THE USE OF NUTRACEUTICALS IN CHRONIC LIVER DISEASE: MYTHS, FACTS AND DANGERS

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Impact of HCV Infection in the US

Approximately 4.0 million persons are chronically infected with HCV

20% will develop cirrhosis (+/- 780,000 patients)

4% will develop liver cancer (+/- 31,000 patients)

Di Bisceglie, Hepatology, 2000
Chronic hepatitis C is a major health care problem

Projected prevalence of cirrhosis and its complications in the US over the next 20 years

<table>
<thead>
<tr>
<th>Cirrhosis / Complication</th>
<th>Year</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2000</td>
<td>2020</td>
</tr>
<tr>
<td>HCV infection</td>
<td>2,940,678</td>
<td>2,681,556</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>472,103</td>
<td>858,788</td>
</tr>
<tr>
<td>Decompensated cirrhosis</td>
<td>65,294</td>
<td>134,743</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>7,271</td>
<td>13,390</td>
</tr>
<tr>
<td>Liver-related death</td>
<td>13,000</td>
<td>36,483</td>
</tr>
<tr>
<td>Patients listed for transplant</td>
<td>10,893</td>
<td>~30,000</td>
</tr>
<tr>
<td>Transplants performed</td>
<td>4893</td>
<td>unknown</td>
</tr>
<tr>
<td>Transplants performed for HCV</td>
<td>1920</td>
<td>unknown</td>
</tr>
</tbody>
</table>

Organ Procurement and Transplantation Network (OPTN) Database
Davis GL et al., Liver Transplant 2003
The prognosis of HCV-induced cirrhosis is poor

<table>
<thead>
<tr>
<th>Annual incidence of complications (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical decompensation</strong></td>
<td>3.6 – 6.0</td>
</tr>
<tr>
<td>• Hepatocellular carcinoma</td>
<td>1.4 – 2.6</td>
</tr>
<tr>
<td>• Ascites</td>
<td>2.2</td>
</tr>
<tr>
<td>• Variceal bleeding</td>
<td>0.5</td>
</tr>
<tr>
<td>• Hepatic encephalopathy</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5-year survival</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Compensated cirrhosis</td>
<td>91%</td>
</tr>
<tr>
<td>After 1&lt;sup&gt;st&lt;/sup&gt; major complication</td>
<td>50%</td>
</tr>
</tbody>
</table>

Fattovich G et al., Gastroenterology 1997
Benvegnú L et al., Gut 2004
Why should we treat HCV patients?

Short term endpoints
- Eradicate HCV
- Reduce/Stop necroinflammation
- Reduce/stop fibrosis progression

Ultimate aims
- Prevent/delay cirrhosis
- Prevent/delay liver decompensation
- Reduce the risk of HCC
Limits to successful antiviral therapy

- High viral replication rate
- High mutation frequency
- Selection of resistance

- Low potency
- Short term efficacy
- Poor pharmaco-kinetic & -dynamic
- Poor tolerability
- Difficult to take
- Selection of resistance

- Adherence <100%
- Intolerance
Treatment of Chronic Hepatitis C

Evaluating Factors Associated With Poor Response to HCV Therapy

<table>
<thead>
<tr>
<th>Fixed Factors</th>
<th>Correctable Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV genotype</td>
<td>Pretreatment</td>
</tr>
<tr>
<td>Race</td>
<td>– Prescription of optimal course of therapy</td>
</tr>
<tr>
<td>Patient age</td>
<td>– Substance abuse</td>
</tr>
<tr>
<td>Serum HCV RNA level</td>
<td>– Fatty liver disease</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>– Obesity/metabolic syndrome</td>
</tr>
<tr>
<td>Morbid obesity</td>
<td>– Psychiatric comorbidities</td>
</tr>
<tr>
<td></td>
<td>– Other comorbidities</td>
</tr>
<tr>
<td></td>
<td><strong>On treatment</strong></td>
</tr>
<tr>
<td></td>
<td>– Noncompliance with treatment</td>
</tr>
<tr>
<td></td>
<td>– Management of adverse effects</td>
</tr>
</tbody>
</table>

Hepatitis C

Metabolic factors (IR)

Hepatic steatosis

Hepatic fibrosis

Complications

Liver injury

Immune

Hepatic steatosis, IR and chronic hepatitis C infection

Yes

Yes

Yes: Gen 3

Hepatic steatosis, IR and chronic hepatitis C infection

Liver injury

Immune

Hepatic steatosis

AICAH 15-20% - HBV 27-51% - HCV 50-70%

Hepatic fibrosis

Complications
Evidence: Metabolic Syndrome, Steatosis, and HCV

Insulin Resistance in HCV\textsuperscript{[1]}

Mean HOMA-IR Score

\begin{center}
\begin{tabular}{ll}
<table>
<thead>
<tr>
<th></th>
<th>HCV-Infected Patients (n = 121)</th>
<th>Healthy Controls (n = 137)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR Score</td>
<td>2.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>
\end{tabular}
\end{center}

\textit{P} = .002

Steatosis in HCV Patients\textsuperscript{[2]}

Patients With Steatosis (%)

\begin{center}
\begin{tabular}{ll}
<table>
<thead>
<tr>
<th></th>
<th>Nonobese (n = 91)</th>
<th>Obese* (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients With Steatosis (%)</td>
<td>16</td>
<td>40</td>
</tr>
</tbody>
</table>
\end{tabular}
\end{center}

\textit{P} = .02

*BMI \geq 30.

### Fibrosis

<table>
<thead>
<tr>
<th>Fibrosis Score</th>
<th>Description of Fibrosis</th>
<th>Patients Progressing to Cirrhosis by Year 10, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1.9 (n = 27)</td>
<td>None; too mild to alter portal tract size</td>
<td>29.6</td>
</tr>
<tr>
<td>2.0-2.9 (n = 28)</td>
<td>Portal/periportal \pm portal-portal bridging</td>
<td>42.9</td>
</tr>
<tr>
<td>3.0-3.45 (n = 15)</td>
<td>Septal + regions of partial nodular regeneration</td>
<td>100</td>
</tr>
</tbody>
</table>

### Inflammation

<table>
<thead>
<tr>
<th>Change in Fibrosis Score According to Necrosis Score at Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piecemeal Necrosis Score at Baseline</td>
</tr>
<tr>
<td>0-1</td>
</tr>
<tr>
<td>Patients, n</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>Mean change in fibrosis score per yr</td>
</tr>
<tr>
<td>0.05</td>
</tr>
</tbody>
</table>
Evidence: Steatosis and Fibrosis Progression in HCV-Infected Patients

- Younossi, et al (N = 122)
  - Predictors of advanced fibrosis: higher BMI, superimposed NASH

- Fartoux, et al (N = 135 paired liver biopsies, untreated patients)
  - After 6 yrs follow-up, steatosis was only independent predictor of progressive fibrosis

