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Pentagalloylglucose, a highly bioavailable polyphenolic compound present in Cortex moutan, efficiently blocks hepatitis C virus entry

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ABSTRACT

Approximately 142 million people worldwide are infected with hepatitis C virus (HCV). Although potent direct acting antivirals are available, high costs limit access to treatment. Chronic hepatitis C virus infection remains a major cause of orthotopic liver transplantation. Moreover, re-infection of the graft occurs regularly. Antivirals derived from natural sources might be an alternative and cost-effective option to complement therapy regimens for global control of hepatitis C virus infection.

We tested the antiviral properties of a mixture of different Chinese herbs/roots named Zhi Bai Di Huang Wan (ZBDHW) and its individual components on HCV. One of the ZBDHW components, Penta-O-Galloyl-Glucose (PGG), was further analyzed for its mode of action *in vitro*, its antiviral activity in primary human hepatocytes as well as for its bioavailability and hepatotoxicity in mice.

ZBDHW, its component Cortex Moutan and the compound PGG efficiently block entry of HCV of all major genotypes and also of the related flavivirus Zika virus. PGG does not disrupt HCV virion integrity and acts primarily during virus attachment. PGG shows an additive effect when combined with the well characterized HCV inhibitor Daclatasvir. Analysis of bioavailability in mice revealed plasma levels above tissue culture IC_{50} after a single intraperitoneal injection.

In conclusion, PGG is a pangenotypic HCV entry inhibitor with high bioavailability. The low cost and wide availability of this compound make it a promising candidate for HCV combination therapies, and also emerging human pathogenic flaviviruses like ZIKV.

1. Introduction

Worldwide, approximately 142 million people are infected with HCV which can result in liver damage, the development of cirrhosis and an increased risk for hepatocellular carcinoma (Disease et al., 2016; Lavanchy, 2011; Levrero, 2006). Hence, end-stage liver disease due to HCV is the most common indication for liver transplantation (Brown, 2005). However, re-infection of the graft occurs

Abbreviations: TCM, Traditional Chinese Medicine; CM, Cortex Moutan; PGG, Penta-O-Galloyl-Glucose; HCV, hepatitis C virus; IC_{50}, half maximal effective inhibitory concentration; ZIKV, Zika virus; ConcaA, Concanamycin A; DAAs, direct-acting antivirals; EGCG, Epigallocatechin-Gallate; ZBDHW, Zhi Bai Di Huang Wan.

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in almost all transplanted individuals and can lead to the need of re-transplantation (Rubin et al., 2011). While the treatment of choice against HCV consisted of interferon/ribavirin for more than a decade, now an array of direct-acting antivirals (DAAs) have been licensed by the Food and Drug Administration (FDA) and show drastically improved potency in almost all infected individuals (Sarrazin, 2016). Despite of this, these new treatments face several obstacles that need to be overcome in the near future. While treatment of genotype 1 infected individuals with the new DAAs displayed improved response rates relative to interferon/ribavirin-based therapy, treatment efficacy is lower in genotype 3 infected individuals (Petta and Craxi, 2015). Another limitation is that special cases demand alternative therapies, such as when individuals have end-stage renal/liver disease or have recently undergone liver transplantation (Lam et al., 2015). Furthermore, drug-to-drug interactions between DAAs and certain anticonvulsants, antiarrythmics, antibiotics, antifungals, immunosupressants and even HIV antiretrovirals have been described and narrow treatment options in patients taking these drugs (Lam et al., 2015). Most importantly, accessibility to DAAs is limited due to their high costs, in particular in developing countries, which host the majority of chronically infected people (Messina et al., 2015).

ZBDHW is a mixture of eight different herbs/roots used in Traditional Chinese Medicine (TCM) to treat diseases of the liver, kidney and heart. Cortex Moutan (CM) is the root bark of tree peony and one component of ZBDHW. It is used in TCM as a hepatoprotective and anti-inflammatory herb (Poon et al., 2011). PGG is one constituent of CM that has previously been shown to prevent oxidative DNA damage in particular (Okubo et al., 2000), although anti-diabetic, anti-cancer and antiviral properties have also been reported (Cao et al., 2014).

In this study, we evaluated the antiviral properties of ZBDHW against HCV. We show that out of the different constituents of ZBDHW, an extract of CM revealed the strongest antiviral potency. Testing of different bioactive ingredients of CM revealed that PGG is a potent inhibitor of HCV entry in Huh-7.5 hepatoma cells as well as primary human hepatocytes. Further mode of action analyses showed that PGG acts at an early stage of virus entry into the target cells. Intraperitoneal administration of PGG in mice lead to compound plasma levels above tissue culture half maximal effective concentration (IC_{50}).

