Artemisinin Analogues as Potent Inhibitors of In Vitro Hepatitis C Virus Replication

Susan Obeid1, Jo Alen2, Van Hung Nguyen3, Van Cuong Pham3, Philip Meuleman4, Christophe Pannecouque1, Thanh Nguyen Le3, Johan Neyts1*, Wim Dehaen2, Jan Paeskeuze1

1 Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, 2 Molecular Design and Synthesis, Department of Chemistry, KU Leuven, Leuven, Belgium, 3 Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam, 4 Department of Clinical Chemistry, Microbiology and Immunology, University Ghent, Ghent, Belgium

Abstract

We reported previously that Artemisinin (ART), a widely used anti-malarial drug, is an inhibitor of in vitro HCV subgenomic replicon replication. We here demonstrate that ART exerts its antiviral activity also in hepatoma cells infected with full length infectious HCV JFH-1. We identified a number of ART analogues that are up to 10-fold more potent and selective as in vitro inhibitors of HCV replication than ART. The iron donor Hemin only marginally potentiates the anti-HCV activity of ART in HCV-infected cultures. Carbon-centered radicals have been shown to be critical for the anti-malarial activity of ART. We demonstrate that carbon-centered radicals-trapping (so-called TEMPO) compounds only marginally affect the anti-HCV activity of ART. This provides evidence that carbon-centered radicals are not the main effectors of the anti-HCV activity of the Artemisinin. ART and analogues may possibly exert their anti-HCV activity by the induction of reactive oxygen species (ROS). The combined anti-HCV activity of ART or its analogues with L-N-Acetylcysteine (L-NAC) [a molecule that inhibits ROS generation] was studied. L-NAC significantly reduced the in vitro anti-HCV activity of ART and derivatives. Taken together, the in vitro anti-HCV activity of ART and analogues can, at least in part, be explained by the induction of ROS; carbon-centered radicals may not be important in the anti-HCV effect of these molecules.

Introduction

Worldwide, an estimated 180 million people are chronically infected with the hepatitis C virus (HCV) [1]. The current therapy consists of pegylated interferon α (peg-IFNα), Ribavirin (RBV) in combination with either the protease inhibitor (PI) Telaprevir or Boceprevir. This combination therapy has been reported to be effective in up to 79% of the treated patients infected with HCV [1,2]. PIs and many of the selective inhibitors of HCV replication that target the viral genome (including most of those in advanced clinical development) select rapidly for drug-resistant variants [3]. Alternatively, host targeting antivirals, such as the cyclophilin-binding molecule Alisporivir, have a high barrier to resistance [4,5].

Artemisinin (ART), a sesquiterpene lactone with an endoperoxide function isolated from the plant Artemisia annua L., is widely used as an anti-malarial drug [6-8]. The drug has also been reported to exert anti-bacterial, anti-inflammatory and anti-angiogenic activities [9–12]. However, because of its low solubility and poor oral bioavailability, its therapeutic efficacy is not optimal [11,13]. To combat these hurdles, numerous ART analogues were synthesized and evaluated for their potential anti-microbial effect [14]. Interestingly, some of these compounds exhibited, in vitro, anti-herpes viruses, anti-human cytomegalovirus, anti-human immunodeficiency virus and anti-hepatitis B virus activity [15–19]. We reported earlier that ART inhibits in vitro HCV replicon replication at concentrations that have no effect on host cell growth [24].

Here we report on the discovery of ART analogues that are more potent and selective inhibitors of HCV replication than the parent compound and propose by which mechanism they may do so.

Materials and Methods

Compounds

Artemisinin, Hemin and TEMPO compounds were purchased from Sigma (Bornem, Belgium). Artemisinin analogues (Fig. 1 and 2) were synthesized by methods that will be reported elsewhere [20].

HCV Replicon Assay

Cells carrying HCV replicons I 389luc-ubi-neo/NS3-3′/5.1 (Huh 5-2) were kindly provided by Prof. R. Bartenschlager (University of Heidelberg, Germany). Cells were cultured in Dulbecco’s modified Eagle’s Medium (DMEM, Gibco, Merelbeke, Belgium) supplemented with 10% heat-inactivated fetal bovine serum (Integro, Zaandam, The Netherlands), 1× non-essential amino acids, 100 IU/mL penicillin (Gibco), 100 μg/mL strepto-
mycin (Gibco), and 250 μg/mL G418. Cell cultures were maintained at 37°C with 5% CO2.

