Evaluation of a Novel Lipid Based Nanoparticulate System for Ocular Drug Delivery

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ABSTRACT SUMMARY

A novel drug delivery system based on Nanostructure Lipid Carriers (NLCs) coated with low molecular weight chitosan (LCH) was developed for the ocular delivery of Acyclovir (ACV) (Seyfoddin and Al-Kassas 2012). The in vitro and in vivo drug release studies from NLCs indicated that the system releases the drug in a controlled manner which could increase the ocular bioavailability of ACV. Moreover, NLCs were found not to be toxic in a number of in vitro eye irritancy assays.

INTRODUCTION

Ocular drug delivery is a challenge for pharmaceutical scientists because of the complex nature and structure of the eye. Conventional ocular delivery systems such as eye drops and ointments have low bioavailability and require large doses. Systemic administration of drugs to target the posterior chamber of the eye requires continuous exposure to very high, often toxic doses and therefore, there is a need to develop novel drug delivery carriers which would increase ocular bioavailability and decrease cytotoxicity (Seyfoddin and Al-Kassas 2012).

The only available ophthalmic ACV formulation is in the form of ophthalmic ointment which has poor ocular bioavailability as low as 5-10 %. By providing controlled drug release, corneal penetration enhancement, and longer precorneal resident time, NLCs are theoretically bound to increase the ocular bioavailability of poorly soluble drugs (Müller, Mäder et al. 2000).

The reliability of this hypothesis was determined by in vitro drug release studies and an in vivo drug permeation study on New Zealand albino rabbits. The aim of this study was to modify the NLCs to cationic particles by coating them with chitosan which is a natural and non-toxic polymer. We also aimed to characterize the developed formulations and test their ocular toxicity/irritancy potential.

EXPERIMENTAL METHODS

The NLCs were prepared by a hot microemulsion technique reported previously (Heydenreich, Westmeier et al. 2003; Vighi, Ruozzi et al. 2007; Seyfoddin and Al-Kassas 2012) and then coated with the mucoadhesive low molecular weight chitosan polymer (LCH) to reverse the negative zeta potential and to increase bioadhesive properties. Initially, a 1% solution of LCH was prepared in a 0.2 M acetate buffer pH-5. The solution was stirred over night for complete dissolution. 50 mg of freeze dried NLCs were dispersed in 5 mL of different concentrations of LCH solutions and magnetically stirred at 600 rpm for 20 minutes at ambient temperatures.

For fresh NLCs, the ultra-centrifuged mass was dispersed in 5 mL milliQ water. Subsequently, 1 mL of NLCs dispersion was added to 5 mL of appropriate LCH solution as above. The nanoparticles were pharmaceutically characterized and the in vitro drug release profiles were obtained. The in vivo drug permeation studies were carried out on male New Zealand white rabbits (3-4 kg) and 6 animals were euthanized according to Auckland University’s ethical protocols at each time point. The concentration of drug in aqueous humor samples were analyzed by HPLC. The ex vivo bovine eye (BE) assay, histopathological examinations of bovine corneas, and MTT cell proliferation assay were used to assess cytoxicity and/or irritancy potential of NLCs (Bruner, Kain et al. 1991).

RESULTS AND DISCUSSION

Table 1 shows the physiochemical properties of freshly prepared chitosan coated and uncoated NLCs. The original NLCs were 323.33 ± 14.6 nm in diameter. The diameter of NLC increased as a result of surface coating with chitosan. At relatively low concentration of chitosan (0.1%), the particle size increased sharply because, the induced positive charge was not strong enough to stabilize the NLCs suspension (Li, Zhuang et al. 2009). However, as the concentration of chitosan increased, the positive surface charges also increased. This led to the formation of a stable suspension and therefore the particle size was increased just slightly compared to uncoated NLCs.

Table 1 Physiochemical properties of fresh NLCs coated with different LCH concentration (n =3 ± SD).

