A new silybin-vitamin E-phospholipid complex improves insulin resistance and liver damage in patients with non-alcoholic fatty liver disease: preliminary observations

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medium chain triglyceride oil, corn oil, soy lecithin, vitamins, minerals, fructooligosaccharides, lactose, gluten, and growth factor free) ingested by the mice was similar throughout the week (15 ml/day). Body weight did not differ between the Perative fed and chow fed groups. Northern blot analysis of total RNA extracted from the jejunum and ileum, using an oligo probe specific to cryptdin 1, cryptdin 2, and cryptdin 3 (>93% nucleotide identity), or cryptdin 4, revealed that levels of cryptdins 1–3 (fig 1A) and cryptdin 4 (fig 1B) increased by 1.6-fold (Student’s t test, p<0.05).

To further study the direct effect of Perative on Paneth cells, we isolated primary Paneth cells’ from mouse jejunum and tested the effect of different concentrations of Perative (0.0025%, 0.01%, 0.025%), isoleucine, and arginine (25, 100, 250 μg/ml) on cryptdin expression by real time polymerase chain reaction.5 Whereas different concentrations of arginine and isoleucine did not stimulate cryptdin expression (Student’s t test, p>0.05), 0.025% Perative increased significantly expression of cryptdins 1–3 (Student’s t test, p=0.003 (fig 1C) and cryptdin 4 (Student’s t test, p<0.0001) (fig 1C). The ability of similar concentrations of isoleucine and arginine to induce human β-defensin in HCT-116 cells’ stems, most probably, from the difference in the mechanism regulating expression of cryptdins (α-defensins) versus β-defensins. Different control of α- and β-defensin expression is further reiterated by the fact that spleens challenged with Listeria monocytogenes, lungs infected with influenza A, and kidneys challenged with Candida albicans, all β-defensin expressing tissues, revealed no differences in the cure rate when mice were fed Perative or other immune enhancing formulas.6

To further determine whether expression of cryptdin mRNA was concomitant with protein secretion in primary Paneth cells, we analysed the medium of primary Paneth cells stimulated with the aforementioned concentrations of isoleucine, arginine, and Perative. Cryptdins were isolated from the medium using a method to denature, renature, and concentrate small cationic proteins7 followed by reverse phase high performance liquid chromatography. Protein data of the primary Paneth cell media correlated with that of RNA (that is, higher concentrations of secreted cryptdins were found in the 0.025% Perative treatment—data not shown). Furthermore, eluting fractions exhibited defensin characteristics, such as a size of 3 kDa on sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and activity against Escherichia coli DH5a (data not shown).

In summary, our results demonstrate that immune enhancing formulas, such as Perative, can upregulate cryptdin expression in Paneth cells. Further study is needed to delineate the molecular pathways by which nutrients lead to cryptdin upregulation. As defensins display antimicrobial activity as well as recruit the adaptive immunity by cytokine secretion and recruitment of lymphocytes, their secretion could be the first step in a cascade that leads to a general bolstering of the immune system.

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**References**


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Non-alcoholic fatty liver disease (NAFLD) may occur as an expression of a metabolic syndrome or in association with hepatitis C virus (HCV) chronic infection. The contemporaneous presence of NAFLD in this later group of patients may negatively affect the progression of fibrosis and the response to antiviral treatment.8 It has been suggested

Table 1 Results in the two groups; group A had primitive non-alcoholic fatty liver disease and group B had hepatitis C virus (HCV) related chronic hepatitis C in combination with NAFLD, all HCV genotype 1b, and were non-responders to previous antiviral treatment

