Development of a mucoadhesive nanoparticulate drug delivery system for a targeted drug release in the bladder

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Introduction

Diseases of the urinary bladder

- cancer
- inflammation, infection
- incontinence

- treated by oral administration of pharmaceutical compounds
  ➔ systemic delivery

- Intravesical Drug Delivery (IDD)

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Introduction

Intravesical drug delivery (IDD) limitations

- periodical voiding of urine dilutes and washes out the drug
- reduces the residence time of drug and lead to a new administration
- repeated catheterizations increase potential for infections
- very low permeability of the urothelium in the diseased state

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Introduction

Purpose of the present study

- development of a mucoadhesive nanoparticulate drug delivery system for local use in intravesical therapy
  - retarding release of the drug
  - prolong the residence time of the drug in the bladder
- trimethoprim (TMP) was used as an effective local therapy from cystitis in the bladder
  - fluorescein diacetate (FDA) was used as fluorescent marker

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Methods & Results

Preparation of the matrix of the drug delivery system

Chitosan

Chitosan - Thioglycolic acid (TGA)

Thioglycolic acid

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Preparation of the drug delivery system

Chitosan or chitosan-TGA in 0.01 M acetic acid /sodium acetat buffer 0.5% (w/v) pH 6.2

TPP solution in demineralised water 0.2% (w/v)

Nanoparticles (NP)

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Preparation of the drug delivery system

Different amounts of 0.5% (v/v) H₂O₂ solution
**Methods & Results**

**Characterization of the drug delivery system**

**Table 1.**
Mean particle diameter and zeta potential of chitosan-TGA nanoparticles obtained by ionic gelation with TPP and followed by different oxidation with H$_2$O$_2$, respectively. Indicated values are means ± SD (n ≥ 3).

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>Mean particle diameter [nm]</th>
<th>Polydispersity index</th>
<th>Zeta potential [mV]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ionically crosslinked</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chitosan</td>
<td>266 ± 64</td>
<td>0.44</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Chitosan-TGA</td>
<td>197 ± 24</td>
<td>0.38</td>
<td>7 ± 1</td>
</tr>
<tr>
<td><strong>Covalently crosslinked</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chitosan TGA (ox1)</td>
<td>183 ± 7</td>
<td>0.31</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>Chitosan TGA (ox2)</td>
<td>186 ± 6</td>
<td>0.29</td>
<td>12 ± 1</td>
</tr>
</tbody>
</table>
Methods & Results

Characterization of the drug delivery system

Fig. 2. Size distribution of ionically crosslinked nanoparticles as well as covalently crosslinked nanoparticles. Indicated values are means ± SD of last three experiments.

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Characterization of the drug delivery system

Table 2.
Amount of thiol groups and disulfide bonds immobilised on the basic thimer Chitosan-TGA and nanoparticles after ionic gelation with TPP and different degrees of oxidation with H₂O₂, respectively. Indicated values are means ± SD (n ≥ 3).

<table>
<thead>
<tr>
<th></th>
<th>H₂O₂ [μmol]</th>
<th>-SH [μmol/g]</th>
<th>-S-S- [μmol/g]</th>
<th>Σ-SH [μmol/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan-TGA</td>
<td>-</td>
<td>1456</td>
<td>136</td>
<td>1728 ± 62</td>
</tr>
<tr>
<td>Chitosan-TGA NP</td>
<td>-</td>
<td>1391</td>
<td>178</td>
<td>1747 ± 36</td>
</tr>
<tr>
<td>Chitosan-TGA NP (ox1)</td>
<td>10.60</td>
<td>903</td>
<td>426</td>
<td>1753 ± 55</td>
</tr>
<tr>
<td>Chitosan-TGA NP (ox2)</td>
<td>21.21</td>
<td>641</td>
<td>559</td>
<td>1758 ± 27</td>
</tr>
</tbody>
</table>

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Characterization of the drug delivery system

Fig. 3. Transmission electron microscopy images of the spherical shape of nanoparticles based on chitosan [A], chitosan-thioglycolic acid [B], chitosan-thioglycolic acid with 426 µmol/g disulfide bonds [C] and chitosan-thioglycolic acid with 559 µmol/g disulfide bonds [D]. Displayed bar represents 4.0 µm.

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**Methods & Results**

**Fig. 4.** Payload of trimethoprim [white bars] and fluorescein diacetate [black bars] loaded nanoparticles based on chitosan, chitosan-thioglycolic acid and thioglycolic acid with 426 µmol/g (ox1) and 559 µmol/g (ox2) disulfide bonds. Indicated values are means ± SD (n ≥ 3). * Differs from TMP, p < 0.05.
Methods & Results

**In vitro mucoadhesion studies on porcine urinary bladders**

- **Methods & Results**
- **FDA loaded NP**
- **Instillation of 8 mg prehydrated NP**

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In vitro mucoadhesion studies on porcine urinary bladders

Fig. 5. Percentage of fluorescein diacetate remaining on porcine urinary bladders as a function of time. Studies were carried out with chitosan-thioglycolic acid nanoparticles [black bars] and unmodified chitosan nanoparticles [white bars] as control. Indicated values are means ± SD (n ≥ 3). * Differs from unmodified chitosan nanoparticles, p < 0.05.

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In vivo evaluation of particles with rats

- female Sprague-Dawley rats, average body weight 250 g
- rats were fasted but had free access to water
- anesthetized by an injection of ketamine (20 mg/kg)/xylazine-hydrochloride (4 mg/kg) mixture
- before urethral catheterization animals were positioned in supine position, and micturition was induced through mild caudal abdominal massage
- 500 µl of each formulation was administered

<table>
<thead>
<tr>
<th>Animal grouping</th>
<th>Administered formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>FDA suspension</td>
</tr>
<tr>
<td>Group 2</td>
<td>FDA loaded unmodified chitosan nanoparticles</td>
</tr>
<tr>
<td>Group 3</td>
<td>FDA loaded chitosan-TGA nanoparticles</td>
</tr>
</tbody>
</table>

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In vivo evaluation of particles with rats

Fig. 6. Amount of fluorescein diacetate remaining on rats bladders. Fluorescein diacetate was applied without any excipients [black bars] or incorporated in unmodified chitosan nanoparticles [grey bars] or chitosan-thioglycolic acid nanoparticles [white bars]. Indicated values are means ± SD (n ≥ 3).

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Mucoadhesion on mucosa of urinary bladders

Mechanism of disulfide bond formation between thiomers and mucus glycoproteins
Methods & Results

Release studies

OXIDATION

H₂O₂
Fig. 7. Release properties of trimethoprim nanoparticles among simulated conditions with artificial urine as a function of crosslinking. Studies were carried out with nanoparticles based on chitosan [□], chitosan-thioglycolic acid [◇], chitosan-thioglycolic acid with 426 µmol/g disulfide bonds [▲] and 559 µmol/g [●] disulfide bonds. Indicated values are means ± SD (n ≥ 3). Differs from chitosan nanoparticles, p < 0.05.
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Conclusion

- intravesical drug delivery system based on thiolated chitosan offers an adequate release profile besides its mucoadhesive properties
- chitosan-TGA NP showed:
  1. greater stability
  2. superior mucoadhesion
  3. more sustained and controlled release

Finally, chitosan-TGA intravesical drug delivery system might be a useful tool for a local drug application in the urinary bladder, which allows:

1. prolonged residence time at the target site
2. enables sustained drug delivery of trimethoprim over a longer time span
THANK YOU FOR ATTENTION!