Silibinin dihydrogensuccinate for the prevention of hepatitis C recurrence after liver transplantation and treatment of non-responders to anti-HCV therapy

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Introduction

- Silymarin is a flavonolignan extract from the seeds of milk thistle (carduus marianus), used for centuries as a remedy for liver disorders.
- The standardized silymarin extract is the active ingredient of a prescription drug (Legalon®-Rottapharm|Madaus), approved in Europe and elsewhere for toxic liver damage and as a supportive treatment for chronic inflammatory liver diseases and cirrhosis. Lower quality extracts are available as OTC/dietary supplements.
- Silymarin is a flavonolignan mixture, whose main and pharmacologically active component is silibinin.
Major components of silymarin (flavonolignans)

MW=482.4 g/mol

- **Silibinin A** (25-30%)
  - Synonyms: “Silybin” and “Silybinin”

- **Silidianin** (ca. 15%)

- **Silibinin B** (25-30%)

- **Isosilibinin A** (ca. 5%)

- **Isosilibinin B** (ca. 5%)

- **Silicristin** (ca. 20%)

- **Silidianin** (ca. 15%)
Silbinin dihydrogensuccinate

Silbinin-C-2ʻ;3 dihydrogensuccinate disodium salt is the hydrosoluble chemical derivative of pure silibinin (A and B), exclusively contained in Legalon®SIL (Rottapharm|Madaus), for intravenous infusions.

Legalon®SIL is approved in Europe and is an experimental drug in the USA for the acute treatment of Amanita phalloides mushroom intoxication.

The pioneering studies by P. Ferenci recently showed that i.v. silibinin is a potent direct antiviral agent against HCV.
Dose-response viral load decline in HCV-infected non-responders to SOC (PEG+riba), after silibin infusion alone (7 days) and with SOC (+7 days)

(Ferenci et al. Gastroenterology 2008;135:1561-7)
Inhibitory effect of different silymarin components on HCV replication in the subgenomic replicon model, in the JFH1 infectious model in Huh7 cells, and HCV RNA polymerase activity in a cell-free enzyme assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Genotype 1b Replicon EC50 (µM)</th>
<th>Genotype 2a JFH1 model EC50 (µM)</th>
<th>Genotype 1b RNA Polymerase IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silibinin A</td>
<td>0.4±0.3</td>
<td>23.4±7.5</td>
<td>96.4±15.9</td>
</tr>
<tr>
<td>Silibinin B</td>
<td>1.0±0.6</td>
<td>39.3±12.5</td>
<td>97.2±24.1</td>
</tr>
<tr>
<td>Silibinin A + B</td>
<td>0.5±0.2</td>
<td>25.4±6.7</td>
<td>82.0±22.3</td>
</tr>
<tr>
<td>Legalon® SIL</td>
<td>0.6±0.2</td>
<td>16.8±10.2</td>
<td>74.5±5.5</td>
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<tr>
<td>Isosilibinin A</td>
<td>1.5±0.5</td>
<td>45.8±5.5</td>
<td>211.6±34.9</td>
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<tr>
<td>Isosilibinin B</td>
<td>1.3±0.2</td>
<td>20.9±6.6</td>
<td>165.5±17.4</td>
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<tr>
<td>Silichristin</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Silidianin</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

Ahmed-Belkacem et al, Gastroenterology 2010;138:1112-22
Silibinin mechanism of action (MoA) against HCV

Silibinin antiviral effects span from low µM efficiency in the subgenomic replicon model, to 20-30 µM in cell cultures and up to 70-100 µM in a RNA polymerase cell-free assay, without major differences between A and B isomers, or their dihydrogensuccinate derivatives (Ahmed-Belkacem et al, Gastroenterology 2010).

Even the highest concentrations are achieved by intravenous infusion of 20 mg/kg Legalon®SIL (Ferenci et al, in preparation 2012), thus suggesting that direct NS5B polymerase inhibition contributes to the MoA in patients.

Modeling analysis of HCV-RNA decay kinetics in patients (Dahari et al, Hepatology 2011) and in vitro studies (Wagoner et al, PLoS One 2011) suggest that both direct antiviral (i.e. by polymerase inhibition) and virus entry/release mechanisms contribute to the anti-HCV effects.
HCV re-infection after liver transplantation

✓ Liver transplantation (LT) is the treatment of choice for HCV-infected patients with end-stage liver disease or hepatocellular carcinoma.

✓ Re-infection of the graft universally occurs within hours or days after LT and hepatitis C recurrence/cirrhosis is faster and more severe.

✓ Current standard of care (PEG+riba± protease inhibitors) has low applicability/efficacy pre-LT and, overall, after relapse post-LT. In addition, interferon cannot be administered immediately following LT and protease inhibitors have severe side effects/drug-drug interactions discouraging use for months after LT.

✓ Silibinin dihydrogen succinate is well tolerated and showed efficacy in published case reports in the peri-LT setting.

