Research Article

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Viral Hepatitis

Soraphen A: A broad-spectrum antiviral natural product with potent anti-hepatitis C virus activity

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Background & Aims: Soraphen A (SorA) is a myxobacterial metabolite that inhibits the acetyl-CoA carboxylase, a key enzyme in lipid biosynthesis. We have previously identified SorA to efficiently inhibit the human immunodeficiency virus (HIV). The aim of the present study was to evaluate the capacity of SorA and analogues to inhibit hepatitis C virus (HCV) infection.
Methods: SorA inhibition capacity was evaluated *in vitro* using cell culture derived HCV, HCV pseudoparticles and subgenomic replicons. Infection studies were performed in the hepatoma cell line HuH7/Scr and in primary human hepatocytes. The effects of SorA on membranous web formation were analysed by electron microscopy.
Results: SorA potently inhibits HCV infection at nanomolar concentrations. Obtained EC_{50} values were 0.70 nM with a HCV reporter genome, 2.30 nM with wild-type HCV and 2.52 nM with subgenomic HCV replicons. SorA neither inhibited HCV RNA translation nor HCV entry, as demonstrated with subgenomic HCV replicons and HCV pseudoparticles, suggesting an effect on HCV replication. Consistent with this, evidence was obtained that SorA interferes with formation of the membranous web, the site of HCV replication. Finally, a series of natural and synthetic SorA analogues helped to establish a first structure–activity relationship.
Conclusions: SorA has a very potent anti-HCV activity. Since it also interferes with the membranous web formation, SorA is an excellent tool to unravel the mechanism of HCV replication.

Keywords: HCV/HIV co-infection; HCV replication inhibitor; Lipid metabolism; Broad-spectrum antiviral; Natural product.
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Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; VLDL, very low density lipoprotein; LDL, low density lipoprotein; ACC, acetyl-CoA carboxylase; SorA, Soraphen A; DMVs, double membrane vesicles; HCVcc, cell culture-derived HCV; TOFA, 5-(tetradecyloxy)-2-furoic acid; MOI, multiplicity of infection; EC_{50}, half maximal effective concentration; CC_{50}, half maximal cytotoxic concentration; SI, selectivity index; 2′-*C*-Met, 2′-*C*-methyladenosine; PHHs, primary human hepatocytes; $TCID_{50}$, tissue culture infectious dose 50; SGR, subgenomic replicon; HCVpp, HCV pseudoparticles; IRES, internal ribosomal entry site; EM, electron microscopy; FDA, Food and Drug Administration; DAAs, direct-acting antivirals; Sol, solubility; JFH1, Japanese fulminant hepatitis 1; EMCV, encephalomyocarditis virus; DMSO, dimethyl sulfoxide; UTR, untranslated region.

Introduction

The hepatitis C virus (HCV) and the human immunodeficiency virus (HIV) have infected around 170 million and 35 million individuals worldwide, respectively. Due to overlapping acquisition modes, the number of individuals co-infected with HCV and HIV has risen up to 7 million [1]. In developed countries, HIV-infected individuals who inject drugs and HIV-infected men who have sex with men comprise the majority of HCV/HIV co-infected patients. Amongst the latter, multiple HCV outbreaks and reinfections have recently been described [2].

HCV mono-infection and HCV/HIV co-infection treatment(s) have changed dramatically in the era of direct-acting antivirals (DAAs) [3]. Currently approved DAAs include NS3-4A protease inhibitors (telaprevir, boceprevir and simeprevir) NS5A inhibitors (daclatasvir and ledipasvir) and the NS5B polymerase inhibitor sofosbuvir. Regimens containing these DAAs have brought major progress in the treatment, primarily of the HCV mono-infection patients group and secondarily in the group of HCV/HIV co-infected patients. However, some constraints still remain. First, the therapeutic efficiency of the DAAs towards the different

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HCV genotypes varies. Second, although some DAAs present high barrier to resistance (i.e. sofosbuvir), in general this property is low. Third, the deleterious interactions between some anti-HCV and anti-HIV drugs and the associated liver toxicities [4]. Forth, therapy costs are extremely high and represent a significant financial burden even for countries with developed economies. Treatment simplifications that may include broad-spectrum antiviral drugs targeting both viruses could help in solving some of these issues.

Due to their genome simplicity, viruses rely on a network of host-cell factors to multiply. Host-targeting drugs inhibiting cellular factors required for HCV to replicate are already in clinical trials. These include the cyclophilin inhibitors alisporivir and SCY-635, the miR-122 inhibitor miravirsen and the SR-B1 receptor blocker ITX-5061 [5,6]. Since some host factors are exploited by different viruses, conceptually, it would be possible to inhibit several viruses by targeting shared host factors. Given the genetic stability of the host, the development of such broad-spectrum antivirals would have the additional advantage of rendering selection of drug resistant virus mutants less likely, a major problem of conventional drugs that target viral proteins [7]. Initial efforts in this direction are showing promising results [8,9]. Predicted good targets for the development of antiviral drugs that act on HCV and HIV are the proteins that modulate the lipid metabolism. HCV particles that circulate in the blood of infected patients are associated with very low and low density lipoproteins (VLDL and LDL), resembling the so-called lipo-viral particle. Lipids have been shown to play an important role during entry (apolipoproteins), replication (fatty acids) and during budding and egress (VLDLs and LDLs) [10]. Thus, the HCV life cycle is intimately linked to intracellular membranes and host-cell lipid synthesis. The role of lipids in the HIV life cycle is also quite profound. HIV virion assembly, encapsidation and budding from the cell surface of the infected cell require a lipid milieu enriched in cholesterol and phosphatidylinositol-4,5-bisphosphate [11]. In addition, modifiers of the cellular lipid metabolism have been shown to affect HIV propagation [12].

