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REVIEW ARTICLE

Antiviral Effects of *Glycyrrhiza* species

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Historical sources for the use of *Glycyrrhiza* species include ancient manuscripts from China, India and Greece. They all mention its use for symptoms of viral respiratory tract infections and hepatitis. Randomized controlled trials confirmed that the *Glycyrrhiza glabra* derived compound glycyrrhizin and its derivatives reduced hepatocellular damage in chronic hepatitis B and C. In hepatitis C virus-induced cirrhosis the risk of hepatocellular carcinoma was reduced. Animal studies demonstrated a reduction of mortality and viral activity in herpes simplex virus encephalitis and influenza A virus pneumonia. *In vitro* studies revealed antiviral activity against HIV-1, SARS related coronavirus, respiratory syncytial virus, arboviruses, vaccinia virus and vesicular stomatitis virus.

Mechanisms for antiviral activity of *Glycyrrhiza* spp. include reduced transport to the membrane and sialylation of hepatitis B virus surface antigen, reduction of membrane fluidity leading to inhibition of fusion of the viral membrane of HIV-1 with the cell, induction of interferon gamma in T-cells, inhibition of phosphorylating enzymes in vesicular stomatitis virus infection and reduction of viral latency.

Future research needs to explore the potency of compounds derived from licorice in prevention and treatment of influenza A virus pneumonia and as an adjuvant treatment in patients infected with HIV resistant to antiretroviral drugs. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: licorice; glycyrrhizin; glycyrrhetinic acid; influenza; hepatitis; SARS.

INTRODUCTION

Glycyrrhiza glabra is a perennial herb, native to central and South-Western Asia, as well as to the Mediterranean region and cultivated in temperate and sub-tropical regions of the world, including Europe and Asia. The root, dried and processed, is called *licorice* and has a characteristic odour and sweet taste ('licorice' derives from the Greek words *γλυκυσ*, 'sweet', and *ριζα*, 'root').

Licorice is one of the most widely used medicinal plants, found in traditional formulas since antiquity (Armanini *et al.*, 2002; Fiore *et al.*, 2005). The use of the plant can be traced back to ancient Assyrian, Egyptian, Chinese and Indian cultures, and was appreciated by ancient Greeks and Romans. Licorice was used in Arabic medicine during the Middle Ages, as documented by the *Canone* of Ibn Sina (980–1037 AD), a summary of Hippocrates and Galen's medicine. All sources mention its use for symptoms attributable to viral respiratory tract infections such as dry cough or hoarse voice and for the symptoms of hepatitis. For the past 25 years the uses of Glycyrrhiza compounds in antiquity and traditional herbal medicine have been investigated scientifically.

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The main chemical constituents of licorice root are triterpene saponins. Glycyrrhizin is the major component, with a concentration varying between 1% and 9%, depending on the species, geographical location and methods of extraction (Barnes *et al.*, 2002; Blumenthal *et al.*, 2000). Glycyrrhizin is a glycoside, occurring as a mixture of calcium, sodium and potassium salts of glycyrrhizinic acid (also named glycyrrhizic acid) (Fig. 1). On hydrolysis it releases two molecules of D-glucuronic acid and the aglycone 18 β-glycyrrhetinic acid (also called glycyrrhetic acid), a pentacyclic triterpene derivative of the β-amyrin type (Robbers *et al.*, 1996; Blumenthal *et al.*, 2000; Evans, 2002; Baltina, 2003).

The aim of this review was to summarize data from medical research into the effects of licorice-derived compounds in viral infections and corresponding *in vitro* data analysing the underlying mechanisms.

RESULTS

Data from studies in humans

Use of licorice in viral hepatitis. Glycyrrhizin has been used in Japan for more than 20 years as a treatment for chronic hepatitis (van Rossum *et al.*, 1998; Shibata, 2000). Following the first preliminary reports, a large number of studies have been conducted on the effects

