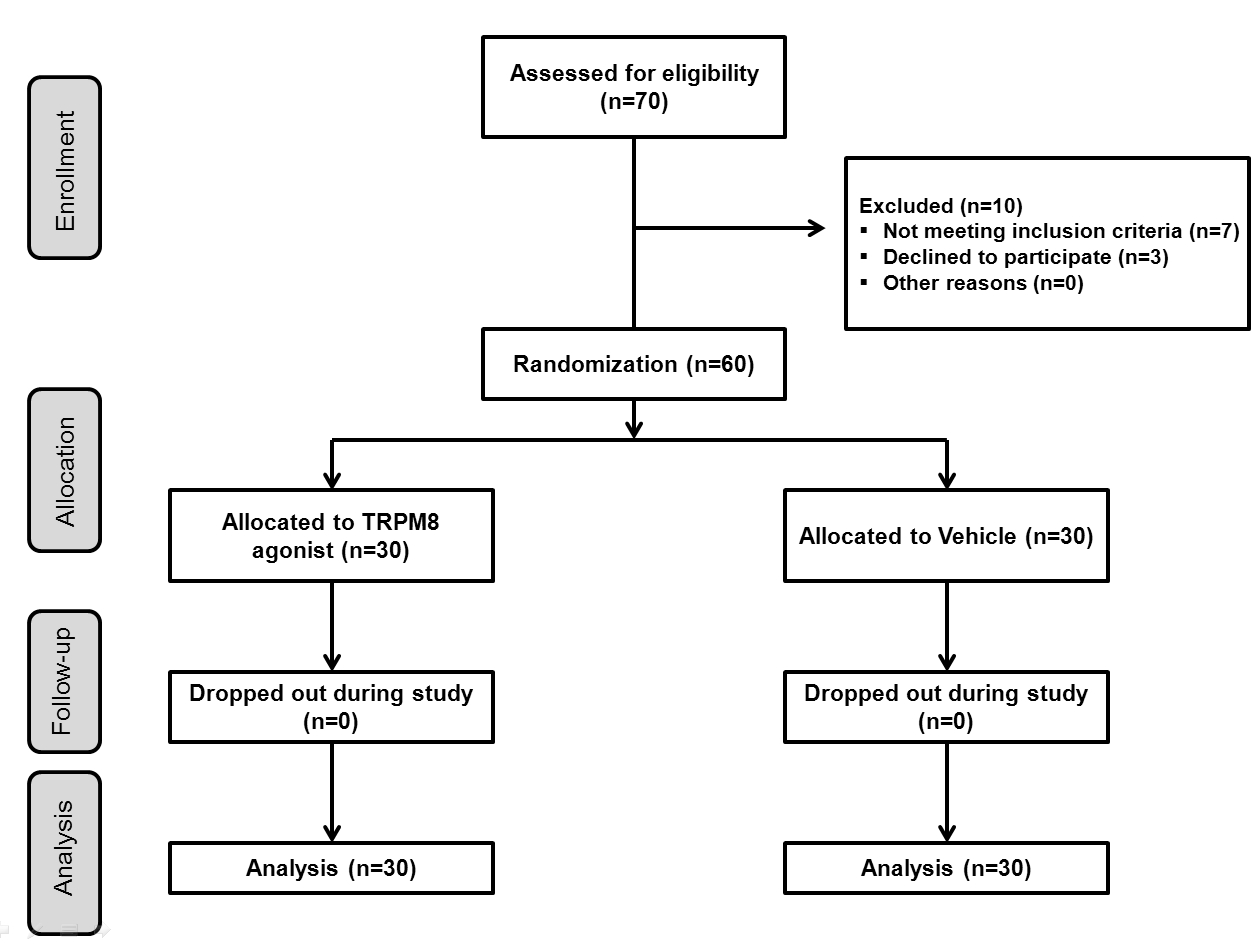
**Additional file 1.**

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**Fig. S1. Cornea, but not conjunctiva, is highly innervated by TRPM8 sensory fibers.** (A) TRPM8-expressing sensory fibers (green) densely innervate the cornea from *Trpm8EGFPf/+* transgenic mice. (B) Conjunctiva is seldom innervated by TRPM8+ fibers. White arrows indicate *Trpm8EGFPf/+* fiber axons go through the conjunctiva under the mucosa layer. Scale bar: 250μm.

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**Fig. S2. The cell bodies of sensory fibers innervating the upper eyelid and cornea are located in the V1 ophthalmic branch of the trigeminal ganglion.** (**A**) WGA-Alexa Fluor® 555 labeled trigeminal neurons for upper eyelid. (**B**) WGA-Alexa Fluor® 488 labeled trigeminal neurons for the cornea. (**C**) Merge of the fluorescence in the same trigeminal ganglion section. Scale bar: 100μm.

**Fig. S3. Flow diagram for the design of Expt 1.** Subjects received a single application of C3, 2 mg/mL, wiped across the upper eyelid with a gauze pad.

