

Chemical Aspects of Coumarin Compounds for the Prevention of Hepatocellular Carcinomas

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Abstract: The normalization of plasma alanine aminotransferase (ALT) has been proved to be a strategy for preventing the development of hepatocellular carcinoma (HCC) in hepatitis C virus (HCV)-infection. Glycyrrhizin, a plant medicine, normalizes plasma ALT and prevents HCC. However, glycyrrhizin is administered intravenously and thereby chemical which is effective on oral administration is required. Coumarin compounds are active components of herbs used for the treatment of various diseases. The ability of coumarin compounds to lower plasma ALT were examined using mice concanavalin A-induced hepatitis and mice anti-Fas antibody-induced hepatitis. Furanocoumarins pd-Ia, pd-II and pd-III lower plasma ALT, but they are large molecules that are hardly absorbed on oral administration. Furocoumarin effectively lowers plasma ALT, but the safety range between the effective and toxic dosages is narrow. In contrast, osthole, a simple coumarin, causes strong reduction of plasma ALT and also inhibits caspase-3 activation. Furthermore, this chemical is quite safe upon large dose administration. In the structure of osthole, the methoxy group at position-7 and the 3-methyl-2-butenyl group at position-8 were elucidated to be essential for the beneficial effect of this chemical. We conclude that osthole will become a leading chemical for synthesizing a compound which prevents HCC on oral administration.

Key Words: Hepatocellular carcinomas, coumarin, prevention, hepatoprotection.

INTRODUCTION

The prevention of the development of hepatocellular carcinomas (HCC) in chronic hepatitis caused by hepatitis C virus (HCV)-infection is a worldwide issue. Interferon (IFN)-based therapy is a standard treatment for virus-induced chronic hepatitis. However, IFN treatment has limited effectiveness [1, 2], and side effects are common [3]. Thus, another strategy is required as well. Recently, the normalization of plasma alanine aminotransferase (ALT) in chronic viral hepatitis was proved to be another strategy for preventing the development of HCC [4, 5]. Glycyrrhizin, a plant medicine, has been used to treat virus-induced hepatitis. Glycyrrhizin decreases the plasma ALT level and thereby prevents the development of HCC [6, 7]. However, glycyrrhizin is administered intravenously, and a drug that is effective on oral administration is preferable for patients. Thus, the finding of small molecules that are effective in preventing HCC on oral administration is necessary for the complete prevention of HCC. Coumarin compounds are active components of herbal medicines. We have conducted research on coumarin compounds to find an appropriate chemical for preventing HCC. In the present review, the chemical aspects of coumarin compounds for the prevention of HCC are described.

PREPARATION OF CHEMICAL COMPOUNDS

Dr. S. Yoshida prepared coumarin compounds by both isolation from plants and synthesis. Imperatorin was isolated from *Notopterygium incisum*, and osthole from *Cnidium*

monniery. Pd-Ia, Pd-II and Pd-III were isolated from *Angelica praeruptorum*. Y355, HP-21, 22, 23, 24, 25 and osthenol were synthesized.

SCREENING SYSTEM FOR CHEMICALS

To study the chemical aspects of coumarins, mice concanavalin A-induced hepatitis [8] and mice anti-Fas antibody-induced hepatitis [9] were used. Mice concanavalin A-induced hepatitis is a hepatitis in which liver injury is initiated by T-cell activation. In chronic hepatitis caused by HCV-infection, the Fas system plays an important role in liver cell injury, and mice anti-Fas antibody-induced hepatitis reflects a part of the liver injury on chronic hepatitis caused by viral-infection.

NORMALIZATION OF PLASMA ALT AS A STRATEGY FOR PREVENTING HCC

IFN-Therapy has Limited Effectiveness

IFN administration is a standard therapy for the treatment of chronic hepatitis caused by HCV-infection. However, complete virus elimination is obtained only in 30-40 % of the treated patients [1, 2]. Although the use of ribavirin in combination with IFN increases the effectiveness [10, 11], most patients are not cured with IFN treatment. Furthermore, IFN-caused side effects such as neuropsychiatric symptoms, influenza-like symptoms and hematological abnormalities are common [3]. Thus, in addition to viral elimination therapy, another therapeutic option is required.