- Hui et al (N = 117)
  - Fibrosis progression predicted by HOMA-IR, serum cholesterol

- Conjeevaram et al (N = 399 GT 1 patients)
  - Bridging fibrosis or cirrhosis in patients with vs without steatosis (45% vs 23%, respectively; P < .0001)

Factors Associated With Advanced Fibrosis


- Retrospective study of 460 pts with chronic hepatitis C (41% F3-4)
- Multivariate analysis of factors associated with F3-4

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Adjusted Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at entry (≥ 60 years)</td>
<td>3.444</td>
<td>.0334</td>
</tr>
<tr>
<td>Duration of infection (≥ 25 years)</td>
<td>1.750</td>
<td>.0378</td>
</tr>
<tr>
<td>BMI (≥ 30)</td>
<td>1.917</td>
<td>.0173</td>
</tr>
<tr>
<td>History of diabetes</td>
<td>2.251</td>
<td>.0304</td>
</tr>
<tr>
<td>AST (≥ 80 U/L)</td>
<td>4.032</td>
<td>.0087</td>
</tr>
<tr>
<td>AFP (≥ 15 μg/L)</td>
<td>3.875</td>
<td>.0383</td>
</tr>
<tr>
<td>Grades 2 and 3 steatosis</td>
<td>2.790</td>
<td>.0378</td>
</tr>
</tbody>
</table>
**Independent predictors of fibrosis stage**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol: past</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Portal inflammation</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT</td>
<td>0.04</td>
</tr>
<tr>
<td>Platelets (negative association)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (negative association)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Steatosis, fibrosis and necroinflammation in chronic hepatitis C: a Meta-Analysis of Individual patient Data (The HCV MAID Study)

Leandro G et al
Future Therapy of Hepatitis C

Treatment Strategies to Enhance Response to Current Therapies

New strategies: molecular based therapy

Therapeutic Strategies

- To Reduce Liver Injury
- To Reduce Progression of Fibrosis
- To Decrease Hepatocytes Proliferation
Treatment of hepatitis C

*Unsolved issues*

- Clinical heterogeneity of hepatitis C
- Over-treatment
- Tailoring of dose/duration
- Role of PEG-IFNs monotherapy
- **Non-responders to IFN and to IFN-ribavirin**
- Drug toxicity
- Co-morbidities
- Special patient populations
- The financial issue
<table>
<thead>
<tr>
<th>Absolute</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>Cirrhosis, compensated</td>
</tr>
<tr>
<td>Decompensated cirrhosis</td>
<td>Controlled psychiatric disease</td>
</tr>
<tr>
<td>End stage kidney disease</td>
<td>Mild anemia/leukopenia</td>
</tr>
<tr>
<td>Severe or uncontrolled psychiatric disease</td>
<td>Renal insufficiency</td>
</tr>
<tr>
<td>Cardiopulmonary disease</td>
<td>Mild autoimmune disease</td>
</tr>
<tr>
<td>Severe Autoimmune disease</td>
<td></td>
</tr>
<tr>
<td>Severe anemia</td>
<td></td>
</tr>
<tr>
<td>Noncompliance</td>
<td></td>
</tr>
</tbody>
</table>
Epidemiology of CAM

- Prevalence of the use of complementary and alternative medicine (CAM) in US adults
  - 1990  2.5%
  - 1997  12.1%
  - 2002  18.9%
  - 2012  > %

- 2009 Estimated sales >$4 billion in the US

- Worldwide, underdeveloped countries

- Europe
  - Regulate herbs as prescription or nonprescription medicines available only through a pharmacist
  - German physicians receive medical school training in medicinal herbs (and must pass a test to become licensed)
Percent of Patients Using CAM

Liver Clinics

Seeff et al. Hepatol. 2001

Patients Using CAM

CAM
Herbal
Milk Thistle

0 5 10 15 20 25 30 35 40 45 50 %
Appeal of CAM
Among Patients With HCV Infection

- A *chronic* illness with limited treatment success
- Frustration with *uncertainty* of prognosis
  - Limited information available from providers
  - Absence of signs and symptoms
- Lack of symptoms vs *side effects* of conventional treatment
- Desire for a “*holistic*” approach to therapy
Evidence to support important interactions between NAFLD and Metabolic Syndrome

- Evidence #1: NAFLD and Metabolic Syndrome co-exist
- Evidence #2: Metabolic Syndrome affects progression of NAFLD
- Evidence #3: Treating Metabolic Syndrome influences the outcome of NAFLD
From the spectrum of NAFLD, only those patients with NASH have convincingly been shown to progress.

- Teli 1995
- Matteoni 1999

NAFLD Spectrum

Steatosis alone

NASH

Cirrhosis

50% of diabetics: 2012
15% progression/1 year: 2012

Ludwig 1980
Diehl 1988
Powell 1990
Bacon 1994
Caldwell 1999
Angulo 1999
Matteoni 1999
Younossi 2000
Ong 2001
Ratziu V 2002
Harrisson 2003
HCV: a metabolic disease? Common pathways with NASH. Koike et al. JSH (Japan Society Hepatology) meeting, October, 2004

There is a growing body of evidences suggesting the role of free radical generation and oxidant injury in the pathogenesis of liver fibrosis, NASH and NAFLD.
Oxidative stress and hepatitis C viral infection

Kazuhiko Koike*, Hideyuki Miyoshi

Hepatology Research 34 (2006)
Modulating leukocyte DNA damage and cytokines by nutraceuticals in HCV-CLD: a fermented papaya preparation vs vitamin E

% reduction over baseline

Marotta et al. J Gastroenterol Hepatol 2006
abundant evidence suggests that antioxidants can effectively attenuate the oxidative and nitrosative stress in liver injury, ultimately improving inflammation and fibrosis progression.

It is worth testing these drugs in future clinical trials including CHC patients, mainly those who present negative predictive factors of sustained virological response to standard antiviral regiments.

But not any antioxidant naively!

Nakamura M et al.. An antioxidant resveratrol significantly enhanced replication of hepatitis C virus. World J Gastroenterol 2010
Regression of fibrosis and cirrhosis

IFN alpha is a potent inhibitor of experimental fibrosis
Inaki Y et al., Hepatology 2003

IFN reverses fibrosis in clinical studies
Poynard T et al., Gastroenterology 2002

Whether structural changes of cirrhosis are reversible is still unclear
Desmet VJ et al., J Hepatol 2004
Interferon alpha decreases development of HCC in patients with hepatitis C

Meta-analysis, 11 studies, 2178 patients

HCC: 21% → 8%
Odds ratio 3.0

HCC: 22% → 9%
Odds ratio 2.7

HCC: 9% → 1%
Odds ratio 3.7

Papatheodoridis GV et al., Aliment Pharmacol Ther 2001
Therapies for hepatic fibrosis: real hope or just academic exercise?
(Pinzani 2004)

- **Immunomodulatory & Antinflammatory**
  - Corticosteroids, IL-1 receptor antagonist, TNF antagonist, IL-10

