Silibinin mode of action(s) against HCV: A controversy yet to be resolved

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Dear Sir,

Ahmed-Belkacem et al.1 suggested that silymarin components (such as silibinin A and silibinin B or Legalon-SIL (SIL), a commercially available intravenous (IV) preparation of silibinin) mainly inhibit HCV NS5B RNA-dependent RNA polymerase (RdRp) activity. Here we note the HCV kinetics observed in patients treated with SIL2-4 and the HCV-RdRp inhibitor RG7128 (5 and manuscript in preparation) have similarities and suggest further studies to better understand SIL’s MOAs in vivo.

To our best knowledge three clinical studies have reported the viral response during SIL therapy: one clinical trial2 with 20 patients receiving ascending doses of SIL from 5mg/kg to 20mg/kg and two case reports with patients treated with 20mg/kg3, 4. In these three studies patients were infected with HCV genotype 1 and were non-responders to Peg-IFN+ribavirin; the patient in4 was HCV/HIV coinfected. The protocols were similar and consisted of daily injection of SIL for 7 days followed by Peg-IFN+ribavirin; however in3 ribavirin was administered before and during silibinin treatment. Viral decline after the initiation of SIL was monophasic until day 7 (which led to approximately 3 log decline in viral load from baseline) in the two case reports and in the majority of subjects in 2 (Fig. 1, red squares). Interestingly, a monophasic pattern of viral decline (Fig. 1, blue curves) was also observed in about half of patients (N=31) given 14 days of monotherapy with RG7128, a nucleoside HCV-RdRp inhibitor (manuscript in preparation), and in 3 subjects (N=5) in Le Pogam et al. (Fig. 1A in5). This monophasic decline is strikingly different from the biphasic viral decline typically observed in patients treated with protease inhibitors or (pegylated)interferon-α-based therapies (Fig 1, triangles; reviewed in 6). The fact that both SIL and RG7128 led to a monophasic HCV decline in some patients is interesting and tends to support, in part, Ahmed-Belkacem et al1 findings in vitro. Further studies are needed to reveal why only a portion of subjects treated with SIL or RG7128 monotherapy had a monophasic viral decline during the first week of treatment.

Very recently, however, it was suggested by Wagoner et al7, 8 that silymarin components (including SIL), have a profound effect on HCV entry and cell-to-cell spread in vitro with only marginally suppression of HCV-RdRp activity. While this controversy needs further attention, modeling viral kinetics in vivo may bring new insights into SIL’s mode of action(s). According to the standard HCV infection model9, a monophasic viral decline pattern suggests that viral infection is blocked. On the other hand, one can also predict a monophasic decline of virus if one assumes in the standard viral kinetic model a gradual reduction in blocking viral production (rather than an immediate high antiviral effectiveness in reducing viral production as it is the case with interferon-α or protease inhibitors) (manuscript in preparation). This gradual reduction in viral production could be related to drug pharmacokinetic and pharmacodynamic (PK/PD) properties and/or gradual destabilization of viral replication components that are still not known. Such PK/PD properties and/or...
intracellular processes leading to a progressive reduction in viral production could explain the similarity in the pattern of viral decline observed under treatment with SIL and RG7128 and possibly will shed light on why some patients treated with either of these two agents had a monophasic viral decline pattern.

In conclusion, Ahmed-Belkacem et al.¹ findings that SIL inhibits HCV RdRp activity in vitro might be in resonance with in vivo observations as similar viral decline patterns were observed in patients treated with SIL and with RG7128, an HCV-RdRp-nucleoside inhibitor (Fig. 1). Pharmacokinetic and pharmacodynamic studies of SIL are needed to better understand the nature of the observed monophasic viral decline. If SIL resistant strains can be identified, the nature of the resistance mutations would provide information about the MOA. If resistance mutations are found in the HCV polymerase it would favors an HCV-RdRp inhibitor mechanism, whereas if resistance mutations are in HCV E1/E2 it would support an entry inhibitor mechanism. Further in vitro experiments¹⁰ that include detailed kinetics of both intracellular and extracellular HCV RNA during treatment with silymarin components (such as SIL) are likely to provide more insights into their MOAs against HCV with the ultimate goal of developing better anti-HCV therapeutic regimens.

References:

**Figure 1:** Representative serum HCV RNA decline from baseline during the first week of treatment with silibinin monotherapy (red squares), RG7128 1500-mg BID (blue circles; manuscript in preparation), daily 10MIU IFN (black triangles) and telaprevir+PegIFN (gray triangles). Solid lines were used to emphasize plausible phases of viral decline.

**Conflicts of interest:** The authors disclose no conflicts.

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