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Anti-hepatitis B virus effect of matrine-type alkaloid and involvement of p38 mitogen-activated protein kinase and tumor necrosis factor receptor-associated factor 6

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ABSTRACT

The matrine-type alkaloid, oxymatrine inhibits hepatitis B virus (HBV) replication but very little is known about these effects in other matrine-type alkaloids, including sophoridine and sophocarpine. Therefore, we compared the in vitro anti-HBV effects of matrine, oxymatrine, sophocarpine, and sophoridine by treating an HBV-transfected cell line (HepG2.2.15) with 0.4–1.6 mM of the compounds for 24 or 72 h. The levels of the HBV surface antigen (HBsAg) and e antigen (HBeAg) in the culture medium, as well as the intracellular and extracellular HBV DNA levels, were determined. Metabolomic analysis and detection of the mRNA level of p38 mitogen-activated protein kinase (MAPK), tumor necrosis factor receptor-associated factor (TRAF) 6, extracellular signal-regulated kinase (ERK) 1, NOD-like receptor family pyrin domain containing 10 (NLRP10), and caspase-1 were conducted in sophoridine-treated HepG2.2.15 cells. HepG2.2.15 cell exposure to 0.4–1.6 mM sophocarpine or sophoridine for 24 h reduced the HBsAg level of the medium more effectively than exposure to matrine and oxymatrine did, and reduced the HBeAg levels more effectively than these compounds did at 1.6 mM. Sophoridine (0.4–1.6 mM) reduced the cell medium HBV DNA levels more than the same concentrations of matrine, oxymatrine, or sophocarpine did. After 72 h, 0.4 and 0.8 mM sophoridine reduced HBsAg and intracellular HBV DNA levels more potently than matrine, oxymatrine, or sophocarpine did. Furthermore, sophoridine (0.8 mM) potently reduced the cell medium HBeAg levels while the metabolomic analyses revealed that HepG2.2.15 cells exposed to 0.8 mM sophoridine for 72 h exhibited reduced cycloleucine and phytosphingosine levels. In addition, the mRNA expression analyses revealed that HepG2.2.15 cells exposed to 0.8 mM sophoridine showed reduced levels of p38 MAPK, TRAF6, ERK1, NLRP10, and caspase-1. Sophoridine produced more potent anti-HBV effects than matrine, oxymatrine, and sophocarpine did. These effects may be related to the sophoridine-mediated reduction of p38 MAPK and TRAF6 levels.

1. Introduction

Hepatitis B virus (HBV) is the pathogen causing chronic hepatitis B (CHB), and long-term infection with this virus is associated with cirrhosis and hepatocellular carcinoma, which pose serious threats to human health. The inhibition of HBV replication is the most effective treatment for CHB (Gish et al., 2012). Potent anti-HBV drugs include nucleoside and nucleotide analogs (NAs) and

Abbreviations: HBV, hepatitis B virus; CHB, chronic hepatitis B; NAs, nucleoside and nucleotide analogues; p38 MAPK, p38 mitogen-activated protein kinase; TRAF6, tumor necrosis factor receptor-associated factor 6; ERK, extracellular signal-regulated kinase; HBsAg, hepatitis B virus surface antigen; HBeAg, hepatitis B virus e antigen.

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interferon. Oral NAs such as lamivudine, adefovir dipivoxil, and entecavir are HBV reverse transcriptase inhibitors that have been widely used clinically and have a potent activity that lowers the serum HBV titer in patients with CHB. However, long-term therapy with lamivudine or adefovir dipivoxil can induce mutation of the reverse transcriptase gene and is associated with a high rate of antiviral resistance in treatment-naïve patients with CHB. In addition, 57% of lamivudine-resistant patients with CHB also showed resistance to entecavir after 60 months of treatment, resulting in treatment failure (Zoulim and Locarnini, 2009).

New antiviral drugs were developed and introduced as anti-HBV treatments in an attempt to overcome HBV resistance. For example, oxymatrine, a matrine-type alkaloid extracted from the traditional Chinese herb, *Sophora flavescens* Aiton or *Sophora subprostrata*, showed high clinical efficacy in inhibiting lamivudine-resistant HBV replication (Wang et al., 2011). Furthermore, oxymatrine produces its anti-HBV effect by destabilizing heat stress cognate 70 mRNA and reducing the levels of this protein in the liver cells, which differs from the mechanism of the NAs (Wang et al., 2010). In addition to oxymatrine, the matrine-type alkaloids matrine, sophocarpine, and sophoridine are also important compounds isolated from *S. flavescens* Aiton or *S. subprostrata*. These four alkaloids have a similar chemical structure with the common molecular feature of O═C═N—C—C—C—N functional groups. Furthermore, they have all shown multiple biological effects including anti-inflammatory and antiviral. However, few studies investigating the anti-HBV effects of sophocarpine or sophoridine have been performed, and the anti-HBV activities of these four alkaloids have not been compared previously.