- **Reduce activated HSC profibrogenic actions**
  - Proliferation: small molecule tyrosine kinase inhibitors, fumigillin, glitazones
  - Contractility: endot helin antagonists, NO
  - Fibrogenesis: TGF antagonists, halofigunone
  - Inflammatory effects: inhibitors of chemokine binding/action, NF-kB inhibition

- **Antiviral**
  - Ribavirine, Lamivudine and derivatives; Amantadine, Rimantadine; Thimosine 1; Viral enzyme inhibitors (NS3 protease; NS3 helicase; NS5B polymerase); HCV monoclonal antibodies; E1 therapeutic vaccine

- **Promote Collagen Degradation**
  - MMPs, uPA, TIMP-1 antisense

- **Antioxidant Membrane Protective**
  - Vit E, SAMe, phosphatidylcholine, rosvaterol, sylmarin

- **Promote HSC apoptosis**
  - Gliotoxin, RGD peptides, sulphasalazine, death receptor ligands
Nutritional Genomics And Biomarker Discovery

Nutrients to Modify Disease Risk

- Vitamins A
- Vitamin D
- Vitamin E
- Vitamin C
- Folic Acid
- Calcium
- Selenium
- Lycopene
- Resveratrol
- Tea Polyphenols
- Curcumin
- Genistein
- Sulforaphane
- Macronutrients
  - Carbohydrates
  - Fat
  - Protein
  - Fiber & Water

Regulation by Diet

Gene Expression Process

- DNA
  - Transcription, RNA Processing and Stability

- RNA
  - Translation, Modification, and Stability

- Protein

Health

Functional Genomic Techniques

- Genomics (Gene or Promoter Sequences and Polymorphisms)

- Transcriptomics (DNA Arrays)

- Proteomics

- Metabolomics, Metabonomics, Bioassays

BMJ 2002; 324:1438.
FibroTest: Estimates Anti-Fibrotic Impact

FibroTest

Baseline | EOF
---|---
F234 NR n=110: 0.71 | 0.64
  -10%
F234 SVR n=65: 0.68 | 0.47
  -31%

Poynard et al Hepatology, 2003
Reversal of cirrhosis in 75 (49%) of patients

Peginterferon Alfa-2a and Ribavirin in Patients With Chronic Hepatitis C Who Have Failed Prior Treatment

HALT-C trial

- Multicenter, 604 patients
- 233 cirrhosis, 371 bridging fibrosis
- Peginterferon alfa-2a + ribavirin 20 + 28 weeks
- **Cirrhosis is a negative predictor of therapy response**

Sustained viral response

<table>
<thead>
<tr>
<th>Cirrhosis</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>11%</td>
<td>23%</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p<0.001

Shiffman ML et al., Gastroenterology 2004
<table>
<thead>
<tr>
<th>Compound</th>
<th>Putative Biological Mechanism*</th>
<th>Targeted Liver Disease†</th>
</tr>
</thead>
</table>
| Silymarin (milk thistle)17,18,24,27,28 | Biologically active compound—silibinin  
Acts as an antioxidant and free radical scavenger  
In animals, prevents glutathione depletion free radical formation in the liver  
May also be antifibrotic through undeterminate mechanism(s)                                                                 | Cirrhosis  
In Europe—chronic liver disease, digestive disorders, and gallbladder disease                           |
| Glycyrrhizin29,30,31,35       | Licorice root—multiple constituents appears to inhibit enzyme 11-beta-hydroxysteroid dehydrogenase, thus anti-inflammatory in inhibiting prostaglandin production and modifies arachidonic acid metabolism  
Also antioxidant properties—induces glutathione-S-transferase and catalase | Used traditionally for cough, bronchitis, gastritis, liver inflammation  
Fibrosis                                                                                         |
| Plantago asiatica seed39,40  | Aucubin—active ingredient, iridoid glycoside  
Transient inhibition of viral replication                                                                                                                                                                                      | Hepatitis B virus                                                                                   |
| Herbal Medicine 86140,42     | Herbal mixture, blocks stellate cell activation through inhibiting cell cycle progression                                                                                                                                               | Fibrotic liver disease                                                                             |
| TJ-9 (Sho-saiko-to)44-48     | Herbal mixture, blocks stellate cell activation  
Inhibits lipid peroxidation in hepatocytes and stellate cells                                                                                                                                                                      | Fibrotic liver disease                                                                             |
| TJ-4150,51                   | Herbal mixture, induces cellular apoptosis via P 53.                                                                                                                                                                                  | Hepatocellular carcinoma                                                                          |
| TJ-10851                     | Herbal mixture with active compound gomisin A. Antiviral                                                                                                                                                                             | Hepatitis C virus                                                                                 |
| Liv-5252                     | Herbal mixture—hepatoprotective                                                                                                                                                                                                       | In India, alcohol-induced liver disease                                                            |
| Phyllanthus amarus54,55      | Extract inhibits hepatitis B viral polymerase by inhibiting the virus enhancer I activity—complexes transcription factors                                                                                                              | Hepatitis B virus                                                                                 |
some Clinical studies

Plantago
1997 10mg/kg/day i.v. x 4-month: 10-40% ↓ HBV-DNA;

Compound 861
1995 2-years, CHB: 83% subj. improv., ↓ 41% spleen size, ↓ AST,ALT (73% to normal range), PIIINP;
1998 6-month, CHB: histological improvement (infl. & fibrosis);

CH-100 1998 RCT - HCV pts: significant ALT reduction;

TJ-9 1995 5-year study, 260 cirrhotics, ↑ survival, ↓ HCC;
TJ-108 2000 ↓ HCV-RNA in 21% HCV +ve patients;

YHK/K-17.22 1998-2004 HCV pts.: ↓ ALT;
Compound 861 in HBV CLD
Salviae miltiorrhizae (丹参): Reversal rate

<table>
<thead>
<tr>
<th></th>
<th>861 Reverse(%)</th>
<th>Placebo Reverse(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>s3 + s4</td>
<td>66*</td>
<td>33*</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>20</td>
</tr>
</tbody>
</table>

* p<0.05

Liu EY et al. Chin J Hepatol 1993
Shanti Wasser et al. J Hepatol, 1998
Chinese Herbal Medicine and Interferon in the Treatment of Chronic Hepatitis B: A Meta-Analysis of Randomized, Controlled Trials

Am J Publ Health, 2002
Traditional Chinese medicine causing hepatotoxicity in patients with chronic hepatitis B infection: a 1-year prospective study

Traditional Chinese medicine-related hepatotoxicity resulted in high mortality in chronic hepatitis B patients.

Prospective RC trials with the same stringent criteria as western medicine clinical trials are required for Chinese medicines, to document their efficacies and safety before they can be advocated for the treatment of patients.

