In Vitro Effect of Peruvian Antimicrobial Agents on *Borrelia burgdorferi*

by

Priyanka A.S. Theophilus M.S.
Postgraduate Research Fellow
Advisor
Eva Sapi Ph.D.

Lyme Disease Research Group, Department of Biology and Environmental Sciences, University of New Haven, West Haven, CT 06516
Lyme Disease – The fastest growing epidemic

Lyme Disease – The most common vector-borne infectious disease in the Northern United States

Number of people diagnosed with Lyme Disease each year in the US ≈300,000

Rapidly spreading throughout Europe and northeast Asia (The Centers for Disease Control and Prevention)

Source: Picture taken from the Centers for Disease Control and Prevention
How long did we know about Lyme disease?

The autopsy of Ötzi the Iceman (5,300-year-old mummy) revealed the presence of DNA sequences of *Borrelia*

New discoveries of ticks fossilized in amber show that the bacteria which cause it may have been lurking around for 15 million years

Source: Picture taken from google image library
Borrelia burgdorferi

Exists in different morphological forms

Round body and biofilm form under stressful conditions
(Sapi E et al; 2011)

In favorable conditions, round body form revert back to spirochetes

Round body and biofilm forms show higher resistance to antimicrobials (Sapi E et al; 2012)

Source: Picture taken from the UNH Lyme Disease Research Group. Scale bar-100 μm
**Conventional Antibiotics – Advantage/Disadvantage**

**Doxycycline** - Choice of Lyme disease treatment

Doxycycline failed to cure infected mice (Moody K. D *et al*; 1994)

Spirochetes are capable of persisting in doxycycline treated mice and nonhuman primate hosts (Hodzic E *et al*; 2014) (Embers M. E *et al*; 2012)

Our research group has proven that all forms have different sensitivities to antimicrobial agents (Sapi E *et al*; 2011)

*Borrelia* frequently becomes resistant to antibiotic treatment

Known to have unfavorable side effects (Chang E.T *et al*; 2005)
Alternative therapies for Lyme disease

Enzyme therapy
Homeopathics
**Herbal antimicrobial agents**
Bee venom therapy
Chi machine/light beam generator
Hyperbaric oxygen therapy
Detox therapy
Photon therapy
Ozone therapy

Source: Picture taken from Google image library
Clinical study on Peruvian Herbal extracts

Utilizes several herbal extracts from the Cowden support program designed to eliminate microbes in advanced Lyme disease patients

(Dr. Richard Horowitz, ILADS, 2010)

Study conducted on over 100 Lyme disease patients who did not respond to standard antibiotic treatment

Samento and Banderol plus 8 other natural products used for the first 78 days

Later, 4 other antimicrobial agents (Enula, Mora, Cumanda, Houttuynia) are used in rotation along with Samento and Banderol

Effective for more than 80% of the patients
Another independent European clinical study

Dr. Armin Schwarzbach – Study conducted on 20 advanced Lyme disease patients (1st Ireland Lyme Disease conference, 2012)

The herbal extracts from the Cowden support program markedly improved symptoms in **80% of the patients**

Improved laboratory test results in **90% of the patients**

The doses were equivalent to thousands of times the recommended doses for humans

Caused no acute or chronic organ damage or any adverse effects
Can we find additional effective antimicrobial agents from the list of Peruvian herbs for *Borrelia burgdorferi*?
Peruvian Medicinal Antimicrobial Agents - Benefits

Have significant antimicrobial, antiprotozoal and antiviral properties

Safer to use

Cost effective

Might provide effective therapeutic options

<table>
<thead>
<tr>
<th>Name</th>
<th>Species</th>
<th>Activity of the agent</th>
<th>Reported effectiveness against the following pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mora</td>
<td>Premix of 3 plant extracts: <em>Rubus fruticosus</em>, <em>Achillea millefolium</em> and <em>Calycophyllum spruceanum</em></td>
<td>Antibacterial</td>
<td><em>Staphylococcus aureus</em>, <em>Streptococcus pneumonia</em> and <em>Clostridium perfringens</em></td>
</tr>
<tr>
<td>Enula</td>
<td><em>Inula heleneium</em>, <em>Mirabilis jalapa</em> and <em>Vitis vilifolia</em></td>
<td>Antibacterial and antifungal</td>
<td><em>Bacillus cereus</em>, <em>Pseudomonas aeruginosa</em>, <em>Staphylococcus aureus</em>, <em>Escherichia coli</em>, <em>Klebsiella pneumonia</em> and <em>Aspergillus parasiticus</em></td>
</tr>
<tr>
<td>Stevia</td>
<td><em>Stevia rebaudiana</em></td>
<td>Antimicrobial</td>
<td><em>Staphylococcus aureus</em>, <em>Bacillus subtilis</em>, <em>Vibrio cholera</em>, <em>Bacillus subtilis</em>, <em>Micrococcus luteus</em>, <em>Serratia marcescens</em>, <em>Pseudomonas aeruginosa</em>, <em>Bacillus megaterium</em>, <em>Escherichia coli</em> and <em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>Cumanda</td>
<td><em>Campsiandra angustifolia</em></td>
<td>Anti-parasitic, anti-fungal, anti-viral</td>
<td><em>Plasmodium falciparum</em></td>
</tr>
<tr>
<td>Houttynia</td>
<td><em>Houttynia cordata</em></td>
<td>Antibacterial</td>
<td><em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td>Samento</td>
<td><em>Uncaria tomentosa</em></td>
<td>Antibacterial</td>
<td><em>Borrelia burgdorferi</em> and <em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>Banderol</td>
<td><em>Otoha species</em></td>
<td>Antibacterial</td>
<td><em>Borrelia burgdorferi</em></td>
</tr>
<tr>
<td>Takuna</td>
<td><em>Cecropia strigosa</em></td>
<td>Antibacterial</td>
<td><em>Escherichia coli</em> and <em>Bacillus subtilis</em></td>
</tr>
<tr>
<td>Barberry</td>
<td><em>Mahonia aquifolium</em></td>
<td>Antimicrobial, antifungal</td>
<td><em>Staphylococcus aureus</em> and <em>Candida sp</em></td>
</tr>
<tr>
<td>Lakato</td>
<td><em>Echinacea angustifolia</em></td>
<td>Antibacterial</td>
<td><em>Escherichia coli</em>, <em>Proteus mirabilis</em>, <em>Pseudomonas aeruginosa</em> and <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Quina</td>
<td><em>Cinchona calisaya</em></td>
<td>Antimalarial</td>
<td><em>Plasmodium falciparum</em></td>
</tr>
</tbody>
</table>

