Using Novel In Vitro NociOcular Assay Based on TRPV1 Channel Activation for Prediction of Eye Sting Potential of Baby Shampoos

Anna Forsby,*† Kimberly G. Norman,‡ Johanna EL Andaloussi-Lilja,* Jessica Lundqvist,* Vincent Walczak,† Rodger Curren,† Katharine Martin,‡ and Neena K. Tierney‡

*Department of Neurochemistry, The Arrhenius Laboratories for Natural Science, Stockholm University, SE-10691 Stockholm, Sweden; †Institute for In Vitro Sciences, Gaithersburg, Maryland 20878; and ‡Johnson & Johnson Consumer and Personal Products Worldwide, Skillman, New Jersey 08558

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The transient receptor potential vanilloid type 1 (TRPV1) channel is one of the most well-characterized pain-inducing receptors. The purpose of this study was to predict human eye stinging of 19 baby bath and shampoo formulations by studying TRPV1 activity, as measured by increase in intracellular free Ca2+. The NociOcular test, a novel recombinant neuronal in vitro model with high expression of functional TRPV1 channels, was used to test formulations containing a variety of surfactants, preservatives, and fragrances. TRPV1-specific Ca2+ influx was abolished when the TRPV1 channel antagonist capsazepine was applied to the cells prior to shampoo samples. The positive control, an adult shampoo that contains cocamide monoethanolamine (CMEA), a known stinging ingredient, was the most active sample tested in the NociOcular test. The negative control, a marketed baby shampoo, was negative in the NociOcular and human tests. Seven of the formulations induced stinging in the human test, and of those six were positive in the NociOcular test. Twelve formulations were classified as nonstinging in the NociOcular test. There was no correlation between the clinical stinging results for the baby formulations and the data generated from other in vitro eye irritation assays (cytensorsor microphysiometer, neutral red uptake, EpiOcular, transepithelial permeability). Our data support that the TRPV1 channel is a principal mediator of eye-stinging sensation induced by baby bath and shampoo formulations and that the NociOcular test may be a valuable in vitro tool to predict human eye–stinging sensation.

Key Words: Eye sting; in vitro assay; nociception; TRPV1; baby shampoo; NociOcular

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assay (NRU), cytosensor microphysiometer assay, transepithelial permeability assay (TEP), and assays using human cell constructs such as EpiOcular assay (Landin et al., 2007; Martin and Stott, 1992; Stern et al., 1998; Wallace et al., 1992). However, none of these in vitro assays have previously been shown effective as sensitive biomarkers for stinging sensation or noiception, symptoms that are not necessarily correlated to mild eye irritation.

For sensory irritation such as the ocular stinging sensation, various predictive animal and in vitro models have been proposed (Beuerman et al., 1992; Inoue et al., 1996; Laden, 1973; Lenoir et al., 2011; Shanahan and Ward, 1975). Although Inoue et al. (1996) have shown that certain cosmetic ingredients, which are known or expected to cause ocular stinging, affect the electrical properties of cultured neuronal cells, to date, no study has demonstrated the ability of a single in vitro assay to be capable of predicting the in vivo human stinging or pain response of personal care formulations containing a variety of ingredients that may cause stinging. Therefore, the standard approach today is to use in vivo human studies to assess any sting or pain associated with the use of mild personal care products, such as baby shampoos and baths. Human clinical testing can be time consuming and expensive; thus, the identification of an in vitro assay that can effectively identify ocular stinging potential especially among mild personal care products would be immensely beneficial in preclinical screening of new product formulations, especially in the early development stages. In addition, because in vitro testing can be more readily conducted, a robust ocular stinging assay can be a tool to advance the understanding of the relative contributions to ocular sting of various ingredients within personal care products.

In this study, 20 personal care cleansing products provided by Johnson & Johnson were tested to determine the potential of the NociOcular in vitro assay in predicting ocular sting. For each of the 19 mild baby bath and shampoo formulations, human clinical ocular instillation testing to determine eye-stinging responses had been previously performed (described later in the Materials and Methods section). The test products were then blind coded and sent to the testing lab (Department of Neurochemistry, Stockholm University, Sweden), and results were compiled and sent to an independent lab (Institute for In Vitro Services, Gaithersburg, MD) for decoding and comparison between the clinical eye sting data and TRPV1-activating responses.

