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

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Dept. of Human Nutrition & Food Science, Texas University, USA
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 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 pharmacokinetic & dynamic
- Poor tolerability
- Difficult to take
- Selection of resistance

- Adherence <100%
- Intolerance
## Evaluating Factors Associated With Poor Response to HCV Therapy

### 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>
</tbody>
</table>

**On treatment**
- Noncompliance with treatment
- Management of adverse effects

---


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Hepatic steatosis, IR and chronic hepatitis C infection

Hepatitis C → Metabolic factors (IR) → Hepatic steatosis → Hepatic fibrosis → Complications

Liver injury Immune

Hepatitis C (Yes)

Metabolic factors (IR) (Yes)

Liver injury Immune (AICAH 15-20%, HBV 27-51%, HCV 50-70%)

Complications
### 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 ± 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></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Patients, n</td>
</tr>
<tr>
<td>30</td>
</tr>
</tbody>
</table>

Mean change in fibrosis score per yr:

- 0-1: 0.05
- 2-3: 0.19
- > 4: 0.37
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>
Steatosis, fibrosis and necroinflammation in chronic hepatitis C: a Meta-Analysis of Individual patient Data (The HCV MAID Study)  

Leandro G et al

### 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>

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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

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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
Contraindications to therapy

Absolute
- Pregnancy
- Decompensated cirrhosis
- End stage kidney disease
- Severe or uncontrolled psychiatric disease
- Cardiopulmonary disease
- Severe Autoimmune disease
- Severe anemia
- Noncompliance

Relative
- Cirrhosis, compensated
- Controlled psychiatric disease
- Mild anemia/leukopenia
- Renal insufficiency
- Mild autoimmune disease
Percent of Patients Using CAM

Liver Clinics

Seeff et al. Hepatol. 2001
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
Non-alcoholic Fatty Liver Disease

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.

- **NAFLD Spectrum**
  - Steatosis alone
  - NASH
  - Cirrhosis

- Teli 1995
- Matteoni 1999

- 50% of diabetics: OP4
- 15% progression/1 year: PP15

- Ludwig 1980
- Diehl 1988
- Powell 1990
- Bacon 1994
- Caldwell 1999
- Angulo 1999
- Matteoni 1999
- Younossi 2000
- Ong 2001
- Ratziu V 2002
- Harrisson 2003

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Natural antiox/anti-inflammatory compounds in NASH: any relevant role?

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
Hepatoprotective effects of antioxidants in chronic hepatitis C

R Moreno-Otero, M Trapero-Marugán

World J Gastroenterol 2010

...... 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 regimens.

**But not any antioxidant naively!**

Nakamura M et al. An antioxidant resveratrol significantly **enhanced replication** of hepatitis C virus. *World J Gastroenterol* 2010

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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, halofuginone
- Inflammatory effects: inhibitors of chemokine binding/action, NF-kB inhibition

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

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

**Antioxidant Membrane Protective**
- Vit E, SAMe, phosphatidylcholine, resveratrol, sylimarin

**Promote HSC apoptosis**
- Gliotoxin, RGD peptides, sulphasalazine, death receptor ligands


**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), PIHNP;
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;**

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Compound 861 in HBV CLD
Salviae miltiorrhizae (丹参): Reversal rate

Cpd861 (50)

S1: 0%
S2: 39%
S3: 53%
S4: 78%

placebo (50)

S1: 0%
S2: 14%
S3: 25%
S4: 42%

reverse: score ↓ > 2
worse: score ↑ > 2
no change: score < 2

<table>
<thead>
<tr>
<th></th>
<th>861</th>
<th>placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse(%)</td>
<td>66*</td>
<td>33*</td>
</tr>
<tr>
<td>s3+s4</td>
<td>52</td>
<td>20</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
F. Marotta®
Chinese Herbal Medicine and Interferon in the Treatment of Chronic Hepatitis B: A Meta-Analysis of Randomized, Controlled Trials

