Chitosan nanoparticles improves antibacterial activity of ceftazidime against antibiotic resistance *Burkholderia pseudomallei*

Presented by

Nuttaya Thonglao 647070018-7

Department of Microbiology, Faculty of Medicine, Khon Kaen University
Chitosan nanoparticles improve antibacterial activity of ceftazidime against antibiotic resistance *Burkholderia pseudomallei*

**Rationale**

*Burkholderia pseudomallei*
- Gram negative, motile rod-shaped bacteria
- Southeast Asia, Northern Australia
- Caused melioidosis
- Ceftazidime (CAZ)

*Chitosan (CS)*
- Antibacterial activity
- Antibiofilm activity
- Non-toxic
- Biological molecule

**Bp biofilms**
- High conc. of antibiotic treatment
  - (Anutrakunchai et al., 2018)
- Relapsing of melioidosis
  - (Limmmathurotsakul et al., 2014)

**Ciprofloxacin-DNase loaded CSNPs reduced* P. aeruginosa biofilm**
- (Patel et al., 2020)

**CS-TPP-Ungeremine eradicated* P. roqueforti**
- (Moeini et al., 2018)

**CSNPs contained NEO against *E. coli & S. aureus***
- (Bagheri, Ariaii, & Motamedzadegan, 2021)

**Chitosan kill* B. pseudomallei (5 mg/ml)***
- (Kamjumphol, Chareonsudjai, & Chareonsudjai, 2018)

**Chitosan-streptomycin conjugate**
- Eradicate bacterial biofilm formation
  - (Zhang et al., 2013)

**dCS/CAZ eradicate planktonic and biofilm cells of *B. pseudomallei***
- (Thonglao et al., in press)
Objectives

- To prepare and characterize chitosan nanoparticles (CSNPs) using tripolyphosphates (TPP)
- To cross-link CSNPs with ceftazidime (CAZ)
- To assess the antimicrobial and antibiofilm activities of CSNP-CAZ against antibiotic resistance *B. pseudomallei*.
- To examine the ability of CSNPs-CAZ to prevent *B. pseudomallei* internalization on human epithelial cells
The optimization of concentration and ratio of CS to TPP will be revealed.

Antimicrobial and antibiofilm activity of CPNPs-CAZ on *B. pseudomallei* will be demonstrated.

Improve susceptibility of *B. pseudomallei* biofilm to CAZ.

CPNPs-CAZ may prevent *B. pseudomallei* infection to human cells.
Chitosan nanoparticle (CSNPs)

- Cause cell wall leakage
- Disperse/inhibit biofilm formation

Ceftazidime (CAZ)

- Inhibit cell wall synthesis

Facilitate infection

Persist to host immune response

Resist to antibiotics

Relapse melioidosis

B. pseudomallei biofilms

Treated B. pseudomallei biofilms

Conceptual framework

Biofilm formation inhibition/dispersion

Facilitate antibiotic accessibility to Bp

Increase antibiotic susceptibility to Bp

Reduce Bp infection/internalization
Investigation of antimicrobial and antibiofilm activity of chitosan nanoparticle

**Part I**
Preparation and characterization of chitosan nanoparticle

**Part II**
Investigation of antimicrobial and antibiofilm activity of chitosan nanoparticle

**Part III**
Investigation of chitosan nanoparticle potential against Bp internalization

Study design

- Preparation and characterization of dCS nanoparticles with containing of CAZ
- Ionic crosslinking using TPP addition under stirring
- Zeta sizer
  - particles size
  - PDI
  - zeta potential
  - morphology
- SEM
- Loading of CAZ into the dCS-TPP using ionotropic gelation to generate dCS-TPP-CAZ
The modification and characterization of chitosan nanoparticles

Thesis progression
Preparation of chitosan nanoparticle (CSNPs)

1. 0.25% or 0.5% chitosan (CS)
   - Dissolve in acetic acid
   - pH 5.6
   - Stir at RT, 160 rpm, O/N