In conclusion, we report that an extract of CM and in particular the constituent PGG harbor potent anti-HCV properties. Thus, PGG could be a promising alternative for the generation of a more affordable antiviral treatment or as an add-on to current treatment options in specific cohorts, including transplanted individuals.

2. Material and Methods

2.1. Cell culture

Huh-7.5 cells (Blight, 2000), Huh-7.5 cells stably expressing firefly luciferase (Gentzsch et al., 2011) and Vero B4 cells (kindly provided by M. Müller, University of Bonn Medical Centre) were grown in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Karlsruhe, Germany) supplemented with 2 mM L-glutamine, non-essential amino acids, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 10% fetal calf serum. Human hepatocytes were isolated by a modified 2-step collagenase perfusion technique (Kleine et al., 2014).

2.2. Viruses

The chimeric 2a/2a Jc1 virus (pFK-Jc1), the monocistronic renilla luciferase reportervirus JcR2a (Haid et al., 2012; Pietschmann et al., 2006), the bicistronic firefly Jc1 virus (pFK-Luc-Jc1) (Koutsoudakis et al., 2006) and the monocistronic renilla luciferase reporter chimeric virus genomes H77c/1a/R2a, J4/1b/R2a, JcR2a, J8/2b/R2a, S52/3a/R2a, ED43/4a/R2a, SA13/5a/R2a, HK6a/6a/R2a and QC69/7a/R2a (Haid et al., 2012) have been previously described. Virus stocks were produced by electroporation of cells with *in vitro* transcribed RNA as described (Perin et al., 2016). The circulating Zika virus (ZIKV) strain from Puerto Rico (PRVABC59) has been obtained from Culture Collections of Public Health England (UK) and passaged twice on *A. albopictus* C6/36 before used in experiments.

2.3. Virus titration by immunohistochemical staining and luciferase assays

Virus titration by limiting dilution assay was determined according as previously described (Vieyres and Pietschmann, 2013). For immunohistochemical detection we used the monoclonal antibody targeting NS5A (9E10) (Lindenbach et al., 2005).

2.4. Quantitative detection of viral RNA

RNA was isolated from cells using the Nucleo Spin RNA II kit (Macherey-Nagel; Düren, Germany) according to the manufacturer's instructions. A one-step RT-PCR LightCycler 480 RNA Master Hydrolysis Probe kit (Roche; Mannheim, Germany) was used. The PCR protocol and primers were previously reported (Menzel et al., 2012). ZIKV RNA was isolated using the NucleoMag® VET kit (Macherey-Nagel; Düren, Germany) according to the manufacturer's instructions. Viral RNA was transcribed into cDNA using the M-MLV Reverse Transcriptase (Promega) according to the manufacturer's instructions. The Taqman Fast Universal Master Mix 2X (Applied Biosystems) was used for RT-PCR using the following primers and probes: forward primer CGYTGCCCAACACAAGG, reverse primer 5′-CCACYAAYGTTCTTTTGCABACAT-3′, probe 5′-FAM-AGCCTACCTTGAYAA-GCARTCAGACACYCAA-BHQ1-3′.

2.5. Binding and fusion of R18 labeled viruses

HCV JFH-1 virions were labeled with 0.5 μM R18 as recently described (Colpitts and Schang, 2014; Colpitts et al., 2013). Fusion with Huh-7.5 cells was triggered by increasing the temperature and lowering the pH and measured by R18 dequenching as previously described (Anggakusuma et al., 2014; Colpitts et al., 2013).

2.6. Fusion at the plasma membrane assay

The assay was performed as previously described (Tscherne et al., 2006). As controls, the CD81 antibody JS-81 and the fusion inhibitor flunarizine (Perin et al., 2016) were used.

2.7. Pharmacokinetic and cytotoxicity study of PGG in mice

Evaluation of bioavailability of PGG was conducted in a small proof-of-concept study in SCID mice. Five animals were intraperitoneally injected with a single dose of PGG (50 mg/kg), dissolved in PBS containing 5% ethyl alcohol. Blood was taken at 2, 4 or 8 as well as 24 and 96 h post treatment and analyzed for PGG concentrations. The concentration of PGG in mouse plasma was determined by LC-MS/MS as described before (Li et al., 2011). Epigallocatechin-Gallate (EGCG) was used as internal standard. For evaluation of cytotoxicity, ten Balb/c mice were similarly treated with PGG. Blood was drawn before treatment as well as day 1, 2 and 6 after treatment and analyzed for ALT as a marker of liver cytotoxicity. Six mice were treated with vehicle alone as control.

Further supporting “Material and Methods” can be found in the