MATERIALS AND METHODS

Test products. Baby shampoo and bath test products were formulated with standard surfactants, conditioning agents, thickening agents including polymers, preservatives, fragrances, pH adjusters, and in some cases other skin benefit agents. It is well understood that for certain combinations and at high levels, some surfactants, conditioning agents, preservatives, and fragrances can result in ocular sting or pain. Example ingredients within these test products included sodium laureth sulfate, trideceth sulfate, cocamidopropyl betaine, sodium lauroamphocetocet, cocoglucoside, polyquaternium-10, PEG 8 sorbitan laurate, sodium benzoate, quaternium-15, and phenoxethanol.

Of the 20 test products tested in this study, 18 of them were new product formulations, which were designed to be baby shampoo and bath products that have a mild ocular stinging profile. The test products were formulated with various combinations of the ingredients described in the previous paragraph and represented a range of baby shampoo and bath formulation design. Additionally, a commercially available adult shampoo that does not claim non-stinging and that contains cocamide monoethanolamine (CMEA), a known stinging ingredient, was used as a positive control. A commercially available baby shampoo that has been shown to be nonstinging through evaluation in the human ocular instillation clinical study described below, Johnson’s baby shampoo, was used as the negative control. The test products, including the positive and negative controls, were tested in the in vitro tests as described below. In addition, the 18 new product formulation test products and the marketed baby shampoo negative control were tested in the human ocular instillation clinical study (described below). The positive control was not tested in human ocular instillation clinical studies because it contained the stinging ingredient CMEA and also was shown to have a stronger eye irritation response when evaluated in the other in vitro tests.

The NociOcular assay. Human neuroblastoma SH-SY5Y cells have been stably transfected by using a plasmid containing the rat trpv1. A constitutively high expression of functional receptors was previously shown by RT-PCR, Western blot, immunofluorescence, and capsaicin-induced Ca2+ increase in the cytoplasm (Lilja et al., 2007). The SH-SY5Y-TRPV1 cells were plated in Eagle’s minimal essential medium with Earle’s salts, 10% fetal bovine serum, 1% nonessential amino acids, 2mM L-glutamine, 100 µg streptomycin/ml, 100 units penicillin/ml, and 0.4 µg/ml puromycin (all from Gibco, Life Technologies, Stockholm)—in transparent 96-well plates (Corning) at a density of 3 × 104 cells/well. The TRPV1 activity was monitored in confluent cell cultures as an increase in the intracellular free Ca2+ concentration, [Ca2+]i, using Fura 2-AM (Sigma), which is hydrolyzed intracellularly to cell membrane-impermeable Fura 2. The cells were incubated in the cell culture medium with 2µM Fura-2/AM for 30 min in 37°C followed by one washing step in Krebs-Ringer HEPES (KRH) buffer (125mM NaCl, 5mM KCl, 1.2mM MgSO4 × 7H2O, 2mM CaCl2, 1.2mM KH2PO4 × 2H2O, 25mM HEPES [free acid], and 6mM glucose, pH adjusted to 7.4 with 1M NaOH) followed by incubation in KRH buffer for 10 min to achieve complete hydrolysis. The Fura 2 fluorescence is increased at an excitation wavelength of 340 nm and decreased at 380 nm (em 510) upon binding Ca2+. The ratio between the two excitation wavelengths gives a sensitive relative measurement of the intracellular free Ca2+ concentration, which is independent of the cell density. An automatic fluorescence reader FlexStation II (Molecular Devices, Sunnyvale, CA) was used to monitor the change in [Ca2+]i every 2 s without interruption for 17 s to obtain a baseline registration before addition of the test products and during the automatic addition of the test products and for the following 100 s to register the full response of the products. Test products, personal care formulations, were diluted in eight concentrations using KRH buffer, with 6% as the highest concentration. KRH was used as a reference, and 600µn capsaicin was prepared as the positive control (set to 100%). Twenty microliters of each concentration was added automatically to each well containing Fura 2–loaded cells and 100 µl KRH buffer only or KRH buffer with 30µM capsazipene, the specific TRPV1 antagonist. Every concentration of the test products or capsaicin was applied to six of the wells in a column: the three upper wells in KRH only and the lower three wells in KRH containing capsazipene. Hence, the final concentrations applied to the cells were one sixth of the initial dilution series. The effect of the test products with or without capsaizipene was determined as the area under the curve of the monitored fluorescence intensity (FI) during the registration (SoftMax, Molecular Devices) and expressed as the percent increase in comparison with the FI induced by capsaicin, as a function of concentration. A difference between the two concentration-effect curves indicated whether the test product induces TRPV1-specific Ca2+ fluxes and thus a possible pain induction/neurogenic inflammation in vivo.

