Experimental studies of oxymatrine and its mechanisms of action in hepatitis B and C viral infections

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OBJECTIVE: Viral hepatitis B is a worldwide public health problem; in China, chronic hepatitis B patients account for 30–40 million people and chronic hepatitis C accounts for 16% of all chronic hepatitis patients. Interferon-alpha (IFNα) is rather expensive and has many serious side-effects, which makes practitioners prudent in its use.

METHOD/RESULTS: A multicenter study held in China showed that the negative seroconversion of HBV DNA by oxymatrine and IFNα-1b was 42.3% and 40.7%, respectively, and that of HBeAg was 36.5% and 38.9%, respectively, after 3 months treatment with each in patients with chronic hepatitis.

CONCLUSIONS: Oxymatrine has a similar efficacy to IFNα, but with no adverse effects apart from slight injection pain, and it is much less expensive.

KEY WORDS: chronic hepatitis B, chronic hepatitis C, efficacy, experimental studies, interferon, oxymatrine.

INTRODUCTION

The past several years have witnessed dramatic advances in our understanding of the mechanisms of action of oxymatrine (OM), which is a product of its mother substance, matrine (containing >98% of OM), an alkaloid extracted from a Chinese plant, Sophora alopecuraides L. It has the formula C15H24N2O2 and a molecular weight of 264.4, whereas matrine is C15H24N2O and has a molecular weight of 258.4. Experimental and clinical studies have revealed that OM has antiviral, antifibrotic, hepatoprotective and immunomodulating effects, especially against hepatitis B and C viral infections, which has received great interest nationally.

An in vitro study has shown that within a certain range of concentration and duration of culture OM had an inhibitory effect on HBV DNA transfected HepG 2.2.15 cells with regard to secretion of HBsAg and HBeAg. In HBV transgenic mice, OM had a significant inhibitory effect on serum HBV. These results create an important and challenging problem, which together with the drug's effectiveness against antiduck hepatitis B, have initiated further investigations of OM in chronic hepatitis B and C.

EXPERIMENTAL STUDIES

Antiviral effect on HBV

Xue et al. used a HBV transfected HepG 2.2.15 cell line as the target cells for observing the effect of OM on the concentrations of HBsAg and HBeAg at different concentrations and different time points. They also recorded the cytotoxicity of OM in the cultured cell supernatant and then calculated the inhibition rates of the HBV antigens and the therapeutic index (TI) for comparison with acyclovir as an assessment of the in vitro efficacy of OM. Their results showed that the
HBsAg and HBeAg secreted by HepG 2.2.15 cells were inhibited at the 50–2000 µg/mL range of OM concentration, and the effect was dose- and time-dependent, the inhibition rate gradually increasing and reaching a peak at 20 days at a rate of 93% and 63%, respectively. The TI was 6.3 and 6.2, respectively, whereas in the case of acyclovir, the inhibition rates of HBsAg and HBeAg were 96% and 60% at a concentration of 1000 µg/mL, but with a higher cytotoxicity, and the TI were lower than those of OM. Using PCR-ELISA to determine quantitatively the HBV content in the HepG 2.2.15 cell supernatant, they calculated the inhibition rate of HBV DNA when OM was at the concentration of 1000 µg/mL and found that the highest inhibition rate was 79.6%, which indicated that OM could inhibit the replication of HBV DNA.

In another study, Chen et al. constructed a complete genomic HBV transgenic mice model ICR (TgN, HBV 1.2 copy) and used ELISA, immunohistochemistry and electron microscopy to detect the changes in the intrahepatic HBV antigens at different dosages of OM and different time points as an investigation of its anti-HBV effect. They found the OM could reduce the intrahepatic HBsAg, HBeAg and HBCAg concentrations of the HBV transgenic mice. At a dosage of 200 mg/kg for 30 days, the intrahepatic HBsAg and HBeAg became negative in six mice, but with prolonged treatment, they returned to positive, probably because of immune tolerance.

Antiviral effect on HCV

An in vitro study used pBK-HCV transfected SMMC-7721 cells for 12 days to detect quantitatively the intracellular HCV RNA by bDNA method. OM at the concentration of 100–1000 µg/mL could decrease the level of recombinant HCV RNA in the transfected cells and the inhibition rate increased with increasing concentration of OM. Concomitantly, the MTT method was used to detect the HCV RNA activity of the transgenic cells and only at the high concentration of 2000 µg/mL could the inhibitory effect be visualized (P < 0.05). This result indicated that the inhibitory effect of OM on HCV RNA was specific, and without toxic effects on the target cells, which was an important finding.

