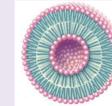


Antisense DNA against *Helicobacter pylori* urease impairs urease expression



Maysoon A. Mahmoud, Jelena L. Holovati and Monika M. Keelan

Department of Laboratory Medicine and Pathology, University of Alberta



Abstract

Background: *Helicobacter pylori* is a bacterial pathogen that may cause gastritis, peptic ulcers or gastric cancer and can be very difficult to treat. *H. pylori* produces urease, which neutralizes gastric pH by hydrolyzing urea to CO₂ and NH₃. Antisense DNA against the urease gene may inhibit its expression. Cationic liposomes can enhance the delivery of antisense DNA into bacterial cells. We investigated the ability of urease antisense DNA to impair *H. pylori* urease expression in the presence and absence of cationic liposomes (DOTAP®).

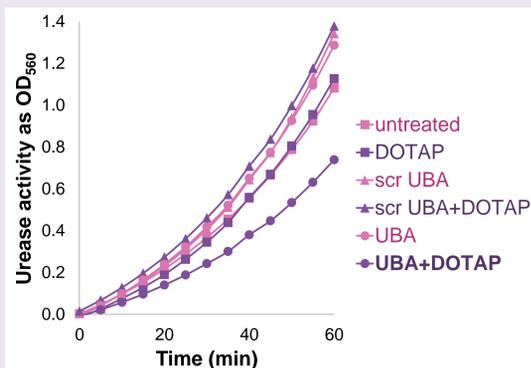
Methods: An antisense DNA oligo (UBA) was designed based on the *ureB* gene sequence of *H. pylori* 26695, along with a scrambled sequence (scr UBA, negative control). *H. pylori* A64 (strong urease producer) was cultured on 3.7% brain heart infusion / 0.5% yeast extract / 5% horse serum media at 37°C under microaerobic conditions, first on agar plates for 24 hr, then in broth (pH 7.4) for 24 h (at 100 rpm) with UBA (20 nM) or scr UBA (20 nM) ± DOTAP® (1.6 µM) added at 0, 6 and 10 hr. At 24 h, growth (OD₆₀₀, cfu/ml) and cell lysate urease activity (DA₅₆₀/min) were determined. Urease activity was also assessed for UBA (1-5 µM) ± DOTAP®, and DOTAP® (0.4-6.4 µM) ± UBA (20 nM).

Results: UBA±DOTAP® did not affect *H. pylori* growth. UBA+DOTAP® suppressed urease activity by 40% versus untreated control, UBA or, scr UBA ± DOTAP®. At 4 µM UBA, urease activity was maximally inhibited by 59%. Increasing DOTAP® concentration beyond 1.6 µM did not further increase urease inhibition.

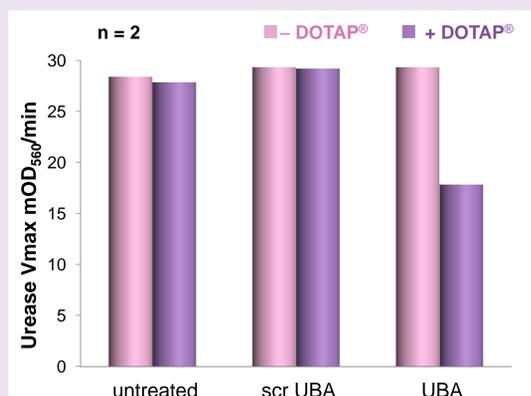
Conclusion: Cationic liposomes can successfully deliver *ureB* gene antisense DNA to *H. pylori* and impair urease expression at pH 7.4 without affecting growth. Impairing urease expression may decrease *H. pylori* survival under the acidic conditions of the stomach and offer a novel approach for treatment.