<table>
<thead>
<tr>
<th></th>
<th>Treated</th>
<th></th>
<th>Not treated</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6 months</td>
<td>12 months</td>
<td></td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (ng/ml)</td>
<td>79 (51)</td>
<td>40 (15)*</td>
<td>59 (5)*</td>
<td>54.2 (20)</td>
</tr>
<tr>
<td>&lt;ALT (ng/ml)</td>
<td>75 (112)</td>
<td>59 (100)*</td>
<td>60 (33)*</td>
<td>48.3 (17)</td>
</tr>
<tr>
<td>Insulinemia (μIU/ml)</td>
<td>41.5 (34)</td>
<td>29.6 (26.4)*</td>
<td>30.6 (15.4)*</td>
<td>13 (6)</td>
</tr>
<tr>
<td>HOMA</td>
<td>12.3 (6.4)</td>
<td>6.2 (3.9)*</td>
<td>6.4 (3.2)*</td>
<td>42.2 (504)</td>
</tr>
<tr>
<td>Hyaluronic acid (mg/l)</td>
<td>383 (627)</td>
<td>176 (184)*</td>
<td>331.1 (293.2)*</td>
<td>160 (183)</td>
</tr>
<tr>
<td>MMP-2 (ng/ml)</td>
<td>51 (132)</td>
<td>141 (84)*</td>
<td>158.2 (165.5)*</td>
<td>41.2 (22)</td>
</tr>
<tr>
<td>TGF-β (ng/ml)</td>
<td>45.3 (17.3)</td>
<td>32.9 (24.3)*</td>
<td>42.9 (22)</td>
<td>2 (2–3)</td>
</tr>
<tr>
<td>US score (random range)</td>
<td>2 (2–3)</td>
<td>1 (1–2)*</td>
<td>1 (1–2)</td>
<td>2 (2–3)</td>
</tr>
</tbody>
</table>

**Group B**

|                      |         |          |          |         |          |          |         |
| ALT (ng/ml)          | 69 (28) | 45 (18)* | 62 (16) | 47.8 (30) | 50.3 (32) | 60.3 (33) |         |
| <ALT (ng/ml)         | 118 (70) | 56 (20)* | 83 (24) | 115 (60) | 100 (53) | 150 (13) |         |
| Insulinemia (μIU/ml) | 36.2 (1.5) | 27.4 (1.5)* | 28.9 (6.5)* | 35.4 (3) | 34.2 (5.7) | 38.2 (4.7) |         |
| HOMA                 | 8.4 (7.3) | 5 (4.8)* | 6.2 (5.1)* | 8.8 (5.6) | 8.4 (5.1) | 8.2 (4.8) |         |
| Hyaluronic acid (mg/l) | 1295 (220) | 625 (122)* | 582.7 (496.2)* | 1180 (708) | 1372 (956) | 1362 (428) |         |
| MMP-2 (ng/ml)        | 292 (201) | 137 (33)* | 196.6 (94.2)* | 280 (300) | 311 (278) | 299 (123) |         |
| TGF-β (ng/ml)        | 54.1 (21.7) | 27 (12.2)* | 21.2 (17.4)* | 53.3 (18.3) | 45.2 (28.5) | 49.6 (30) |         |
| US score (random range) | 2 (2–3) | 2 (2–3)| 2 (2–3) | 2 (2–3)| 2 (2–3)| 2 (2–3)|         |

*p<0.05, **p<0.01 versus basal values.

Values are reported as the mean (SD).

ALT, alanine aminotransferase; <ALT, gamma-glutamyl-transpeptidase; MMP-2, metalloproteinase 2; TGF-β, transforming growth factor β; US, ultrasonography; nv, normal value.
that in the future a therapeutic approach to chronic liver disease would consist of a number of complementary approaches considering the multitude of pathogenic mechanisms. Silybin is a natural flavonoid that has been conjugated to vitamin E and phospholipids to improve its bioavailability, and antioxidant and antifibrotic activity.