Clinical testing of eye sting. Human ocular instillation clinical studies were performed with approval from an independent institutional review board, under supervision of an ophthalmologist, and following the principles of the Declaration of Helsinki. A panel of adult subjects, aged 18–70, with
no history of chronic or ocular diseases, signed a written informed consent form prior to study initiation. Diluted test product and sterile distilled water were randomly instilled into each eye, with ophthalmologist and human subjects both blinded to the randomization. The baby shampoo and bath test materials were diluted with sterile distilled water and warmed to 37°C–38°C immediately prior to the ocular instillation study. While the lower eyelid was extracted downward, one drop of each diluted test material was instilled into the inferior cul de sac. Within 30 s following instillation, eyes were examined and scored by the ophthalmologist. Eyes were examined and assessed again by the ophthalmologist at 15 min and 1 h postinstillation. Examinations at each time point also included scoring of subject-perceived ocular stinging or pain (0–3 scale, 0 = within normal limits, 3 = severe) compared to sterile distilled water control on the contralateral eye.

Neutral red uptake bioassay. The NR cytotoxicity assay is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and accumulate neutral red (NR, obtained from Sigma) into functional lysosomes, thereby allowing discrimination between viable or dead cells. Normal human epidermal keratinocytes (obtained from Lonza, Walkersville, MD) were treated with test samples diluted over a range of concentrations in culture medium for 48 h according to procedures described in Wallace et al. (1992). The amount of incorporated NR was measured spectrophotometrically, and data were analyzed to determine an NRU50 value, which is the test article concentration that induces a 50% decrease in metabolic rate) was identified or until the high-

Cytosensor microphysiometer bioassay. The cytosensor microphysiometer (Molecular Devices Corporation, Menlo Park, CA) measures the extracellular acidification rate of cell cultures, which is indicative of cellular toxicity. Procedures were performed according to those previously described in Landin et al. (2007). Each study sample was dissolved in low-buffered Dulbecco’s Modified Eagle’s Medium and administered directly onto the cells. L929 cells were selected because they have been shown to provide a sensitive indication of toxic effects. Cells within a capsule cup were exposed to each concentration of study sample for ~810 s, after which the study sample was rinsed out of the sensor chamber with fresh medium. The acidification rate was immediately measured after washout of the sample. Dosing was conducted by testing lower concentrations first and gradually increasing the dose until the MD50 (the dose that induces a 50% decrease in metabolic rate) was identified or until the highest concentration was dosed.

EpiOcular assay. The EpiOcular assay was used to evaluate the potential toxicity of the study samples, using stratified human epithelial cell cultures (MatTek, Ashland, MA) according to methods described in Stem et al. (1998). These cell models were exposed to topically applied study samples, and cell viability was determined by conversion of 3-[4,5-dimethylthiazol-2-y1]2,5-di phenyltetrazolium bromide (MTT) in the treated cultures and was expressed as a percentage relative to negative control–treated cultures. The endpoint of the EpiOcular bioassay is the ET90 which is the time of exposure to test article required to reduce cell viability (MTT metabolism) to 50% of negative control levels as calculated from time-response curves.

TEP assay. The TEP assay was used to measure the chemical-induced loss of transmembrane impermeability of a confluent epithelial monolayer. The TEP assay was performed on a confluent monolayer of nonreplicating cells, which are grown on permeable insert. Madin-Darby canine kidney (MDCK) renal tubular epithelial cells are used because they form tight junctions and desmosomes similar to those found in the corneal epithelium. Following exposure to the test product, the monolayer is thoroughly rinsed and the marker dye sodium fluorescein is placed into the upper well of the insert (apical surface). During a short incubation period, the dye will pass through areas of the monolayer where the tight membrane junctions have been disturbed. Damage is evaluated spectrophotometrically by measuring the amount of marker dye that leaks through the cell layer and microporous membrane to the lower well. Various concentrations of each formulation are evaluated in the assay. Each concentration is tested in duplicate, and the optical density readings of the receiver fluid (lower well) are recorded. The collected data are analyzed by probit analysis, and an EC50 value is generated for each formulation and used to predict the eye irritation potential.