Results

UBA + DOTAP® suppressed urease activity by 40%

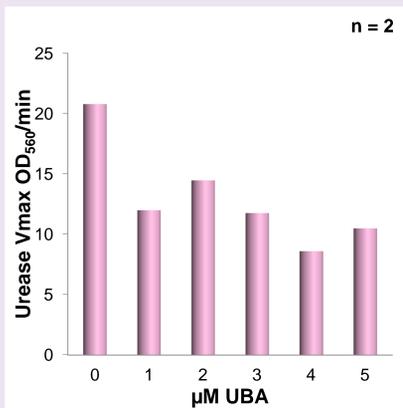


- urease activity of *H. pylori* in cell lysates after 24 h incubation following treatment (n=2)
- scr UBA, DOTAP® and UBA, did not affect *H. pylori* growth at neutral pH



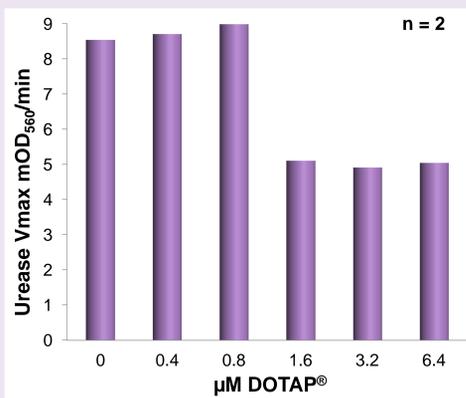
Vmax = maximum urease activity per min after 60 min incubation

4 µM UBA maximally suppressed urease activity



Without DOTAP®, 4 µM UBA suppressed urease activity by 59%

DOTAP® concentration > 1.6 µM did not further suppress urease activity



1.6 µM DOTAP® is required with 20 nM UBA to suppress urease activity

Summary

Treatment	Urease inhibition
scr UBA	none
DOTAP®	none
20 nM UBA	none
1-5 µM UBA	42-59%
20 nM UBA + 0.4-0.8 µM DOTAP®	5-6%
20 nM UBA + 1.6-6.4 µM DOTAP®	40-42%

In the absence of DOTAP®, 50 times more UBA is needed to achieve the same level of urease inhibition

Conclusion

Cationic liposomes deliver *ureB* gene antisense DNA (UBA) more efficiently than UBA alone to impair urease expression, which may decrease *H. pylori* survival under the acidic conditions of the stomach and offer a novel approach for treatment.

Future Directions

- To assess the decrease in urease mRNA level caused by UBA + DOTAP®
- To investigate suppression of urease expression by siRNA
- To design acid stable cationic liposomes to be loaded with UBA or siRNA
- To incorporate a targeting molecule within liposome structures to specifically interact with *H. pylori* and not other gut bacteria
- To investigate the effect of UBA / siRNA loaded liposomes on *H. pylori* survival in an acidic medium to mimic the stomach environment

Acknowledgements



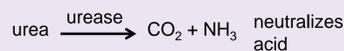
Megan Burlet
Jun Li



Background

Helicobacter pylori

- Gram negative microaerophilic bacteria
- causes gastritis, peptic ulcer, gastric adenocarcinoma & MALT lymphoma
- requires urease to colonize stomach



- treatment failure is 20-25%

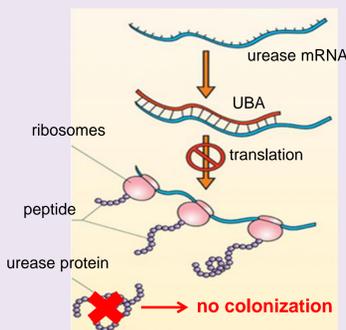
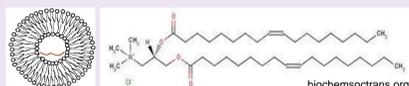
Could urease gene be a target for therapy?

ureB Antisense DNA (UBA)

- ssDNA oligo complimentary to *ureB* gene sense strand may inhibit transcription and/or translation

Cationic liposomes (DOTAP®)

- can be loaded with UBA to enhance delivery to *H. pylori* and inhibit urease production



Objective

To investigate the ability of urease antisense DNA to impair *H. pylori* urease expression in the presence and absence of DOTAP® liposomes

Methods