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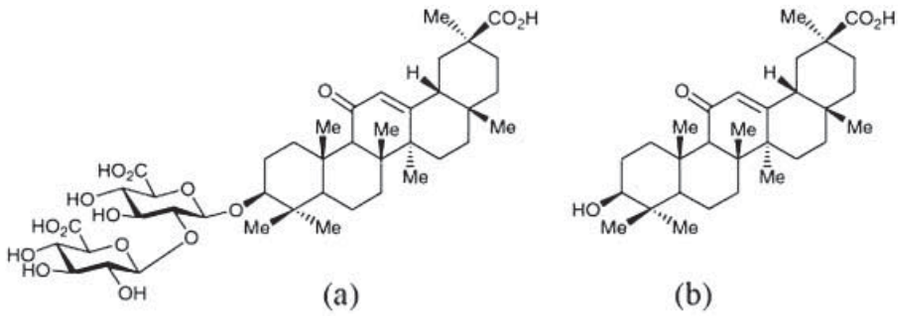

Figure 1. Chemical structure of glycyrrhizinic (glycyrrhizic) acid (a) and glycyrrhetinic acid (b).

of licorice extracts against viral hepatitis. In randomized controlled trials, glycyrrhizin, usually administered intravenously, induced a significant reduction of serum liver enzymes and caused an improvement in liver histology in comparison with placebo.

Among the most recent studies, the effect of a formulation (Stronger Neo-Minophagen C, SNMC) containing 40 mg glycyrrhizin, administered by injection to patients with chronic viral hepatitis should be mentioned. The formulation was evaluated at different doses and frequency of administration, and the overall short-term therapeutic response consisted of a dose-dependent effect suppressing alanine aminotransferase (ALT) levels in patients with chronic viral hepatitis (Miyake *et al.*, 2002). SNMC, although without specific activity against hepatitis virus, showed an antiinflammatory effect and was able to improve the clinical condition of patients with liver disease at various stages (Acharya *et al.*, 1993).

Glycyrrhizin has been used to treat chronic hepatitis B virus infection, and the drug may improve liver function with occasional complete recovery from hepatitis (Takahara *et al.*, 1994; Sato *et al.*, 1996). Daily intravenous administration of 100 and 40 mL of the glycyrrhizin-containing preparation SNMC for 4 weeks was a safe and efficacious treatment in lowering or normalizing ALT levels in patients with chronic hepatitis B (Zhang and Wang, 2002). Intravenous administration for 1 year of glycyrrhizinic acid in patients with chronic viral hepatitis B was able to produce a positive effect on the evolution of the disease, with a 30%–40% success rate, comparable to the results obtained with interferon (Eisenburg, 1992).

In patients with chronic hepatitis C glycyrrhizin has been shown to reduce transaminase levels in a randomized phase II trial (Orient *et al.*, 2006).

Following the use of SNMC, a retrospective study was conducted in Japan in order to evaluate the effect of glycyrrhizin on hepatocellular carcinoma development (Arase *et al.*, 1997). Of 453 patients diagnosed with chronic hepatitis C a group of 84 patients was treated with SNMC (100 mL daily for 8 weeks, then 2–7 times a week for 2–16 years, median 10.1 years). Another group of 109 patients was not treated with SNMC or interferon for a long period of time (median 9.2 years) and received other herbal medicine (including vitamin K). The 15th-year rates of cumulative hepatocellular carcinoma incidence were 12% and 25% in the two groups, respectively, indicating a relative risk of 2.49 (estimated by Multivariate Cox Regression Analysis; 95% confidence interval: 1.01–6.12, $p = 0.044$) in patients not treated with SNMC (Arase *et al.*, 1997). This result was later confirmed in another longitudinal cohort study conducted over 10 years in which 100 mL (median daily dose) of SNMC was injected intravenously daily for a median period of 4.3 years. Crude carcinogenesis rates at 10 years in the treated and untreated group were 21.5% and 35.5%, respectively ($p = 0.02$). Proportional hazard analysis disclosed that glycyrrhizin significantly decreased the hepatocarcinogenesis rate (hazard ratio 0.49, 95% confidence interval 0.27–0.86) (Ikeda *et al.*, 2006). On the basis of clinical and histological markers, it was concluded that SNMC can suppress liver necrosis and inflammation in patients with chronic hepatitis C, suggesting that a long-term treatment with the product might be useful in preventing liver cirrhosis and hepatocellular carcinoma (Kumada, 2002).

Trials of glycyrrhizin in patients with HIV infection. In 1987, Gotoh *et al.* conducted a long-term study with SNMC (5 mg glycyrrhizin/kg) by drip infusion to AIDS patients with high CD4/CD8 ratios before treatment. In this clinical study the count of CD4 lymphocytes and the CD4/CD8 ratio in asymptomatic carriers (AC) or patients with AIDS-related complex (ARC) showed an increase. Significant clinical improvement was achieved in almost half of the treated patients (Gotoh *et al.*, 1987).

