Lyceum Chinense and Calcium Phosphate Nanoparticles for Ophthalmic Drug Delivery

Q. He[1, 2], Zhenqing Fang[2], M. Wang[2], Q.W. Xu[2], R Chen[2], D. Li[2], X.Z.Zhang[1], W.M. Yaun[1], D.M Zhau[1], Jian Wu[3]

[1]Nanjing Carrier Nano-Biotech Inc, [2]Nanjing Medical University, and [3]Nanjing High Technology Industry Development Zone.

ABSTRACT

Glaucoma, the leading cause of irreversible blindness in the world, is estimated to be affected by 67 million people. It is characterized by an increase in intraocular pressure (IOP). Carbonic anhydrase inhibitors (CAIs) are orally used for controlling IOP. Since CA II/IV/XII is present in many other tissues, systemic CAIs possess undesired side effects. In order to avoid these undesired side effects, we have developed topically methazolamide using nanoparticles consisting lyceum chinense and calcium phosphate as a delivery system. Preliminary studies showed strong and long-lasting IOP lowering, being more effective than clinical used brinzolamide. The maximum lowering in normal white and pigmented rabbits were about 6.14±0.96mmHg and 4.20±1.38mmHg (mean±SEM) respectively. HPLC results showed the peak concentration in the aqueous humor appeared in 15 minutes (0.3μg/ml). Clinical and toxicity evaluation indicated there is no irritation and inflammation on the experimented eyes compared with the control group by histology examination and slit lamp. Also, there is no toxic effect in the acute toxicology and allergic experiment. Based on our findings, we suggest that lyceum chinense and calcium phosphate nanoparticles may favor the localized ocular delivery of CAIs for the treatment of glaucoma.

Keyword: lyceum Chinense, IOP, nanoparticles

1 INTRODUCTION

Carbonic anhydrase inhibitors (CAIs) such as acetazolamide and methazolamide were and still are widely used systemic anti-glaucoma drugs. Their mechanism of action consists in inhibition of CA present in ciliary processes of the eye, with the consequent reduction of bicarbonate and aqueous humor secretion, and of elevated IOP characteristic of this disease. Since CA is present in many other tissues, systemic CAIs possess undesired side effects such as metallic taste; depression; fatigue; malaise; weight loss; decreased libido; gastrointestinal irritation; metabolic acidosis; renal calculi and transient myopia. Therefore, the topically effective CAIs have been highly needed. To achieve this goal, we introduced a new nanoparticles delivery system consisting of lyceum chinense, calcium phosphate, and methazolamide. The efficiency and toxicity of the new formulation were investigated and evaluated.

2 MATERIALS AND METHODS

2.1 Preparation and Characterization of methazolamide nanoparticles

Nanoparticles were made of calcium chloride, dibasic sodium phosphate and Lyceum Chinense (Fisher Scientific USA). The particle size and the zeta-potential were measured using Zetasizer Nano ZS90 (Malvern instruments limited, China). The Dispersion Technology Software was used for analysis effective diameter.

2.2 Intraocular Pressure (IOP) Measurements

Twenty-four white rabbits (NZW) and twenty-four pigment rabbits (Nanjing anlimo company) were randomly divided to 4 groups (methazolamide nanoparticles, brinzolamide, nanoparticles, and control). 100ul of Experimental drugs were topically administrated. Intraocular pressure (IOP) was measured at appropriate times using an impression tonometer (YJ1 Mingren Co. China).

2.3 Pharmacokinetic analysis

The aqueous humor samples were carried out at appropriate times after the drug was administered in NZW rabbits and analyzed with Hitachi HPLC system.

2.4 Tolerance and irritation assay in vivo and pathology analysis

The left eye of NZW rabbits were exposed to nano-mathazolamide 3 times per day for 7 days. Ocular irritation was evaluated every day by slit lamp. The right eyes were used as the control group. Experimental rabbits were sacrificed at day 7 and the eyeballs were collected and fixed in 10% methanol for histology analysis.