Funded by a grant from the Hepatology Research Fund, The University of Hong Kong
Glycyrrhizin

1991 4-wks Gly + 4 wks IFN: 70% loss of HbeAg after 6 months;
1994 Gly + IFN vs IFN: 33% vs 13% HCV-RNA negativization;
1997 80mg x 2 weeks → AST, ALT in >60% of CAH pts;
1997 2-7/weekly i.v. Gly x 10 years: 2.5-fold decrease of HCC and 1.5-fold decrease of cirrhosis;
Mechanism of Pharmacological Action of Glycyrrhizin (GL)

Glycyrrhizin binds to selectin

Selectin

Glycyrrhizin

Inhibition of Infiltration and Chemotaxis of Leukocytes

Anti-inflammation

SLe^x (sugar chain)

Inhibition of Cell-Cell Adhesion

Glycyrrhizin binds to selectin

signal transduction

Rao BN. et al., JBC 269, 19663-19666, 1994
Chronic Hepatitis C Trial
Indian Council Medical Research

Multicenter Double-Blind Randomized controlled

130pts, HCV+ve, ALT >60IU, HAI >3

IFN + Ribavirin vs IFN + SNMC

Genotype 3 – 1 – 4: 71%, 27%, 2%

median ALT: 100.5 IU vs 38.0 IU p<0.0001

Sustained Virological Response: Genotype 1:100%, Gen. 3: 70%, Gen 4: 100%

Viral Load: Genotype 1: all -ve, Gen 3: Gen 4: -ve

HCV-RNA +ve: 25% - HCV-RNA -ve: 75%
Therapeutic Effect of SNMC to IFN Non-responders in Patients with Chronic Hepatitis C
Van Rossum TGJ. et al. Am. J. Gastroenterol. 2001

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>SNMC 3 times/W (40, 80, 120 mL)</th>
<th>SNMC 6 times/W (100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>13</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td>Male/Female</td>
<td>13/0</td>
<td>32/9</td>
<td>12/3</td>
</tr>
<tr>
<td>White/Other</td>
<td>8/5</td>
<td>23/18</td>
<td>11/4</td>
</tr>
<tr>
<td>Median age* (yr) (range)</td>
<td>47 (37-60)</td>
<td>46 (32-60)</td>
<td>49 (30-70)</td>
</tr>
<tr>
<td>Noncirrhosis/Cirrhosis</td>
<td>7/6</td>
<td>24/17</td>
<td>7/8</td>
</tr>
<tr>
<td>Previous interferon (ribavirin) Yes/No</td>
<td>12/1</td>
<td>32/9</td>
<td>13/2</td>
</tr>
<tr>
<td>Median ALT ULN** (range)</td>
<td>3.1 (1.5-6.8)</td>
<td>2.6 (1.4-11.8)</td>
<td>3.0 (1.6-12.5)</td>
</tr>
<tr>
<td>Median HCV-RNA Mgeneq#/mL (range)</td>
<td>4.5 (1.4-39.2)</td>
<td>14.9 (0.2-104)</td>
<td>14.1 (0.7-76.3)</td>
</tr>
<tr>
<td>Genotype-1/Genotype non-1</td>
<td>7/6</td>
<td>20/21</td>
<td>7/8</td>
</tr>
</tbody>
</table>

*at start of treatment  **upper limit of normal  #Mega genome equivalent

The mean percentages ALT change from baseline

Distribution of ALT at the end of treatment

- Placebo (n=13)
  - 8% ALT normal
  - 92% ALT ≤ 1.5 × ULN
  - 0% ALT > 1.5 × ULN
- SNMC 3 times/W (n=41)
  - 10% ALT normal
  - 63% ALT ≤ 1.5 × ULN
  - 27% ALT > 1.5 × ULN
- SNMC 6 times/W (n=15)
  - 20% ALT normal
  - 53% ALT ≤ 1.5 × ULN
  - 27% ALT > 1.5 × ULN
The Long-Term Efficacy of SNMC in Chronic Hepatitis C Patients
Y. Arase et al., Cancer, 1997

<table>
<thead>
<tr>
<th></th>
<th>SNMC(+)</th>
<th>SNMC(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>84</td>
<td>109</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47(31-64)</td>
<td>48(30-64)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>73/11</td>
<td>92/17</td>
</tr>
<tr>
<td>Transfusion(+/-)</td>
<td>39/45</td>
<td>48/61</td>
</tr>
<tr>
<td>Histology (F1/F2 or F3)</td>
<td>51/33</td>
<td>61/48</td>
</tr>
<tr>
<td>HCV genotype (1b2a or 2b)</td>
<td>60/16</td>
<td>62/21</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>200(100-726)</td>
<td>186(104-698)</td>
</tr>
<tr>
<td>ICG R15 (%)</td>
<td>14(9-24)</td>
<td>15(8-26)</td>
</tr>
</tbody>
</table>

\[a: \text{Data are expressed as the median value(range)}\]

**Cumulative HCC appearance rate with or without SNMC administration**

- Group B [SNMC(-): n=109]  
P = 0.0319

- Group A [SNMC(+): n=84]

**Cumulative HCC appearance rate based on the average ALT after SNMC administration**

- ALT > 50 IU: n=54  
P = 0.08

- ALT ≤ 50 IU: n=30
Silymarin

- Extract of crushed milk thistle seeds:
  - Milk Thistle:
    - Silymarin:
      - Extract from seeds of Milk Thistle
      - a complex of at least 7 flavonolignans and 1 flavonoid that comprise 65-80% of milk thistle extract
  - Prevents liver disease in many experimental animal models
  - Used widely by HCV patients as a hepatoprotectant
  - Clinical studies indicate that Silymarin is very well tolerated and safe
Immunomodulatory

Antiviral

Antioxidant

Antiinflammatory

Hepatoprotection

Immunomodulatory
Milk Thistle (Silymarin)

- Choose a brand that has silibin and phosphotidyl choline
  - Better absorbed

- Typical dose 140-420 mg per day in divided doses of 2-3 times per day of 70-80% silymarin

- Large doses can cause loose stools
Silymarin

1978 expedites recovery after acute A or B hepatitis;
1980 expedites recovery in alcohol-related hepatitis;
1982 2-fold decrease of death rate due to Amanita intoxication;
1989 41 months follow-up: higher survival in cirrhotics;
1998 previous data not confirmed!

