBV activity and the TI of astragaloside IV was less than 1.94 (Table 1).

Furthermore, clinical evaluation of *Radix Astragali* was performed in 208 patients with chronic viral hepatitis B (*43*). The treatment group (n = 116) was treated with the Astragali compound (AC), containing *Radix Astragali* and adjuvant components, and the control group (n = 92) was treated with other drugs regularly used for viral hepatitis. Negative conversion rates of HBeAg and HBV DNA were significantly higher in the treatment group than in the control group (Table 2).

In summary, the major saponin of *Radix Astragali*, astragaloside IV, has been shown to possess anti-HBV activity and *Radix Astragali* appears to have a marked clinical efficacy in the treatment of patients with chronic viral hepatitis B.

3.4. *Rheum palmatum L.*

The herbal plant *Rheum palmatum* L. (Zhangyedahuang), a TCM, is widely distributed in mainland China and has a long history of treatment for gastroenteritic and liver diseases (*44*).

Some reports demonstrated that *R. palmatum* L. extracts could inhibit coxsackie virus and herpes simplex virus (*45,46*) and *R. palmatum* L. volatile oil could inhibit the expression of HBV antigens (HBsAg and HBeAg) (*47*). Moreover, some reports showed that the aqueous extracts of *R. palmatum* L. decreased the extracellular HBV DNA levels at concentrations ranging from 64 to 128 μg/mL, inhibited HBsAg secretion and HBV DNA polymerase activity *in vitro* (*48,49*), and showed a potential antiviral effect against duck hepatitis B virus (*50*).

Recently, Li *et al.* (*51*) evaluated the anti-HBV activities of *R. palmatum* L. ethanol extract (RPE) and its isolated anthraquinones in HepG2.2.15 cells. They found that RPE inhibited HBV-DNA production and HBsAg expression in a dose-dependent manner and its TI was 7.67 (Table 1). They also found that the only combined anthraquinone chrysophanol 8-*O*-β-D-glucoside (Figure 1C) exhibited significant activity against HBV DNA production with an IC_{50} of 36.98 μg/mL and antigen expression with an IC_{50} of 237.4 μg/mL for HBsAg and 183.41 μg/mL for HBeAg and its TI was more than 270.42 (Table 1). Furthermore, they observed that chrysophanol 8-*O*-β-D-glucoside was a potential inhibitior of HBV-DNA polymerase. Therefore, they concluded chrysophanol 8-*O*-β-D-glucoside is the major active compound in RPE and could be a promising candidate for the development of new anti-HBV drugs in the treatment of HBV infection.

In summary, *R. palmatum* L. extracts possess anti-HBV activity in *in vitro* and *in vivo* assays and its major active compound may be chrysophanol 8-*O*-β-D-glucoside.

3.5. *Oxymatrine*

Oxymatrine (OM) is an alkaloid extracted from two kinds of Chinese plants, *Sophora alopecuraides* L. (Kudouzi) and the root of *Sophora flavescesn* Ait. (Kushen). The chemical structure of OM is shown in Figure 1D. OM was reported to possess antiviral, antifibrotic, hepatoprotective, and immunomodulating effects, especially against hepatitis B (*52*).

In vitro and *in vivo* assays suggested that OM possessed anti-HBV activity. Xu *et al.* (*53*) found that in HepG2.2.15 cells 1,000 μg/mL of OM inhibited HBV DNA production 79.6%. The TI of OM was more than 2 (Table 1). They also showed that OM inhibited the secretion of HBsAg and HBeAg from HepG2.2.15 cells according to dose- and time-dependence and the maximal inhibition rates were 93% and 63%, respectively. Furthermore, Chen *et al.* (*54*) demonstrated that in a complete genomic HBV transgenic mice model ICR (TgN, HBV 1.2 copy) OM decreased the intrahepatic HBsAg, HBeAg, and HBcAg concentrations and caused the intrahepatic HBsAg and HBeAg to become negative in six mice at a dosage of 200 mg/kg after a 30-day treatment. However, the intrahepatic HBsAg and HBeAg returned to positive with prolonged treatment, probably due to immune tolerance.

In a randomized double-blind and placebo-controlled multi-center trial (*55*), treatment with OM capsules resulted in seroconversion rates of 38.61% for HBV DNA and 31.91% for HBeAg and treatment with OM injections resulted in seroconversion rates of 43.33% for HBV DNA and 39.29% for HBeAg by the end of a 24 week treatment course. Both OM groups were significantly better than the placebo in seroconversion rates. There was no statistically significant difference among OM capsule, OM injection, and placebo in side-effects. In another trial (*56*), negative conversion rates of HBeAg (42.0%) and HBV DNA (44.0%) in the OM capsule treatment group were significantly higher than those (4.0%, 4.0%, respectively) in the control group (Table 2).