3 RESULTS

3.1 Characterization of methazolamide nanoparticles The size of particles ranged from 80 to 620 nm with 85% of the particles between 85nm-150 nm. The mean of zeta potential was -22mV

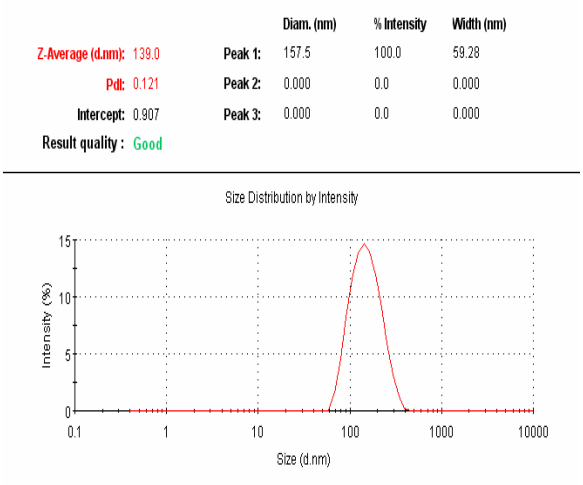


Figure1: Distribution of size of nano-methazolamide

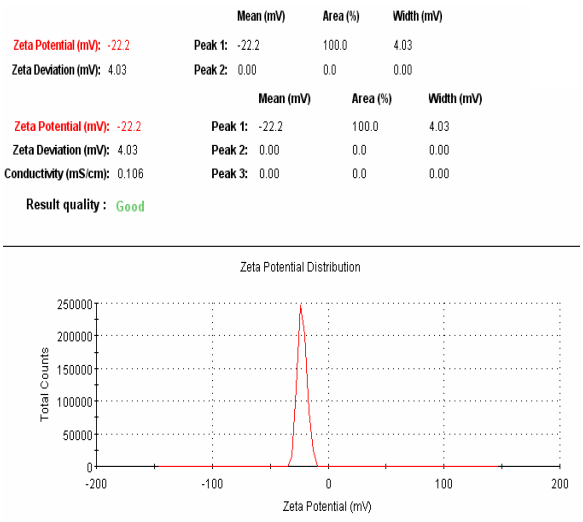


Figure2: The zeta potential of nano-methazolamide

3.2 Efficiency of methazolamide nanoparticles on IOP in NZW and pigmented rabbits

As shown in Figure 3 and 4, IOP lowering was more pronounced in 0.2% nano-methazolamide group than the 1%brinzolamide in both animals. The maximum IOP lowering was about 6mmHg and 4mmHg respectively.

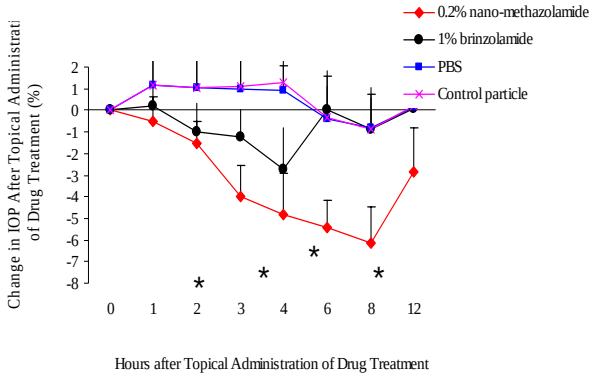


Figure 3. IOP in NZW rabbits (n=10). * p<0.05.

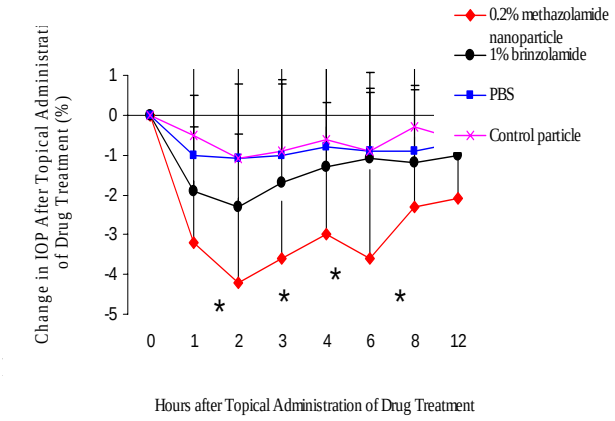


Figure 4. IOP in pigmented rabbits (n=10). * p<0.05.

