

# A new safety concern for glaucoma treatment demonstrated by mass spectrometry imaging of benzalkonium chloride distribution in the eye, an experimental study in rabbits

Brignole-Baudouin F, Desbenoit N, Hamm G, Liang H, Both JP, Brunelle A, Fournier I, Guerineau V, Legouffe R, Stauber J, Touboul D, Wisztorski M, Salzet M, Laprevote O, Baudouin C

INSERM, U968, Paris, France ; UPMC Univ Paris 06, UMR\_S 968, Institut de la Vision, Paris, France ; CNRS, UMR\_7210, Paris, France ; Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, INSERM-DHOS CIC 503, Paris, France ; Chimie Toxicologie Analytique et Cellulaire, EA 4463, Faculté des Sciences Pharmaceutiques et Biologiques, Université Paris Descartes, Paris, France.

We investigated in a rabbit model, the eye distribution of topically instilled benzalkonium\_(BAK) chloride a commonly used preservative in eye drops using mass spectrometry imaging. Three groups of three New Zealand rabbits each were used: a control one without instillation, one receiving 0.01%BAK twice a day for 5 months and one with 0.2%BAK one drop a day for 1 month.

After sacrifice, eyes were embedded and frozen in tragacanth gum. Serial cryosections were alternately deposited on glass slides for histological (hematoxylin-eosin staining) and immunohistological controls (CD45, RLA-DR and vimentin for inflammatory cell infiltration as well as vimentin for Müller glial cell activation) and ITO or stainless steel plates for MSI experiments using Matrix-assisted laser desorption ionization time-of-flight.

The MSI results were confirmed by a round-robin study on several adjacent sections conducted in two different laboratories using different sample preparation methods, mass spectrometers and data analysis softwares. BAK was shown to penetrate healthy eyes even after a short duration and was not only detected on the ocular surface structures, but also in deeper tissues, especially in sensitive areas involved in glaucoma pathophysiology, such as the trabecular meshwork and the optic nerve areas, as confirmed by images with histological stainings. CD45-, RLA-DR- and vimentin-positive cells increased in treated eyes.

Vimentin was found only in the inner layer of retina in normal eyes and increased in all retinal layers in treated eyes, confirming an activation response to a cell stress. This ocular toxicological study confirms the presence of BAK preservative in ocular surface structures as well as in deeper structures involved in glaucoma disease.

The inflammatory cell infiltration and Müller glial cell activation confirmed the deleterious effect of BAK. Although these results were obtained in animals, they highlight the importance of the safety-first principle for the treatment of glaucoma patients.

PLoS One. 2012;7(11) :e50180. doi: 10.1371/journal.pone.0050180. Epub 2012 Nov 27.

PMID: 23209668

<http://www.ncbi.nlm.nih.gov/pubmed/23209668>