Normalization of Plasma ALT Is a Strategy

In a patient with chronic hepatitis induced by HCV-infection, persistent elevation of the plasma ALT level is

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correlated with the development of HCC. On the other hand, sustained low ALT levels ensure the patient survives without developing HCC. Thus, the normalization of plasma ALT is accepted as a strategy for preventing the development of HCC [4, 5]. In viral hepatitis, injury to the liver increases the chance of developing HCC. Elevation of plasma ALT is a marker of liver injury, and normalization of plasma ALT reflects protection of the liver.

Ability of Glycyrrhizin to Normalize Plasma ALT

Glycyrrhizin is a main gradient of licorice (*Glycyrrhiza glabra*) roots. Glycyrrhizin is a glycoside of glycyrrhetic acid (Fig. 1), and is a large molecule that is hardly absorbed on oral administration. In Japan, glycyrrhizin administration is almost common for the treatment of chronic hepatitis C. In patients with chronic hepatitis caused by HCV-infection, the administration of glycyrrhizin significantly reduces plasma ALT and thereby improves the liver histology [6, 7, 12, 13]. Long term administration of glycyrrhizin results in prevention of the development of HCC, regardless of the lack of elimination of the virus. For Europeans, the administration of glycyrrhizin to patients with chronic hepatitis caused by HCV-infection resulted in lowering of plasma ALT, and this drug is expected to be effective for preventing HCC in Europe also [14-16]. However, glycyrrhizin is administered intravenously, and a drug that is effective on oral administration is preferable for patients.

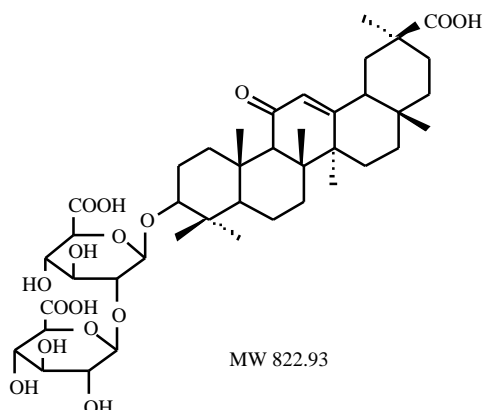


Fig. (1). Chemical structure of glycyrrhizin.

Mechanism of Prevention of Hepatitis by Glycyrrhizin

Although glycyrrhizin is effective in preventing HCC, the mechanism by which it lowers plasma ALT was not fully understood. Mice concanavalin A-induced hepatitis is a hepatitis model initiated by T-cell activation. Treatment of mice with glycyrrhizin prevents concanavalin A-induced hepatitis, but cytokine expression as a result of T-cell activation is not affected [17]. Thus, glycyrrhizin prevents mice concanavalin A-induced hepatitis by mechanism other than inhibition of T-cell activation. Fas ligand, a type II membrane protein, is an inducer of apoptosis, and the Fas system plays a major role in virus-induced liver injury [18-20]. Glycyrrhizin has been shown to prevent mice anti-Fas antibody-induced hepatitis [21]. Thus, the inhibitory effect of glycyrrhizin against Fas-mediated apoptosis may participate

in the lowering of plasma ALT in the chronic hepatitis caused by HCV-infection. Long term inhibition of the immune system does not allow the prevention of HCC, and these results for glycyrrhizin may explain the fewer side effects of this drug.

Caspase-3 in Hepatitis

Caspase-3 is a cystein protease involved in the development of apoptosis. Intravenous injection of anti-Fas antibodies into mice causes liver injury by mediating caspase-3 activation [22], and glycyrrhizin has been revealed to inhibit anti-Fas antibody-induced elevation of caspase-3 activity [21]. Since caspase-3 is a key enzyme inducing apoptosis in the Fas-system, glycyrrhizin might inhibit caspase-3 activity in viral-induced chronic hepatitis.

Drugs Other Than Glycyrrhizin

Cyclosporine A is an immunosuppressant clinically used for the treatment of organ transplant rejection and skin diseases. The administration of cyclosporine A is reportedly effective for the treatment of fulminant hepatitis. Experimental results indicate that cyclosporine A has an inhibitory effect on Fas-mediated hepatitis [23], and this effect may facilitate the curing of fulminant hepatitis in man. However, due to its immunosuppressive effect, this drug is not appropriate for long term administration in virus-induced chronic hepatitis.

Calcium channel blockers are used for a variety of purposes. Calcium channel blocker prevents mice concanavalin A-induced hepatitis [24], and this drug is effective for the treatment of fulminant hepatitis in man [25]. However, blood pressure is depressed by this drug.

