Using TRPV1 Channel Activity to Predict the Eye Stinging Potential of Baby Cleansers

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INTRODUCTION

- **The TRPV1 channel is a well characterized pain–inducing receptor that is expressed in sensory nociceptors which can be activated by chemical stimuli**
- **Corneal and mucosal tissue in conjunctiva are rich in innervations which express TRPV1 channels**
- **Our hypothesis is that TRPV1 may be a general mediator of chemically induced pain on the surface of the eye**
- **We sought to test our hypothesis by evaluating the eye sting (pain) potential of 19 baby shampoos which had been previously evaluated in human clinical eye sting tests**
- **No current *in vitro* assay has demonstrated the ability to predict the human sting potential of personal care products which may come in contact with the eyes**
- **An *in vitro* assay capable of identifying the eye sting potential of personal care products would be very beneficial as a pre-clinical screening tool**

"NOCIOCULAR" *IN VITRO* ASSAY

A TRPV1 expressing clone of the human SH-SY5Y neuroblastoma cell line (Figure 1) was obtained by stable transfection, using puromycin-containing selection medium. Prior to Ca^{2+} measurements the TRPV1-SH-SY5Y cells were cultured in 96-well plates to confluency. Acute increase in the intracellular free Ca^{2+} level was measured in a semi-HTS fluorescence reader (FlexStation II, Molecular Devices) using Fura-2/AM. The ratio of fluorescence at 340 (Ca^{2+}-bound Fura-2)/380 (Fura-2) nm excitatory wavelengths was registered without interruption before and during the 2 min exposure to the test compounds. The mean value (% increase of basal Ca^{2+} level) from triplicate wells in the 96-well plate was monitored for each concentration from each experiment. The TRPV1 antagonist capsazepine was added simultaneously with each concentration of the chemicals in three sister wells to confirm TRPV1-mediated Ca^{2+} influx. The intracellular Ca^{2+} increase induced by the specific TRPV1-agonist capsaicin was set to 100% response for each experiment and the effect of the test products was calculated as percent of the capsaicin induced response. All test compounds were diluted in HKR-buffer and the addition to the cells was performed robotically during measurements by the FlexStation II reader.

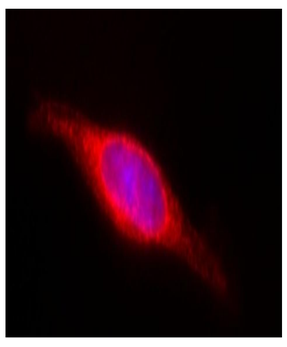

Figure 1. TRPV1-SH-SY5Y cell. TRPV1 expression is visualized by primary TRPV1 antibodies and Alexa fluor red 568-conjugated secondary antibodies (red); the nucleus is stained with Hoechst (blue).

TEST SAMPLES

For the 20 test products tested in this study, 18 were new product formulations designed to be baby shampoo or bath products with a mild eye sting profile. The test samples were formulated with standard surfactants, conditioning agents, and thickening agents including polymers, preservatives, fragrances, pH adjusters, and in some cases other skin benefit agents. Example ingredients within these test products included:

sodium laureth sulfate, trideceth sulfate, cocoamidopropylbetaine, sodium lauroamphoacetate, cocoglucoside, polyquaternium-10, PEG80 sorbitan laurate, sodium benzoate, quaternium-15, and phenoxyethanol.

Negative Control: A commercially available baby shampoo that has been shown to be non-stinging in human ocular instillation studies (JOHNSON'S® baby shampoo).

Postive Control: A commercially available adult shampoo that does not claim non-stinging and that contains cocoamide MEA (CMEA), a known stinging ingredient.

The negative control and 18 new product formulations had been previously tested in human ocular instillation studies. The negative and positive control and several of the new product formulations were also tested in a battery of sensitive *in vitro* eye irritation assays. None of the *in vitro* eye irritation assays had a positive correlation with the human clinical sting data for the products tested.

DATA ANALYSIS

Table 1. Criteria for classification of a product to be stinging to the eye by using the NociOcular Assay.