2. 1% sodium tripolyphosphate (TPP)
   - 3 ratios of CS:TPP (7:2, 4:1, 6:1)
   - Stir at RT, 2 h

3. Centrifuge at 18,000 x g, 10 min
   - Discard supernatant
   - Redisperse by deionized water

4. Nanoparticle characterization
   - 0.25% & 0.5% CSNPs
     - Under stirring
   - Particle size
     - Zeta sizer
   - Polydispersity index (PDI)
     - Zeta sizer
   - Zeta potential
     - Zeta sizer
   - Morphology
     - Scanning electron microscope (SEM)
Nanoparticle characterization

Particle size

Figure 1. Particle size of 0.25% and 0.5% chitosan nanoparticle. The 1% of TPP was dispersed into 0.25% (black bar) and 0.5% (gray bar) chitosan (CS) solution with different ratios (ratio CS:TPP = 7:2, 4:1 and 6:1) under mechanical stirring and then purified by centrifugation. The particles size was evaluated using ZetaSizer Nano ZS, Malvern instruments, UK (MAL 1227435).
Figure 2. Polydispersity index (PDI) of 0.25% and 0.5% chitosan nanoparticle. The 1% of TPP was dispersed into 0.25% (black bar) and 0.5% (gray bar) chitosan (CS) solution with different ratios (ratio CS:TPP = 7:2, 4:1 and 6:1) under mechanical stirring and then purified by centrifugation. The PDI values were evaluated using ZetaSizer Nano ZS, Malvern instruments, UK (MAL 1227435).
Nanoparticle characterization

Zeta potential

Figure 3. Zeta potential of 0.25% and 0.5% chitosan nanoparticle. The 1% of TPP was dispersed into 0.25% (black bar) and 0.5% (gray bar) chitosan (CS) solution with different ratios (ratio CS:TPP = 7:2, 4:1 and 6:1) under mechanical stirring and then purified by centrifugation. The zeta potential were evaluated using ZetaSizer Nano ZS, Malvern instruments, UK (MAL 1227435).
Figure 4. SEM images 0.25% and 0.5% chitosan nanoparticles. The SEM visualization of 0.25% (A) and 0.5% (B) chitosan nanoparticles with different ratio of chitosan and TPP were demonstrated under SEM, Quanta 450 FEI (100,000 x). All images indicating the aggregation of nanoparticles with spherical shape. The scale bar represents 500 nm.
# Conclusion

## 0.25% CSNPs

<table>
<thead>
<tr>
<th>CS:TPP ratio</th>
<th>Particle size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
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<tbody>
<tr>
<td>7:2</td>
<td>46.97</td>
<td>0.37</td>
<td>+18.30</td>
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<tr>
<td>4:1</td>
<td>68.16</td>
<td>0.41</td>
<td>+15.10</td>
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<tr>
<td>6:1</td>
<td>86.97</td>
<td>0.64</td>
<td>+14.70</td>
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## 0.5% CSNPs

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<th>CS:TPP ratio</th>
<th>Particle size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
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<tr>
<td>7:2</td>
<td>51.49</td>
<td>0.69</td>
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<td>4:1</td>
<td>72.75</td>
<td>0.79</td>
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<td>6:1</td>
<td>98.10</td>
<td>0.81</td>
<td>+16.50</td>
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Further works

Part I
Preparation and characterization of chitosan nanoparticle

Part II
Investigation of antimicrobial and antibiofilm activity of chitosan nanoparticle

Part III
Investigation of chitosan nanoparticle potential against Bp internalization

- MIC assay
- MBC assay
- Using broth microdilution method
- Performed on antibiotic resistant bacteria
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<td>Part I: Preparation of CSNPs</td>
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<td>Part I: Characterization of CSNPs</td>
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<td>Part I: Formulation of CSNPs-CAZ</td>
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<td>Part III: Evaluation of CSNPs potential on Bp internalization</td>
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<td>Part III: Cytotoxicity of CSNPs</td>
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- **Done**
- **On process**
- **Further work**

**Registered 13 credits**
Acknowledgement

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THANK YOU