The results were confirmed in another study (Mori *et al.*, 1989) in haemophilia A patients with HIV infection but with AC status. The authors found that glycyrrhizin not only possesses an inhibitory effect on HIV replication, but also has interferon-inducing and natural killer (NK)-enhancing effects. The authors concluded that the administration of glycyrrhizin to HIV-positive hemophilia patients seemed to be effective in preventing the development of AIDS by raising the number of CD4-positive T-lymphocytes (Mori *et al.*, 1989).

Animal experiments

Effects in the influenza mouse model. The principal component of licorice, glycyrrhizin, has been evaluated experimentally in the mouse model against influenza virus (Utsonomiya *et al.*, 1997). When mice were treated intraperitoneally with 10 mg of glycyrrhizin/kg body weight 1 day before exposure to 10 LD_{50} (lethal dose killing 50% of animals) of the influenza virus A_2 and 1 and 4 days after the infection, all of the animals survived over the experimental period of 21 days. Conversely, the mean survival time in control mice was 10.5 days, and there were no survivors. The grade of

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pulmonary consolidation and the virus titers in the lung tissues of infected mice treated with glycyrrhizin were significantly lower than those in the lung tissues of infected mice treated with saline. An interesting finding was that when splenic T cells from glycyrrhizin-treated mice were transferred to mice exposed to influenza virus, all the recipients survived, while no survivor was seen in recipient mice inoculated with native T cells, or with splenic B cells and macrophages from glycyrrhizin-treated mice. The administration of glycyrrhizin to infected mice in combination with anti-gamma interferon monoclonal antibody did not produce any antiviral effect. The results obtained by the authors indicated that glycyrrhizin may protect mice exposed to a lethal dose of influenza virus through the induction of interferon-gamma production by T cells (Utsonomiya *et al.*, 1997). Other previously reported studies indicated that in mice glycyrrhizin and glycyrrhetinic acid were able to induce the production of interferon (Abe *et al.*, 1982), suggesting this as a possible mechanism of action against viral infection.

Effects in murine herpes encephalitis. The antiviral effect of glycyrrhizin was evaluated in murine herpes encephalitis (Sekizawa *et al.*, 2001). Intraperitoneal administration of glycyrrhizin increased the survival rate of the animals by about 2.5 times, and the viral replication in the brain was reduced to 45.6% of the control.

In vitro studies of antiviral effects

The first report of an antiviral property of licorice constituents dates to the year 1979 (Pompei *et al.*, 1979). Following screening investigations of plant extracts, the authors found that a component of licorice roots, identified as glycyrrhizic acid, had antiviral activity inhibiting the growth and cytopathic effect of several DNA and RNA viruses, such as vaccinia, herpes simplex type 1, Newcastle disease and vesicular stomatitis viruses *in vitro*. The drug did not affect cell activity. The concentrations of glycyrrhizic acid able to inhibit both the growth and cytopathic effects of the viruses were in the range 2–8 mM, added to infected cell cultures soon after incubation at 37 °C (Pompei *et al.*, 1979).

Effects on herpesviridae. Following this landmark study glycyrrhizin was evaluated for any *in vitro* antiviral action against varicella-zoster virus (Baba and Shigeta, 1987). In human embryonic fibroblast cells inoculated with five strains of the virus, glycyrrhizin produced an inhibitory effect on viral proliferation with an IC_{50} (inhibitory concentration reducing activity to 50% of controls) of 0.71 mM. The selectivity index, defined as the ratio of IC_{50} for host-cell DNA synthesis to IC_{50} for virus replication, was estimated to be 30 (this value is not as high as for the most commonly used antiviral drugs, the selectivity index for acyclovir is close to 600 [Machida *et al.*, 1995]). Pretreatment of cells with the drug 24 h before inoculation was able to inhibit replication of the virus. Incubation of the virus for 30 min with a concentration of 2.4 mM glycyrrhizin was effective in inactivating more than 99% of the virus particles, and glycyrrhizin demonstrated an additive effect with other conventional antiviral drugs such as acyclovir, and also with human beta-interferon.

In studies demonstrating the inhibition of HSV-1 by glycyrrhizic acid *in vitro* a synergism of the inhibitory effect with the endogenous antiviral substance lactoferrin was found (Lampi *et al.*, 2001).