.......lack of reliable formulations, erratic pharmacokinetics
Molecular Profile of Silymarin

Silybum marianum seeds

HPLC Fingerprint of Standardized Milk Thistle Product (MK-001)
Silymarin Inhibits HCV Infection

HCVcc, (m.o.i. 0.01)

Polyak et al., Gastroenterology. 2007. 132(5):1925-36

Therapeutic Design

US Pharmacopoeia Milk Thistle
HCV RNA Synthesis

Therapeutic Design

HCVcc, (m.o.i. 0.01)
Infectious Virus Release

Supes From 48 Hours Post-Treatment

DMSO

Silymarin

Huh7.5.1 & Huh7
Intravenous Silymarin Reduces Viral Loads in IFN Nonresponders

Ferenci et al., Gastroenterology 2008
A novel ISO-controlled nutraceutical: YHK

CCL₄ Model

YHK-Treated

Untreated
### Hydroxyproline content of the liver

<table>
<thead>
<tr>
<th>weeks</th>
<th>Control</th>
<th>CCL(_4)</th>
<th>CCL(_4) + YHK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>367 ± 75</td>
<td>344 ± 87</td>
<td>401 ± 110</td>
</tr>
<tr>
<td>10</td>
<td>389 ± 93</td>
<td>839 ± 147*</td>
<td>563 ± 132*§</td>
</tr>
<tr>
<td>20</td>
<td>343 ± 61</td>
<td>1190 ± 205*</td>
<td>718 ± 151*§</td>
</tr>
</tbody>
</table>
# Serum markers of fibrosis

<table>
<thead>
<tr>
<th>weeks</th>
<th>Control</th>
<th>CCL$_4$</th>
<th>CCL$_4$ + YHK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Hyaluronic acid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.3 ± 4.3</td>
<td>4.6 ± 3.7</td>
<td>6.2 ± 4.0</td>
</tr>
<tr>
<td>10</td>
<td>6.7 ± 3.6</td>
<td>133.8 ± 55.6*</td>
<td>67.8 ± 24.7*§</td>
</tr>
<tr>
<td>20</td>
<td>11.3 ± 5.4</td>
<td>224.6 ± 77.5*</td>
<td>15.5 ± 7.2§</td>
</tr>
<tr>
<td></td>
<td><strong>Type IV collagen 7s</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.3 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>4.2 ± 0.2</td>
<td>4.2 ± 0.5</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>20</td>
<td>4.3 ± 0.6</td>
<td>4.9 ± 0.4</td>
<td>4.7 ± 0.1</td>
</tr>
</tbody>
</table>
Control  Fibrosis Model  Fibr. Model + YHK
EFFECT OF YHK AND SYLIBIN ON LDH LEAKAGE DUE TO METAL IONS DAMAGE IN CULTURED HEPATOCYTES

LDH leakage in medium

IU/L/mg protein

control | Test | YHK 100μM | YHK 200μM | Sylibin 100μM

Fe 100μM

Cu 100μM

V 100μM

* §
Inhibiting activity of YHK and silybin on FeSO$_4$-, Cu SO$_4$- and VCl$_3$ -induced lipid peroxidation in normal hepatocytes (*mean ± SD*)

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>YHK</th>
<th>Silybin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100µM</td>
<td>200µM</td>
</tr>
<tr>
<td>FeSO$_4$</td>
<td>15.6 ± 4.6$^§$</td>
<td>12.2 ± 4.4$^§$ *</td>
</tr>
<tr>
<td>Cu SO$_4$</td>
<td>7.9 ± 0.3</td>
<td>6.7 ± 0.7</td>
</tr>
<tr>
<td>VCl$_3$</td>
<td>8.7 ± 0.99</td>
<td>9.4 ± 0.85</td>
</tr>
</tbody>
</table>

Values represent the concentrations that inhibit lipid peroxidation by 50% (IC50, µM). IC50 is calculated from the concentration-activity curves.

$^§$ p<0.05 vs Cu SO4 and VCl3.  * p<0.05 vs Silybin
Nutritional Modulation of Carcinogenesis

Prevention Strategies
- Alter carcinogen metabolism
- Enhance carcinogen detoxification
- Scavenge electrophiles/ROS
- Enhance DNA repair

Prevention Strategies
- Scavenge ROS
- Alter gene expression
- Decrease inflammation
- Suppress proliferation
- Induce differentiation
- Encourage apoptosis

Additional Genetic Alterations

Increased cell proliferation

Neoplastic

Preneoplastic

Initiated

DNA Damaging Agents

Normal

Nutritional Oncology pp. 91, 1999
Phytotherapeutic Compound YHK Exerts an Inhibitory Effect on Early Stage of Experimentally-Induced Neoplastic Liver Lesions

Marotta F et al

Hepato-Gastroenterology Dept., S.Giuseppe Hospital, Milan, Italy
MHC Hospital, Tokyo, Japan
Hepato-GI Unit, University of Sao-Paulo, Brazil

NUMBER AND SIZE OF **GST-P-POSITIVE HEPATIC LESIONS IN DEN-INDUCED HEPATOCARCINOGENESIS: EFFECT OF CONCOMITANT SUPPLEMENTATION WITH YHK**

<table>
<thead>
<tr>
<th>Group</th>
<th>DEN</th>
<th>DEN + YHK 50mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>No./cm²</td>
<td>12 ± 4</td>
<td>6 ± 3 *</td>
</tr>
<tr>
<td>Mean area (mm²)</td>
<td>0.32 ± 0.04</td>
<td>0.25 ± 0.03 *</td>
</tr>
<tr>
<td>No./cm³</td>
<td>2012 ± 133</td>
<td>1545 ± 109 *</td>
</tr>
<tr>
<td>Mean vol. (mm³)</td>
<td>0.17 ± 0.03</td>
<td>0.14 ± 0.02 *</td>
</tr>
<tr>
<td>Foci/tissue %</td>
<td>28.2 ± 2.5</td>
<td>21.7 ± 2.1 *</td>
</tr>
</tbody>
</table>

* p<0.01 vs DEN-only treated rats
Western blotting and Northern blot hybridization of GST-P mRNA in the liver: effect of YHK
## Incidence, Number, Size and Volume of DEN-Induced Hepatocellular Carcinoma: Effect of Concomitant Supplementation with YHK

<table>
<thead>
<tr>
<th>Group</th>
<th>DEN</th>
<th>DEN + YHK 50mg/kg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats with HCC (%)</td>
<td>96 ± 4</td>
<td>71 ± 4 *</td>
</tr>
<tr>
<td>Mean area (mm²)</td>
<td>1.40 ± 0.47</td>
<td>0.17 ± 0.09 * *</td>
</tr>
<tr>
<td>No./cm³</td>
<td>1.3 ± 0.3</td>
<td>0.8 ± 0.2 *</td>
</tr>
<tr>
<td>Mean volume (mm³)</td>
<td>0.79 ± 0.28</td>
<td>0.02 ± 0.01 * *</td>
</tr>
<tr>
<td>HCC/tissue %</td>
<td>0.7 ± 0.2</td>
<td>0.2 ± 0.1 *</td>
</tr>
</tbody>
</table>

* p<0.01 vs DEN-only treated rats
Small-multiple GST-P Foci

Large GST-P Foci

DEN

DEN + YHK
IS THERE ANY ROLE FOR SUPPORTIVE NUTRACEUTICALS IN HCC?

