REVIEW —Current Perspective—

Hepatoprotective Drugs for the Treatment of Virus-Induced Chronic Hepatitis: From Hypercarcinogenic State to Hypocarcinogenic State

Toshihiro Okamoto\textsuperscript{1,2}, Kazunori Kajino\textsuperscript{2} and Okio Hino\textsuperscript{2,*}

\textsuperscript{1}Research Laboratories, Nippon Chemiphar Co., Ltd., Saitama 341-0005, Japan
\textsuperscript{2}Department of Experimental Pathology, Cancer Institute, Tokyo 170-8455, Japan

Received May 18, 2001 Accepted August 10, 2001

ABSTRACT—Interferon (IFN)-based therapy is a standard treatment for chronic hepatitis caused by hepatitis C virus (HCV) infection. This treatment is effective in approximately 30 – 40% of the patients and using ribavirin in combination with IFN increases the rate of sustained virologic clearance. For the remaining patients, glycyrrhizin is often used. Glycyrrhizin is known to prevent the development of hepatocellular carcinoma (HCC), but glycyrrhizin is usually administered intravenously. Drugs that are effective by oral administration are convenient for patients for long-term administration, and development of more effective drugs than glycyrrhizin is preferable. However, studies on drugs for the treatment of hepatitis are not actively conducted, and promotion of the study of drugs in this area is encouraging. For that reason, we show our approach to study drugs for the treatment of hepatitis. We analyzed the effect of glycyrrhizin on hepatitis as a standard chemical using the mouse liver injury model. Based on this, we screened drugs and found that a coumarin derivative seems to be one of model chemicals for the treatment of hepatitis.

Keywords: Prevention, Hepatocellular carcinoma, Drug, Hepatoprotective, Hepatitis

1. Introduction

The number of patients with chronic hepatitis caused by hepatitis C virus (HCV) infection is increasing in the world (1). Chronic hepatitis caused by HCV infection finally develops into a hepatocellular carcinoma (HCC). For the treatment of HCV-induced chronic hepatitis, interferon (IFN) is administered as a standard therapy (2, 3), and this treatment is effective in 30 – 40% of patients (4). Furthermore, recent reports indicated the effectiveness of using ribavirin in combination with IFN to increase the rate of sustained virologic clearance (5). However, treatment of patients with IFN has side effects such as anorexia, fatigue, general malaise and bone marrow suppression (6). Thus, for patients who do not respond to IFN or in whom the amount of IFN should be reduced due to side effects, other treatment options are necessary. Inhibition of chronic inflammation in the liver with chronic hepatitis leads to prevention of the development of HCC (4). Glycyrrhizin is a type of hepatoprotective drug and administration of glycyrrhizin to patients with HCV infection is reported to prevent the development of HCC (7), but glycyrrhizin is usually administered intravenously. Drugs that are effective by oral administration are convenient for patients on long term administration, and development of a more effective drug than glycyrrhizin is preferable. However, studies on drugs for the treatment of hepatitis are not actively conducted, and promotion of the study of drugs in this area is encouraging. In chronic hepatitis, many factors including cytokines and Fas-systems are involved in the development of liver injury. We use the mouse concanavalin A (Con A)-induced liver injury model and mice anti-Fas antibody-induced liver injury model to evaluate drugs, since these liver injury models may reflect the part of liver injury caused by cytokines and the Fas-system in chronic hepatitis, respectively.

In this review we present our approach to study drugs for the treatment of hepatitis.

2. Effectiveness and limited usage of IFN-based therapy

For HCV infection, IFN-based therapy is used as a standard treatment to clear the virus from the body. Recent reports indicated the effectiveness of using ribavirin in combination with IFN to increase the rate of sustained
The mechanisms of action of IFN are not completely understood, but prevention of de novo infection of susceptible cells may be the most important factor, and IFN treatment reportedly prevented progression of liver fibrosis (8). IFN therapy also has adverse effects, including anorexia, fatigue, general malaise and bone marrow suppression (6). The dose of IFN is standarized, and for patients who develop adverse effects by this treatment, frequency of IFN use should be reduced. IFN-based therapy is effective only in approximately 30–40% of patients with HCV infection. Thus for the remaining patients, other therapeutic options are required.

