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(54) Title: MODULATION OF TRPV EXPRESSION LEVELS

(57) **Abrégé/Abstract:**

The present invention relates to methods and compositions for the treatment and/or the prevention of conditions related to high levels of expression and/ or activity of the transient receptor potential vanilloid-1 (TRPV1). Amongst others, the conditions to be treated are eye conditions such as discomfort and altered sensitivity of the cornea following refractive surgery, use of contact lenses, dry eyes and diabetic retinopathy.



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(54) Title: MODULATION OF TRPV EXPRESSION LEVELS

(57) Abstract: The present invention relates to methods and compositions for the treatment and/or the prevention of conditions related to high levels of expression and/ or activity of the transient receptor potential vanilloid-1 (TRPV1). Amongst others, the conditions to be treated are eye conditions such as discomfort and altered sensitivity of the cornea following refractive surgery, use of contact lenses, dry eyes and diabetic retinopathy.



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Modulation of TRPV expression levels

FIELD OF THE INVENTION

The present invention relates to methods and compositions for the treatment and/or the prevention of conditions related to high levels of expression and/ or activity of the transient receptor potential vanilloid-1 (TRPV1). Amongst others, eye conditions such as discomfort and altered sensitivity of the cornea following refractive surgery, use of contact lenses, dry eyes and diabetic retinopathy, are to be mitigated.

Methods and compositions for the treatment and/or the prevention of hair follicle and skin abnormal conditions mediated by high levels of expression and/ or activity of TRPV1, such as alopecia, are also provided. In preferred embodiments, the invention relates to the use of RNAi technology to downregulate the expression of TRPV1.

BACKGROUND OF THE INVENTION

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing mediated by short interfering RNAs (siRNA). After the discovery of the phenomenon in plants in the early 1990s, Andy Fire and Craig Mello demonstrated that double-stranded RNA (dsRNA) specifically and selectively inhibited gene expression in an extremely efficient manner in *Caenorhabditis elegans* (Fire et al., 1998, Potent and specific genetic interference by double stranded RNA in *Caenorhabditis elegans*. *Nature*, 391:806). The sequence of the first strand (sense RNA) coincided with that of the corresponding region of the target messenger RNA (mRNA). The second strand (antisense RNA) was complementary to the mRNA. The resulting dsRNA turned out to be several orders of magnitude more efficient than the corresponding single-stranded RNA molecules (in particular, antisense RNA).

The process of RNAi begins when the enzyme, DICER, encounters dsRNA and chops it into pieces called small-interfering RNAs (siRNA). This protein belongs

to the RNase III nuclease family. A complex of proteins gathers up these RNA remains and uses their code as a guide to search out and destroy any RNAs in the cell with a matching sequence, such as target mRNA (see Bosher & Labouesse, 2000, RNA interference: genetic wand and genetic watchdog. *Nat Cell Biol*, 2000, 2(2):E31, and Akashi et al., 2001, Suppression of gene expression by RNA interference in cultured plant cells. *Antisense Nucleic Acid Drug Dev*, 11(6):359).

In attempting to utilize RNAi for gene knockdown, it was recognized that mammalian cells have developed various protective mechanisms against viral infections that could impede the use of this approach. Indeed, the presence of extremely low levels of viral dsRNA triggers an interferon response, resulting in a global non-specific suppression of translation, which in turn triggers apoptosis (Williams, 1997, Role of the double-stranded RNA-activated protein kinase (PKR) in cell regulation. *Biochem Soc Trans*, 25(2):509; Gil & Esteban, 2000, Induction of apoptosis by the dsRNA-dependent protein kinase (PKR): mechanism of action. *Apoptosis*, 5(2):107-14).