3.3 Concentration of mathazolamide in the aqueous humor of NZW rabbits'

The peak time of mathazolamide concentration in the aqueous humor appeared in 15 minutes (0.3μg/ml) (n=5).

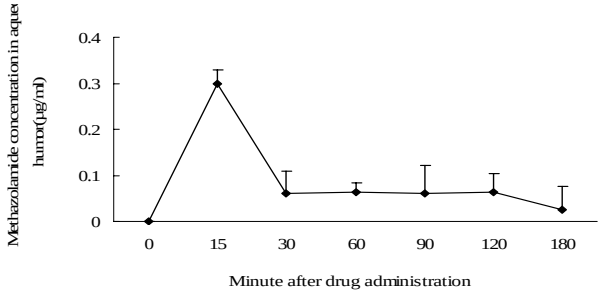


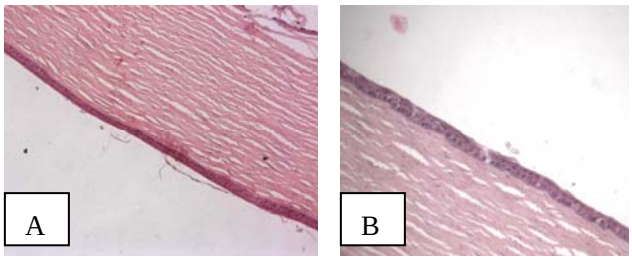
Figure5 the methazolamide concentration in the rabbits' aqueous humor after drug administered

3.4 Preclinical toxicity evaluation:

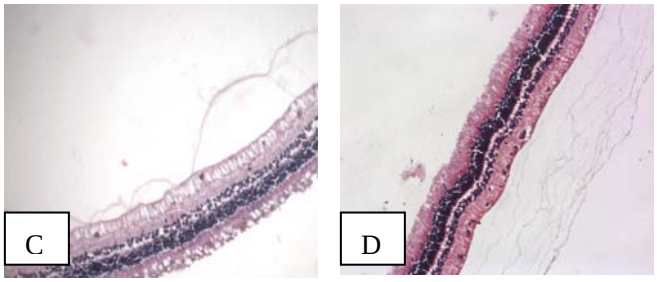
Figure 6 indicated that there were no irritation and

inflammation on the experimental eyes compared with the control eyes by slit lamp. The histological examination showed no severe inflammation responses (n=10).

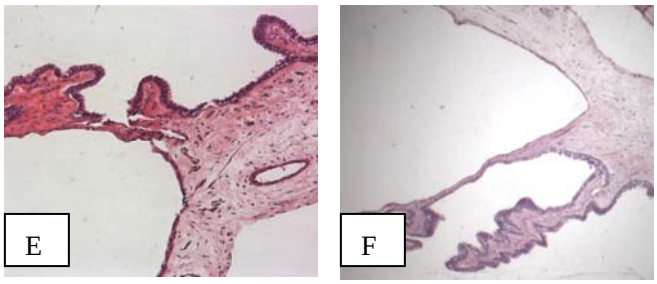
Cornea tissue



Retina tissue



Ciliary tissue



Lens tissue

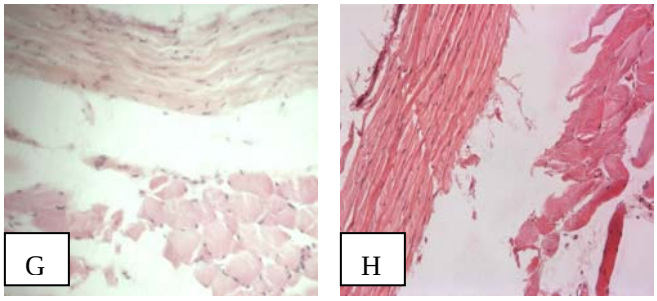


Figure 6: histological results showed no difference between the experimented eyes. A, C, E, G is control tissues. B, D, F, H is nano-methazolamind treated tissues.