3. Normalization of plasma transaminase leads to prevention of hepatocarcinogenesis: from the hypercarcinogenic state to the hypocarcinogenic state

In viral hepatitis, chronic inflammation of the liver increases the chance of developing HCC, and this state is designated as the hypercarcinogenic state (4). Upon liver injury, alanine aminotransferase (ALT) in hepatocytes is released into plasma and usually determined as a marker of liver injury. We have proposed that a shift from the hypercarcinogenic state to a hypocarcinogenic state through normalization of plasma ALT may lead to prevention of the development of HCC (9). Recently, it was reported that persistent elevation of the plasma ALT level is correlated with the development of HCC (10, 11). Thus, drugs that normalize the plasma ALT level may lead to a shift from the hypercarcinogenic state to a hypocarcinogenic state and thus result in prevention of the development of HCC. Although steroid is effective for normalizing an elevated plasma ALT level, it could not be used continuously due to its immunosuppressive effects (12).

4. Effect of glycyrrhizin on mouse liver injury: analysis of the effect of glycyrrhizin as a standard chemical

Intravenous injection of glycyrrhizin into patients with chronic hepatitis caused by HCV infection lowers the plasma ALT level and prevents the development of HCC (7, 13). We used glycyrrhizin as a standard chemical and determined the effect of glycyrrhizin on hepatitis using Con A-induced mouse liver injury (14) and anti-Fas antibody-induced mouse liver injury (15).

Intraperitoneal injection of glycyrrhizin at a dose of more than 200 mg/kg inhibits Con A-induced elevation of the plasma ALT level (16). Con A-induced liver injury is dependent on inflammatory cytokines such as IFN-γ or TNF-α. Glycyrrhizin, however, does not affect the Con A-induced release of these cytokines (16).

In anti-Fas antibody-induced liver injury, intraperitoneal injection of glycyrrhizin at a dose of more than 200 mg/kg inhibited anti-Fas antibody-caused elevation of the plasma ALT level. Caspase-3 is critically involved in the development of anti-Fas antibody-induced liver injury, and glycyrrhizin inhibits anti-Fas antibody-caused caspase-3 activation (17). The mechanism for how glycyrrhizin prevents Con A-induced liver injury is not fully understood, but the effects of glycyrrhizin on anti-Fas antibody-induced liver injury suggest that this drug may inhibit the apoptotic pathway in Con A-induced liver injury. Based on this, a drug that can inhibit the anti-Fas antibody-induced apoptotic pathway while having few effects on cytokine expression would be one example of a model chemical for the treatment of hepatitis.

5. Coumarins

Coumarins are compounds present in many plants used as herbal medicines. Although many pharmacological properties of coumarins have been reported, there is only limited information on the effect of coumarins on hepatitis. The effects of the coumarin derivatives osthole, imperatorin, pd-la, pd-II and pd-III (Fig. 1) on Con A-induced liver injury were examined. In Con A-induced hepatitis, osthole, imperatorin, pd-la, pd-II and pd-III show stronger effects than glycyrrhizin to decrease Con A-induced elevation of plasma ALT. Furthermore, a morphological study also showed the effectiveness of these coumarins (not shown in Fig. 1).
shown) (18). Of these coumarins, osthole and imperatorin have the strongest protective effects. Osthelenol is an osthole derivative with substitution of a 7-methoxy group for the 7-hydroxy of osthole, whereby it loses its protective effect (18). Thus, the 7-methoxy group of osthole plays an important role in protecting the liver against injury. Furthermore, osthole, even at the dose of 200 mg/kg, has a minimal effect on Con A-induced gene expression of cytokines such as TNF-α and IL-2 (18). Blockade of Ca²⁺ channels or phosphodiesterases (19) might be involved in the protective effect of coumarins on liver injury. Oral administration of osthole also had an protective effect on Con A-induced liver injury. Osthole also protected mice from anti-Fas antibody-induced liver injury (not shown).