In 2000 dsRNA was reported to specifically inhibit 3 genes in the mouse oocyte and early embryo. Translational arrest, and thus a PKR response, was not observed as the embryos continued to develop (Wianny & Zernicka-Goetz, 2000, Specific interference with gene function by double-stranded RNA in early mouse development. *Nat Cell Biol*, 2(2):70). Research at Ribopharma AG (Kulmbach, Germany) demonstrated the functionality of RNAi in mammalian cells, using short (20-24 base pairs) dsRNA to switch off genes in human cells without initiating the acute-phase response. Similar experiments carried out by other research groups confirmed these results. (Elbashir et al., 2001, RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev*, 15(2):188; Caplen et al., 2001, Specific inhibition of gene expression by small double stranded RNAs in invertebrate and vertebrate systems. *Proc. Natl. Acad. Sci. USA*, 98: 9742). Tested in a variety of normal and cancer human and mouse cell lines, it was determined that short hairpin RNAs (shRNA) can silence genes as efficiently as their siRNA counterparts (Paddison et al, 2002, Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian

cells. *Genes Dev*, 16(8):948). Recently, another group of small RNAs (21-25 base pairs) was shown to mediate downregulation of gene expression. These RNAs, small temporally regulated RNAs (stRNA), regulate timing of gene expression during development in *Caenorhabditis elegans* (for review see Banerjee & Slack, Control of developmental timing by small temporal RNAs: a paradigm for RNA-mediated regulation of gene expression. *Bioessays*, 2002, 24(2):119-29 and Grosshans & Slack, 2002, Micro-RNAs: small is plentiful. *J Cell Biol*, 156(1):17).

Scientists have used RNAi in several systems, including *Caenorhabditis elegans*, *Drosophila*, trypanosomes, and other invertebrates. Several groups have recently presented the specific suppression of protein biosynthesis in different mammalian cell lines (specifically in HeLa cells) demonstrating that RNAi is a broadly applicable method for gene silencing in vitro. Based on these results, RNAi has rapidly become a well recognized tool for validating (identifying and assigning) gene function. RNAi employing short dsRNA oligonucleotides will yield an understanding of the function of genes that are only partially sequenced.

The transient receptor potential vanilloid-1 (TRPV1), also called Vanilloid receptor 1 (VR-1), is a capsaicin-responsive ligand-gated cation channel, that was first discovered in 1997 (Caterina et al. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 1997 Oct 23;389(6653):816-24). TRPV1 is mainly expressed on sensory neurons and serves as a molecular detector for heat, capsaicin, protons, and endovanilloids (Caterina MJ & Julius D. The vanilloid receptor: a molecular gateway to the pain pathway. *Annu Rev Neurosci.*, 2001;24:487-517; Montell et al. Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. *Genes Dev.*, 2002, 16(8):948-58.; Baumann TK & Martenson ME. Extracellular protons both increase the activity and reduce the conductance of capsaicin- gated channels. *J Neurosci*. 2000;20:RC80).

When TRPV1 is activated by agonists such as capsaicin and other factors such as heat, acidosis, lipoxygenase products or anandamide, calcium enters the cell

and pain signals are initiated. Activation of the channel induces neuropeptide release from central and peripheral sensory nerve terminals, resulting in the sensation of pain, neurogenic inflammation, and sometimes, in smooth muscle contraction and cough. Recent evidence suggests a role of TRPV1 in pain, cough, asthma and urinary incontinence (Jia et al., TRPV1 receptor: a target for the treatment of pain, cough, airway disease and urinary incontinence. *Drug News Perspect.* 2005 Apr;18(3):165-71).

Due to the fact that both the sensitivity and the density of expression of TRPV1 are enhanced during inflammatory conditions (Di Marzo et al., Endovanilloid signaling in pain. *Curr Opin Neurobiol.* 2002 Aug;12(4):372-9), downregulation of TRPV1 expression and/or activity is a promising therapeutic strategy for novel analgesic drugs. As a matter of fact, intraperitoneal administration of selective TRPV1 blockers into mice proved to attenuate chemical and thermal nociception and hyperalgesia (García-Martínez et al., Attenuation of thermal nociception and hyperalgesia by VR1 blockers. *Proc Natl Acad Sci U S A.* 2002 Feb 19;99(4):2374-9).