Test parameter	Cut off level
Emax (% of capsaicin response)	≥ 24
EC50 (concentration inducing 50% effect of Emax)	≤ 0.03
Effect at the concentration 0.032 %	≥ 22

RESULTS

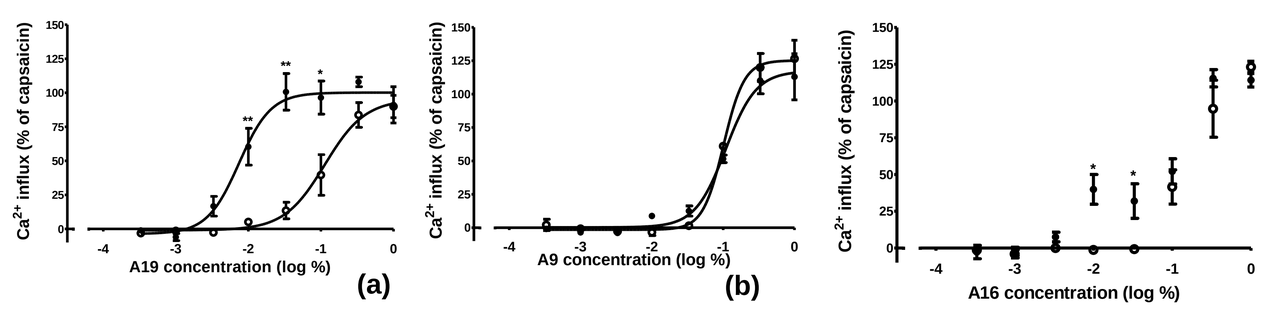

Figure 2. Representative concentration-dependent effect curves illustrating Ca^{2+} influx for (a) one significant eye-stinging product and (b) one non-stinging product. Filled circles show the effect of the product without capsazepine, open circles show the effect of the product with capsazepine. Data are presented as mean +/- SEM of three or four independent experiments, each performed in triplicates. *p≤0.05, **p≤0.01 as compared to +capsazepine.

Figure 3. One representative concentration-effect curve of a product displaying a bi-phasic response in the Ca^{2+} influx. Filled circles show the effect of the product without capsazepine, open circles show the effect of the product with capsazepine. Data are presented as mean +/- SEM of three or four independent experiments, each performed in triplicates. *p≤0.05 as compared to +capsazepine

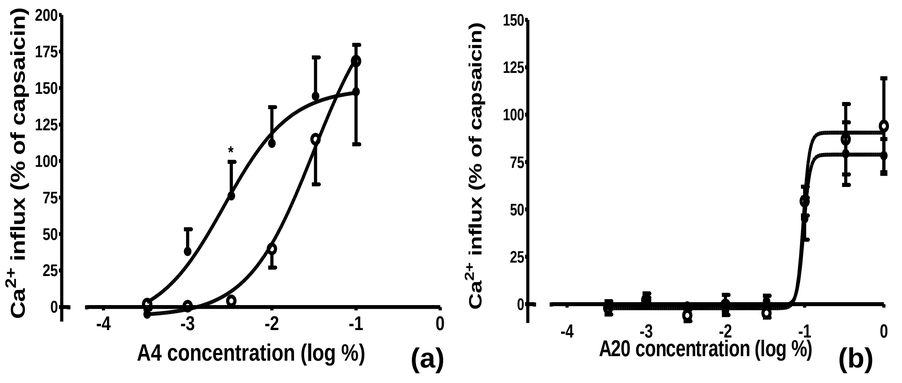

Figure 4. The concentration dependent effect of (a) the positive control (A4) and (b) the negative control (A20/JOHNSON'S® baby shampoo) eye stinging control product. Filled circles show the effect of the product without capsazepine, open circles show the effect of the product with capsazepine. Data are presented as mean +/- SEM of three or four independent experiments, each performed in triplicates. Only one direction bars are shown in a) for clarity. *p≤0.05 as compared to +capsazepine.

Table 2. Quantitative effects of the products on the TRPV1 channel activity. Clinical sting positives in red text. False positives highlighted in orange, false negative in blue.