An effect of glycyrrhizic acid was also reported against Epstein-Barr virus (EBV), which produces infectious mononucleosis (Lin, 2003). The inhibition of EBV replication *in vitro* is dose-dependent; the IC_{50} values for viral inhibition and cell growth were 0.04 and 4.8 mM, respectively, and the selectivity index was 120 (Lin, 2003). It has been suggested that the drug interferes with an early step of EBV replication, possibly penetration, without any effect on viral adsorption, or inactivation (Lin, 2003). Investigation of the effects of glycyrrhizin on cytomegalovirus infection of human monocytic and human embryonic lung cell lines showed that it inhibited viral antigen expression (Numazaki *et al.*, 1994).

Licorice and influenza virus. New strategies for the cure of influenza are needed, since conventional antiviral agents, such as amantadine and ribavirin, are not very effective and have toxic side effects.

Glycyrrhizic acid has been shown to inhibit the recovery of hemagglutinins from influenza virus-infected embryonated hen eggs (Pompei *et al.*, 1983). The substance did not affect viral viability nor impair hemagglutinating activity of the virions, but was able to affect the growth of viruses in embryonic tissues, particularly at the late viral replication steps.

A recent study (Ko *et al.*, 2006) was conducted using *Glycyrrhiza uralensis* ethanol extract in a culture of A549 human bronchial epithelial cells infected with influenza virus H1N1. The extract produced an inhibitory effect on the production of RANTES, the potent chemotactic cytokine for monocytes, basophils and T cells, typically detected in nasal secretions of patients with upper respiratory tract infection, and involved in the epithelial cell-mediated inflammatory process. The licorice extract was evaluated at concentrations in the range 20–200 μg/mL; at a maximal concentration, a 97.0 ± 1.8% inhibition in RANTES production was observed (Ko *et al.*, 2006), suggesting that compounds derived from *Glycyrrhiza* spp. may be beneficial for the treatment of inflammatory processes related to viral infection.

Effects of glycyrrhizin on hepatitis viruses. The effect of glycyrrhizin in ameliorating chronic hepatitis, as indicated by the reduction of the plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in patients with chronic hepatitis (van Rossum *et al.*, 2001), has been demonstrated by experimental investigations on animal cells (Shiki *et al.*, 1992; Shibata, 2000). In hepatitis B the virustatic effect could depend on inhibition of the intrahepatic transport and sialylation of the hepatitis B-virus (HBV) surface antigen (HBsAg) observed *in vitro* (Sato *et al.*, 1996). In isolated rat hepatocytes glycyrrhizin suppressed the release of transaminase in the presence of either anti-liver cell membrane antibody or phospholipase A_2 (Shiki *et al.*, 1992). The results indicated that the treatment of liver cells with antibody activates phospholipase A_2 in cell membranes leading to disintegration of the cell membrane and cell death, which resulted in the release of transaminases. Glycyrrhizin, suppressing the increase in phospholipase A_2 activity, inhibited the release of transaminases, which demonstrated its anticytolytic

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effect (Shiki *et al.*, 1992). Results of *in vitro* and animal (rat) studies supported further that glycyrrhizin inhibits lipid peroxidation, thereby protecting the rat hepatocytes (Kiso *et al.*, 1984; Jeong *et al.*, 2002). It was shown that glycyrrhizin inhibits immunomediated cytotoxicity against hepatocytes and the murine NFκB activity in the murine liver injury induced by CCl_4-ethanol. Moreover, glycyrrhizin inhibited anti-FAS antibody-induced elevation of ALT in mice (Shibata, 2000). Another group demonstrated that glycyrrhizin reduced ALT levels, steatosis and fibrosis in the mouse model of liver injury induced by CCl_4 and ethanol. This experiment showed a concomitant reduced nuclear factor-kappa B binding (Wang *et al.*, 1998).

In vitro experiments demonstrated that glycyrrhizin suppresses the secretion of hepatitis B surface antigen (HBsAg) by inhibiting the intracellular transport of HBsAg at the trans-Golgi area after O-linked glycosylation and before sialylation (Takahara *et al.*, 1994). Other studies confirmed that glycyrrhizin binds to hepatocytes and modifies the expression of HBV-related antigens on the hepatocytes and suppresses sialylation of HBsAg (Sato *et al.*, 1996).