TRPV1 channel function is upregulated by several endogenous mediators present in inflammatory conditions, which decrease the threshold for activation of the channel. Thus, it has recently been demonstrated that acute pain-related behaviour evoked by elevated ionic strength is abolished in TRPV1-null mice and inhibited by iodo-resiniferatoxin, a potent TRPV1 antagonist (Ahern et al., Extracellular cations sensitize and gate capsaicin receptor TRPV1 modulating pain signaling. *J Neurosci.* 2005 May 25;25(21):5109-16). Further, Prostaglandin E₂ (PGE₂) and Prostaglandin I₂ (PGI₂) have proven to increase or sensitize TRPV1 responses through their respective receptors EP₁ or IP (Moriyama et al., Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. *Mol Pain.* 2005 Jan 17;1(1):3), suggesting for the first time that sensitisation of TRPV1 activity through EP₁ or IP activation might be one important mechanism underlying the peripheral nociceptive actions of PGE₂ or PGI₂. WO 2004/042046 shows that siRNA targeted against VR1 can be used in the treatment of chronic pain, sensitivity

disfunctions linked to the VR1 receptor and VR associated inflammation, tumours urinary incontinence and pruritus.

Polymodal nociceptors are the most abundant nociceptor type found in the cornea. There exists pharmacological evidence that these receptor fibers express the TRPV1 receptor because they respond to capsaicin, heat and acid. Moreover, high doses of capsaicin inactivate the activation of corneal polymodal nociceptors to heat and acid whereas mechanical responsiveness remains unaffected. This suggests that TRPV1 receptors present in corneal polymodal nerve endings were selectively inactivated. Therefore, it is likely that an important part of the acute nociceptive response to corneal injury and the sustained pain sensations that accompany inflammatory and irritative processes in this tissue are mediated by TRPV1 activation.

Recent evidence also demonstrates that both insulin and IGF-I enhance TRPV1-mediated membrane currents in heterologous expression systems and cultured dorsal root ganglion neurons (Van Buren et al., Sensitization and translocation of TRPV1 by insulin and IGF-I. *Mol Pain*. 2005 Apr 27;1(1):17). Enhancement of membrane currents results from both increased sensitivity of the receptor and translocation of TRPV1 from cytosol to plasma membrane. An increase of IGF-1 has been found in the serum (Merimee et al., Insulin-like growth factors. Studies in diabetics with and without retinopathy. *N. Engl. J. Med.*, 1983; 309:527-530; Grant et al., Insulin-like growth factors in vitreous. Studies in control and diabetic subjects with neovascularization. *Diabetes*, 1986; 35:416-420) and the vitreous body and intraocular fluid (Grant et al., 1986; Inokuchi et al., Vitreous levels of insulin-like growth factor-I in patients with proliferative diabetic retinopathy. *Curr. Eye Res.*, 2001; 23:368-371) of patients with diabetic retinopathy. Further, vitreous IGF-I levels correlate with the presence and severity of ischemia-associated diabetic retinal neovascularization (Meyer-Schwickerath et al., Vitreous levels of the insulin-like growth factors I and II, and the insulin-like growth factor binding proteins 2 and 3, increase in neovascular eye disease. Studies in nondiabetic and diabetic subjects. *J Clin Invest.*, 1993;92(6):2620-5). However, the source of increased ocular IGF-1 in retinopathy is controversial, and the relative contribution of

either endogenous IGF-1 or serum IGF-1 is unknown (Ruberte et al., Increased ocular levels of IGF-1 in transgenic mice lead to diabetes-like eye disease. *J Clin Invest.* 2004 Apr;113(8):1149-57). Modulation of TRPV1 levels could aid in the control of diabetic retinopathy mediated by IGF-I.

Although originally described on sensory neurons, TRPV1 has now been detected in several human skin cell populations and epithelial compartments of the human hair follicle (HF), mainly the outer root sheath (ORS) and hair matrix (Bodo et al., A hot new twist to hair biology: involvement of vanilloid receptor-1 (VR1/TRPV1) signaling in human hair growth control. *Am J Pathol.* 2005 Apr;166(4):985-98). Stimulation of TRPV1 in organ culture and cultured human ORS keratinocytes inhibits proliferation, induces apoptosis, elevates intracellular calcium concentration, up-regulates known endogenous hair growth inhibitors, and down-regulates known hair growth promoters, thus supporting TRPV1 as a significant novel player in human hair growth control (Bodo et al., 2005).