In summary, OM has many different activities and is much cheaper than INF-α for the treatment of chronic hepatitis B, which makes it an attractive therapeutic option and warrants further clinical trials.

3.6. *Artemisinin and artesunate*

Artemisinin (Figure 1E) is a sesquiterpene lactone derived from the TCM plant *Artemisia annua* (Qinghao) and has been used for centuries in TCM as a remedy for chills and fever (*57*). The semisynthetic derivative of artemisinin, artesunate (Figure 1F), had better anti-HBV effects than artemisinin. Romero *et al.* (*58*) showed that artesunate suppressed HBsAg secretion with an IC_{50} of 0.88 μg/mL and HBV DNA production with an IC_{50} of 0.19 μg/mL and its TI was 40.47 (Table 1). They also found that synergistic anti-HBV effects existed by combining artesunate and lamivudine. Moreover, there are no known serious side-effects with artemisinin and its derivatives because none have been seen in their use in large populations for their antimalaria properties (*59*). Therefore, artemisinin and artesunate deserve to be further investigated for their anti-HBV activities.

3.7. *Wogonin*

Wogonin (Figure 1G) is a monoflavonoid derived from the TCM herb *Scutellaria radix* (Huangqin), which has been widely used for treatment of inflammatory and liver diseases for thousands of years in Asia (*60*). In recent years, wogonin has been found to have antiviral activity. Huang *et al.* (*61*) demonstrated that wogonin suppressed HBsAg secretion in a HBV-transfected liver cell line without cytotoxicity. Guo *et al.* (*62*) showed that wogonin effectively suppressed the secretion of both HBsAg and HBeAg with an IC_{50} of 4 μg/mL and reduced HBV DNA levels in a dose-dependent manner in HepG2.2.15 cells. The TI of wogonin was more than 50 (Table 1). They also found that wogonin dramatically inhibited DHBV DNA polymerase with an IC_{50} of 0.57 μg/mL in DHBV-infected ducks and significantly improved duck livers in histopathological evaluations. Moreover, they observed that wogonin significantly reduced plasma HBsAg levels in human HBV-transgenic mice. Although only a limited number of observations have been performed on the anti-HBV activity of wogonin, preliminary results suggested that wogonin might be a candidate as a new antiviral drug.

4. Development strategy of TCM with potential anti-HBV activity

The use of TCM to treat HBV infections has a long tradition and is common in China and India. However, TCM has not yet become a widely acceptable treatment modality for hepatitis B around the world. This eventuality is held back by the lack of the following factors: standardization of TCM and identification of its active ingredient(s), randomized controlled clinical trials (RCTs), and toxicological evaluation (*63*). The above-mentioned drugs, including *Phyllanthus*, *Salvia miltiorrhiza*, *Rheum palmatum* L., *Radix Astragali*, oxymatrine, artemisinin and artesunate, and wogonin, to a greater or less degree, lack assessment in these areas.

Recently, enormous efforts have been directed towards the scientific basis and clinical evaluation of TCM (*64*,*65*) as a result of a growing interest in therapeutic agents derived from TCM. Some advanced and interdisciplinary technology and methodology can facilitate standardization of TCM and identification of its active ingredient(s) (*66*). Modern pharmacological disciplines, including phytochemistry, pharmacognosy, and phytotherapy, can promote more significant breakthroughs and scientific achievements through scientific technology and methodology (*67*). The information about all aspects (herbal formulations, constituent herbs, herbal ingredients, molecular structure and functional properties of active ingredients, therapeutic and toxic effects, clinical indications and applications) of TCM in several databases is available and makes the scientific evaluation of TCM easier (*68*). In addition, a herbogenomics approach, defined as the process during which functional genomics and proteomics can identify target molecules affected by TCM has been started. Thus researchers can study critical signaling pathway cascades resulting in effective recovery of patients with HBV infections, and the information described can be used to understand the mechanisms of action of TCM (*69*). Rigorously designed TCM treatment and long-term monitoring by a standardized and effective report system can promote the toxicological evaluation of TCM (*70*).

5. Conclusions

Continuous development of new agents to treat HBV infections is urgently needed because, to date, only a few drugs have been approved. Although the use of TCM provokes debate in its current and future role in health care and evidence for both efficacy and safety, many TCMs have been recognized for their promising and potent anti-HBV activities. More information is needed regarding TCM, including preparation, standardization, identification of active ingredients, and toxicological evaluation. Thus, TCM development needs to apply advanced and interdisciplinary technology and methodology. Further experimental and clinical investigations will allow a better understanding of mechanisms of action, therapeutic effects, and the safety profile of TCM.

Acknowledgements

This project was supported by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan and JSPS and CAMS under the Japan-China Medical Exchange Program.

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(Received January 25, 2010; Revised March 18, 2010; Accepted March 23, 2010)