STUDY OF BENZALKONIUM CHLORIDE DISTRIBUTION IN RABBIT EYES BY MASS SPECTROMETRY IMAGING TECHNIQUES

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Introduction

Benzalkonium chloride (BAK), the most commonly used preservative in eye drops is generally composed of benzododecinium (C22) and myristalkonium (C16) (Figure 1) and is known to increase penetration of active compounds. However, numerous studies have reported its toxic effects on the ocular surface, especially in long-term treatments of maladies such as glaucoma. In this experiment, Mass spectrometry imaging (MSI) is used to characterize the BAK spatial distribution and evaluate its physiopathological impact at the molecular level.

Materials and Methods

Animal preparation and sacrifice
New Zealand albino rabbits were treated with a 0.01% BAK solution (65.7% BAK C22 and 30.7% BAK C16) 2 times 2 drops/day. Rabbits were sacrificed, followed by eye enucleation at T0 (rabbit control), 1h30, 1 and 5 months after the start of treatment. The eyes were stored at -80°C in traganth gum.

Tissue Sectioning
Eye sections of 20µm were obtained by cryostat CM3050S (Leica, Germany) and applied to indium tin oxide (ITO) coated conductive glass slides (Bruker Daltonik GmbH, Bremen, Germany).

Matrix deposition
The MALDI matrix, α-cyano-4-hydroxycinnamic acid (CHCA) matrix was deposited with a pneumatic spray system. The matrix solution was CHCA (10mg/mL) in ACN/TFA 0.1%, (7:3, v/v). After imaging, every section was washed with 100% methanol to eliminate the matrix before H.E. (Hematoxylin/Eosin) staining.

Instrument
After matrix deposition, analyses of eye sections were performed in an Ultraflex III TOF-TOF instrument (Bruker Daltonik GmbH, Bremen, Germany) and an AutoFlex Speed (Bruker Daltonik GmbH, Bremen, Germany). The Ultraflex is equipped with a LIFT III cell and Smartbeam laser with a repetition rate up to 200 Hz. The AutoFlex Speed contains a YAG laser with a repetition rate of 1000Hz.

Results

BAK distribution in different ocular surface structures:

- BAK ions were detected in different structures: cornea, iris, conjunctiva, limbus, retina and near the optic nerve at the optic disc.
- Figure 4 shows the distribution of two BAK ions in three different types of eye samples.
- Control sample revealed no BAK distribution (unlike 1 month sample).
- At 5 months we observed a concentration of BAK in the iridocorneal angle region, which shows a time-dependent accumulation of BAK in specific ocular structures, such as the trabecular meshwork, known as inflammatory area.
- Additional experiments have been done on this area and are presented in Figure 6.

High lateral resolution and FAST-SRM Mode:

- To reach high lateral resolution in MALDI-TOF MS imaging, a laser focus diameter of about 30-20 µm (Smartbeam II) was used. A lateral resolution of 50 µm allowed the detection of the precise localization of the BAK C22 ion and demonstrated its accumulation at the sclerocorneal junction (Figure 6a & 6b).
- To achieve univocal characterization of BAK distribution, the FAST-SRM (Single Ion Monitoring) mode was used. This new method is divided into three parts: (1) Select the parent ion of interest in a precursor ion selector, (2) fragment the drug molecule ions and (3) monitor a single fragment in a very narrow mass spectrum.
- Figure 5a shows the fragmentation spectrum of the BAK C22 ion from a standard eye drop solution, our precursor ion, and this fragment at m/z 212.42 corresponding to a quaternary ammonium alkyl chain.
- Figure 6c shows the distribution of a specific BAK C22 fragment at m/z 212.42 corresponding to the neutral loss of C4H4. Comparison of BAK C22 ion distribution (Figure 6b & 6c) demonstrates the co-localization of parent and daughter ion and validates our earlier results.

Conclusion

We report here the uses of mass spectrometry imaging in the study of the specific distribution of benzalkonium chloride in the eye. We demonstrate the utility of the FAST-SRM mode to obtain identification of interest ion and to decrease the influence of complex background signals. These mass spectrometry techniques offer a powerful tool to investigate the distribution of these kinds of compounds with known deleterious effects and are therefore useful in pharmacological and toxicological preclinical studies.

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