The CapsuLight project: from *in vitro* studies to intra-gastric robotic device prototyping for antibacterial phototherapy against *Helicobacter pylori*

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Overview

• *Helicobacter pylori* infection

• The idea: *H. pylori* PDT by an ingestible luminous pill

• Preliminary *in vitro* measurements

• Semi-theoretical action spectrum determination for *in vivo* *H. pylori* photokilling

• The pill prototypes

• Preliminary animal testing
**Helicobacter pylori**

A Gram-negative bacterium
A first class cancer-causing agent
It colonizes the gastric mucosa with a high worldwide prevalence of the infection

Credit: World Health Organization
Current therapy

Pharmacological eradication therapy
(antisecretive drug + 2-3 antibiotics per os, bid or quid for 7-10-14 days)

Eradication therapy failure is due to:
- The Side-effects
- The increasing rate of ANTIBIOTIC RESISTANCE

<table>
<thead>
<tr>
<th>Area</th>
<th>Amoxycillin</th>
<th>Clarithromycin</th>
<th>Metronidazole</th>
<th>Tetracycline</th>
<th>Levofoxacin</th>
<th>Multidrugs</th>
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<td>America</td>
<td>8/352</td>
<td>118/402</td>
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<td>11/393</td>
<td>NA</td>
<td>53/352</td>
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<tr>
<td></td>
<td>(2.2%)</td>
<td>(29.3%)</td>
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<td>(15.0%)</td>
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<tr>
<td>Africa</td>
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<td>(18.9%)</td>
<td>(37.1%)</td>
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<td>Europe</td>
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<td>(0.5%)</td>
<td>(11.1%)</td>
<td>(21.0%)</td>
<td>(2.1%)</td>
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<tr>
<td>Overall</td>
<td>184/1,640</td>
<td>2,014/11,697</td>
<td>948/3,549</td>
<td>94/1,580</td>
<td>254/1,562</td>
<td>278/2,876</td>
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<td>(26.7%)</td>
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<td>(9.6%)</td>
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</table>

O’Connor A et al., Helicobacter 2013;18 (Suppl 1)
De Francesco et al., J Gastrointestin Liver Dis 2010: 19 (4)
Megraud F et al. Gut 2013; 62
O’Connor A et al., Helicobacter 2015; 20 (Suppl 1)
The beginning of the story

Helicobacter pylori Accumulates Photoactive Porphyrins and Is Killed by Visible Light

Michael R. Hamblin, Jennifer Viveiros, Changming Yang, Atosa Ahmadi, Robert A. Ganz, and M. Joshua Tolkoff

Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts; Department of Dermatology, Harvard Medical School, Boston, Massachusetts; Sercia LLC, Minneapolis, Minnesota; Department of Gastroenterology, Abbott-Northwestern Hospital, Minneapolis, Minnesota; and Seedling Ventures LLC, Boston, Massachusetts

Received 21 September 2004/Returned for modification 22 September 2004/Accepted 7 March 2005

Helicobacter pylori colonizes the mucosa layer of the human stomach and duodenum, causes chronic gastritis, gastric ulcer, and is a risk factor for gastric adenocarcinoma. There is a 20% failure rate in antibiotic therapy, which is increasingly due to antibiotic resistance and necessitates the search for alternative antimicrobial methods. We have discovered that H. pylori when cultured in liquid medium, accumulates significant quantities of coproporphyrin and protoporphyrin IX, both in the cells and secreted into the medium. These photoactive porphyrins lead to cell death (up to 5 logs) by photodynamic action upon illumination with low doses of visible light, with blue/violet light being most efficient. The degree of killing increases with the age of the culture and is greater than that found with Propionibacterium acnes (another bacterium known to be photosensitive due to porphyrin accumulation). Both virulent and drug-resistant strains are killed. The data suggest that phototherapy might be used to treat H. pylori infection in the human stomach.

Treatment of Helicobacter pylori Infection With Intra-Gastric Violet Light Phototherapy: A Pilot Clinical Trial

Anthony J. Lembo, MD, Robert A. Ganz, Sunil Sheth, MD, David Cave, MD, PhD, Ciaran Kelly, MD, Philip Levin, PhD, Peter T. Kazlas, PhD, Paul C. Baldwin III, BS, William R. Lindmark, BS, Jonathan R. McGrath, BS, and Michael R. Hamblin, PhD
The PhotoDynamic Theraphy (PDT)

Based on the presence of 3 factors:

1. Light
2. Photosensitizer (PS)
3. Oxigen

→ Production of cyto-toxic species (ROS)
→ Cell death

*Helicobacter pylori* naturally produces porphyrins, a natural photosensitizer
→ Bacterium death
The idea: an ingestible pill

To kill *H. pylori* by light

*H. pylori* produces porphryines = natural photo-sensitizers

To perform photo-dynamic therapy

NO medical intervention

Intragastric, swallable device

With an ingestible illuminating pill
Reduced costs

Better patient compliance

NO drug resistance like antibiotics

NO adverse effects, as porphyrines are endogenous molecules

NO need for a medical doctor for the administration

NO need for monitoring devices during use

NO endoscopy!

Reduced costs
*In vitro* tests: porphyrins characterization

- Bacterial strains purchase
- Growth and biofilm formation

- Porphyrins extraction for spectroscopic characterization
- Biofilm fluorescence imaging

Battisti et al., Biophysical Chemistry 2017, 229
*In vitro* tests: photokilling

- Photokilling proof-of-principle with LED sources
- *ad hoc* illumination support
- Illumination in microaerophilic conditions
Action spectrum for *in vivo* photokilling (i)

Stomach wall illumination geometry

Calculation of a local effective mucosa penetration depth $d_{\text{eff}}$

Calculation of an average effective mucosa penetration depth $D_{\text{eff}}$ to find a mean transmittance spectrum of the gastric wall
Action spectrum for *in vivo* photokilling (ii)

2 main peaks: 500 nm and 630 nm
Endoscopic capsules

Minimally invasive endoscopic capsules are being commercialized and developed for diagnosis purposes since about 15 years.

No THERAPEUTIC endoscopic capsules have been developed and commercialized till now!

Credit (2,3,4,5): F Cubeddu, SSSA Pisa
Inside the pill

Single treatment ~ 20’ - 30’ (repeatable)

light sources: low cost LEDs

+ a battery and a switch

transparency casing broad angle emission

NO CCD, NO pointing system!
The capsule prototypes

First prototype with red and blue LEDs

Model with green, red and blue LEDs with more battery power and illumination efficiency

Temperature and pH sensors for use safety
Animal testing

- Test of the pill safety in Minipig animal model
- Animal tests made in CNR – Fondazione Monasterio laboratories, Pisa
- Verify and optimize the administration procedure
Conclusions

I. A minimally-invasive device for intragastric PDT (swallowable illuminating pill) has been built and characterized

II. Preliminary *in vitro* irradiation has been performed for photokilling tests

III. Action spectrum for *H. pylori* photokilling has been calculated, with main peaks at ~630 nm and ~500 nm

IV. Preliminary animal tests have been made on minipigs, verifying the device safety and administration procedure
Aknowledgments

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