Effect of YHK on HepG2 cell proliferation

Marotta F et al. Annal Hepatol 2007
Effect of YHK on cell cytotoxicity in HepG2 cells

Marotta F et al. Annal Hepatol 2007
Effect of YHK on Cell cycle and apoptosis of HepG2 cells

Marotta F et al. Annal Hepatol 2007
A pilot clinical study of YHK in *HCV*-related CLD

<table>
<thead>
<tr>
<th>Fibrosis score</th>
<th>Necro-Inflamm. score</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁ → F₀₋₁</td>
<td>A₂ → A₀</td>
<td><em>improved</em></td>
</tr>
<tr>
<td>F₂ → F₀₋₁</td>
<td>A₃ → A₂</td>
<td><em>improved</em></td>
</tr>
<tr>
<td>F₁ → F₁</td>
<td>A₂ → A₂</td>
<td><em>no change</em></td>
</tr>
<tr>
<td>F₀ → F₀</td>
<td>A₂ → A₀₋₁</td>
<td><em>improved</em></td>
</tr>
<tr>
<td>F₂ → F₃</td>
<td>A₂ → A₂</td>
<td><em>progression</em></td>
</tr>
<tr>
<td>F₁₋₂ → F₁₋₁</td>
<td>A₃ → A₁₋₂</td>
<td><em>improved</em></td>
</tr>
</tbody>
</table>
A pilot clinical study of YHK in HCV-related CLD
Yo jyo hen shi ko, a novel Chinese herbal, prevents nonalcoholic steatohepatitis in ob/ob mice fed a high fat or methionine-choline-deficient diet.

**de Lima YM, de Oliveira CP, Sawada LY, Barbeiro HV, de Mello ES, Soriano FG, Alves VA, Caldwell SH, Carrilho FJ.**

Department of Gastroenterology (LIM 07), University of São Paulo School of Medicine, São Paulo, Brazil.

Yo Jyo Hen Shi Ko (YHK) improves transaminases in nonalcoholic steatohepatitis (NASH): a randomized pilot study.

**Chande N, Laidlaw M, Adams P, Marotta P.**
Factors Affecting HCC Risk

Active disease
- Elevated ALT
Persistently elevated AFP
Low platelet count
HBV DNA level

Histologic changes
- Dysplasia
- Geographic morphologic changes
- PCNA positive

Use of TIPS (?)

Cirrhosis (Non-HBV) Suitable for HCC Surveillance*

- **Hepatitis C**
  - Incidence of HCC ~ 2% to 8% per year
- **Primary biliary cirrhosis**
- **Alcoholic cirrhosis**
- **Genetic hemochromatosis**
- **Nonalcoholic steatohepatitis**
- ? Alpha1-antitrypsin deficiency
- ? Autoimmune hepatitis
- ? Cryptogenic cirrhosis

*Populations with an annual HCC incidence of ≥ 1.5%.

Drug Development is NOT Easy

### Clinical Trials – Timeline for new drug development

<table>
<thead>
<tr>
<th>Years</th>
<th>Preclinical Testing</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
<th>FDA</th>
<th>Total Years</th>
<th>Phase IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.5</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2.5</td>
<td>12</td>
<td>Post-marketing</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test Population</th>
<th>Purpose</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Laboratory &amp; animal studies</td>
<td>20 to 80 healthy volunteers</td>
<td>100 to 300 patient volunteers</td>
<td>1000 to 3000 patient volunteers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Assess safety and biological activity</td>
<td>Determine safety and dosage</td>
<td>Evaluate effectiveness, look for side effects</td>
<td>Verify effectiveness, monitor adverse reactions from long-term use</td>
<td></td>
</tr>
</tbody>
</table>

HCV ADVOCATE
www.hcvadvocate.org
HCV Drugs in Development
(as of April 21st, 2009)

- 23 drugs against HCV targets:
  - 12 targeting NS3/4a protease
  - 8 targeting NS5B polymerase
  - 2 targeting NS5A
  - 1 entry inhibitor

- 15 general drugs:
  - 6 against cellular targets: cyclophilin, miRNAs, caspases, glucosidase, phospholipids
  - 9 Immunomodulators (stimulators/inhibitors): TLR9 agonists, A3AR agonists, anti-inflammatory, anti-fibrotic

- 6 Interferons:
  - IL-29, oral IFN, albuferon, consensus IFN

- 6 vaccines

- 4 liver cancer drugs

- 42 studies cancelled

http://www.hcvadvocate.org/hepatitis/hepC/HCVDrugs.html
Risks of CAM

Indirect risks
- Delay/avoidance of effective treatment

Direct health risks
- Toxic reactions
- Pharmacologic effects
- Mutagenic effects
- Drug interactions
- Contamination
- Substitutions or adulteration of ingredients
Hidden risks: Ginger

- Beneficial for nausea
- Be careful if you have gallstones
- Can worsen blood clotting!
Herbals supplements implicated in causing hepatotoxicity

- Atractylis gummifera
- Black cohosh
- Callilepis laureola
- Chaparral

Chinese herbal medicines
- Chaso and Onshido
- Sho (Do)-saiko-to
- Jin Bu Huan
- Ma huang
- Shou-wa-pian

- Comfrey/pyrrolizidine alkaloids
- Germander
- Greater celandine
- Kava
- Mistletoe
- Pennyroyal
- Skullcap and valerian
- Centella Asiatica
- Red yeast

Leonard B. Seeff, MD, Clinics in Liver Disease, August 2007
Common Chinese Herbs with potentially liver-toxic substances

- An Gong Niu Huang Wan
- Bi Tong Pian
- Bi Yan Pian
- Dendrobum Moniliforme
- Farfunoeiminkam Wan
- Gan Mao Ling
- High Strength Yin Cheng
- Huang Lien Shang Ching Pian
- Ma Hsing Zhe Ke Pian
- Marguerite Acne Pills
- Aconite or aconitum
- Acorus

- Comfrey
- Crotalaria
- Eupatorium
- Germander
- Groundsel
- Heliotropium
- Jin Bu Huan
- Mentha pulegium
- Mistletoe
- Pennyroyal oil
- Hedeoma pulegoides
- Sassafras
- Senicio species
- Senna
- Sophora

- Night Sight Pills
- Niu Huang Chiang Ya Wan
- Pe Min Kan Wan
- Da Huo Luo Wan
- Shen Ling Bai Zhu Pian
- Ta Huo Lo Tan
- Tsai Tsao Wan
- Yin Chiao Chieh Tu Pian
- Zhi Sou Ding Chuam Wan
- Zhong Gan Ling
- Amanita mushroom
- Chaparrel

In general, combination ingredient supplements are more likely to cause serious adverse events than single ingredient supplements!
CAM Can Be Beneficial in HCV

- 40% use in liver patients suggests benefit
- Preliminary data promising
- Need more scientific data
  - May *ameliorate side effects* of conventional therapy
  - Use in those *in whom therapy is contraindicated*
  - Use in *cirrhotics*
  - Use in *non-responders*
  - Potential *synergy* with conventional therapy
  - Bridge pending advances in conventional therapy
How Do We Counsel Patients Using Alternative Therapies?