**Antiviral Assay in HCV Replicon Cells**

The antiviral assay was performed as described [21,22]. Briefly, cells were seeded at a density of 5 × 10^3 cells per well in 96-well cell culture plates in DMEM containing 250 μg/mL G418 at 37°C (5% CO2). After 24 hours of incubation, medium was replaced with fresh DMEM (without G418) and serial dilutions of the test compounds. Replicon RNA levels were determined by a quantitative reverse transcription polymerase chain reaction (qRT-PCR) or quantified by measuring the firefly luciferase activity in 96-well cell culture plates (Safire, Tecan, Austria).

**Antiviral Assay in the HCV Infectious System**

The highly infectious HCV JFH-1/CS-N6 described by Delgrange et al [23] was used for the antiviral assays. A total of 7.2 × 10^3 Huh 7.5.1 cells per well of a 96-well cell culture plate were incubated with the virus at specific infectivity of about 400 (400 HCV RNA copies per foci-forming unit [24]) and at the same time with serial dilutions of compounds. Following 3 days of incubation, medium was removed and cells were washed once and lysed to extract the intracellular RNA with the RNeasy kit (Qiagen). HCV RNA was quantified by means of qRT-PCR [25].
qRT-PCR Assay
A qRT-PCR mixture contained: cellular RNA extract, HCV JFH-1 forward primer SF-JFH186 [5'-TGG CGT TAG TAT GAG TGT CGT ACA GCC TTC A-3'], reverse primer SR-JFH194 [5'-AAA GGA CCC AGT CTT CCC GGC AAT T-3'], and probe [5'-FAM-TGG TCT GGG GAA CCG GTG AGT ACA CC-TAMRA-3'], was performed at 50°C for 30 min, subsequent 15 min at 95°C and PCR amplification of 40 cycles of denaturation at 94°C for 20 s and annealing and extension at 60°C for 1 min in an ABI 7500 Taqman (Life Technologies).

Cytostatic Assay
Cells were seeded at a density of 5×10³ or 7.2×10³ cells per well in a 96-well plate in complete DMEM in serial dilutions of the test compounds for Huh 5-2 and Huh 7.5.1 cells, respectively. After three days of incubation, cell viability was determined by MTS/PMS method (Promega). The 50% cytotoxic concentration (CC50) was defined as the concentration that inhibited the proliferation of exponentially growing cells by 50%.

Drug Combination Studies
The effects of drug combinations were evaluated in a checkerboard format using the method of Prichard and Shipman [26]. The theoretical additive effect was calculated from the dose–response curves of individual compounds by the equation Z = X + Y - 1, where X represents the inhibition produced by first compound alone and Y the inhibition by the second compound alone. Z, represents the effect produced by the combination of the first with the second compound. The theoretical additive surface is subtracted from the actual experimental surface.

Results
Novel Analogues of ART with Improved in vitro anti-HCV Activity
ART inhibits, as we demonstrated earlier, the in vitro replication of HCV subgenomic replicons (genotype 1b) in a selective and dose-dependent manner [27]. Here, we studied whether ART is also effective in hepatoma cells infected with the infectious HCV JFH-1. ART was found to inhibit HCV replication in a dose-dependent manner with EC50 value of 167±38 μM. At the highest concentration tested (400 μM), the host cell proliferation and cell viability were not affected (Fig. 3 and Table S1). Well known derivatives of ART such as Artesunate (ARS), Artemether (ARM) and Dihydroartemisinin (DHA) were found to be highly toxic in our hepatoma cell cultures (CC50<6 μM).

We next assessed the antiviral activity of novel ART derivatives (all were recently synthesized with the aim to improve the antimalarial properties of this class of drugs [20]) belonging to three different categories (AJ, TVN and DW). The chemical structures of ART and the compounds of category AJ are depicted in Figure 1. All compounds of this group were found to be more active against HCV-cell culture (HCVcc) than ART (Fig. 3, Table S1). The EC50 values were 26.5±2, 15±2, and 16±4 μM for AJ-001, AJ-002 and AJ-004, respectively. The antiviral effect of these analogues was next assessed in the subgenomic HCV replicon system (Huh 5-2). The compounds exert anti-HCV activity against the genotype (1b) subgenomic replicon with AJ-001 and AJ-004 being the most potent with EC50 values of 8.8±2.7 and 3.2±2.4 μM, respectively (Table 1). Category TVN consists of 3 analogues (Fig. 2), of which TVN4 inhibits the replication of the infectious HCV at EC50=59±6 μM (Fig. 3) while inhibiting the subgenomic replicon at EC50=36±16 μM (Table 1). TVN2 and TVN6 had weak activities against the infectious HCV JFH (>70 μM). Of the 30 compounds of category DW (Figure S1), only one, i.e. DW 13, exerts anti-HCV activity at non-toxic concentrations in the HCVcc system and the subgenomic replicon assay (EC50 value ~30 μM). All DW 13 related analogues proved toxic to the cells at ~10 μM (Figure S1).