After approval of the ethics committee and individual consent, 85 outpatients were consecutively enrolled in the study: 59 were affected by primitive NAFLD (group A) and 26 by HCV related chronic hepatitis C in combination with NAFLD, all HCV genotype 1b. All patients were referred to previous antiviral treatment (group B). All patients with a diagnosis of liver disease in the two years prior to the study, according to histological criteria, were enrolled over six consecutive months and further divided into two treated groups using a systematic random sampling procedure: 53 (39 NAFLD and 14 HCV) were treated with 4 pieces/day of the complex silybin-vitamin E-phospholipids (Realasil RA; IBI-Lorenzini Pharmaceutical, Italy) for 26 weeks (12 weeks of treatment and 12 weeks of follow-up). One piece contained 94 mg of silybin, 194 mg of phosphatidylcholine, and 90 mg of vitamin E. At 0, 6, and 12 months, we evaluated: body mass index (BMI), liver stiffness by ultrasonography in assessing diffuse parenchymal liver disease, and by the Pearson bivariate correlation test, comparison of liver histology with liver fibrosis in both treated groups, with a percentage of overweight patients decrease (absence of a placebo treatment and no significant percentage of overweight patients decrease in the group B). We report the case of a 49 year old male who presented signs of acquired factor VIII inhibitor associated with chronic hepatitis C virus infection. We report that our case report provides further evidence in favour of the association between chronic hepatitis C virus infection and the development of acquired factor VIII inhibitors, it raises several important questions too. Firstly, what is the real incidence of this phenomenon in patients treated for HCV infection? Are these inhibitors so rare as initially believed or are they misdiagnosed? Are they clinically relevant, what is the natural history of the coagulative alteration (that is, do they require treatment or do they tend to disappear spontaneously), and which is the best treatment? In our opinion, a response to these concerns may be obtained only through a careful coagulation study (especially aPTT ratio) of these patients during and after IFN treatment.

**References**


**Conflict of interest:** None declared.

**Is the periodic repetition of a coagulation check necessary during anti-hepatitis C virus therapy?**

Peginterferon α and ribavirin have become the mainstay of treatment for chronic hepatitis C virus (HCV) infection. However, chronic use of interferon (IFN)-α has been associated with the development of autoimmune disorders, such as systemic lupus erythematosus, autoimmune haemolytic anaemia, and autoimmune thyroiditis. In particular, some reports have documented the development of acquired factor VIII inhibitors in patients receiving IFN-α, including those treated for chronic HCV infection.1,2 We report the case of a 49 year old male patient treated for chronic active HCV related cirrhosis. He received a six month course of peginterferon 224 at a dose of 180 µg weekly and ribavirin at a dose of 800 mg daily for three months only, due to a marked decrease in haemoglobin level, obtaining a complete viral response. Before therapy, activated partial thromboplastin time (aPTT) and prothrombin time-international normalised ratio (PT-INR) values were in the normal range (aPTT ratio 1.28 and INR 1.11). Due to abnormal bleeding during a routine dental treatment performed at the end of antiviral treatment in September 2005, the patient presented a coagulation screening which revealed a prolonged aPTT (2.14; normal range 0.85–1.17). Coagulant factor VIII level was reduced (FVIII:C 17%; normal range 50–150%) and a mixing study demonstrated the presence of a two titre inhibitor (1.6 Bethesda units/ml). Response to subcutaneous injection of desmopressin at a dose of 0.3 mg/kg was satisfactory as FVIII:C increased to 70% and aPTT normalised four hours after injection. Recent tests performed three months after the end of antiviral treatment (November 2005) were unchanged. If our case report provides further evidence in favour of the association between chronic hepatitis C virus infection and the development of acquired factor VIII inhibitors, it raises several important questions too. Firstly, what is the real incidence of this phenomenon in patients treated for HCV infection? Are these inhibitors so rare as initially believed or are they misdiagnosed? Are they clinically relevant, what is the natural history of the coagulative alteration (that is, do they require treatment or do they tend to disappear spontaneously), and which is the best treatment? In our opinion, a response to these concerns may be obtained only through a careful coagulation study (especially aPTT ratio) of these patients during and after IFN treatment.

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