The above-mentioned evidence points to inhibition of TRPV1 as an efficient treatment for eye conditions that mediate with an excess of expression and /or activity of TRPV1, such as discomfort and altered sensitivity of the cornea following refractive surgery, use of contact lenses and dry eyes. The functional relationship between TRPV1 and IGF-I highlights the importance of downregulation of TRPV1 for the treatment of diabetic retinopathy mediated by high levels of IGF-I. The role played by TRPV1 in human hair follicle growth and keratinocytes targets TRPV1 as a good candidate to be inhibited for the treatment of hair follicle and skin abnormal conditions such as alopecia.

SUMMARY OF THE INVENTION

In the present invention we describe a method for the treatment and/or prevention of conditions related to high levels of TRPV1, comprising eye and hair follicle abnormal conditions. The method is based on the downregulation of expression of one or more splice forms of the TRPV1 gene. Inhibition

(downregulation) may be effected by the use of double stranded nucleic acid moieties, named siNA or small interfering NA that are directed at interfering with the mRNA expression of either one or more splicing forms of the TRPV1 gene. The siNA are preferably siRNA, although modified nucleic acids or similar chemically synthesised entities are also included within the scope of the invention.

The TRPV1 receptor, like other membrane proteins and channels is manufactured inside the cell and transported to the periphery by centrifugal axonal transport. Nevertheless, the possibility exists that TRPV1 is also synthesized at the sensory nerve terminals, being inserted locally into the membrane of the transducing portion of the ending. Therefore, without wishing to be bound by theory, it is suggested that blockade of the local synthesis of TRPV1 through topical administration of siNA directed to specifically silence the gene in charge of TRPV1 expression might lead to a partial or complete inactivation of polymodal nociceptor fibers of the cornea to chemical stimuli by exogenous or endogenous stimuli and to a reduction or elimination of their impulse activity associated to injury and inflammation.

In a first aspect of the present invention relates to the use of siNA in the preparation of a medicament for use in a method of treatment of an eye and/or hair follicle abnormal condition characterised by increased expression and/or activity of TRPV1.

A second aspect of the present invention relates to a siNA compound targeted to TRPV1.

Another aspect of the present invention relates to a pharmaceutical composition comprising a siNA compound targeted to TRPV1.

A further aspect of the present invention provide method of treatment of a disease characterised by increased expression and/or activity of TRPV1, comprising administering siNA to inhibit expression of TRPV1 gene in a patient, wherein the disease condition is selected from the group comprising an

abnormal eye condition, such as altered sensitivity of the cornea following refractive surgery, use of contact lenses, dry eyes, diabetic retinopathy, and other eye pathologies, as well as a hair follicle abnormal condition such as alopecia.

DESCRIPTION OF THE DRAWINGS

Figure 1 displays GenBank Accession Numbers corresponding to the four TRPV1 transcripts produced by alternative splicing.

Figure 2 shows short DNA fragments of the target gene sequence chosen as preferred target sequences of the siNA of the invention.

Figure 3 shows preferred siNA molecules of the invention.

Figure 4. Effect of topical application of a 10 μ L drop of 0.1mM capsaicin solution in the right eye and of 10 μ L of sterile isotonic saline in the left eye of guinea pigs. Scratching and wiping movements (A), Tearing (B) and Conjunctival hyperemia (C) were analysed.

Figure 5. Attenuating effect of ON3 on the behavioural response to topical capsaicin 0.1mM. Scratching and wiping movements (A), Tearing (B) and Conjunctival hyperemia (C) were analysed.

Figure 6. Average reduction in the number of scratching/wiping movements in all the studied animals, presented in absolute values (upper graph) and as percentage of reduction in the treated side from the control side, at the various days after treatment.