6. Conclusion

We have proposed that a shift of the hypercarcinogenic state in the liver with chronic hepatitis to a hypocarcinogenic state through lowering of plasma ALT would lead to prevention of the development of HCC (9). Our strategy for developing drugs is based on the above concept. Recent reports showed that elevated plasma ALT level is correlated with the incidence of HCC development in virus-induced hepatitis (10, 11), supporting our strategy. We have proposed that a shift of the hypercarcinogenic state in the liver with chronic hepatitis to a hypocarcinogenic state through lowering of plasma ALT would lead to prevention of the development of HCC (9). Our strategy for developing drugs is based on the above concept. Recent reports showed that elevated plasma ALT level is correlated with the incidence of HCC development in virus-induced hepatitis (10, 11), supporting our strategy.

In HCV-induced chronic hepatitis, intravenous administration of glycyrrhizin lowers the plasma ALT level and prevents the development of HCC (7). Long term administration of drugs by means of intravenous injection makes a patient’s life difficult. Thus, drugs that are effective by oral administration are preferable. We analyzed the way glycyrrhizin, as a standard chemical, inhibits hepatitis. Glycyrrhizin inhibits both Con A-induced liver injury and anti-Fas antibody-induced liver injury (16, 17). TNF-α plays a role in inducing hepatic injury and also acts to protect the liver (20). Furthermore, IL-2 is an important factor in the immune system. Thus complete inhibition of cytokines may have adverse effects. Glycyrrhizin does not affect cytokine expression and this may explain its fewer side effects. Glycyrrhizin inhibits anti-Fas antibody-induced liver injury by affecting a process upstream of caspase-3 protease, suggesting that glycyrrhizin may have the ability to block the hepatocyte apoptotic pathway. Thus, drugs that inhibit the anti-Fas antibody-induced apoptotic pathway with little effect on cytokine expressions might be model chemicals for the treatment of hepatitis. We studied drugs using the mouse liver injury models and found that coumarins show an inhibitory action similar to that of glycyrrhizin. Coumarins are plant medicines with therapeutic potential (21) and are active components of traditional medicines used in both Europe and Asia. Although many pharmacological and biochemical properties of coumarins have been reported (21–23), their effect on hepatitis has not been determined. We examined the effects of coumarins on hepatitis using mouse liver injury models and obtained results suggesting that coumarins may be useful as drugs for the treatment of chronic hepatitis. In chronic hepatitis, the activity of drug-metabolizing enzymes is suppressed (24), and the administration of drugs at high doses easily produces adverse effects. Thus the safety of drugs that are used to treat chronic hepatitis is one of the important points to consider and coumarins seem to be suitable for this purpose due to their safety (21).

In virus-induced chronic hepatitis, prevention of the development of HCC is required. Although IFN-based therapy is a standard treatment, other therapeutic options are required. Drug development involves many steps. We are studying drugs for the treatment of hepatitis and have found that coumarins including osthole seem to be model chemicals for this purpose.

Acknowledgment

We wish to thank Mr. N.J. Halewood for critically reading this manuscript.

REFERENCES

1 Alter MJ: Epidemiology of hepatitis C. Hepatology 26, Suppl 1, 62 – 65 (1997)
2 Poynaud T, Leroy V, Cohard M, Thevenot T, Mathurin P, Opolon P and Zarski JP: Meta-analysis of interferon randomized trials in the treatment of viral hepatitis: effects of dose and duration. Hepatology 24, 778 – 789 (1996)
3 Reichard O, Norkrans G, Fyden A, Braconier JH, Sonnerborg A and Weiland O: Randomised, double-blind, placebo-controlled trial of interferon alpha-2b with and without ribavirin for chronic hepatitis C. Lancet 351, 83 – 87 (1998)
4 Hino O, Yamamoto T and Kajino K: Inflammation-mediated hepatocarcinogenesis-hypercarcinogenic state of chronic liver disease. Prog Hepatol 4, 35 – 39 (1998)
5 Souvignet C and Zarski JP: Combination treatment for chronic hepatitis C: what is the role of ribavirin? Fundam Clin Pharmacol 14, 321 – 325 (2000)
6 Zdilar D, Franco-Bronson K, Buchler N, Locala JA and Yoinossi ZM: Hepatitis C, interferon alfa, and depression. Hepatology 3, 1207 – 1211 (2000)
7 Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, Suzuki Y, Saitoh S, Kobayashi M and Kumada H: The long term efficacy of glycyrrhizin in chronic hepatitis C patients. Cancer 79, 1494 – 1500 (1997)
8 Sobesky R, Mathurin P, Charlotte F, Moussali J, Olivi M, Vidaud M, Ratziu V, Opolon P and Poynaud T: Modeling the impact of interferon alfa treatment on liver fibrosis progression in chronic hepatitis C: A dynamic view. Gastroenterology 113, 378 – 386 (1999)
9 Hino O: Origin of cancer. Mol Med 32, 1140 – 1144 (1995)
10 Tarao K, Rino Y, Ohkawa S, Shimizu A, Tamai S, Miyakawa K, Aoki H, Imada T, Shindo K, Okamoto N and Totsuka S: Association between high serum alanine aminotransferase levels and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. Cancer 86, 589 – 595 (1999)
11 Tarao K, Rino Y, Takemiya S, Tamai S, Ohkawa S, Sugimasa Y, Miyakawa K, Morinaga S, Yoshida M, Shibuya A, Kokubu S, Kaita A and Endo O: Close association between high serum ALT and more rapid recurrence of hepatocellular carcinoma in hepatectomized patients with HCV-associated liver cirrhosis and hepatocellular carcinoma. Intervirology 43, 20 – 26 (2000)