Hemin Potentiates the Anti-HCV Activity of ART and Derivatives
The malaria parasite is enriched in Hemin which results from the digestion and degradation of haemoglobin. Hemin was demonstrated to exert its anti-malarial activity, in part, by binding to the ART molecule forming Hemin-ART adducts from which radicals are released [28]. Hemin alone inhibits the replication of the HCV infectious virus in a dose dependent manner as measured by means of qRT-PCR (EC50=8.0±0.6 μM) and is not toxic to Huh 7.5.1 at concentrations >50 μM. At 5 μM, Hemin potentiates the antiviral activity of ART in the HCVcc system by a factor 2-fold and in the replicon model by a factor 8. The anti-HCV activity (in the subgenomic replicon system) of AJ-002, but not of AJ-004, was potentiated 15-fold by Hemin (Table 1). The combined treatment with Hemin was selective and did not increase the toxicity profile at the concentrations tested.

Carbon-centered Radicals are not Crucial for the in vitro Anti-HCV Activity for ART and Analogues in Cultures
Formation of carbon-centered radicals has been reported to be critical for the in vitro anti-malarial activity of Artemisinin [29]. To study whether these radicals are or are not required for the anti-HCV activity of ART and its analogues, we combined a nitroxide radical spin trap, 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) compound with either ART or TVN4 in the HCV replicon system. TEMPO alone had no effect on the replication of HCV at concentrations up to 200 μM. The combination of ART or TVN4 with TEMPO resulted only in a marginal antagonistic effect (Figure S2).

ART and its most Potent Analogues Partially Inhibit the in vitro Replication of HCV by Induction of Reactive Oxygen Species (ROS)
A possible mechanism by which ART and analogues may exert their activity may be by the induction of reactive oxygen species (ROS) [30]. If so, the addition of an anti-oxidant should reduce their anti-HCV activity. We therefore combined in the HCV subgenomic replicon (Huh 5-2) assay ART with L-N-acetylcycteine (L-NAC); a compound that reduces reactive oxygen species (ROS) formation. Whereas L-NAC alone has no effect on the HCVcc replication at the concentration tested (HCV RNA replication was 98%±11% of UTC), the anti-HCV activity of ART and its analogues (TVN4, AJ-001, AJ-002 and AJ-004) was reduced by a factor 2 to 5 following the addition of L-NAC (Table 1, Figure S3).

Discussion
Artemisinin (ART), a natural product isolated from the plant Artemesia annua L, was originally discovered during the Vietnam
War as a potent treatment for malaria [10]. Besides its anti-malarial properties, ART also exerts in vitro anti-bacterial, anti-inflammatory and anti-angiogenic activity [15,27,31]. ART also inhibits the in vitro replication of the human cytomegalovirus (HCMV) and the hepatitis B virus (HBV) [10,16,32] and its derivative Artesunate inhibits the in vitro HIV replication [33]. We demonstrated previously that ART inhibits the in vitro HCV replicon replication [27]. Here, we report that ART inhibits also the replication of infectious HCV JFH-1.

ART derivatives such as Artesunate, Artemether and Dihydroartemisinin [34–37], that are currently being used to treat malarial infections, proved in our hands highly toxic in hepatoma cell cultures (Huh 7.5.1) and were not considered for further study. Interestingly, we were able to identify analogues that proved markedly more potent as HCV inhibitors than the parent compound.

In the subgenomic replicon system, the ART dimer (AJ-004) was to be about 3-fold more efficient in inhibiting the in vitro HCV replicon replication as compared to the monomer AJ-001, and was -10-fold more potent than the monomer AJ-002 (benzyl aldehyde derivative of ART). In cells infected with HCV JFH-1, the ART monomers were roughly equipotent to the dimer. This may suggest that the antiviral activity in the HCVcc-infected cells may be determined by properties other than those related to the endoperoxide bridge.