Experimental studies have indicated that OM can act directly on HCV and suppress the replication of HCV RNA. OM may also have antifibrotic and immunomodulating effects. It is thought that OM might act through interference at the transcription level, inducing intracellular nuclease in the degradation of viral RNA and hence, we can speculate that OM has a direct anti-HCV effect. In addition, matrine can increase the number of CD4+ cells and the CD4+:CD8+ ratio, augment the NK activity, promote cellular immunity and induce interferon production, thereby eventually decreasing the inflammatory reaction. In the future, OM may be a new anti-HCV drug for clinical use. In the case of IFN-α2b, it also significantly inhibited recombinant HCV RNA at the intracellular level, at the concentration of 5 × 10⁴ U/mL; the intracellular HCV RNA was reduced from 2.2085 mmol/mL to 0.5455 mmol/mL (P < 0.05), which was consistent with the results from a report on the study of cultured HCV.

Antifibrotic effect

Another surprising finding comes from a CCl₄-induced hepatic damage model that was set up for the study of the effect of OM on hepatic fibrosis. The parameters were serum alanine aminotransferase (ALT), type IV collagen (IV-C), hyaluronic acid (HA), tumor necrosis factor-alpha (TNF-α) and hepatic histopathology via the Tomographic Imaging Analysis System. By analyzing the proliferative status of intrahepatic fibrotic tissue, it was shown that in the OM group, the serum concentrations of ALT, IV-C, HA and TNF-α, as well as the intrahepatic inflammatory activity and extent of proliferative fibrous tissue, were all less than the results for the model group; those of the groups with the larger dosages of OM were even less than those of the smaller doses group, indicating that OM can ameliorate the intrahepatic inflammatory activity and inhibit the synthesis of intrahepatic collagens.

Using NIH3T3 fibroblasts as the target cells and the MTT method, western and northern blotting, and immunocytochemistry showed that when the OM concentration was 62.5 µg/mL, the proliferative activity of fibroblasts was significantly inhibited, in a dose-dependent phenomenon. The inhibited fibroblasts had a small cell volume, scarce cytoplasm, fusiform or round shape, and the nucleus was also small with few mitotic figures. A recent study also showed that OM could significantly inhibit the expression of both procollagen III (PCIII) and transforming growth factor-1-beta (TGF-1β), suggesting that these were possible mechanisms of the inhibitory effect because TGF-1β is of crucial importance in liver fibrosis.

Blockage of abnormal apoptosis of hepatocytes

Wang et al. used a murine experimental model to induce hepatocyte apoptosis by giving and then withdrawing phenobarbital, in order to observe the
influence of OM and glycerrhizic acid on apoptosis. The ratio of liver to body mass, hepatic DNA content, hepatic histopathology, in situ hepatocyte apoptosis and a TUNEL-labeled marker were the parameters. The dosage of OM was 150 mg/kg and that of glycerrhizic acid was 50 mg/kg, and each was injected intraperitoneally. The liver:body mass ratio and hepatic DNA content in the model mice fell by 16.3% and 32.4%, respectively, and in the glycerrhizic acid group, they fell by 16.1% and 28.9%, whereas in the OM group there was no fall in either parameter. Histology and the TUNEL-labeled marker showed typical apoptotic hepatocytes in the model group and glycerrhizic acid group, but not in the OM group. These results showed that OM can block the induced hepatocytic apoptosis.

The LO2 hepatocyte cell line is a primary culture of normal human liver cells obtained through serial passages by some special treatment. The standards for its growth and death are similar to those of normal human liver cells, and thus it is most suitable for studying the effects of OM in human beings. A large number of LO2 cells underwent apoptosis when exposed to TNF-α at a concentration of 15 ng/mL for 24 h, but when OM was added simultaneously, the cellular incorporation of 3H-thymidine (3H-TdR) and the rate of cell growth were both significantly higher than in the TNF-α group; the negative trypan blue staining rate in both groups did not differ statistically. With increasing concentrations of OM, the inhibition rate was enhanced significantly, indicating a dose-related blocking effect of OM on the cytotoxic reaction of TNF-α. That conclusion was drawn from the appearance of a sub G1 peak (apoptosis peak) on flow cytometry.

The possible mechanisms of the antiapoptotic effect are: (i) OM stabilizes the cellular and lysosomal membrane; (ii) OM induces the proliferation of liver microsomes, with increment of the microsomal cytochrome P450 concentration; or (iii) OM can eliminate hydroxy free radical (OH.) in a dose-responsive manner.

Hepatoprotective effect in experimental hepatic failure

Zhang et al. used OM to treat endotoxin-induced activation of murine intraperitoneal macrophages and found that OM had a significant inhibitory effect on the secretion of TNF-α by the activated macrophage, which was related to the inhibition of activated protein kinase C. Shan et al. used endotoxin coupled with D-aminogalactosamine to successfully construct a murine model of fulminant hepatic failure. In that model, they gave 50 mg/kg OM intraperitoneally twice daily, 3 days before the experiment. The death rate of OM treated mice was only 10%, far less than that of the model group (84%), and the degree of both hemorrhage and necrosis was significantly ameliorated. In the same experiment, the TNF-α secreted by the intraperitoneal macrophages induced by endotoxin was also inhibited significantly by OM in a dose-dependent manner. It is possible that the protective effect of OM on the damage induced by endotoxin is related to its inhibitory effect on the macrophagic release of cytokines, such as TNF-α, interleukin-1 and IL-6, the blocking of hepatocyte apoptosis and an antioxidant effect. A similar model and experiment showed that the death rate of the 50 mg/kg OM group was 38.1%, lower than that of the 25 mg/kg group and similar to that given hepatic stimulating substance (40%), but the death rate of the 100 mg/kg OM group approached that of the model group, indicating that an extra large dose of OM was not beneficial and in fact had an adverse effect.