Orphan Drug designation was obtained from both EMA and FDA for the «Prevention of hepatitis C recurrence after liver transplantation»
Effect of Silibinin dihydrogensuccinate on prevention of HCV-RNA recurrence in LT recipients

Time course of the effect of SHS on HCV viremia [real-time PCR assay (Cobas AmpliPrep Taqman®, Roche Diagnostics, Germany) - upper panel] and on aminotransferase (ALT) and bilirubin levels [as relative values of the upper limit of normal (ULN) - lower panel]

Neumann UP et al: J Hepatol, 52: 951-2, 2010

Time course of the effect of SHS on HCV viremia [real-time PCR assay (Cobas Taqman®, Roche Diagnostics, Pleasanton, CA, US)]

Proof-of-concept study (LTX-02): Effects of i.v. silibinin dihydrogensuccinate monotherapy on viral load and safety in patients re-infected and non-responders to SOC after LT

✓ Study design
  • Single center, randomised (3:1 ratio, active: placebo), double-blind, placebo-controlled

✓ Study population
  • 20 patients re-infected with HCV after OLT (>1 year), non-responders to SOC (PEG+riba)

✓ Study Flow-chart

OLT (> 1 year) → 20 → Silibinin 20 mg/kg/d
0.9% saline [15] [5] → [15] [5]

Days 1 14 30 90 180 365

Long-Term Follow-up (ongoing) → Short-Term Follow-up
Proof-of-concept study (LTX-02):

Effects of i.v. silibinin dihydrogensuccinate (SHS) monotherapy (20 mg/kg for 14 consecutive days) on viral load in patients re-infected after LT and non-responders to SOC

HCV genotype
1b: SHS 10; Plac 2
1a: SHS 2; Plac 2
2: SHS 1; Plac 0
3a: SHS 1; Plac 0
3: SHS 1; Plac 0

Rendina M et al, AASLD 2012
Proof-of-concept study (LTX-03): Effects of i.v. silibinin dihydrogensuccinate monotherapy on viral load and safety in the immediate peri-LT period

**Study design**
- Single center, randomized (3:1 ratio, active: placebo), double-blind, placebo-controlled

**Study population**
- 14 HCV-infected cirrhotic or hepatocellular CA patients awaiting OLT per MELD criteria

**Study Flow-chart**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>-3</th>
<th>-2</th>
<th>-1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>24</th>
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<tr>
<td>HCV-RNA</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>

**Per-protocol analysis**
- A total of 7 patients on silibinin and 3 on placebo completed the study as planned
LTX-03: Selected patients viremia throughout the study

<table>
<thead>
<tr>
<th>Subject</th>
<th>Genotype</th>
<th>Treatment</th>
<th>Scr</th>
<th>Treat</th>
<th>Fup</th>
<th>Scr</th>
<th>Treat</th>
<th>Fup</th>
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<tbody>
<tr>
<td>S14</td>
<td>1b</td>
<td>Placebo</td>
<td>9</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>S10</td>
<td>1a</td>
<td>Placebo</td>
<td>9</td>
<td>7</td>
<td>5</td>
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<td>2</td>
</tr>
<tr>
<td>S13</td>
<td>1b</td>
<td>Legalon SIL</td>
<td>9</td>
<td>7</td>
<td>5</td>
<td>3</td>
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<td>Legalon SIL</td>
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<td>S11</td>
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<td>S12</td>
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<td>2</td>
</tr>
</tbody>
</table>

Proof-of-concept study (LTX-03): Effects of i.v. silibinin dihydrogensuccinate (SHS) monotherapy on viral load in the immediate peri-LT period

Silibinin dihydrosuccinate in the liver transplant setting: Safety

- The drug was well tolerated in the two proof-of-concept studies, with the known pattern of AEs:
  - Transient hot flushes and GI symptoms (diarrhea, abdominal pain, nausea) with the first infusions
  - Increase in bilirubin without signs of cholestasis or hepatocellular damage
- No changes in blood levels nor dosage adjustments necessary for concomitant immunosuppressants (tacrolimus, ciclosporin, micophenolate, everolimus, sirolimus, azathioprine), including corticosteroids.
Silibinin dihydrogensuccinate as a directly acting antiviral agent: Viral resistance issues

- Short-term i.v. silibinin dihydrogensuccinate does not select for RNA-dependent RNA polymerase (NS5B) substitutions (Chevaliez et al, EASL 2010)
- Preliminary data indicate that the drug may select for one mutation in NS4B conferring partial resistance (Essez-Nobis et al, EASL 2012), whose significance (clinical and MoA) should be further explored.
Silibinin dihydrogen succinate i.v. for the prevention of recurrent hepatitis C after liver transplantation: Ongoing plans

✓ A phase II/III clinical program in the peri-transplant setting was recently discussed with both EMA and FDA, with studies being now set-up.

✓ In the impossibility to plan a pre-transplant treatment period of a fixed duration (with an i.v. medication), studies are focusing in the immediate post-transplant period (anhepatic phase start), extended up to 4 weeks continuous administration.

✓ Treatment goal is viral suppression during treatment, sustained thereafter and/or with satisfactory histological endpoints.
Silibinin dihydrogensuccinate i.v. and other HCV-related indications

- HCV/HIV co-infected patients (studies planned with existing cohorts, ± HCV triple therapy)
- Rescue treatment of incomplete responders, including triple therapy (Rutter et al, 2011; Biermer et al 2012)

*Rutter K et al, Antivir Ther 2011; 16:1327-33*
Silibinin dihydrogensuccinate (SHS) and incomplete responders: 65% viral suppression and 25% SVR12 after 2 days SHS: (Biermer et al, J Viral Hepat 2012;19:547-53)

Studies ongoing with the aim to improve SVR with extended (short-term) treatment duration

Viral rebound after protease inhibitors and continued SOC

Incomplete response to SOC

Protracted decline but persistent viremia with SOC
Conclusions

- Intravenous silibinin dihydrogensuccinate (Legalon®SIL) is a directly acting antiviral agent with a new activity profile against HCV.
- Pilot studies indicate potential in the prevention of hepatitis C recurrence in liver transplant recipients, for which the drug obtained orphan designation by both EMA and FDA.
- The preliminary efficacy and safety profile (including apparent lack of clinical interaction with immunosuppressants) proposes it as a unique treatment opportunity in the peri-transplant period, currently being studied in a phase II/III clinical trial program.
- Among other HCV-related indications, studies are running in HCV/HIV co-infected patients and as rescue treatment in incomplete responders to standard or triple therapy.