Release and corneal permeation of 2-oxothiazolidine-4-carboxylic acid from chitosan nanoparticles for cataract treatment

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ABSTRACT SUMMARY

Chitosan nanoparticles loaded with 2-oxothiazolidine-4-carboxylic acid (OTZ) were formulated by ionic gelation method. The in vitro release of OTZ from the nanoparticles was quantified over 96 hours. Permeation of OTZ through excised bovine cornea mounted on a Franz cell diffusion apparatus was measured using HPLC. The lag time and the flux were 0.2 h and 3.05 µg/cm².h respectively.

INTRODUCTION

It has been estimated that more than 18 million people are affected by cataract (1). Senile cataract is an age related condition that affects the lens of the eye causing visual impairment (1). One of the reasons for cataract formation is the low levels of glutathione (GSH) which is the main endogenous antioxidant in the lens. Cysteine is a GSH precursor and its supplementation may increase the GSH level in the lens (1). However, cysteine is very unstable and toxic. A recent study suggested that OTZ, which is in turn a precursor of cysteine, can increase the cellular level of GSH (2).

Chitosan nanoparticles have many propitious properties such as the availability of chitosan polymer, simple to formulate and scale up methods, biodegradability, biocompatibility and lack of toxicity (1). In addition, chitosan nanoparticles are potentially good ophthalmic delivery systems; this is because of the mucoadhesive properties, of chitosan arising from possible electrostatic interactions between the negatively charged mucin and the positively charged chitosan. This interaction increases the residence time of drug-loaded nanoparticles, reducing drainage, thus increasing drug bioavailability.

We report on the formulation, in vitro release and transcorneal permeation of OTZ released from chitosan nanoparticles through excised bovine cornea. This is a promising system for delivery OTZ to the lens thereby increasing the cellular level of GSH and potentially preventing or delaying the formation of age-related cataract.

EXPERIMENTAL METHODS

OTZ-loaded chitosan nanoparticle formulation

Following previous in-house experiments, an optimized method was employed to formulate chitosan nanoparticles by an ionic gelation. OTZ (0.6mg/mL) was added to a 5 mL chitosan solution (1.75 mg/mL); the solution was stirred for 1 hour. Sodium tripolyphosphate (TPP; 2 mL) was added to this solution drop-wise. This resulted in a particulate dispersion which was covered in an ice bath and was sonicated for 30 seconds. The preparation, now with a reduced particle size, was centrifuged at 40,000 g for 30 min. this enabled the separation of nanoparticles from the clear supernatant that may have contained un-entrapped OTZ. The nanoparticles obtained were re-dispersed in water.

In vitro release study of OTZ from chitosan nanoparticles

A dialysis membrane (10,000 Dalton MWCO) was soaked overnight in simulated aqueous humor (SAH). A Franz cell diffusion apparatus (Logan instruments Corp., Somerset, USA) was used to conduct the study. The receptor compartment of the Franz cell was filled with SAH and a dispersion of chitosan nanoparticles loaded with OTZ (2 mL) was transferred to the donor compartment. The two compartments were separated with the dialysis membrane. The temperature of the cell was maintained at 35 ± 0.5 °C. At hourly intervals, an aliquot (400 µL) was withdrawn from the receptor compartment for analysis; this volume was replaced with fresh SAH. The OTZ released from the nanoparticles was quantified by HPLC.

Ex vivo permeation study of OTZ from chitosan nanoparticles

The lag time and the flux were 0.2 h and 3.05 µg/cm².h respectively.
The corneal permeation of OTZ was investigated using a Franz cell diffusion apparatus. The receptor compartment was filled with freshly prepared SAH (12 mL). Chitosan nanoparticles loaded with OTZ (2 mL) were placed in the donor compartment. The two compartments were separated by a freshly excised bovine cornea (endothelium facing the receptor compartment). The temperature of the system was maintained at 35 ± 0.5 °C. A sample (400 µL) was withdrawn from the receptor compartment at set time points (every 1 hour) and replaced with fresh SAH the withdrawn samples were analyzed by HPLC.

RESULTS AND DISCUSSION

The particle size and zeta potential of the prepared nanoparticles were measured and found to be 150.1 ± 5 nm and +26 ± 0.5 mV respectively. Figure 1 illustrates the in vitro release of OTZ from the chitosan nanoparticles over a period of 96 hours. The figure shows that a burst release of OTZ was initially achieved and this resulted in nearly 8.0% drug release within the first 6 hours. This was, followed by a release with a more sustained profile over the following 90 hour period. The burst release of OTZ can be attributed to the possible surface adsorption of drug on the nanoparticles, while the sustained release was from the drug entrapped within the core of the chitosan nanoparticles.

Figure 1: In vitro release of OTZ from chitosan nanoparticles. Results are expressed as a mean value ± SD, (n = 6)

The amount of OTZ released from chitosan nanoparticles over the period of the experiment was 14.7% of the total amount of used OTZ. This amount agrees with a previous study which showed a similar percentage of a drug release from chitosan nanoparticles (3). This finding suggests that a significant amount of drug is being retained in the nanoparticle system presumably due to a strong electrostatic interaction between the positively charged chitosan and the negatively charged OTZ. Nevertheless, the amount of released OTZ is still the within therapeutic window.

Figure 2 shows the amount of OTZ that permeated through excised bovine cornea after bring released from the chitosan nanoparticles. The profile is linear, indicating that the corneal epithelium limits the permeation of OTZ. The lag time and the flux were calculated and found to be 0.2 h and 3.05 µg/cm².h respectively.

Figure 2: Permeation of OTZ (loaded into chitosan nanoparticles) through excised bovine corneas. Results are expressed as a mean value ± SD, (n = 6)

CONCLUSION

Chitosan nanoparticles loaded with OTZ were formulated using an ionic gelation method. In vitro studies showed the release of therapeutic amounts of OTZ. Also, its permeation through excised bovine cornea did not appear to have been hindered by the corneal tissue. The use of chitosan nanoparticles for the ophthalmic delivery of OTZ is promising; further investigations are underway.

REFERENCES

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