Glycyrrhizin, as an immunomodulatory agent, given intravenously in combination with lamivudine was also useful in the treatment of subacute hepatitis due to hepatitis B (Tandon *et al.*, 2001). In 1990 Crance *et al.* (Crance *et al.*, 1990) found a complete concentration-dependent inhibition of the expression of the hepatitis A virus (HAV) antigen and HAV infectivity by glycyrrhizin in the human hepatoma cell line PLC/PRF/5. The mechanism of this antiviral effect was the inhibition of the penetration and endocytosis in liver cells. Proposed mechanisms were the induction of a decrease in the negative charge on the cell surface and/or a decrease of membrane fluidity.

Licorice and HIV. Glycyrrhizin inhibited the cytopathic effect and the virus-specific antigen expression in HIV-infected MT-4 cells. Furthermore glycyrrhizin inhibited giant cell formation caused by HIV-infection in Molt-4 cells, which are sensitive to HIV and fuse to giant cells after infection, providing a parameter for determining the cytopathic effect of HIV (Ito *et al.*, 1987; Baba *et al.*, 1988). Glycyrrhizin sulphate was found to both inhibit cell-free viral infection and cell to cell infection (Tochikura *et al.*, 1989). Some of these effects may be due to its ability to reduce membrane fluidity. Reduced membrane fluidity by glycyrrhizin could explain how it can inhibit cell-to-cell fusion by suppression of the formation of virological synapses (Harada, 2005). It was found that HIV-1 reverse transcriptase (rRT) functioned as an effective phosphate acceptor for recombinant human casein kinase II (rhCK-II) *in vitro*; this phosphorylation was inhibited by the glycyrrhetinic acid derivative, quercetin and a high dose (100 μg) of glycyrrhizin. RNA-Dependent DNA-polymerase (RDDP) activity was stimulated by about 2.5-fold after full phosphorylation of rRT by rhCK-II (Harada *et al.*, 1998).

Recent investigations have evaluated the effect of glycyrrhizin on HIV replication in cultures of peripheral blood mononuclear cells (PBMC) from HIV-infected patients (Sasaki *et al.*, 2002–2003). In 31% of the samples, glycyrrhizin inhibited more than 90% of HIV replication, including a non-syncytium-inducing variant of HIV (NSI-HIV). Glycyrrhizin induced the production of CC chemokine ligand (CCL)4 and CCL5 in a dose-dependent manner, suggesting that the drug possesses the potential to inhibit NSI-HIV by stimulating the production of beta-chemokines (Sasaki *et al.*, 2002–2003) which compete with the chemokine receptor mediated infection of cells by HIV.

Among a variety of natural products described as anti-HIV agents, glycyrrhizin was found to have a mechanism of action which may at least in part be attributed to interference with virus–cell binding (De Clercq, 2000). More recently, an increasing quantity of data suggested that the antiviral effects of glycyrrhizin are linked to the induction of endogenous interferon gamma (Thyagarajan *et al.*, 2002). Further, glycyrrhizin affects other cellular signalling pathways such as protein kinase II, casein kinase II and transcription factors such as activator protein1 and nuclear factor κB (Wang *et al.*, 1998).

Licorice and SARS related coronavirus. A new coronavirus has been identified in patients with severe acute respiratory syndrome (SARS), and the disease has drawn enormous attention and caused fear worldwide since early 2003. Although the disease is now under control, the possibility of a return of the pathology has stimulated the search for a remedy. Several studies have been reported, but a specific treatment for SARS has not yet been established. Various pharmacological treatments have been suggested, such as steroids, ribavirin, interferon and also glycyrrhizin (Fujii *et al.*, 2004; Chen *et al.*, 2004).

Glycyrrhizin inhibits SARS-associated coronavirus (SARS-CoV) replication in Vero cells with a selectivity index of 67 (Cinatl *et al.*, 2003). In addition to inhibition of virus replication, glycyrrhizin is able to inhibit adsorption and penetration of the virus during the early steps of the replicative cycle. The activity of glycyrrhizin is lower when added during the adsorption period than after virus adsorption (EC_{50} is 600 mg/L vs 2400 mg/L, respectively). Glycyrrhizin has been found to be most effective when given both during and after the adsorption period. The mechanism of the activity of glycyrrhizin against SARS-CoV is unclear. The studies from Cinatl *et al.* (2003) show that glycyrrhizin induces nitrous oxide synthase in Vero cells and that virus replication is inhibited when a nitrous oxide donor (DETA Nonoate) is added to the culture medium.