By screening a library of secondary metabolites produced by *Myxobacteria*, we discovered recently that soraphens possess anti-HIV activities [13]. Soraphens are a group of polyketide natural products that have been first identified due to their potent antifungal activity [14] and more recently received much attention due to their anti-cancer [15], insulin-sensitizing [16] and immunoregulatory functions [17]. Soraphens mediate their function via inhibition of acetyl-CoA carboxylase enzyme (ACC) [18] that plays a key role in the cellular homeostasis of fatty acids. In humans exist two ACC isoforms (ACC1 and ACC2, a cytosolic and mitochondrial isoform, respectively) that are encoded by separate genes [19]. As soraphens are known to alter the lipid metabolism of treated cells [20], and the HCV life cycle critically depends on lipids [10], we tested soraphen A (SorA) for its anti-HCV activity. Here, we show that SorA is a highly potent HCV inhibitor that is active in the low nanomolar range. It exert its activity, at least in part, via interfering with the formation of double membrane vesicles (DMVs) that are the sites of HCV replication. A structure/activity analysis conducted in this study suggested options for structural modifications to optimize the pharmacological properties of SorA.

Materials and methods

Materials and methods were done as previously described (Ref. [21,22]). A detailed description of the methods used in this manuscript is provided in the Supplementary material section.

Results

SorA inhibits HCVcc infection

We have recently identified SorA as an HIV inhibitor [13]. To assess the inhibitory effects of SorA (Fig. 1A) on HCV infection we used the HCV cell culture (HCVcc) system [23–25]. Unless otherwise stated, we used the HuH7/Scr and the HuH7/Lunet [26] cells, which are highly permissive for HCV propagation *in vitro*. Our experiments were performed in the context of genotype 2a (Jc1 chimera [27]) HCVcc virus. To facilitate the quantification of infection, we used the bicistronic Jc1 luciferase reporter construct, designated Luc-Jc1 [28]. Cell viability was monitored in parallel by a commercial ATP assay [29]. The commercial ACC inhibitor 5-(tetradecyloxy)-2-furoic acid (TOFA), which has been previously described to inhibit HCV replication [30], was used for comparison. The NS3–4A serine protease inhibitor VX-950 [31], a known HCV replication inhibitor, was used as positive control. As shown in Fig. 1B, Luc-Jc1 virus infection in HuH7/Scr cells (at an MOI of 0.03 $TCID_{50}$/cell) was strongly inhibited by SorA at concentrations in the 1–10 nM range. Viability assays show no toxicity of the compounds up to 10 μM. The half maximal effective concentration (EC_{50}) for SorA was estimated to be 0.70 nM [the half maximal cytotoxic concentration (CC_{50}): ~98.52 μM, and the selectivity index (SI): 140,742], as compared with an EC_{50} of 1.01 μM for TOFA [CC_{50}: ~33.87 μM, SI: 33.53] and an EC_{50} of 18.92 nM for VX-950 [CC_{50}: ~94.51 μM, SI: 4995] in this assay. To demonstrate the specific inhibitory activity of SorA against HCV at a higher MOI, Jc1 virus (which does not carry a reporter gene) was challenged in similar experiments with increasing doses of SorA. As shown in Fig. 1C, SorA also inhibited Jc1 infection at an MOI of 1 $TCID_{50}$/cell in an equipotent manner. The EC_{50} value was 2.30 nM [$CC_{50} \geqslant 10$ μM, SI $\geqslant$ 4357]. A 2'-modified nucleoside analogue (2′-*C*-methyladenosine, 2′-*C*-Met) [32] was used as a positive control [EC_{50}: 0.101 μM, CC_{50}: 12.42 μM, SI: 122.97].

Although HuH7 cells are a well-accepted cell culture model for HCV infection studies, these cells might present differences in lipid metabolism compared to primary human hepatocytes (PHHs). To further corroborate our findings in a more natural system, PHHs were inoculated with the Jc1 virus (at an MOI of 0.2 $TCID_{50}$/cell) in the presence of SorA or VX-950. 24 and 48 h post-infection cell culture supernatants were harvested and the amount of virus production by the PHHs was quantified by an endpoint dilution assay ($TCID_{50}$). As shown in Fig. 1D, Jc1 infection of PHHs was also inhibited by SorA. Similar to HuH7/Scr cells, SorA was not toxic to PHHs up to 10 μM (data not shown). Conclusively, our *in vitro* results using human hepatoma carcinoma-derived cell line HuH7/Scr as well as PHHs demonstrate that SorA is a very potent non-toxic inhibitor of HCV infection.

SorA inhibits HCV subgenomic replicons

To investigate the impact of SorA in HCV RNA translation and/or replication, we transfected HuH7/Scr cells with a subgenomic JFH1 luciferase replicon (SGR-JFH1; Fig. 2A, top) [33]. These HCV subgenomes do not contain structural proteins and therefore cannot sustain HCV particle production. However, they possess the ability to replicate autonomously in cell culture, rendering them powerful tools for HCV translation and replication studies. As shown in Fig. 2A, SorA inhibits HCV RNA