Malotilate is approved as a drug for improving protein metabolism in the liver. In some experimental hepatitis models, such as carbontetrachloride-induced rat hepatitis and paracetamol-induced mice hepatitis, malotilate has a protective effect. In the clinical situation of virus-induced hepatitis, this drug is not employed for treatment so much.

In the present clinical situation in virus-induced chronic hepatitis, intravenous injection of glycyrrhizin is most widely used to normalize plasma ALT.

COUMARIN COMPOUNDS

Coumarins are active components of herbal medicines used for the treatment of many diseases. Coumarins possess various pharmacological features and have potential pharmaceutical properties. Coumarin compounds are classified into several types according to their chemical structures, i.e including simple coumarins, furocoumarins and furanocoumarins (Fig. 2).

Furanocoumarin

In mice concanavalin A-induced hepatitis, glycyrrhizin at the dose of 200 mg/kg (i.p.) causes 45-60 % inhibition of elevated plasma ALT (Table 1) [26]. Furanocoumarins pd-Ia, pd-II and pd-III (Fig. 3) inhibit more than 90% of the elevated ALT in mice concanavalin A-induced hepatitis (Table 1) [26]. However, these coumarins are hardly

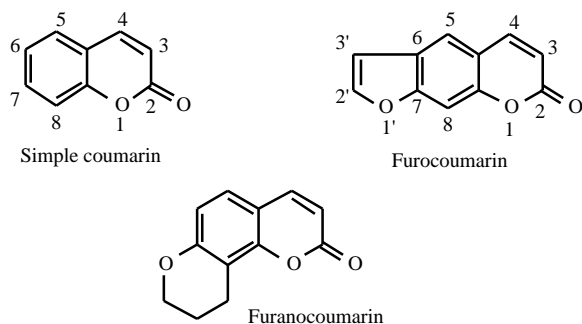


Fig. (2). Structure of coumarin skeletons.

absorbed in the intestine on oral administration. Since furanocoumarins are large molecules, they seem not to be appropriate as leading chemicals for producing a compound effective for preventing HCC on oral administration.

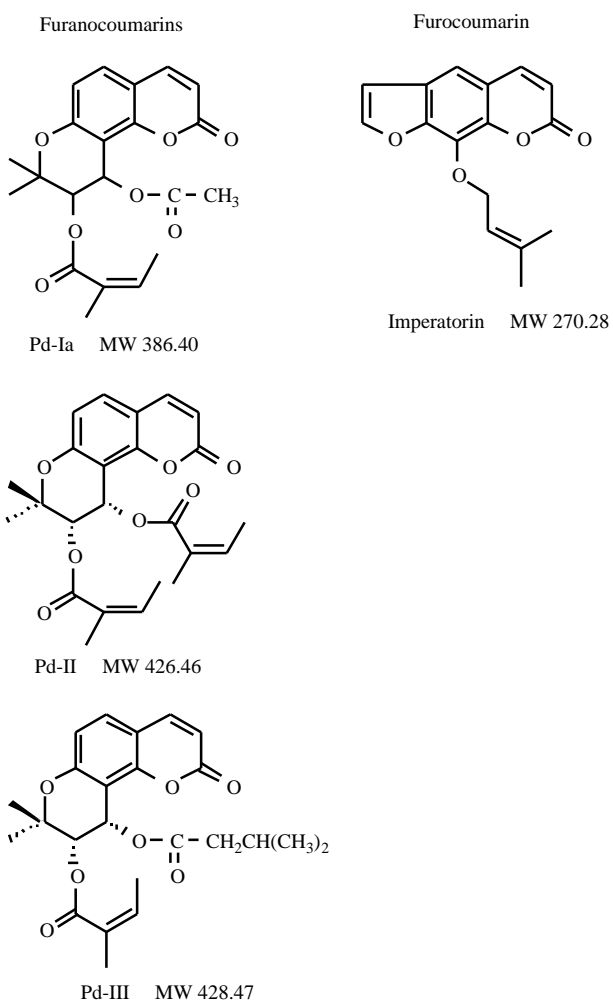


Fig. (3). Naturally occurring furanocoumarins and furocoumarins.