Am J Publ Health, 2002

Chinese Herbal Medicine alone vs IFN-α

Hepatitis B surface antigen

- Dai F
- Li CQ
- Li GY
- Li J
- Lu L
- Song TW
- Wang MS
- Zhao XW
- Overall

Favors IFN-α
Favors CHM

Chinese Herbal Medicine combined with IFN-α vs IFN-α

Hepatitis B surface antigen

- Fu QP
- Hao LP
- Huang L
- Li MY
- Qian JH
- Shen GQ
- Wang RR
- Wu YH
- Zhang YL
- Zhang YX
- Overall

Favors IFN-α
Favors CHM + IFN-α
Traditional Chinese medicine causing hepatotoxicity in patients with chronic hepatitis B infection: a 1-year prospective study

*Aliment Pharmacol Ther, 2006*

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**
Mechanism of Pharmacological Action of Glycyrrhizin (GL)

Glycyrrhizin binds to selectin

Inhibition of SLe\(^{\alpha}\) (sugar chain) Cell-Cell Adhesion

Inhibition of Infiltration and Chemotaxis of Leukocytes

Anti-inflammation

Rao BN. et al., JBC 269, 19663-19666, 1994

Inflammation \(\downarrow\)
Chronic Hepatitis C Trial
Indian Council Medical Research

Multicenter Double-Blind Randomized controlled

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

IFN + Ribavirin  →  IFN + SNMC

Genotype 3 – 1 – 4: 71%, 27%, 2%

median ALT: 100.5 IU  →  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%

F. Marotta®
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 (32-69)</td>
<td>49 (39-70)</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
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</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: Data are expressed as the median value (range)

- Cumulative HCC appearance rate with or without SNMC administration

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

P = 0.0319
P = 0.08
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
Hepatoprotection

Antiviral

Antiinflammatory

Antioxidant

Immunomodulatory
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
Silymarin Inhibits HCV Infection

HCVcc, (m.o.i. 0.01)

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

Therapeutic Design

M=mock
E=EtOH
I=IFN

US Pharmacopoeia Milk Thistle

F. Marotta®
Infectious Virus Release

Supes From 48 Hours Post-Treatment

DMSO

Silymarin

Huh7.5.1 & Huh7
HCV RNA Synthesis

HCVcc, (m.o.i. 0.01)

Therapeutic Design
Intravenous Silymarin Reduces Viral Loads in IFN Nonresponders

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

CCL$_4$ Model

Untreated

YHK-Treated

F. Marotta®
### Hydroxyproline content of the liver

<table>
<thead>
<tr>
<th>weeks</th>
<th>Control</th>
<th>CCL&lt;sub&gt;4&lt;/sub&gt;</th>
<th>CCL&lt;sub&gt;4&lt;/sub&gt; + 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>

F. Marotta®
# Serum markers of fibrosis

<table>
<thead>
<tr>
<th>weeks</th>
<th>Control</th>
<th>CCL\textsubscript{4}</th>
<th>CCL\textsubscript{4} + YHK</th>
</tr>
</thead>
<tbody>
<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>
</tbody>
</table>

**Hyaluronic acid**

<table>
<thead>
<tr>
<th>weeks</th>
<th>Control</th>
<th>CCL\textsubscript{4}</th>
<th>CCL\textsubscript{4} + YHK</th>
</tr>
</thead>
<tbody>
<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>

**Type IV collagen 7s**

F. Marotta®
EFFECT OF YHK AND SYLIBIN ON LDH LEAKAGE DUE TO METAL IONS DAMAGE IN CULTURED HEPATOCYTES

IU/L/mg protein

$\$\$

LDH leakage in medium

control  Test  YHK 100$\mu$M  YHK 200$\mu$M  Sylirbin 100$\mu$M

$\text{Fe}$  $\text{Cu}$  $\text{V}$

F. Marotta®
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$\S$</td>
<td>12.2 ± 4.4$\S$ *</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.