Tumor necrosis factor receptor-associated factor 6 (TRAF6) is a member of the tumor necrosis factor (TNF) receptor superfamily and activates p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK), which are the MAPKs associated with antiviral effects (Kashiwada et al., 1998; Yamashita et al., 2008). Downregulation of TRAF6 expression and stimulation of its degradation inhibit HBV proliferation (Kang et al., 2013). However, its involvement in the anti-HBV effect of matrine-type alkaloid is unknown. The NOD-like receptor family pyrin domain containing 10 (NLRP10) is a receptor that is essential for the initiation of adaptive immunity and is highly expressed in the liver (Lech et al., 2010). Reduced NLRP10 levels diminished the *Shigella*-induced expression and activation of p38 at early time points (Lautz et al., 2012). Suppression of p38 MAPK phosphorylation inhibits HBeAg and HBsAg syntheses and secretion, as well as HBV replication (Chang et al., 2008). However, the relationship between NLRP10 levels and HBV replication is unknown. Therefore, the present study evaluated the in vitro anti-HBV effects of matrine, oxymatrine, sophocarpine, and sophoridine, and compared them with those of lamivudine in a human HepG2.2.15 HBV-transfected cell line (Fig. 1, Bedse et al., 2009; Zhao et al., 2009). Furthermore, metabolomic and mRNA expression analyses of p38 MAPK, TRAF6, ERK1, NLRP10, and caspase-1 were performed to investigate the sophoridine-induced changes in HepG2.2.15 cells.

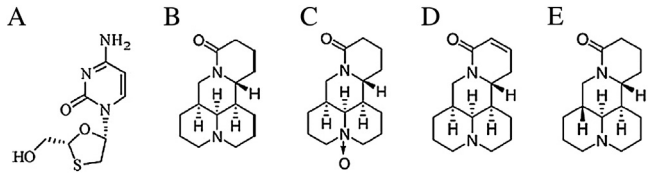

Fig. 1. Chemical structures of tested compounds (A) lamivudine, (B) matrine, (C) oxymatrine, (D) sophocarpine, and (E) sophoridine.

2. Material and methods

2.1. Drugs and reagents

Matrine, oxymatrine, sophocarpine, and sophoridine (all with >98% purity) were purchased from Undersun Biomedtech Co., Ltd. (Xian, Shanxi, China). Lamivudine was purchased from GlaxoSmithKline (Brentford, UK). The drugs were dissolved in Dulbecco's modified Eagle's medium (DMEM, GE Healthcare HyClone, Logan, Utah, USA) before use. The primers and probes to p38-α (Hs00176247-m1), TRAF6 (Hs00371512-g1), ERK1 (Hs00946872-m1), NLRP10 (Hs00738590-m1), caspase-1 (Hs00354836-m1), and β-actin (Hs99999903-m1) were purchased from Applied Biosystems (Foster City, CA, USA).

2.2. Cell culture

HepG2.2.15 cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS, Gibco, Langley, OK, USA), 100 U/mL penicillin, 100 μg/mL streptomycin, and 380 μg/mL G418, at 37 °C in an atmosphere of 5% CO_2. The cells were seeded in the culture plates in the medium at a density of 2×10^5 cells/mL and incubated for 24 h prior to incubation with the indicated concentrations of the drugs.

2.3. Cytotoxicity assay

Drug cytotoxicity was assessed using the cell counting kit-8 (CCK-8, Dojindo, Tokyo, Japan) according to the manufacturer's protocol. Briefly, HepG2.2.15 cells were seeded at a density of 2×10^4 cells/well in 96-well plates and incubated for 24 h. The culture medium was then replaced with fresh culture medium containing 0.2, 0.4, 0.8, 1.6, 3.2, or 6.4 mM matrine, oxymatrine, sophocarpine, sophoridine, or lamivudine and cultured for 3 days in the presence of these drugs. Then, the medium was removed, and the cells were incubated with the CCK-8 solution for 2 h prior to measuring the absorption at a wavelength of 450 nm. The following formula was used to calculate the percentage of viable cells: (mean absorbance of drug-treated wells – mean absorbance of blank wells)/(mean absorbance of control wells – mean absorbance of blank wells) × 100%.

2.4. Evaluation of the antiviral effects of drug treatments

HepG2.2.15 cells were seeded in 24-well plates (2×10^5 cells/well) and incubated for 24 h. Then, DMEM containing 0.4, 0.8, or 1.6 mM matrine, oxymatrine, sophocarpine, or sophoridine was added to the wells while lamivudine (0.4 and 0.8 mM) was the positive control. After incubation for 24 or 72 h, the culture medium was collected to determine the concentrations of HBsAg, HBeAg, and HBV DNA while the cells were processed for the detection of intracellular HBV DNA.

2.5. Measurement of HBsAg and HBeAg

HBsAg and HBeAg levels in the conditioned medium were determined using enzyme-linked immunosorbent assay (ELISA) kits (Kewei Clinical Diagnostic Reagents Co., Ltd., Beijing, China) according to the manufacturer's protocol.

2.6. HBV DNA quantification

HBV DNA was quantified in the conditioned medium and intracellularly using real-time quantitative polymerase chain reaction (qPCR) using a quantitative PCR Diagnostic kit for HBV DNA (Sino-MDgene Technology Co., Ltd., Beijing, China), and an ABI 7500