Since glycyrrhizin was shown to be able to inhibit SARS-CoV replication *in vitro*, the activity of several glycyrrhizic acid derivatives was evaluated (Hoever *et al.*, 2005). The introduction of 2-acetamido-beta-D-glucopyranosylamine into the glycoside chain of glycyrrhizin produced a 10-fold increase of the anti-SARS-CoV activity. Other compounds, such as amides and conjugates of glycyrrhizin with two amino acid residues presented up to 70-fold increased activity against the virus (Hoever *et al.*, 2005); however, the cytotoxicity increased as well in those derivatives, resulting in a decreased selectivity index.

Effects on other viruses. Glycyrrhizin was tested *in vitro* for antiviral activities against several pathogenic flaviviruses involved in diseases such as dengue, Japanese encephalitis, mammalian tick-borne encephalitis and yellow fever (Crance *et al.*, 2003). Glycyrrhizin was found to be able to inhibit the replication of flaviviruses

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at high non-cytotoxic concentrations. Moreover, glycyrrhizin inhibited plaque formation in Japanese encephalitis virus at a concentration of 0.6 mmol/L at 96 h (Badam, 1997). The target for glycyrrhizin action against the vesicular stomatitis virus (VSV) has been identified as enzyme kinase P (Ohtsuki and Iahida, 1988), which is essential for the early stages of viral replication. Glycyrrhizin at low doses was found to selectively inhibit protein phosphorylation by kinase P, without any significant effect on other kinases. It has been reported that this direct binding of glycyrrhizin to the virus-associated kinase results in its inactivation and a reduction of viral infectivity (Ohtsuki and Iahida, 1988). Recently glycyrrhiza GD4, which does not contain glycyrrhizic acid was found to inhibit the cytopathic effect of respiratory syncytial virus in HeLa cells (Wang *et al.*, 2006).

Recently, it has been demonstrated that a treatment with glycyrrhizic acid of cells latently infected with Kaposi sarcoma-associated herpesvirus (KSHV) is able to reduce the synthesis of a viral latency protein and to induce apoptosis of infected cells (Curreli *et al.*, 2005). This finding suggests that glycyrrhizic acid may be the key to find a novel way to interrupt latency in infected cells (Cohen, 2005).

Potential adverse effects of treatment with glycyrrhizin. The toxic effects of licorice extract and glycyrrhizinate compounds have been well studied and documented in humans over the past 30–40 years.

Reported adverse effects of glycyrrhizin include aldosterone-like effects (pseudohyperaldosteronism), which are related to its inhibition of conversion of cortisol to cortisone (Armanini *et al.*, 2005). This has been associated with hypokalemia, hypertension, decreased plasma renin and aldosterone levels, myopathies, oedema and/or muscle weakness in people taking excessive amounts of glycyrrhizin containing products. An acceptable daily intake avoiding these effects has been determined as 0.2 mg/kg of glycyrrhizin. Heavy consumption of licorice has also been associated with an increased risk of preterm birth in cross-sectional and retrospective studies (Isbrucker and Burdock, 2006).

CONCLUSIONS

The threat to global public health by pandemics of viral diseases like those induced by influenza and HIV viruses requires the urgent evaluation of herbal drugs which showed promise in traditional herbal medicine. The lack of effective drugs against influenza virus and the increasing problem with multiresistance in HIV infection makes *Glycyrrhiza* sp.-derived compounds important candidates for drug development. The data reviewed showed that several constituents of licorice roots have a potential as effective alternatives in combating a wide variety of respiratory, hepatic and systemic viral diseases by general immune modulatory and membrane effects, as well as specific effects on enzyme activity and expression related to selected viruses (see Table 1). In view of the safety profile established in Japanese trials in patients with viral hepatitis, randomized controlled trials and dose finding studies in the prevention and treatment of influenza virus and HIV infection are justified. Future trials need to address the potential side effects, which have been reported with licorice use, particularly in elderly people with heart disease and on diuretic medication. Further *in vitro* studies working on chemically modified derivatives with greater activity and increased selectivity indices are required.