The malaria parasite has a high content of Hemin as a result of the haemoglobin digestion and degradation. Thus, Hemin may play a critical role in the anti-malarial activity of ART. It was suggested that the iron centre of Hemin attacks the endoperoxide bridge of the trioxane resulting in the cleavage of C3–C4 and the release of radicals. Iron binds O1 (not O2) of ART to form an iron-O-C bond (a Hemin-ART adduct) responsible for the biological activity of the compound [38]. Surprisingly, Hemin did not potentiate the anti-HCV activity of the most potent derivative (AJ-004). In line with previous findings [39–41], we showed that Hemin itself was able to inhibit the HCVcc replication. It is thus possible that the role of iron in the anti-HCV activity of ART and its derivatives may vary with the chemical structure of the compound.

### Table 1. Effect of ART and derivatives on Huh 5-2 HCV replicon replication.

| Compound | EC50 (μM) | CC50 (μM) | +Hemin (5 μM) | +L-NAC (5 mM) |
|----------|----------|----------|---------------|---------------|
| ART      | 75±7     | >400     | 9.3±0.9 (8)   | >400 (–5)     |
| AJ-001   | 8.8±2.7  | >133     | 4.6±2.8 (2)   | 26±2 (–3)     |
| AJ-002   | 30±8     | >133     | 1.9±0.7 (15)  | 68±22 (–2)    |
| AJ-004   | 3.2±2.4  | >133     | 4.0±0.1 (0)   | 17±4 (–5)     |
| TVN2     | 25±13    | 36±7     | 6.3±2 (44)    | nd            |
| TVN4     | 36±16    | 123±14   | 17±6 (42)     | >100 (–3)     |
| TVN6     | 3.6±2.3  | 40±20    | nd            | nd            |

EC50: 50% effective concentration, CC50: 50% cytostatic concentration. Data obtained from the measurement of the firefly luciferase activity, and are mean values ± SD for four independent experiments (expressed in μM). Values between brackets indicate fold-change. At 5 μM, Hemin inhibits HCV replicon replication by 30%.

doi:10.1371/journal.pone.0081783.t001
Based on the observations made for the effect of the combination of ART (as well as the analogue TVN4) with a nitroxide radical spin trap (TEP0) on anti-HCV activity, it is unlikely that carbon centred radicals are as important for the anti-HCV activity of ART as was suggested for the anti-malarial activity of the compound.

The cleavage of the endoperoxide bridge within the ART molecule results in the release of carbon radicals and reactive oxygen species (ROS). The induction of ROS has been demonstrated to regulate the replication of other viruses such as HBV (negatively) [42] or HIV (positively) [43]. For HCV, it was shown that peroxide treatment (which results in ROS induction), at concentrations that were not toxic to the cells, resulted in the disruption of active HCV replication complexes through reduction of the amount of NS3 and NS5A in the replication complexes [42]. The anti-HCV activity of ART induced by peroxides could be negated by L-N-Acetyl-l-cysteine [L-NAC] [the molecule that inhibits ROS generation]. Therefore, we studied the anti-HCV activity of ART or analogues in combination with L-N- Acetyl-l-cysteine [L-NAC]. L-NAC reduced the anti-HCV activity of ART and derivatives (2 to 5 fold) (Table 1).

In conclusion, we identified novel derivatives of ART that are markedly more potent and selective in vitro HCV inhibitors than the parent compound. It is suggested that at least part of the antiviral activity is related to the induction of ROS. Carbon-centred radicals are only marginally involved in the anti-HCV activity of ART and derivatives thereof.

Supporting Information
Figure S1 Structural formulae of Artemisinin and synthetic derivatives belonging to the third category DW.

Figure S2 Combination studies of ART and TVN4 with TEMPO in Huh 5-2 cells: zero plane indicates to additive effect on the z-axis, while all values above zero point to a synergistic effect, and all values below zero indicate an antagonistic effect.

Figure S3 In vitro anti-HCV subgenomic replicon activity (in Huh-5-2) of a. ART, b. AJ-001, c. AJ-002 and d. AJ-004 in combination with hemin or L-NAC.

Table S1 Effect of ART and its analogues on the replication of HCVcc.

Acknowledgments
We thank Katrien Geerts for excellent technical assistance and Dominique Brabant for dedicated editorial help.