Test Sample	EC50±S.E.M. (%)	Emax±S.E.M. (% of capsaicin effect)	Effect at 0.032% ±S.E.M. (% of capsaicin effect)	Stinger according to NociOcular	Human Ocular Instillation Test for Sting (Yes/No)
A1	**0.0077±0.0017**	**60±23**	**54±24**	**Yes**	**Yes**
A2	N.C.	<20	<10	No	No
A3	N.C.	<20	<10	No	No
A4* (+ control)	**0.0029±0.0015**	**159±41**	**144±38**	**Yes**	**Yes***
A5	0.29±0.027	1227±16	95±19	No	No
A6	N.C.	<20	21±15	No	No
A7	N.C.	<20	<10	No	No
A8	N.C.	<20	<10	No	No
A9	N.C.	<20	<10	No	No
A10	**0.0091±0.0009**	**30±7**	**23±8**	**Yes**	**Yes**
A11	0.020±0.011	105±29	75±7	Yes	No
A12	0.043±0.0016	51±15	3±3	No	No
A13	**0.011±0.0011**	**54±21**	**52±24**	**Yes**	**Yes**
A14	**N.C.**	**<20**	**<10**	**No**	**Yes**
A15	0.013±0.0035	87±11	79±6	Yes	No
A16	**0.0056±0.003**	**44±17**	**32±17**	**Yes**	**Yes**
A17	**0.025±0.015**	**42±1**	**22±8**	**Yes**	**Yes**
A18	0.014±0.0032	23±10	21±6	No	No
A19	**0.0084±0.0032**	**101±23**	**101±23**	**Yes**	**Yes**
A20[1] (- control)	N.C.	<20	<10	No	No

*Positive control, an adult shampoo product that does not claim non-stinging and that contains cocoamide MEA (CMEA), a known stinging ingredient. Hypothesized to be stinging, but was not tested in the human ocular instillation test.

[1] Negative control (JOHNSON'S® baby shampoo); N.C. Not converged to curve fit

Table 3. Results of clinical sting test and available *in vitro* eye irritation data for test products. Clinical sting positives highlighted in red text.

Product	Human Ocular Instillation Test for Sting (Yes/No)	EpiOcular ET_{50} (hours)	Cytosensor MRD_{50} (mg/mL)	NRU NRU_{50} (µg/mL)	TEP EC_{50} (%)
A1	**Yes**	7.8	3.99	103	2.29 ± 1.35
A2	No	11.6	2.36	29.8	3.96 ± 0.053
A3	No	22.1	3.72	106	NA
A4*(+)	**Yes**	<1	0.519	16.2	NA
A5	No	11.4	2.05	41.6	4.31 ± 0.59
A6	No	3.0	1.31	53.0	NA
A8	No	9.6	2.17	45.2	NA
A12	No	9.0	2.88	44.3	NA
A13	**Yes**	18.6	5.19	195	6.17 ± 0.59
A18	No	12.0	2.84	132	3.47 ± 0.81
A19	**Yes**	3.3	1.62	55.1	3.11 ± 0.38
A20[1](-)	No	8.3	2.87	80.3	4.19 ± 1.25

*Positive control, an adult shampoo product that does not claim non-stinging and that contains cocoamide MEA (CMEA), a known stinging ingredient. Hypothesized to be stinging, but was not tested in the human ocular instillation test. [1] Negative control (JOHNSON'S® baby shampoo)

CONCLUSIONS

- **Our data show that 6/7 formulations that induced stinging in the human test were positive in the NociOcular assay, as was the positive control; 10/12 that did not induce sting in the human test were negative in the NociOcular assay.**
- **There was no correlation between the clinical sting results and data generated from the four sensitive *in vitro* eye irritation assays.**
- **Our data support that the TRPV1 channel is a principle mediator of eye stinging sensation induced by baby bath and shampoo formulations and that the NociOcular assay may be a valuable *in vitro* tool to predict human eye sting sensation.**

ACKNOWLEDGEMENTS

This work was funded by JOHNSON & JOHNSON Consumer and Personal Products Worldwide.