Data analysis. Concentration–effect curves were obtained using GraphPad Prism 5.0. For calculating quantitative effects in the NociOcular test, only concentrations up to 0.1% were included to avoid unspecific Ca2+ influx, which was related to cytotoxicity. The concentrations generating 50% of the maximum effect (E50), the maximum effect (Emax), and the effect at 0.032% (E at 0.032%, weight/vol) were determined when possible. Student’s t-test was used to analyze statistically significant differences between TRPV1–specific Ca2+ influx, i.e., an effect of the products without or with capsazepine present, at the given concentrations.

For the human ocular instillation test, comparison between the diluted test product and sterile distilled water was conducted at each time point (30 s, 15 min, and 1 h). The median treatment difference in ocular sting rating was tested by a one-sided exact matched pairs Wilcoxon signed-ranks test. A test product was classified as “nonstinging” if the treatment difference in eye stinging had a value > 0.05, when compared with sterile distilled water; a test product was classified as “stinging” if the treatment difference in eye stinging had a p value < 0.05, when compared with sterile distilled water.

Upon completion of the NociOcular testing, the results were sent to an independent laboratory (Institute for In Vitro Sciences, Inc. [IIVS]) for comparison with the human ocular instillation data, which was sent to IIVS from J&J. Given the prediction criterion of the NociOcular assay and the Yes/No results of the human sting test, test samples were identified as correctly or incorrectly predicted by the NociOcular assay. Results of the in vitro assays performed to assess the eye irritation potential of the test samples were ranked from lowest to highest irritation potential and then compared with the results of the clinical sting test to assess the potential of the in vitro eye irritation assays to predict clinical sting response.

RESULTS

All 20 products were tested blindly in the NociOcular assay at eight fixed concentrations ranging from 1 to 0.00032% dilutions in KRH buffer (w/v). Nine of the products were shown to be TRPV1 channel agonists (Fig. 1a). The other 11 products induced Ca2+ influx at considerably higher concentrations, and this effect could not be abolished by capsazepine (Fig. 1b). Hence, the Ca2+ influx induced by these products was related to an unspecific, possibly cytotoxic response. Five of the nine products possessing TRPV1 channel agonist activity induced a biphasic increase in the [Ca2+]i (Fig. 2). The response at low concentrations was abolished by the TRPV1 channel antagonist capsazepine. Hence, a TRPV1 channel–specific effect was evident, whereas the effect could not be blocked at the highest concentration tested. After decoding the test products, it was confirmed that the positive control (the commercially available adult shampoo) induced a TRPV1 channel–specific activity displayed as a Ca2+ influx, which was possible to block with capsazepine. The negative control (the commercially available baby shampoo, Johnson’s baby shampoo) did not induce any TRPV1 channel–specific effect (Fig. 3).

To avoid concentrations generating a nonspecific response when estimating TRPV1 channel–specific EC50 and Emax, the two highest concentrations were omitted in all calculations. The quantitative effects were analyzed as EC50, Emax, and E at
When using the prediction model presented in Table 1, seven of the eight products with clinical eye-stinging activity were positively identified, two products were false positive, and one product was a false negative in the NociOcular assay (Table 2).

In addition, several of the baby shampoo and bath products, plus the negative and the positive control, were evaluated in a battery of well-established in vitro assays used for predicting eye irritation (Table 3). The positive control showed the strongest response in each in vitro eye irritation assays with an ET$_{50}$ value of less than 1 h in the EpiOcular assay, a MRD$_{50}$ value of 0.519 mg/ml for the cytosensor microphysiometer assay, and an NRU$_{50}$ value of 16.2 µg/ml in the NRU assay. This result is not surprising as most adult shampoos would generally be considered to be less mild than baby shampoos or bath products because they are not marketed or formulated to be ultramild or nonstinging. On the other hand, baby products are formulated to be ultramild in terms of ocular irritation and stinging, and it is within this ultramild irritation range that we sought to distinguish stinging products from nonstinging products. For the negative control, the commercially available baby shampoo, Johnson’s baby shampoo, which was known to be nonstinging, the in vitro eye irritation results demonstrate an ultramild irritation potential. Also, the other baby shampoo and bath products for which in vitro eye irritation data are available similarly show an ultramild irritation potential. However, when considering the ability of these in vitro eye irritation tests to predict the human ocular sting response, within this narrow range of irritation responses, no correlations were observed.