MECHANISMS OF ACTION

Immunodulating effects

In viral hepatitis, especially the severe type, the serum concentrations of IL-1, IL-6 and TNF-α are all elevated, which can cause inflammation as well as necrosis. OM can inhibit the secretion of these cytokines and lower their serum concentrations, which in turn decreases the liver damage. The hepatotropic viruses do not damage the liver cells directly. In an immunopathologic study, activated CD8+ cells were proven to be positively correlated with the activity of hepatitis; in the peripheral blood, the CD4+:CD8+ ratio decreased, but both increased gradually during convalescence. Currently, OM is believed to be a bidirectional immunomodulate; that is, at low concentration, it stimulates lymphocytic proliferation, and at high concentration, it inhibits proliferation of lymphocytes. Humoral immunity is involved in the immunopathology of the liver, apart from that caused by cellular immunity. The immunopathologic damage to the liver involves types II, III, and IV hypersensitivity reactions. Viral antigen can induce its corresponding antibody, forming a circulating immune complex that can produce acute or chronic liver damage. When liver cells are infected with hepatitis virus, the membrane-specific lipoprotein (LSP) of the degenerated liver cell can act as an autoimmune antigen, stimulating B lymphocytes to produce anti-LSP antibody. The Fab...
fibrous tissue was much less in the OM-treated group seen in the 60 mg/kg OM group than in the model group, and even less fibrosis was the murine model used by Guo et al.8 Intrahepatic fibrous tissue was much less in the OM-treated group than in the model group, and even less fibrosis was seen in the 60 mg/kg OM group than in the 30 mg/kg OM group; only some enlargement of the portal area was observed at 12 weeks. Furthermore, OM can decrease the expression of both the TGF-1β and PCIII mRNA of fibroblasts.6

Anti-inflammatory activity
IL-1, IL-6 and TNF-α are all inflammatory cytokines. OM can inhibit the macrophagic release of these cytokines and thus ameliorate the inflammatory activity in the liver.

Antifibrotic effect
Oxymatrine can inhibit hepatic collagen synthesis as evidenced by the decrease of HA and IV-C in the murine model used by Guo et al.8 Intrahepatic fibrous tissue was much less in the OM-treated group than in the model group, and even less fibrosis was seen in the 60 mg/kg OM group than in the 30 mg/kg OM group; only some enlargement of the portal area was observed at 12 weeks. Furthermore, OM can decrease the expression of both the TGF-1β and PCIII mRNA of fibroblasts.

Antiviral effect
As already stated, OM inhibited the expression of HBsAg, HBeAg and HBV DNA of HepG 2.2.15 cells, and inhibited the expression of HBV antigen by HBV transgenic mice,5 indicating an antiviral effect of OM. In addition, OM can also inhibit the coxsackie virus, with resolution of viral myocarditis.15,16

Protective effect on hepatocytes
The protective effect of OM can be seen in many models of experimental liver damage, such as with CCL4, d-aminogalactosamine and ethanol, manifesting as a decrease in the concentration of serum ALT and inhibition of the release of inflammatory cytokines by macrophages, thus ameliorating hepatic pathological changes. In mice, OM at a dosage of 100 mg/kg can increase the microsomal cytochrome P450 content, which is where drug metabolizing enzymes and detoxification reactions take place.17 OM can also stabilize biomembranes, including the cell membrane and lysosomal membranes, preventing release of various lysosomal enzymes. Furthermore, it can block the apoptosis of hepatocytes and preserve the residual hepatocytes during massive necrosis by inhibiting TNF-α cytotoxicity and the Fas/Fasl pathway. In addition, a recent study using vascular endothelial cells induced by the liver cancer cell line SMMC-7721, found that OM inhibited growth of the cancer cells without affecting the normal cells,18 causing speculation that OM can inhibit the vascular epithelial growth factor and basic fibroblast growth factor secreted by the liver cancer cells. OM can also increase the number of white cells and platelets, making it a potential new drug for tumor antiangiogenesis and prevention of early tumor metastasis.

CONCLUSION
Oxymatrine has many targets and mechanisms of action, which make it an attractive therapeutic option, warranting further clinical trials. Moreover, it is much less expensive than INF-α2b in the treatment of chronic hepatitis B and C, being only 20% of the cost of interferon therapy, which is important for Chinese patients.

REFERENCES


