

Neurocytometry: A New Method to Assess Ocular Pain-Associated Markers in Trigeminal Ganglion

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Abstract

Purpose : Ocular pain is a common symptom of dry eye disease (DED). However, studying ocular pain at a molecular level has been challenging due to the limited number of cornea-innervating neurons in the trigeminal ganglion (TG), the laborious process to prepare the tissue for immunohistochemistry and challenges in quantifying the results. Here, we introduce a new approach to specifically quantify corneal-innervating TG neurons through retrograde labeling and assess their Fos expression, the molecular marker of neuronal activation, with a flow cytometric approach.

Methods : 6-week-old C57BL/6 female mice were housed under desiccating stress in a controlled environment chamber for 14 days to induce DED. The right eye of the DED animals and healthy control (N= 6 animals/ group) was injected intrastromally with Alexa Fluor™ 647 Conjugated-recombinant Cholera Toxin Subunit B (Ctb), 4 days prior to sacrifice. The ipsilateral trigeminal ganglion was collected and enzymatically digested using papain to prepare single cell suspension, which was subsequently fixed, permeabilized and stained with antibodies against NeuN (neuronal nuclear antigen) and Fos. The cell suspensions were analyzed using BigFoot Spectral Cell Sorter. All data were compared via student T-test.

Results : There were no significant differences among the numbers of retrograde labeled neurons (NeuN⁺ Ctb⁺) between DED animals and healthy controls (286 ± 38 vs. 366 ± 80). There was a significantly higher percentage of Fos-positive retrograde labeled neurons (NeuN⁺ Ctb⁺ Fos⁺) in DED subjects when compared with healthy controls (58.6 ± 0.6% vs. 88.7 ± 1.4%; $P < 0.05$). The mean fluorescence intensity of Fos protein among the traced Fos-positive population was nearly doubled in DED group

compared to the control group (6706.5 ± 297.6 vs. 3620.7 ± 211.1 ; $P < 0.01$).

Furthermore, the frequency of untraced Fos-positive neurons ($\text{NeuN}^+ \text{Ctb}^- \text{Fos}^+$) were similar in the two groups ($40.0 \pm 4.5\%$ vs. $41.8 \pm 3.1\%$). Accordingly, the mean fluorescence intensity of Fos protein among the untraced Fos-positive population had no significant difference either (1007.8 ± 22.2 vs. 1043.4 ± 27.8).

Conclusions : Flow cytometric analysis of specific cornea-innervating neurons in trigeminal ganglion is a relatively efficient and more accurate method to assess expression of proteins associated with ocular pain.

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