- Consider what motivates patients to pursue alternative therapy
  - Educate patients concerning natural history of HCV infection and improving treatment options
- Obtain a thorough history of alternative treatments
- Discuss limited information on efficacy, safety, and potential risks of therapy
- Safe alternative agents are often beneficial for symptoms
Treatment Options for Hepatitis C

**Western (Allopathic) Medicine**

- Pegylated interferon/ribavirin
- Experimental protocols

**Integrated Medicine**

Hepatitis C Specialist

- Western therapy and complementary and alternative medicine

**Complementary and Alternative Medicine**

Hepatitis C Specialist

- Combination of all/some:
  - Ayurvedic medicine
  - Chinese herbs and acupuncture
  - Homeopathy
  - Mind:body medicine
  - Naturopathic treatments
  - Nutrition and lifestyle

Relapse or non-responder: **Try retreatment or use supportive care while waiting for new options. Continue healthcare provider follow-up on a regular basis.**

No treatment or self-treatment

*Discuss possible implications with your hepatitis C specialist/healthcare provider.*

*Understand your risks of cirrhosis or liver cancer.*
Herbogenomics: From Traditional Chinese Medicine to Novel Therapeutics

Novel Platform for TCM Analysis and Application

Traditional Chinese Medicine

Disease regression model

Human Patients
(Chronic diseases)

Animal disease models
Liver cirrhosis
Viral cardiomyopathy
Diabetic nephropathy

Validation of TCM

Mechanistic study

Target cell and molecular study
Affected cells
Modified molecular pathways

Product development

Novel products
Chemical compounds
Molecular products
Fermented Papaya Preparation:
≡15 years of Evidence-Based studies

- L. Packer’s group -UCLA, USA  
  FFP is a potent macrophage activator increasing NO synthesis and TNFα secretion in vitro.
  hydroxyl scavenging and iron-chelating properties of FFP prevents oxidative damage to DNA and proteins.  
  Anticancer Res 2000; 20:2907-14
- F. Marotta et al.  
  FFP promotes an effective protection against ethanol-induced gastric mucosal damage and reduced ox stress and DNA damage in cirrhosis
  FPP reduced precancerous markers of GI lesions  
  Ann NYAS 2004, 2006
- Luc Montagnier et al.  
  Immune-stimul. in imm-NR HIV (in process)
- “Development of Life Living Guidance for Prevention of HIV Progression” Res. Project  
  Dept. of Health Japan, 1998
  FFP enhanced CD8+ cell count in HIV pts. No side effects.
# Fermented Papaya Preparation (100g)

ISO 9001, ISO 14001  Japan Food Res. Lab., report n. 397100396-007

<table>
<thead>
<tr>
<th>Vitamin/Mineral</th>
<th>Amount</th>
<th>Vitamin/Mineral</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD</td>
<td>90.7g</td>
<td>Phenylalanine</td>
<td>11mg</td>
</tr>
<tr>
<td>Moisture</td>
<td>8.9g</td>
<td>Tyrosine</td>
<td>9mg</td>
</tr>
<tr>
<td>Protein</td>
<td>0.3g</td>
<td>Leucine</td>
<td>18mg</td>
</tr>
<tr>
<td>Fat</td>
<td>none</td>
<td>Isoleucine</td>
<td>9mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>2µg</td>
<td>Methionine</td>
<td>5mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>0.24mg</td>
<td>Valine</td>
<td>13mg</td>
</tr>
<tr>
<td>Lysine</td>
<td>6mg</td>
<td>Glycine</td>
<td>11mg</td>
</tr>
<tr>
<td>Histidine</td>
<td>5mg</td>
<td>Proline</td>
<td>8mg</td>
</tr>
<tr>
<td>Aspartic ac.</td>
<td>27mg</td>
<td>Tryptophan</td>
<td>2mg</td>
</tr>
<tr>
<td>Serine</td>
<td>11mg</td>
<td>Threonine</td>
<td>8mg</td>
</tr>
<tr>
<td>Arginine</td>
<td>16mg</td>
<td>Glutam.ac.</td>
<td>37mg</td>
</tr>
</tbody>
</table>
SOME COMMON CONDITIONS WITH IMMUNE SYSTEM-LINKED INFLAMMATORY & OXIDATIVE STRESS PHENOMENA

- Aging per sè
- Chronic Diseases (Liver, Diabetes, etc.)
- Relates stress
- Seasonal (flu, COPD flare up) stress
Nutraceutical supplementation: effect of a fermented papaya preparation on redox status and DNA damage in healthy elderly individuals and relationship with GSTM1 genotype: a randomized, placebo-controlled, cross-over study.

DNA adducts/10^8 nucleotides

8OHDG/10^5 dG

Marotta et al NY Acad Sci 2006
Prevention of Chronic Diseases: the ox stress-inflammation network

Genetics  Environment  Virus  Diet

Development

ROS

NF-κB Activation

Genetic Transcription
Citokines, Chemotactic Factors

Inflammation

ROS

Amplification

Cell Death

Anti-Inflammatories

Antioxidants
Modulating leukocyte DNA damage and cytokines by nutraceuticals in HCV-CLD: a fermented papaya preparation vs vitamin E

% reduction over baseline

Marotta et al. J Gastroenterol Hepatol 2006
Interleukin-6 Promoter Polymorphism Analysis

- GG: 35%
- GC: 45%
- CC: 20%
Effect of FPP supplementation on IL-6, TNF-α and Hsp70 in elderly population

Inv. Corr. with:
- IL-6
- CRP-hs

EFFECT OF A FERMENTED NUTRACEUTICAL ON THIOREDOXIN LEVEL AND TNF-α SIGNALLING IN CIRRHOTIC PATIENTS

Plasma Level of THIOREDOXIN

Marotta et al JBRHA 2010
EX-VIVO LPS-STIMULATION TEST OF TNF-α PRODUCTION FROM MONOCYTES: NUTRACEUTICAL MODULATION.