$\S_p<0.05$ vs Cu SO$_4$ and VCl$_3$. *$p<0.05$ 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

Normal → DNA Damaging Agents → Initiated → Increased cell proliferation → Preneoplastic → Neoplastic

Additional Genetic Alterations
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

F. Marotta®
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><strong>No. of rats with HCC (%)</strong></td>
<td><strong>96 ± 4</strong></td>
<td><strong>71 ± 4</strong> *</td>
</tr>
<tr>
<td><strong>Mean area (mm²)</strong></td>
<td><strong>1.40 ± 0.47</strong></td>
<td><strong>0.17 ± 0.09</strong> * *</td>
</tr>
<tr>
<td><strong>No./cm³</strong></td>
<td><strong>1.3 ± 0.3</strong></td>
<td><strong>0.8 ± 0.2</strong> *</td>
</tr>
<tr>
<td><strong>Mean volume (mm³)</strong></td>
<td><strong>0.79 ± 0.28</strong></td>
<td><strong>0.02 ± 0.01</strong> * *</td>
</tr>
<tr>
<td><strong>HCC/tissue %</strong></td>
<td><strong>0.7 ± 0.2</strong></td>
<td><strong>0.2 ± 0.1</strong> *</td>
</tr>
</tbody>
</table>

* p<0.01 vs DEN-only treated rats

F. Marotta®
Small-multiple GST-P Foci

Large GST-P Foci
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

F. Marotta®
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

#### Biopsy Assessment

<table>
<thead>
<tr>
<th>Fibrosis score</th>
<th>Necro-Inflamm. score</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; → F&lt;sub&gt;0-1&lt;/sub&gt;</td>
<td>A&lt;sub&gt;2&lt;/sub&gt; → A&lt;sub&gt;0&lt;/sub&gt;</td>
<td>improved</td>
</tr>
<tr>
<td>F&lt;sub&gt;2&lt;/sub&gt; → F&lt;sub&gt;0-1&lt;/sub&gt;</td>
<td>A&lt;sub&gt;3&lt;/sub&gt; → A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>improved</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; → F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>A&lt;sub&gt;2&lt;/sub&gt; → A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>no change</td>
</tr>
<tr>
<td>F&lt;sub&gt;0&lt;/sub&gt; → F&lt;sub&gt;0&lt;/sub&gt;</td>
<td>A&lt;sub&gt;2&lt;/sub&gt; → A&lt;sub&gt;0-1&lt;/sub&gt;</td>
<td>improved</td>
</tr>
<tr>
<td>F&lt;sub&gt;2&lt;/sub&gt; → F&lt;sub&gt;3&lt;/sub&gt;</td>
<td>A&lt;sub&gt;2&lt;/sub&gt; → A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>progression</td>
</tr>
<tr>
<td>F&lt;sub&gt;1-2&lt;/sub&gt; → F&lt;sub&gt;1-1&lt;/sub&gt;</td>
<td>A&lt;sub&gt;3&lt;/sub&gt; → A&lt;sub&gt;1-2&lt;/sub&gt;</td>
<td>improved</td>
</tr>
</tbody>
</table>

F. Marotta®
A pilot clinical study of YHK in HCV-related CLD
1: Liver Int. 2007 Mar;27(2):227-34.

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 VM, 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 (?)

References:
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%.


F. Marotta®
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
Treatment Options for Hepatitis C

Western (Allopathic) Medicine
Hepatitis C Specialist

- 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.*
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
Herbogenomics: From Traditional Chinese Medicine to Novel Therapeutics

Novel Platform for TCM Analysis and Application

Traditional Chinese Medicine → Human Patients (Chronic diseases) → Animal disease models → Validation of TCM

Disease regression model

Mechanistic study

Target cell and molecular study

Product development

Novel products

Chemical compounds

Molecular products

Liver cirrhosis
Viral cardiomyopathy
Diabetic nephropathy
- Macronutrients - carbohydrates, fats, and proteins
- Micronutrients - vitamins and minerals
- Dietary fiber

- Phytochemicals (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.