Table 1. Key studies conducted with licorice-derived compounds as antiviral agent

Virus	Study subject	Principal antiviral effects and mechanisms of action	Compound used	References
Hepatitis B virus (HBV)	Humans	Effective in normalizing serum transaminases; immunomodulating effect	Glycyrrhizin (mainly from SNMC, see text)	Takahara *et al.*, 1994 Sato *et al.*, 1996 Eisenburg, 1992 Tandon *et al.*, 2001
	Guinea-pig *in vivo*	Suppression of HbsAg secrection	Glycyrrhizin	Sato *et al.*, 1996
	Rat hepatocytes *in vitro*	Reduction of transaminase release; anticytolytic effect	Glycyrrhizin	Shiki *et al.*, 1992
Hepatitis C virus (HCV)	Humans	Effective in normalizing serum transaminases; reduced risk of hepatocarcinoma; inhibition of immune-mediated cytotoxicity	Glycyrrhizin (mainly from SNMC) glycyrrhetinic acid	Orient *et al.*, 2006 Arase *et al.*, 1997 Ikeda *et al.*, 2006 Kumada, 2002 Van Rossum *et al.*, 2001 Shibata, 2000
Hepatitis A virus (HAV)	Hepatoma cell line	Inhibition of virus expression	Glycyrrhizin	Crance *et al.*, 1990

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Table 1. (*Continued*)

Virus	Study subject	Principal antiviral effects and mechanisms of action	Compound used	References
Human immunodeficiency virus (HIV)	Humans	Improved CD4/CD8 ratio; inhibition of HIV replication	Glycyrrhizin (from SNMC)	Gotoh *et al.*, 1987 Mori *et al.*, 1989
	Cell cultures	Inhibition of cytopathic effect; inhibition of viral infection also via reduction of membrane fluidity and binding to cell; inhibition of reverse transcriptase; inhibition of viral replication; induction of IFN-γ; effects on multiple signaling pathways (including protein kinase and NFκB)	Glycyrrhizin	Ito *et al.*, 1987 Baba *et al.*, 1988 Tochikura *et al.*, 1989 Harada, 2005 Harada *et al.*, 1998 Sasaki *et al.*, 2002–2003 De Clercq 2000 Thyagarajan *et al.*, 2002 Wang *et al.*, 1998
Influenza virus	Mice *in vivo*	Improvement in survival time of animals after infection; increase of IFN-γ production	Glycyrrhizin	Utsonomiya *et al.*, 1997 Abe *et al.*, 1982
	Cell cultures	Inhibition of virus growth; inhibition of inflammatory cytokines	Glycyrrhizic acid *G. uralensis* ethanol extract	Pompei *et al.*, 1983 Ko *et al.*, 2006
SARS-related coronavirus	Cell cultures	Inhibition of virus replication; induction of cellular NO-synthase	Glycyrrhizin glycyrrhizic acid derivatives	Cinatl *et al.*, 2003 Hoever *et al.*, 2005
Herpesviridae family viruses (VZV, HSV-1, EBS, CMV)	Cell cultures	Inhibition of virus replication	Glycyrrhizin glycyrrhizic acid	Baba and Sigeta, 1987 Lampi *et al.*, 2001 Lin, 2003 Numazaki *et al.*, 1994 Machida *et al.*, 1995 Pompei *et al.*, 1979
	Mice (murine herpes encephalitis)	Increase in animal survival rate	Glycyrrhizin	Sekizawa *et al.*, 2001
Vaccinia virus (VV)	Cell cultures	Inhibition of virus growth	Glycyrrhizic acid	Pompei *et al.*, 1979
Newcastle disease virus (NDV)	Cell cultures	Inhibition of virus growth	Glycyrrhizic acid	Pompei *et al.*, 1979
Vesicular stomatitis virus (VSV)	Cell cultures	Inhibition of virus growth; inhibition of enzyme kinase P	Glycyrrhizic acid glycyrrhizin	Pompei *et al.*, 1979 Ohtsuki and Iahida, 1988
Flaviviruses	Cell cultures	Inhibition of virus replication	Glycyrrhizin	Crance *et al.*, 2003
Respiratory syncytial virus (RSV)	Cell cultures	Inhibition of cytopathic effect	Glycyrrhizic acid (Glycyrrhiza GD4)	Wang *et al.*, 2006
Kaposi sarcoma-associated herpes virus (KSHV)	Cell cultures	Reduction of synthesis of a viral latency protein; induction of infected cell apoptosis	Glycyrrhizic acid	Curreli *et al.*, 2005 Cohen, 2005

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