Marotta et al, JBRHA 2010
Vitamin Supplements

- Multivitamin without iron
  - **Excess iron** increases inflammation in the liver
  - Powder capsule formula is best for digestion
  - Can sometimes make people nauseated: take with food

- Fatty acids
  - Decreases muscle aching and fibromyalgia symptoms
  - Get refrigerated type to avoid rancidity
Vitamin Supplements

- Avoid Vitamin A unless you have been documented to be deficient
- Calcium with vitamin D two-three times daily
- Vitamin E: 400-1200 IU per day
  - Can help cell-mediated immune function, skin problems, memory loss
- Vitamin C: improves the immune function
- Lactobacillus acidophilus: aids with digestion
Patients and Methods
A) 39 healthy subjects, sedentary, teetotaller or <20g/day, non-smoking,
B) Stress questionnaire (State Trait Anxiety Inventory), Dr. Padrini’s psycho-emotional questionnaire and Pittsburgh Sleep Quality Index
C) Dietary- and life-style questionnaire

Treatment and controls
A) FPP (Osato Res. Inst., Gifu, Japan) 9g/day (4.5g twice/day) for 1mo.
B) Blood chemistry 2- and 4-wks afterwards
MDA in ERYTHROCYTES

nm/mg

**

start

350
325
300
275
250
225
200

FPP
2wk
FPP
4wk

normality
Urinary Excretion of BOMs (relative values)

- **start**: High value above normality range
- **FPP**: Decrease to within normality range
- **2wk**: Further decrease, still within normality range
- **FPP**: Similar to previous measurement, within normality range
- **4wk**: Similar to previous measurement, within normality range

**Note**: The stars (**) indicate statistical significance.
8-OHdG in leukocytes

**10^5dG**

- **start**:
- **FPP 2wk**: *
- **FPP 4wk**: *

**Normality**

Regenera research group
CAN WE IMPROVE OUR ADAPTATIVE RESPONSE THROUGH “GOOD” GENES ACTIVATION?

**HO-1 / GAPDH mRNA (AU)**

**HO-1 / CD 14 mRNA (AU)**
SALIVARY SECRETION OF IgA: EFFECT OF FPP SUPPLEMENTATION IN DIFFERENT HEALTHY AGE GROUPS

F. Marotta et al, JBRHA, 2012
Potentiation of Phase II detoxification and Antioxidant Gene Expression in epithelial cells from nasal lavage: effect of FPP
Can Nutrition and Nutraceutical Supplementation affect gene expression of our genes?
Pincemail J et al.  Dept. Cardiovascular Surgery, Diabetology, University of Liege, Belgium

600g of fruit & vegetables/day for 2 months to diabetics:

a) Reduction of some lipoperoxidation markers

b) No variation of plasma level of vitamin C and β-carotene!!
No Effect of 600 Grams Fruit and Vegetables Per Day on Oxidative DNA Damage and Repair in Healthy Nonsmokers

Canc Epide m Prev, 2003

Level of 24 h urinary 8-oxodG excretion (mean and SD)

nmol/24h

Before 24 day-END After (4 wk)

vegetable placebo vitamin
Expression of *OGG1* and *ERCC1* mRNA relative to 18S in leukocytes isolated from whole blood
Danese et al. La Clin Ter, 2006

Plasma glucose level decreases as collateral effect of fermented papaya preparation use -

Type II Diabetic patients

- Fasting
- Postmeal

mg/dl

Baseline
After FPP use
Improved function of diabetic wound-site macrophages and accelerated wound closure in response to oral supplementation of a fermented papaya preparation.
Improved function of diabetic wound-site macrophages and accelerated wound closure in response to oral supplementation of a fermented papaya preparation
Age-related susceptibility of erythrocytes to oxidative stress: A preliminary nutraceutical approach

F. Marotta, K Pavasuthipaisit, C. Yoshida, F. Albergati, P. Marandola

ReGenera Res. Group for Aging Intervention, Milano
Institute of Science & Technology, Mahidol University, Thailand
ORI Bioscience Lab., Gifu, Japan
Generation of TBARs in Erythrocyte in vitro: Effect of Aging and FPP Administration

Marotta et al. Rejuvenation Res. 2006
Peroxidation Profile in young and elderly subjects: role for a nutraceutical?

Hydroperoxides (H₂O₂ mg/dl of plasma)

Plasma Antiox. Capacity (lag time needed: min)

Fluoresc. Anisotropy (rₛ = 1v-Ih/Iv+2Ih)

Marotta et al. Rejuvenation Res. 2006
Micro-Channel array Flow Analyzer: old RBC deformability under placebo and FPP treatment
Amelioration of Oxidative Stress in RBC from Patients with β-thalassemia Major and Intermedia and E-β-thalassemia Following Administration of Fermented Papaya Preparation

Eliezer A. Rachmilewitz  
Phytother. Res. 2010
Fermented Papaya Preparation as Redox Regulator in Blood Cells of β-Thalassemic Mice and Patients

Johnny Amer¹, Ada Goldfarb¹, Eliezer A. Rachmilewitz² and Eitan Fibach¹

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![Graph C](image)

- No FPP
- FPP

![Graph](image)

- % RBC lysis
- FPP (mg/ml)
Overcoming Barriers: Antioxidants for Steatosis and Metabolic Syndrome

- Few studies evaluating impact of improved oxidation on SVR
- Phase I trial: antioxidant cocktail* given for 20 weeks (N = 50)[1]
  - ALT normalization: 44%; ≥ 2-point improvement in HAI score: 36%
- Ursodeoxycholic acid given with HCV therapy did not improve SVR rates (N = 52)[2]
- Patients received IFN or IFN + vitamin E for 24 weeks (N = 24)[3]
  - Greater response, reduction in viral load with vitamin E

*Cocktail included glycyrrhizin, schisandra, silymarin, ascorbic acid, lipoic acid, L-glutathione, alpha-tocopherol, glycyrrhizin, ascorbic acid, L-glutathione, and B-complex

3-month weight-loss program resulted in reduced steatosis and liver enzymes, improved fibrosis (N = 19)\(^1\)

- Mean weight loss: 5.9 kg
- Mean fasting insulin reduced (\(P < .002\))
- Reduced steatosis (\(P < .005\)) and Knodell fibrosis score (\(P = .04\))

3-month low calorie diet (n = 15) vs controls (n = 17) before pegIFN/RBV therapy in GT 1 patients\(^2\)

- Reduced insulin resistance in weight-loss group
- Response 60% for weight-loss group vs 17.6% for controls

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Overcoming Barriers: Insulin Sensitizers for Steatosis, Metabolic Syndrome

Thiazolidinediones: pioglitazone, rosiglitazone
- Pioglitazone improved insulin sensitivity through SOCS 3 suppression in mouse model\[^1\]
- 55 NASH patients with impaired glucose metabolism received hypocaloric diet + pioglitazone or placebo for 6 months\[^2\]
  - Diet + pioglitazone superior at improving glucose tolerance, ALTs
  - Diet + pioglitazone led to greater drop in liver fat content, improved steatosis, reduced inflammation

Biguanide: metformin reduces glucose production in liver
- Effects in NASH less robust than thiazolidinediones\[^3\]

Nutraceutical Approach for Preventing Obesity-Related Colorectal and Liver Carcinogenesis

Nutraceutical Approach for Preventing Obesity-Related Colorectal and Liver Carcinogenesis

• Macronutrients—CHD, fats, and proteins
• Micronutrients—vitamins and minerals
• Dietary fiber

**EB-Phytochemicals (FPP, high-quality silibin, modified-YHK)**

- Associated to established IFN + Rib. treatment?
- Only in IFN + Rib. Non-Responders?
- In the place of Rib. if side effects?
- In cirrhosis with ALT > 80 IU?
- In cirrhosis irrespective of ALT?
- In associated NASH Tx
- Post-op liver surgery? etc.