New surface-active polymers for ophthalmic formulations: evaluation of ocular tolerance

Luma Baydoun, Pascal Furrer*, Robert Gurny*, Christel C. Müller-Goymann
Institut für Pharmazeutische Technologie, Technische Universität Carlsberg-Willeminia zu Braunschweig, Mendelssohnstrasse 1, D-38106 Braunschweig
University of Geneva, School of Pharmacy,30, Quai Ernest-Ansermet, CH-1211 Geneva 4

Introduction

Polymeric hydrogels, based mostly on cellulose derivatives, polyvinyl alcohol, sodium hyaluronate and carborner [1], have been widely used to increase viscosity and consequently retention time of eye drops on the ocular surface. Recently, the use of amphiphilic starch of the n-octylsuccinate starch type (AS) has been described for the formulation of anti-inflammatory eye drops [2]. Due to AS’s emulsifying properties, it is mandatory, when its use is intended for the ocular route, to assess in vivo its ocular tolerance. The aim of the present study was to compare, with respect to their ocular tolerance, different AS types of unknown molecular weights and diverse emulsifying properties regarding viscosity, osmotic activity and reduction of surface tension. To evaluate the preparations’, i.e., solutions (S) and emulsions (E), irritation potential, in vivo ocular tolerance tests were carried out in rabbit eyes applying confocal laser scanning microscopy [3].

Additionally, the tested formulations were incubated with excised porcine corneas. Histological cross sections of treated material were evaluated by light microscopy for pathological modifications caused by an irritant substance.

Materials and methods

Materials

Medium chain triglycerides (MCT 812) and thiemmose (Sythpharm, Barsbüttel, Germany), sorbitol (Hänselel AG, Herrnau, Switzerland), sodium hydroxide (Adtech Chaine S.A., Lusanne, Switzerland), sodium fluorescence (Reactolab, Servion, Switzerland), sodium chloride, potassium dihydrogen phosphate and dextrose (Merck, Darmstadt, Germany) were used to prepare isotonic phosphate buffer pH 7.4 (PBS). AS type 100 and type 300, both emulsifying starches, were supplied by National Starch & Chemical (Manchester, United Kingdom) and Roquette frères (Leinzen, France), respectively. Double-distilled water was used for all preparations.

Experimental methods

Characterisation of AS types

Flow measurements

Rheological properties of AS solutions and emulsions were assessed using a thermostatted (20 °C) rheometer equipped with a concentric cylinder measuring geometry. Viscosity data were derived from the linear region of the flow profile.

Osmolarity

Osmotic activities of AS solutions of different concentrations were analysed by vapour pressure osmometry.

Surface tension

The reduction of surface tension caused by AS 100/300 15% (w/w) at pH 6.5 was measured with a thermostatted-controlled (20 °C) tensiometer provided with a Da Nozy ring.

Tolerance evaluation

Preparation of AS formulations

The AS solutions investigated contained 2% and 15% (w/v) AS of either types. AS w/w-emulsions, stabilized with 15% (w/v) AS 100/300, contained 10% (w/v) MCT 812. Emulsion emulsions (DE) around 1 μm were prepared with a high pressure homogeniser. pH values of all formulations tested were adjusted to 6.5. Osmolarity was adjusted with sorbitol and ranged between 280 and 330 mOsm/kg.

Confocal laser scanning microscopy (CLSO)

25 μl of each test solution were repeatedly applied onto a rabbit’s right cornea (New Zealand albino rabbits of either sex) throughout three days, every 2.5 h 4 times per day and once on the fourth day right before the microscopy experiment. The fluorescent images of the treated rabbit corneas were visualised by CLSO [3]. The total areas of corneal lesions were quantified and expressed in percent of the total corneal surface.

Light microscopy of histological preparations

To evaluate the influence on corneal structure and integrity, corneas, removed from fresh pig eyes [2], was incubated at 37 °C for 2 h in the AS-formulations. PBS and a sodium dodecylsulfate (SDS) solution were used as references. Cross sections, stained with haematoxyline and eosine (H & E), were blinded and microscopically observed for pathological modifications.

Results and discussion

AS 100 at a concentration of 15% (w/w) also lowers surface tension of water by a greater value, 34.2 mN/m, than AS 300 which is 28.6 mN/m. Due to a detectable surface activity, all tested AS systems showed a significantly higher corneal surface damage, ranging from 5 to 29%, as compared to a physiological saline solution (Fig. 3). Nonetheless, the total of fluorescent areas with AS treatment, representing harmed corneal tissue, never exceeded 19% (Figs. 3). Figs. 4 a – c show fluorescent images of three different AS preparations, S 2 and E 15% E 15% Damaged corneal tissue is illustrated as fluorescent spots with a diameter of 7.45% (Fig. 4 a), 8.5% (Fig. 4 b) and 15.1% (Fig. 4 c). This indicates a good tolerance for all AS preparations respecting the tested concentrations. Although AS 100 has more pronounced surface properties than AS 300 both types reveal no notable damage when instilled in concentrations independent of the type of the formulation. The degree of polymerisation seems to have an effect on emulsifying properties but not on the extent of corneal irritation. After incubation in PBS corneal elements like epithelium, stroma and nuclei remain unaffected (Fig. 5 a) as compared to material that has been exposed to an irritant like SDS (Fig. 5 b). Treating corneas with the tested AS formulations leaves corneal structure and integrity visibly unaffected (Fig. 5 c).

Conclusions

Both AS types yield a good cornea tissue acceptability as shown in vivo and in vitro. When comparing different AS types, varying in degree of polymerisation and molecular weight distribution, higher concentrations or superior emulsifying properties do not necessarily increase corneal irritation. Polymer stabilized emulsions can be an appropriate alternative carrier system for highly lipophilic and poorly soluble ocular therapeutics. Hence, AS is a promising new excipient for ophthalmic formulations due to satisfactory solubilising and emulsifying properties coupled with a good eye tolerance.

Acknowledgements

We would like to thank K. Vogel and Dr. Kupisch, Institut für Pathologie, Städtisches Klinikum Celler Straße, Braunschweig, Germany, for support in making and judging histological preparations and A. Düvel, Laurens, Germany for providing us with pig eye material.

References


Luma.Baydoun@ph-tu-bs.de