**C:\Users\Administrator\Dropbox\집에서 작업\TRPM8\Prof. Wei manuscript\Figures\Supplemental figure 4.tifFig. S4. Flow diagram for the design for Expt 2.** Subjects received 14 applicators containing 1 mL of C3, 2 mg/mL, and instructions to wipe the applicator across the upper eyelid four times a day at 6 hr intervals.

**Table S1.** **EC50 and relative potency of compounds on TRPM8.** The EC**50** of the more potent compounds, 1-diisopropylphosphoryl-C**7** to C**9** and 1-di-sec-butylphosphoryl-C**5** to C**8** fell within a narrow range with overlapping 95% Confidence Intervals. By contrast, structural modifications to the diisobutylphosphoryl moiety or branching of the alkane group to 3-methyl-pentane resulted in a significant loss of activity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Chemical** | **EC50 µM** | **95% CL** | **I-Menthol** |
|  |  |  |  |
| l-menthol | 3.8 | 2.5 to 5.6 | 1.0 |
| 1-diisopropylphosphoryl-pentane | 5.6 | 4.4 to 7.2 | 0.7 |
| 1-diisopropylphosphoryl-hexane | 2.4 | 1.5 to 4.0 | 1.6 |
| 1-diisopropylphosphoryl-heptane | 0.7 | 0.5 to 1.0 | 5.4 |
| 1-diisopropylphosphoryl-octane | 0.7 | 0.5 to 1.0 | 5.4 |
| 1-diisopropylphosphoryl-nonane | 0.9 | 0.4 to 2.5 | 4.0 |
| 1-di-sec-butylphosphoryl-4 | 14.5 | 7 to 29 | 0.3 |
| 1-di-sec-butylphosphoryl-pentane | 1.7 | 1.0 to 2.9 | 2.2 |
| 1-di-sec-butylphosphoryl-hexane | 0.8 | 0.5 to 1.3 | 4.7 |
| 1-di-sec-butylphosphoryl-heptane | 1.1 | 0.6 to 2.3 | 3.4 |
| 1-di-sec-butylphosphoryl-octane | 1.3 | 0.7 to 2.3 | 2.9 |
| 1-diisobutylphosphoryl-pentane | 24 | 8 to 76 | 0.2 |
| 1-di-sec-butylphosphoryl-3-methyl-butane | 4.2 | 1.6 to 10.8 | 0.9 |

**Table S2. Baseline characteristics of the enrolled subjects in Expt 1**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Vehicle** | **C3, 2 mg/mL** | ***P* value** |
| Age (years) | 28.57 ± 1.23 | 29.70 ± 4.47 | 0.446a |
| Gender (men/women) | 4/26 | 5/25 | 0.718 b |
| Symptom score (0-4) | 1.80 ± 0.12 | 1.87 ± 0.11 | 0.691 a |
| TBUT (seconds) | 4.53 ± 0.26 | 4.63 ± 0.26 | 0.784a |
| Schirmer score (mm/5min) | 5.93 ± 0.24 | 6.33 ± 0.32 | 0.317a |
| Keratoepitheliopathy score | 1.50 ± 0.16 | 1.27 ± 0.17 | 0.331a |
| Corneal sensitivity (mm) | 60.00 ± 0.00 | 59.33 ± 0.46 | 0.155 a |

C3 = cryosim 3, TBUT = tear break-up time

Data are expressed as the mean ± SEM.

aStudent-*t* test.

bChi-square test.

**Table S3. Baseline characteristics of the enrolled subjects in Expt 2**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Vehicle** | **C3, 2 mg/mL** | ***P* value** |
| Age (years) | 22.25 ± 0.56 | 23.75 ± 0.83 | 0.478 a |
| Gender (men/women) | 6/14 | 8/12 | 0.741 b |
| OSDI (0-100) | 27.12 ± 0.72 | 27.45 ± 0.67 | 0.495 a |
| VAS (0-10) | 3.20 ± 0.18 | 3.25 ± 0.18 | 0.841 a |
| Computer vision syndrome score (0-30) | 8.75 ± 0.41 | 8.70 ± 0.40 | 0.925 a |
| TBUT (seconds) | 5.25 ± 0.18 | 5.10 ± 0.43 | 0.883 a |
| Schirmer score (mm/5min) | 6.70 ± 0.24 | 6.65 ± 0.29 | 0.718 a |
| Keratoepitheliopathy score | 1.10 ± 0.13 | 1.30 ± 0.11 | 0.242 a |
| Corneal sensitivity (mm) | 59.50 ± 0.50 | 59.00 ± 0.69 | 0.799 a |

C3 = cryosim 3, OSDI = ocular surface disease index, TBUT = tear break-up time, VAS = visual analogue scale

Data are expressed as the mean ± standard error of mean.

aMann-Whitney U test.

bChi-square test.