Author Contributions
Conceived and designed the experiments: SO JA JW DP. Performed the experiments: SO. Analyzed the data: SO JA JW DP. Contributed reagents/materials/analysis tools: JA VHN VCP MP WD TNL. Wrote the paper: SO JW DP.

References
1. Craxi A, Licata A (2003) Clinical trial results of peginterferons in combination with ribavirin. Semin Liver Dis 23 Suppl 1: 35–46. 10.1055/s-2003-41633 [doi].
2. Fried MW (2011) The role of triple therapy in HCV genotype 1-experienced patients. Liver International 31: 38–61.
3. Thompson AJ, Locarnini SA, Beard MR (2011) Resistance to anti-HCV protease inhibitors. Current Opinion in Virology 1: 599–606.
4. McCown MF, Rajaguru S, Le FS, Ali SK, Jiang WR et al (2008) The hepatitis C virus replicon presents a higher barrier to resistance to nucleoside analogs than to nonnucleoside polymerase or protease inhibitors. Antimicrob Agents Chemother 52: 1604–1612. A01317-07 [pii];10.1128/AAC.01317-07 [doi].
5. Pavlovska J (2012) The science of direct-acting antiviral and host-targeted agents. Antiviral Therapy 17: 1109–1117.
6. Wright GW, Linley PA, Brun R, Winlin S, Hsu E (2010) Ancient Chinese methods are remarkably effective for the preparation of artemisinin-rich extracts of Qing Hao with potent antimalarial activity. Molecules 15: 804–812. 15020804 [pii];10.3390/molecules15020804 [doi].
7. Tscham S, Krensner PG, Moudharr B (2012) Emerging drugs for malaria. Expert Opinion on Emerging Drugs 17: 319–333.
8. Anthony MP, Burrow JW, Dupras S, JMcClelch; J, Wells TNC (2012) The global pipeline of new medicines for the control and elimination of malaria. Malaria Journal 11.
9. De Vries PJ, Den TK (1996) Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. Drugs 52: 818–836.
10. Efferth T, Romero MR, Wolf DG, Stamminger T, Marin JJ et al (2008) The anti-malaria drug artemisinin inhibits replication of cytomegalovirus in vitro and in vivo. Antiviral Res 69: 60–69. S0166-3542(05)00228-7 [pii];10.1016/j.antiviral.2005.10.003 [doi].
11. Shapira MY, Resnick IB, Chou S, Neumann AU, Lurain NN et al (2006) Artemisin as a potent antiviral agent in a patient with late drug-resistant cytomegalovirus infection after hematopoietic stem cell transplantation. Clin Infect Dis 46: 1453–1457. 10.1086/587106 [doi].
12. Arze-Roger R, He R, Chiuo CJ, Lai J, Woodward L, Rosenhalh A et al (2010) Artemisinin-derived dimers have greatly improved anti-cytomegalovirus activity compared to artemisinin monomers. PLoS One 5: e10510. 10.371/journal.pone.0010510 [doi].
13. Wofflath G, Efferth T (2009) Natural products as promising drug candidates for the treatment of hepatitis B and C. Acta Pharmacol Sin 30: 25–30. aps200805 [pii];10.1038/aps.2008.5 [doi].
14. Van Neck T, Van Mierlooo S, Dehaen W (2007) Functionalisation of artemisinin and its ring-contracted derivatives. Molecules 12: 393–403.
15. Delag L, Coenmoof L, Neyts J (2010) Antiviral therapy for hepatitis C virus: beyond the standard of care. Viruses 2: 826–866. 10.3390/8010426 [doi].
16. Villanueva RA, Thomas DL, Wakisaka T, Lemon SM (2006) Production of infectious genotype 1a hepatitis C virus (Hutchinson strain) in cultured human hepatoma cells. Proc Natl Acad Sci U S A 103: 2310–2315. 0510727103 [pii];10.1073/pnas.0510727103 [doi].
17. Ancelof S, Prinsenosev ska ES, Okulifeva EN, Dallmeier K, Durantel D et al (2011) Inhibition of Hepatitis C virus replication by semi-synthetic derivatives of glycopeptide antibiotics. Journal of Antimicrobial Chemotherapy 66: 1287–1294.
18. Plachard MN, Shipman C, Jr. (1996) Analysis of combinations of antiviral drugs and design of effective multiprop drug therapies. Antivir Ther 1: 9–20.
19. Paeshuyse J, Coelmont L, Vliegen I, Van hJ, Vandenkerckhove J et al (2006) Potential application of Pheroid (TM) technology. International Journal of Parasitology 32: 1655–1660.