12 McHutchison JG, Ponnudurai R, Bylund DL, Anguiano A, Pockros PJ, Mondala T and Wilkes LB: Predonisone withdrawal followed by interferon alpha for treatment of chronic hepatitis C infection: result of randomized controlled trial. J Clin Gastroenterol 32, 133 – 137 (2001)

13 Suzuki H, Ohta T, Takino T, Fujisawa K and Hirayama C: Effects of glycyrrhizin on biochemical tests in patients with chronic hepatitis. Double-blind trial. Asian Med J 26, 423 – 438 (1983)

14 Tiegs G, Hentshel J and Wendel A: A T cell-dependent experimental liver injury in mice inducible by concanavalin A. J Clin Invest 90, 199 – 203 (1992)

15 Ogasawara J, Watanabe-Fukuyama R, Adachi M, Matsuzawa A, Kasugai T, Kitamura Y, Itoh N, Suda T and Nagata S: Lethal effect of the anti-Fas antibody in mice. Nature 364, 806 – 809 (1993)

16 Okamoto T and Kanda T: Glycyrrhizin protects mice from concanavalin A-induced hepatitis without affecting cytokine expression. Int J Mol Med 4, 149 – 152 (1999)

17 Okamoto T: The protective effect of glycyrrhizin on anti-Fas antibody-induced hepatitis in mice. Eur J Pharmacol 387, 229 – 232 (2000)

18 Okamoto T, Yoshida S, Kobayashi T and Okabe S: Inhibition of concanavalin A-induced hepatitis by coumarin derivatives. Jpn J Pharmacol 85, 95 – 97 (2001)

19 Teng CM, Lin CH, Ko FN, Wu TS and Huang TF: The relaxant action of osthole isolated from Angelica pubescens in guinea-pig trachea. Naunyn Schmiedebergs Arch Pharmacol 349, 202 – 208 (1994)

20 Takehara T, Hayashi N, Mita E, Kanto T, Tatsumi T, Sasaki Y, Kasahara A and Hori M: Delayed Fas-mediated hepatocyte apoptosis during liver regeneration in mice: hepatoprotective role of TNF-alpha. Hepatology 27, 1643 – 1651 (1998)

21 Hoult JR and Paya M: Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. Gen Pharmacol 27, 713 – 722 (1996)

22 Guh J-H, Yu S-M, Ko F-N, Wu T-S and Teng C-M: Antiproliferative effect in rat vascular smooth muscle cells by osthole, isolated from Angelica pubescens. Eur J Pharmacol 298, 191 – 197 (1996)

23 Yang EB, Zhao YN, Zhang K and Mack P: Daphnetin, one of coumarin derivatives, is a protein kinase inhibitor. Biochem Biophys Res Commun 260, 682 – 685 (1999)

24 Guengerich FP and Turvey CG: Comparison of levels of several human microsomal cytochrome P450 enzymes and epoxide hydrolase in normal and disease states using immunochemical analysis of surgical liver samples. J Exp Pharmacol Ther 256, 1189 – 1194 (1991)