**DISCUSSION**

The structure, composition, and functioning of baby’s eyes continue to develop over the first year of life (Dahl and Dahl, 1985; Isenberg et al., 2003; Kayed et al., 2008; Lawrenson et al., 2005; Nakamori et al., 1997; Toker et al., 2002). Babies blink less often, their eyes are open for a longer time, and tearing of their eyes is less robust. In addition, babies tend to rub their eyes often, and they continue to fine-tune their involuntary blinking strategies (defensive eye closure) up to 5–7 months of life. Because of these reasons, substances can be more easily rubbed into their eyes, and there is a greater potential for their eyes to be inadvertently exposed to shampoo or bath products used during bath time. Thus, for baby personal care products such as shampoo or bath products, it is imperative to evaluate their ocular irritation potential to ensure that they are ultramild and safe and appropriate for use on babies.

Nineteen baby shampoo and bath products were tested in the novel NociOcular assay, which is based on specific TRPV1 activation in recombinant neuroblastoma SH-SY5Y cells. The hypothesis that eye irritation could be correlated to nociceptor...
activation was first proposed by Garle and Fry (2003). A year later, it was shown that soaps and shampoos that were, by experience, painful when applied to the eye could activate the TRPV1 channel in a transiently expressing cell model, whereas products that were marketed as nonstinging or were assumed not to cause pain did not affect the TRPV1 channel (Lilja and Forsby, 2004). The results were later confirmed in a stably TRPV1 channel expressing cell clone (Lilja et al., 2007). For the first time, in this work, we show a correlation between eye nociception in humans and TRPV1 channel activation using an in vitro assay.

For decades, other in vitro assays have been studied with the aim to phase out the Draize’s eye irritation test. The BCOP test and the Isolated Chicken Eye test are now accepted as alternative in vitro assays for estimation of severe eye irritation.

**TABLE 1**
Criteria for Classification of a Product to Be Stinging to the Eye by Using the NociOcular Assay

<table>
<thead>
<tr>
<th>Test parameter</th>
<th>Cutoff level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emax (% of capsaicin response)</td>
<td>≥ 24</td>
</tr>
<tr>
<td>EC50 (concentration inducing 50% effect of Emax)</td>
<td>≤ 0.03</td>
</tr>
<tr>
<td>Effect at the concentration 0.032%</td>
<td>≥ 22</td>
</tr>
</tbody>
</table>

**TABLE 2**
Quantitative Effects of the Products on the TRPV1 Channel Activity

<table>
<thead>
<tr>
<th>Product</th>
<th>EC50 ± SEM (%)</th>
<th>Emax ± SEM (% of capsaicin effect)</th>
<th>Effect at 0.032% ± SEM (% of capsaicin effect)</th>
<th>Stinger according to NociOcular</th>
<th>Stinger, as classified by human ocular instillations test</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.0077±0.0017</td>
<td>60±23</td>
<td>54±24</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>A2</td>
<td>N.C.</td>
<td>&lt; 20</td>
<td>&lt; 10</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>A3</td>
<td>N.C.</td>
<td>&lt; 20</td>
<td>&lt; 10</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>A4 (+ control)</td>
<td>0.0029±0.0015</td>
<td>159±41</td>
<td>144±38</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>A5</td>
<td>0.11±0.12</td>
<td>56±20</td>
<td>21±17</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>A6</td>
<td>N.C.</td>
<td>&lt; 20</td>
<td>21±15</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>A7</td>
<td>N.C.</td>
<td>&lt; 20</td>
<td>&lt; 10</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>A8</td>
<td>N.C.</td>
<td>&lt; 20</td>
<td>&lt; 10</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>A9</td>
<td>N.C.</td>
<td>&lt; 20</td>
<td>&lt; 10</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>A10</td>
<td>0.0091±0.0009</td>
<td>30±7</td>
<td>23±8</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>A11</td>
<td>0.020±0.011</td>
<td>105±29</td>
<td>75±7</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>A12</td>
<td>0.043±0.0016</td>
<td>51±15</td>
<td>3±3</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>A13</td>
<td>0.011±0.0011</td>
<td>54±21</td>
<td>52±24</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>A14</td>
<td>N.C.</td>
<td>&lt; 20</td>
<td>&lt; 10</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>A15</td>
<td>0.013±0.0035</td>
<td>87±11</td>
<td>79±6</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>A16</td>
<td>0.0056±0.003</td>
<td>44±17</td>
<td>32±17</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>A17</td>
<td>0.025±0.015</td>
<td>42±1</td>
<td>22±8</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>A18</td>
<td>0.014±0.0032</td>
<td>23±10</td>
<td>21±6</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>A19</td>
<td>0.0084±0.0032</td>
<td>101±23</td>
<td>101±23</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>A20 (−control)</td>
<td>N.C.</td>
<td>&lt; 20</td>
<td>&lt; 10</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*Note. N.C., not converged to curve fit.