Source: Picture taken from Google image library
Experimental conditions

*Borrelia burgdorferi* B31 laboratory strain

The herbal extracts were prepared in alcohol and formulated by Nutramedix Inc®

Positive control - Doxycycline (10 μg/ml)

Direct counting method – Seeded a concentration of 1 x 10^5 cells for 5 days

Crystal violet assay- Seeded a concentration of 5 x 10^6 cells for 7 days

Treatment regime – Antimicrobial agents treated for every 24 hrs for subsequent 3 days
In vitro evaluation of antimicrobial agents against *Borrelia burgdorferi*

Direct counting method – Counting live spirochetes and round body forms stained with special dyes

Live cells – Green color
Dead cells – Red color

Source: Picture taken from the UNH Lyme Disease Research Group. Scale bar - 100 µm
Qualitative and quantitative analysis of the attached biofilm form after treatment with antimicrobial agents

Crystal violet staining assay

Live dead assay
Live cells – Green color
Dead cells – Red color

Source: Picture taken from the UNH Lyme Disease Research Group. Scale bar 100 μm
RESULTS
Direct counting method – Live spirochetes and round body forms

Antimicrobial agents % reduction – Spirochetes % reduction – Round body forms

- Doxycycline (25 µg/ml) 85% Increase
- Mora 55% 50%
- Cumanda 23% 20%
- Houttuynia 11% 4%
- Enula 44% 32%
- Takuna 12% 27%
- Samento 63% Increase
- Banderol 29% 20%
- Houttuynia + Enula 22% 61%
- Cumanda + Mora 14% 60%
- Samento + Banderol 68% 45%

Effectiveness of antimicrobial agents on the spirochete and round body forms evaluated using direct counting method. (A) Counting live *Borrelia* spirochetes after treatment with antimicrobial agents (B) Counting live round body forms of *Borrelia* after treatment with antimicrobial agents (n=3 ± SD, *p≤ 0.05, **p≤ 0.01)
Do the antimicrobial agents also work on biofilms?

Source: Picture taken from the UNH Lyme Disease Research Group. Scale bar: 100 μm
Quantitative analysis measuring the total biomass after 72 h treatment measured by crystal violet staining technique. 25% grain alcohol (1:50 dilution) was used as a negative control. (n=3 ± SD, *p≤ 0.05, **p≤ 0.01)
Antimicrobial treated *Borrelia* biofilms stained using BacLight viability kit

The micrographs represent the biofilm form of *Borrelia burgdorferi* to the antimicrobial agents after 72 h treatment measured by fluorescent microscopy using BacLight viability kit.

Live cells – Green color
Dead cells – Red color
Clinical studies + \textit{in vitro} studies show the effectiveness of the antimicrobial agents against \textit{Borrelia burgdorferi}

However the efficacy of these antimicrobial agents must be further reinforced by \textit{in vivo} studies

These antimicrobial agents could provide an effective therapeutic approach for Lyme disease patients

<table>
<thead>
<tr>
<th>Agents effective against spirochetes</th>
<th>Agents effective against round bodies</th>
<th>Agents effective against biofilms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mora</td>
<td>Mora</td>
<td>Mora</td>
</tr>
<tr>
<td>Enula</td>
<td>Houttuynia + Enula</td>
<td>Enula</td>
</tr>
<tr>
<td>Samento + Banderol</td>
<td>Cumanda + Mora</td>
<td>Takuna</td>
</tr>
<tr>
<td></td>
<td>Samento + Banderol</td>
<td>Banderol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Houttuynia + Enula</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cumanda + Mora</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Samento + Banderol</td>
</tr>
</tbody>
</table>
References


References


References


- Horowitz, R.I. (2010). Plenary presentation at the ILADS (International Lyme and Associated Diseases Society) conference held at New Jersey, USA.


Acknowledgements

We are grateful to The Joshua foundation and The University of New Haven for all their support
Thank You