Furocoumarin

Imperatorin (Fig. 3), a furocoumarin, at the dose of 200 mg/kg (i.p.) caused more than 90 % inhibition of concan-

Table 1. Effects of Furanocoumarins and Furocoumarins on Concanavalin A-Induced Elevation of Plasma ALT

Compound	n	Percent Inhibition (%)/200 mg/kg (i.p.)
Glycyrrhizin	5	45
Furanocoumarin		
pd-Ia	5	91
pd-II	5	90
pd-III	5	90
Furocoumarin		
Imperatorin	5	92

avalin A-induced elevation of plasma ALT in mice (Table 1). Imperatorin even at the dose of (30 mg/kg, i.p.) inhibits anti-Fas antibody-induced elevation of plasma ALT, and inhibits anti-Fas antibody-induced caspase-3 activity [26]. Y355 has the substitution of (3-methyl-2-butenyl-oxy) at the position-8 of imperatorin by (2-propenyl-oxy) (Fig. 4). Y355 more potently reduces anti-Fas antibody-induced elevation of plasma ALT [27]. Although these two compounds are effective in lowering plasma ALT, the safety ranges between effective and toxic dosages are narrow. Thus, these compounds seem not to be appropriate for chronic administration.

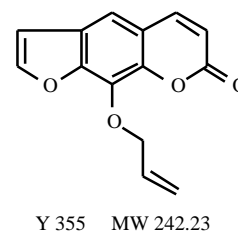


Fig. (4). Chemical structure of Y355.

Simple Coumarins

Three naturally occurring simple coumarins, 7-hydroxycoumarin, frexetin and osthole, were prepared (Fig. 5). At the dose of 200 mg/kg (i.p.), these compounds caused more than 70 % inhibition of concanavalin A-induced elevation of plasma ALT in mice (Table 2A). However, 7-hydroxycoumarin and frexetin lost their inhibitory effect at the dose of 100 mg/kg (i.p.). In contrast, osthole still showed strong inhibition at the dose of 100 mg/kg (i.p.) (Table 2A). Thus, the chemical aspects of osthole were further studied.

CHEMICAL ASPECTS OF OSTHOLE

Methoxy-Group at Position-7

Osthole possesses a methoxy-group at position-7 (Fig. 6). Osthenol, with substitution of the methoxy-group of osthole with a hydroxy-group (Fig. 5), was synthesized. Osthole at the doses of 200 and 100 mg/kg (i.p.) inhibited concanavalin

A-induced elevation of plasma ALT by 94 and 85 %, respectively (Table 2A). In contrast, osthenol at the dose of 200 mg/kg (i.p.) inhibited concanavalin A-induced elevation of plasma ALT by 89%, but this chemical caused only 32 % inhibition at the dose of 100 mg/kg (i.p.) [26]. Thus, the methoxy-group at position-7 is important for osthole to lower plasma ALT.