Artemisinin Analogues as Potent Anti-HCV Drugs

December 2013 | Volume 8 | Issue 12 | e81783
28. Moles P, Oliwa M, Sanchez-Gonzalez A, Salani VS (2010) A topological study of the decomposition of 6,7,8-trioxabicyclo[3.2.2]nonane induced by Fe(II): modeling the artemisinin reaction with heme. J Phys Chem B 114: 1163–1173. 10.1021/jp102077z [doi].

29. Fugi MA, Wittlin S, Dong Y, Vennerstrom JL (2010) Probing the antimalarial mechanism of artemisinin and OZ277 (arterolane) with nonperoxidic isosteres and nitroxyl radicals. Antimicrob Agents Chemother 54: 1042–1046. AAC.01305-09 [pii];10.1128/AAC.01305-09 [doi].

30. Stockwin LH, Han BN, Yu SX, Hollingshead MG, Ellisby MA et al (2009) Artemisinin dimer anticancer activity correlates with heme-catalyzed reactive oxygen species generation and endoplasmic reticulum stress induction. International Journal of Cancer 125: 1266–1275.

31. Chen H, Sun B, Wang S, Pan S, Gao Y et al (2009) Growth inhibitory effects of dihydroartemisinin on pancreatic cancer cells: involvement of cell cycle arrest and inactivation of nuclear factor-kappaB. J Cancer Res Clin Oncol. 10.1007/s00432-009-0731-0 [doi].

32. Romero MR, Efferth T, Serrano MA, Castano B, Macias RI et al (2005) Effect of artemether/artsunate as inhibitors of hepatitis B virus production in an “in vitro” replicative system. Antiviral Res 68: 73–83. 80166-3542(05)00147-6 [pii];10.1016/j.antiviral.2005.07.005 [doi].

33. Birku Y, Mekonnen E, Bjorkman A, Wolday D (2002) Delayed clearance of Plasmodium falciparum in patients with human immunodeficiency virus co-infection treated with artemisinin. Ethiopian Medical Journal 40: 17–26.

34. Van Vugt M, Wilairatana P, Gempferl B, Guthmann I, Phaipun L et al (1999) Efficacy of six doses of artemether-lumefantrine (herlumefan) in multidrug-resistant Plasmodium falciparum malaria. American Journal of Tropical Medicine and Hygiene 60: 936–942.

35. Parry J (2005) Taking a new look at an ancient tradition. Scientist 19: 39–41.

36. Dondorp AM, Maude RJ, Hendriksen IC, Day NP, White NJ (2012) Artesunate Dosing in Severe Falciparum Malaria. Journal of Infectious Diseases 206: 618–619.

37. Nosten F, Ashley E, McGready R, Price R (2006) We still need artesunate monotherapy. British Medical Journal 333: 45.

38. Mavakala BK, Nlandu BB, Mphana PT, Gushimana ZY, Yu ZW (2003) Binding reaction of hemin with chloroquine, quinine and quinidine in water-propylene glycol mixture. Chinese Journal of Chemistry 21: 1022–1025.

39. Fillebeen C, Pantopoulos K (2010) Iron inhibits replication of infectious hepatitis C virus in permissive Huh 7.5.1 cells. Journal of Hepatology 53: 995–999.

40. Fillebeen C, Rivais-Estilla AM, Bisaillon M, Ponka P, Muckenthaler M et al (2005) Iron inactivates the RNA polymerase NS5B and suppresses subgenomic replication of hepatitis C Virus. J Biol Chem 280: 9049–9057. M412687200 [pii];10.1074/jbc.M412687200 [doi].

41. Fillebeen C, Muckenthaler M, Andriopoulos B, Bisaillon M, Mounir Z et al (2007) Expression of the subgenomic hepatitis C virus replicon alters iron homeostasis in Huh 7 cells. J Hepatol 47: 12–22. 80166-6278(07)00107-9 [pii];10.1016/j.jhep.2007.01.035 [doi].

42. Zheng YW, Yen TSB (1994) Negative Regulation of Hepatitis-B Virus Gene Expression and Replication by Oxidative Stress. Journal of Biological Chemistry 269: 8357–8362.

43. Gendron K, Ferbeyre G, Heveker N, Brakier-Gingras L (2011) The activity of the HIV-1 IRES is stimulated by oxidative stress and controlled by a negative regulatory element. Nucleic Acids Research 39: 902–912.