Helicobacter pylori–binding microspheres to prevent gastric cancer

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“Encontros com a Inovação em Saúde”
**Helicobacter pylori infection**

- **World population**
  - 7 billion people

- **Infected with *H. pylori***
  - 50% of the world population
  - 3,5 billion people

- **Develop gastric cancer**
  - 1% of infected individuals
  - 35 million people

- **Have recommendation for *H. pylori* treatment**
  - 20% of symptomatic cases
  - 700 million people

- ***H. pylori* treatment is inefficient**
  - 20% of treated cases
  - 140 million people
Chitosan microspheres that, after oral administration, will adhere to gastric mucosa, bind *H. pylori* and eliminate it through the gastrointestinal tract.

Patent WO2013164652-A2
**Chitosan**

- **Natural polymer**
- **Source:** Squid pen (Chitin)

Mucoadhesive properties

Other sources:

www.ineb.up.pt
Chitosan microspheres

Diameter of ~170 µm

Stable in gastric acidic pH

Mean size (µm)

- pH 7.4: 167
- pH 6.0: 172
- pH 4.0: 175
- pH 2.6: 289
- pH 1.2: 345

0.2 mg Ch Mic

Gastric retention time of ~2h

MKN45 gastric cells

Not cytotoxic

Chitosan microspheres

Chitosan microspheres adhesion to *H. pylori*

<table>
<thead>
<tr>
<th>Strain</th>
<th>BabA Status</th>
<th>SabA Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>J99</td>
<td>BabA+/SabA+</td>
<td></td>
</tr>
<tr>
<td>17875/Leb</td>
<td>BabA+/SabA-</td>
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<tr>
<td>17875 babA1A2</td>
<td>BabA-/SabA+</td>
<td></td>
</tr>
<tr>
<td>097UK</td>
<td>BabA-/SabA-</td>
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</tbody>
</table>

pH 6.0

pH 2.6
The adhesion of *Helicobacter pylori* to the gastric mucosa involves specific interaction between bacteria adhesins and glycosylated receptors of gastric mucins and gastric epithelial cells.

**SabA**
(sialic acid binding adhesin)

binds to inflammation-associated sialyl-Lewis\(^a\) and sialyl-Lewis\(^X\) structures

[Mahdavi et al, 2002]

**BabA**
(blood group antigen binding adhesin)

binds to H-type 1 and Lewis\(^b\) structures expressed in normal gastric mucosa

[Ilver et al, 1998]
"Click Chemistry"
**H. pylori** adhesion to GlyR Ch Mic

- **Ch**
  - BabA+/SabA- *H. pylori*

- **Ch**
  - BabA+/SabA- *H. pylori*

- **Leb**
  - BabA+/SabA- *H. pylori*

- **sLe^x**
  - BabA+/SabA- *H. pylori*
**In vitro** gastric mucosa model

Sections from paraffin embedded stomachs

17875/Leb adhesion to mice gastric mucosa [% in relation to the control]

- Mic Ch
- Mic Ch
- Mic Le
- Mic sLe

17875/Leb adhesion to human gastric mucosa sections [% in relation to the control]

- Mic Ch
- Mic Ch
- Mic Le
- Mic sLe

Welch - ANOVA: Tamhane’s T2 post hoc test (***p<0.001; **p<0.01)
Ex vivo and in vivo mouse gastric mucosa model

**Ex vivo**
- Fresh stomachs

**In vivo**
- 0.2 mg Ch Mic 1x/day for 15 days
- 0.4 mg Ch Mic 1x/day for 15 days

63% reduction of *H. pylori* infection

One-way ANOVA with LSD post hoc (p*<0.05)
**Conclusions**

Ch microspheres are stable in acidic pH (do not dissolve), are non-cytotoxic and are retained in mice stomachs for 2h.

Ch microspheres are able to bind different *H. pylori* strains (unspecifically).

Glycan-modified microspheres bind *H. pylori* specifically through carbohydrate-adhesin binding and remove *H. pylori* adhesion, competing with carbohydrates from the gastric mucosa.

In this panorama, chitosan microspheres should be considered as alternative therapy for *H. pylori* infection treatment.
Advantages

• No problems associated with antibiotics use (bacterial resistance, side effects,...);

• Huge market as *H. pylori* infection treatment:
  • All infected population (3.5 billion people)
  • Patients who have recommendation for treatment (700 million)
  • Patients that do not respond to antibiotherapy (140 million);

• Chitosan microspheres may be used as unspecific treatment, while glycan coated microspheres may provide a personalized treatment for specific bacterial strains.

• Could also be used as preventive treatment or in combination with other treatments, namely as local drug delivery system;
Acknowledgements

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