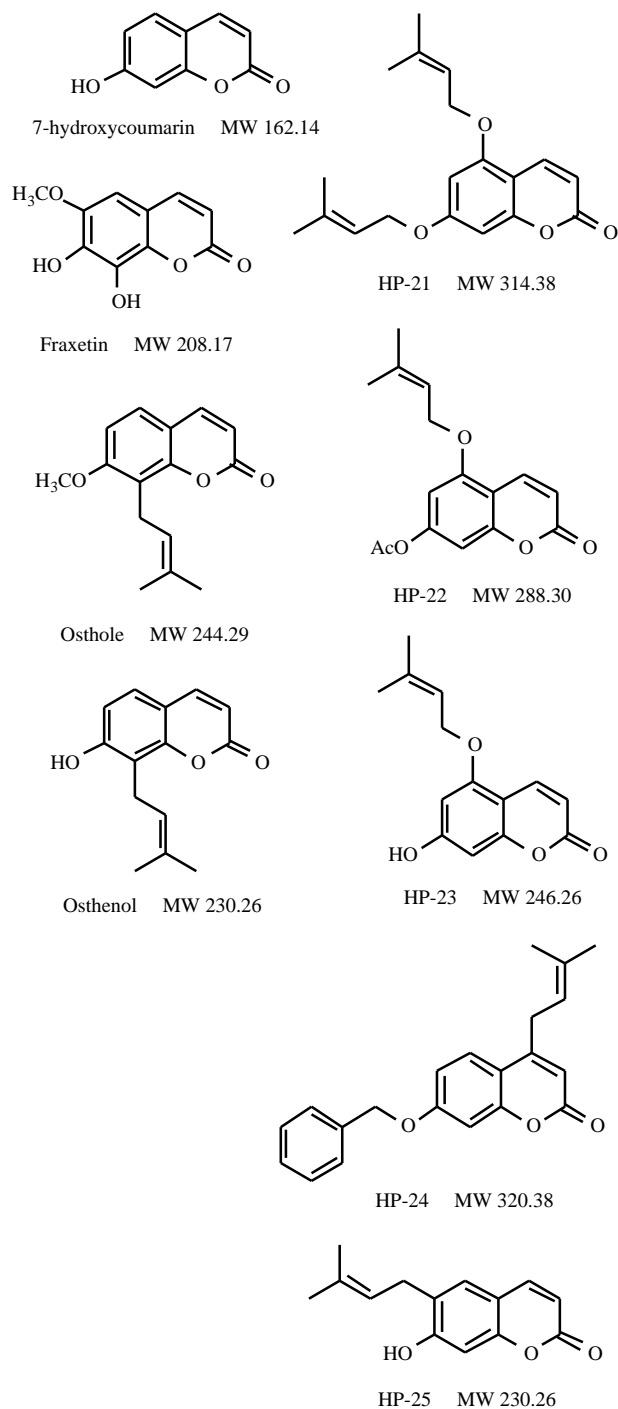


Fig. (5). Chemical structures of simple coumarins.

Table 2. Effects of Simple Coumarins on Concanavalin A-Induced Elevation of Plasma ALT

A

Compound	n	Percent Inhibition (%)	
		200 mg/kg (i.p.)	100 mg/kg (i.p.)
Simple Coumarin			
7-Hydroxycoumarin	5	70	9
Fraxetin	5	79	no inhibition
Osthole	6	94	85

B

Compound	n	Percent inhibition (%) 200 mg/kg (i.p.)
Simple coumarin		
Osthole		90>
HP-21	5	71
HP-22	5	54
HP-23	5	no inhibition
HP-24	5	64
HP-25	5	no inhibition

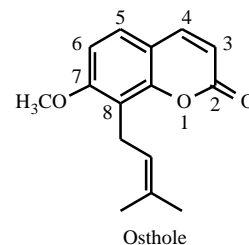


Fig. (6). Chemical structure of osthole.

3-Methyl-2-Butenyl-Group at Position-8

Another unique feature of osthole is the 3-methyl-2-butenyl-group at position-8 (Fig. 6). Dr. S. Yoshida synthesized simple coumarins, HP-21, 22, 23, 24 and 25, which possess a 3-methyl-2-butenyl-group at different positions (Fig. 5). The abilities of these five coumarins to lower concanavalin A-induced elevation of plasma ALT were less potent than that of osthole (Table 2B). HP-25 has the 3-methyl-2-butenyl-group at position- of 7-hydroxycoumarin, and this chemical lost the inhibitory effect even at the dose of 200 mg/kg (i.p.) (Table 2). Thus, the 3-methyl-2-butenyl-group at position-8 might be essential to lower plasma ALT. These results indicate that in the structure of osthole (Fig. 6), the methoxy-group at position-7 and the 3-methyl-2-butenyl-

group at position-8 are essential to lower plasma ALT in hepatitis.

Osthole prevented mice anti-Fas antibody-induced elevation of plasma ALT even at the dose of 10 mg/kg (i.p.) [28]. Furthermore, this chemical inhibits anti-Fas antibody-induced caspase-3 activity and causes improvement of the liver histology. Furthermore, the safety range of osthole between the effective and toxic dosages is wide. In contrast to the crystal form of osthole, making a solid dispersion of this chemical increased its solubility in water by four times. Thus, osthole may become a leading chemical for preventing HCC on oral administration.

CONCLUSION

The normalization of plasma ALT is a strategy for preventing HCC in the chronic hepatitis caused by HCV-infection. Coumarin compounds are the active components of herbal medicines used for the treatment of various diseases. Anti-Fas antibody-induced hepatitis in mice reflects a part of the liver injury in virus-induced chronic hepatitis. Osthole, a simple coumarin, prevents anti-Fas antibody-induced elevation of plasma ALT in mice even at a low dose. Furthermore, this chemical inhibits caspase-3 activity and improves the liver histology. The methoxy-group at position-7 and the 3-methyl-2-butenyl-group at position-8 are essential for the beneficial effect of osthole of lowering of plasma ALT. Making a solid dispersion increased its solubility in water by four times. Thus, osthole may become a leading compound for producing a chemical for the prevention of HCC effective on oral administration.

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