<table>
<thead>
<tr>
<th>Concentration of chitosan</th>
<th>Binding efficiency (%)</th>
<th>Zeta potential (mV)</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-25.5 ± 1.65</td>
<td>323.33 ± 14.6</td>
</tr>
<tr>
<td>0.1</td>
<td>99.90 ± 0.09</td>
<td>5.03 ± 1.01</td>
<td>529.9 ± 86.86</td>
</tr>
<tr>
<td>0.25</td>
<td>99.72 ± 0.17</td>
<td>17.56 ± 0.85</td>
<td>444.73 ± 21.16</td>
</tr>
<tr>
<td>0.5</td>
<td>99.83 ± 0.16</td>
<td>28.1 ± 0.72</td>
<td>457.3 ± 44.38</td>
</tr>
<tr>
<td>1</td>
<td>99.59 ± 0.40</td>
<td>28.2 ± 0.72</td>
<td>467.46 ± 56.25</td>
</tr>
</tbody>
</table>

Figure 1 shows the in vitro release profiles of uncoated and LCH coated NLCs. There was a 25 % decrease in the release rate of the coated nanoparticles. The LCH coat is a water insoluble barrier and has resulted in slower release profiles. Also, coating of NLCs with increasing concentrations of LCH resulted in increased drug permeation through the bovine cornea (data not shown). Based on the results obtained, NLCs coated with 0.5% chitosan were chosen for in vivo drug permeation studies. Figure 2 shows the ACV concentration in aqueous...
humour after topical administration of 0.3 %ACV containing chitosan coated NLCs and ACV eye drop and ointment of matching concentrations as the controls. A threefold increase in the area under the curve (AUC) of ACV was obtained by using NLCs. The bottom-line is that NLCs increased the ocular bioavailability of ACV significantly.

![Figure 1](image1.png)

Figure 1. In vitro release profiles of freshly prepared NLC (NLC), lyophilised NLC (FD NLC), and NLCs coated with 0.1, 0.25, and 0.5% LCH (n=3±SD).

![Figure 2](image2.png)

Figure 2. In vivo aqueous humour permeation profiles of ACV containing chitosan coated NLC, 0.3% ACV eye drop, and 0.3% ACV ophthalmic ointment (n=12±SD).

BE assay, is an ex vivo alternative to Draize test. BE assay, combined with histopathological evaluation of the excised corneas, and cell culture methods, is a reliable strategy to determine the full range of irritation potential of a formulation. The excised corneas retained transparency and integrity in all samples and were completely impermeable to fluorescein. All test substances had a score below 0.5 (See Figure 3) which indicates they do not induce corneal irritation ex vivo.

![Figure 3](image3.png)

Figure 3. Cumulative bovine eye scores for four control solutions (0.5 M NaOH, Acetone, Propylene glycol, and PBS as negative control), and Four formulations (SLN, NLC, NLC containing ACV-BCD complex (NLCBCD), and NLCs coated with 0.5% LCH (NLCLCH)), (n=3 ± SD).

Evaluation of histopathological photomicrographs of the excised corneas indicated that there was no gross toxicity associated with any of the formulation tested even after extended periods of ex vivo exposure. As it can be seen from Figure 5, MTT cell proliferation assay also supported the previous data and that the therapeutic concentration of nanoparticles required is well tolerated by the cells.

![Figure 4](image4.png)

Figure 4 A) Full thickness cornea (10x), B) Epithelium and upper stroma (20x).

![Figure 5](image5.png)

Figure 5 MTT Calorimetric measurement of the proliferation of cells treated with different concentration of ACV containing freeze dried NLCs.

CONCLUSION
NLCs were successfully developed and evaluated as a potential system for enhanced ocular drug delivery. The in vivo drug permeation data had a close correlation with the in vitro drug release data obtained. None of our developed systems were cytotoxic or irritant to ocular membrane ex vivo. We can conclude that NLCs have the potential to enhance ocular drug delivery of many poorly soluble drugs.

REFERENCES