*Adult shampoo product that does not claim nonstinging and that contains CMEA, a known stinging ingredient. Hypothesized to be stinging but was not tested in the human ocular instillation test.
corrosion (OECD TG 437 and OECD TG 438 both accepted in 2009). The NRU, cytosensor microphysiometer assay, EpiOcular, and TEP assays are sensitive in vitro assays, which are able to discriminate between products that have an ultra-mild to mild ocular irritation potential (Landin et al., 2007; Martin and Stott, 1992; Wallace et al., 1992; Stern et al., 1998). Therefore, they have been used for prescreening of baby shampoo and bath products before they are tested in human clinical trials. Although these in vitro assays have shown the ability to discriminate overall ocular irritation potential, the results show that none of these general irritation assays can actually evaluate eye sting potential and that the painful sensation experienced after getting shampoo into the eyes is mediated by specific mechanisms, which are related to nociception. This finding is not surprising because the cornea is densely innervated by fine sensory afferent nerve fibers originating from the ophthalmic or medial branch of the trigeminal ganglion (Marfurt et al., 2001). About 25% of all corneal small diameter neurons in rat have been shown to be TRPV1 positive in immunohistochemical characterization (Nakamura et al., 2007), and as many as 84% of all tested rat cornea sensory neurons were responsive to capsaicin in electrophysiological recordings (Veiga Moreira et al., 2007). Even though other receptors may be involved in chemically induced nociception, the TRPV1 channel should be the major mediator of detergent-induced stinging sensation.

All the tested shampoo and bath products contained a variety of surfactants, polymers, preservatives, and fragrances. It is well understood that for certain combinations and at high levels, these components of shampoo and bath products can result in ocular sting or pain. It has been previously shown that anionic aliphatic surfactants are TRPV1 agonists, whereas nonionic, zwitterionic, and cationic surfactants do not activate the TRPV1 channel (Lindegren et al., 2009). However, the TRPV1 channel is also activated by a moderate decrease in pH (Caterina et al., 1997), and weak acids like citric acid, lactic acid, and benzoic acid are common preservatives in products as tested. Furthermore, it cannot be ruled out that fragrance formulations activate the receptors.

In this work, we have demonstrated that the NociOcular assay has the ability to evaluate a range of shampoo and bath products, with differing formulation design and compositions of a variety of surfactants, polymers, preservatives, and fragrances. The robust predictive capability demonstrated here is of immense benefit in the formulation of mild personal care products, as it would be possible to incorporate the NociOcular in vitro assay as a screening tool, evaluating potential new shampoo and bath formulations earlier in the product development process, rather than solely relying upon the human ocular instillation test to determine the stinging potential of a product. Further studies using this model will include testing of model shampoo and bath formulation systems, which will allow us to determine the relative contribution of various formulation components on ocular stinging.

**CONCLUSION**

Our conclusion is that TRPV1 channel activation may be the principal mechanism for eye-stinging sensation induced by soaps and shampoos and that the NociOcular in vitro assay may be used as a rapid, inexpensive, and simple bioassay for prediction of eye stinging of personal wash-off detergent products, such as soaps, bubble baths, or shampoo products.

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