Table 1: Side effects of mathazolamide nanoparticles

Event	PBS control	brinzolamide	Mathazolamide
Stinging/burning	0	2	0
Conjunctival hyperemia	0	0	0
Itching	0	2	0.2
Superficial punctuate epitheliopathy	0	0	0
Tearing	0	2	0
Conjunctival discharge	0	2	0
Redness	0	3	0

4 CONCLUSIONS

One of the main problems in the ocular drug delivery is related to the presence of the mucus barrier that incorporates the ocular tear film. This defensive mechanism represents the main limitation to the use of liquid formulations for ophthalmic therapy. At present, there is strong evidence that nanoparticles can improve delivery of drugs applied topically onto the eye. In fact, a number of studies have shown the potential of different kinds of nanoparticles, nanocapsules, liposomes, and microparticles to increase the retention of drug on the ocular surface as well as to facilitate penetration across the corneal epithelium. Among the different delivery system nanoparticles hold special promise (8). Therefore, nanoparticles are becoming increasingly investigated as a means of drug delivery and prolonging drug release in and around the eye. They appear to be well tolerated in animal models whether intraocular delivery or injected periocularly or intravitreally. A demonstrated consistency in increasing drug solubility and drug release kinetics is also observed with these drug depots (1, 2, 7). Sustained intraocular therapeutic drug concentrations can be also be achieved without surgical implantation of slow-release drug delivery device or repeated intravitreal/periocular injections (3, 4). It has been shown that intraocular drug concentrations can be typically achieved with

biodegradable and biocompatible nanoparticles (5). Several techniques of nanoparticles preparation and drug encapsulation have been long studied for ophthalmic uses and found to be reliable and safe in those applications.

Clearly, nanoparticles-based methazolamide has achieved better therapeutic effect with low concentration compared with brinzolamide, which is a commercial available topic anti-glaucoma drug. There were no side effects and toxicity according to histological reports. The nanoparticles carrier showed no toxicity and inflammation response to the rabbit eye. Furthermore, the materials that have used for formulating nanoparticles have been proved by Chinese FDA. But the maximum IOP lowering and duration time are different between NZW and pigmented rabbit. The hypothesis is that the differences may be caused by the pigment- drug binding. It is concluded that utilization of Lyceum Chinense and Calcium Phosphate Nanoparticles as a delivery device can enhance the bioavailability of methazolamide in ocular and induce a significant IOP lowering activity. Future studies will be necessary to test this new formulation in humans.

5 REFERANCE

[1] Hoyng, P.F.J. and van Beek, L.M. Pharmacological therapy for glaucoma. Drugs 59:411-434, 2000.

[2] Alward, W.L. Medical management of glaucoma. N. Engl. J. Med. 339:1298-1307, 1998.

[3] Manners, R.M., Alexander, M.M., et. A study of topical carbonic anhydrase inhibitors in ocular hypertensive patients. Glaucoma 15,51-54, 1993.

[4] Li, V.H., Wood, R.W., Kreuter, J., Harmia, T., and Robinson, J.R. Ocular drug delivery of progesterone using nanoparticles. J. Microencapsul. 3:213-8, 1986.

[5] Teh-ching Chu, Qing He, David e. potter. Biodegradable Calcium Phosphate Nanoparticles as a New Vehicle for Delivery of a Potential Ocular Hypotensive Agent. J. Ocular Pharmacology and Therapeutics 18, 507-514, 2002.

[6] De Campos, A.M., Sanchez,A., and Alonso, M.J. Chitosan nanoparticles: a new vehicle for the improvement of the delivery of the delivery of drugs to the ocular surface. Application to cyclosporine A. Int.J.Pharm. 224:159-68, 2001.

[7]. Jean-Louis Bourges, Sandrine E. Gautier, Florence Delie, Riad A. Bejjani, Jean-Claude Jeanny, Robert Gurny, David BenEzra, and Francine F. Behar-Cohen. Ocular Drug Delivery Targeting the Retina and Retinal Pigment Epithelium Using Polylactide Nanoparticles. Investigative Ophthalmology & Visual Science, Vol. 44, No. 8, 3562-3569, 2003.

[8] Andrew A. Moshfeghi , Gholam A. Peyman, Bascom. Palmer Micro- and nanoparticulates. Advanced Drug Delivery Reviews 57, 2047–2052, 2005.

[9] Angela M. De Campos, Alejandro Sa´nchez, Marı´a J. Alons. Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A. International Journal of Pharmaceutics 224, 159–168, 159-168, 2002.