IN VITRO ASSESSMENT OF SHAMPOO EYE STINGING POTENTIAL USING TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 (TRPV1)

INTRODUCTION

Nociceptive sensation is a complex and reflexive change that is usually followed by an inflammatory cascade. This cascade may generate a chemical-induced pain, a general integrator of chemically induced pain in the surface of the eye by direct activation or by a secondary reaction.

In order to evaluate ability of 10 shampoo formulas to activate TRPV1, we used a neuronal-like sensory cell model SH-SY5Y surexpressing TRPV1. These formulas have been selected considering their clinical eye comfort classification. TRPV1 activation was assessed by measurement of cellular calcium influx using Fura2-AM, a fluorescent calcium indicator. In this way, the calcium influx through this channel is increased upon TRPV1 activation.

RESULTS

• The effects of all ten test products on Ca2+ influx in the TRPV1-SH-SY5Y cells have been illustrated in the concentration-effect curves (Figure 3).
• Two treatment groups have been identified, according to the Ca2+ influx results: 1) products without increase in Ca2+ influx, 2) products with increase in Ca2+ influx by TRPV1.
• In fact, the addition of capsaicin addition resulted in an increase in ECS5 associated to these shampoos.
• The 2 last products induce a Ca2+ influx which didn’t been to be mediated by TRPV1 (Figure 3, Case C). Since the addition of capsaicin had no effect on calcium influx profile.

ANALYSIS OF THE IN VITRO DATA

COMPARISON WITH CLINICAL DATA

In order to classify products, we’ve analyzed the effect of 0.1% concentration and we’ve considered the percentage of calcium influx induced by the product compared with the effect of 10 µM capsaicin.

CONCLUSIONS & NEXT STEPS

• These results confirmed that TRPV1 target is an interesting biological target to understand and study eye stinging.
• Good trend in the correlation with the in vivo data.
• It’s important to get a better understanding of TRPV1 independent calcium influx and how such a profile could influence the evaluation.

MATERIALS AND METHODS

1. Cells: The SH-SY5Y-TRPV1 cells were cultured in 96-well plates with transparent bottom at a cell density of 20,000-40,000 cells/well. The medium was changed after 4 days in culture in the two-dose culture.

2. Test products: Two shampoo formulas were evaluated in this study. These formulas had been selected to represent a range of no eye sting and eye sting shampoo concerning clinical data. Each shampoo was diluted in KRB-Hepes buffer (KRB) and evaluated in 8 concentrations.

3. Ca2+ measurements: The cells were loaded with 2 µM Fura2-AM in cell culture medium for 30 minutes in 37°C and then rinsed twice with KRB. To ensure TRPV1 specific responses, a pretreatment of 30µM of capsazepine was realized 30 minutes in darkness before test compound injection.

4. Data analysis: The effect was determined as the maximum fluorescence reached during the registration and expressed as the percentage increase with the fluorescence induced by capsaicin 10µM without capsazepine, as a function of concentration.

• Figure 3: Calcium influx concentration-effect curves from different shampoo formulas.

REFERENCES