Figure 7. Recording chamber configuration.

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect, the invention relates to the use of siNA in the preparation of a medicament for use in a method of treatment of an eye and/or hair follicle abnormal condition characterised by increased expression and/or activity of TRPV1. The method may comprise inhibiting the expression of TRPV1 in a patient. The term inhibition is used to indicate a decrease or downregulation of expression or activity. Preferably, the eye condition is selected from the group comprising discomfort and altered sensitivity of the cornea following refractive surgery, use of contact lenses, dry eyes, diabetic retinopathy, and other eye pathologies. Also preferably, the abnormal hair follicle condition is alopecia.

A gene is "targeted" by a siNA according to the present invention when, for example, the siNA molecule selectively decreases or inhibits the expression of the gene. The phrase "selectively decrease or inhibit" as used herein encompasses siNAs that effects expression of one gene. Alternatively, a siNA targets a gene when the siNA hybridizes under stringent conditions to the gene transcript.

In one embodiment, siNA according to the invention is siRNA.

Four transcript variants corresponding to TRPV1 have been identified. GenBank Accession Numbers corresponding to the four TRPV1 transcripts produced by alternative splicing are displayed in Figure 1. Preferably, the siNA is targeted to a splice form of TRPV1 selected from the group having GenBank Accession Numbers NM_080704, NM_018727, NM_080706, NM_080705.

Selected oligonucleotide sequences against which RNAi is directed according to the first aspect of the invention are shown in Figure 2. Displayed sequences are the DNA sequences targeted by the siNA. Therefore, the invention makes use of NA duplexes with sequences complementary to the indicated DNA sequences. Therefore, in accordance with the first aspect of the invention, siNA is targeted to a sequence selected from SEQ ID NO 1 to SEQ ID NO 81 or to a sequence comprising a sequence selected from SEQ ID NO 1 to SEQ ID NO

81. Thus, the siNA is complementary to a sequence selected from SEQ ID NO 1 to SEQ ID NO 81 or to a sequence comprising a sequences selected from SEQ ID NO 1 to SEQ ID NO 81.

According to the invention, a plurality of siNA species may be used. In one embodiment, the plurality of siNA species may be targeted to the same mRNA species, in another embodiment, it may be targeted to different species.

The sequences displayed in Figure 2 are not limiting. According to the invention, target DNA need not necessarily be preceded by AA or CA. Further, target DNA could be constituted by sequences included in Figure 2 flanked by any contiguous sequence.

In another preferred embodiment, the invention relates to a siNA compound targeted to TRVP1 comprising a nucleotide sequence complementary to a nucleotide sequence selected from SEQ ID NO 1 to 44 or SEQ ID NO 46 to 81 as shown in Figure 2. In one embodiment, the preferred SEQ is SEQ ID NO 65.

In a further embodiment, the invention relates to a siNA compound targeted to TRVP1 comprising a nucleotide sequence complementary to a nucleotide sequence selected from SEQ ID NO 1 to 44 or SEQ ID NO 46 to 81 for use in the treatment of a disease characterized by increased expression and/or activity of TRPV1, the siNA comprising a nucleotide sequence complementary to a nucleotide sequence selected from SEQ ID NO 1 to 44 or SEQ ID NO 46 to 81.

In a further aspect, the invention relates to a pharmaceutical composition comprising a nucleotide sequence complementary to a nucleotide sequence selected from SEQ ID NO 1 to 44 or SEQ ID NO 46 to 81.

In one embodiment, siNA molecules of the present invention comprise nucleotide sequences selected from the group of SEQ ID NO 82 to 162. For example, siNA molecules of the present invention comprise nucleotide sequences selected from the group of SEQ ID NO 82 to 122 or 123 to 162.

In preferred embodiments, siNA molecules comprise overhanging nucleotides.

The invention also relates to a method for inhibiting expression and/or activity of TRPV1 *ex vivo* in cells or tissue comprising treating said cells or tissue with the compound comprising a nucleotide sequence complementary to a nucleotide sequence selected from SEQ ID NO 1 to 44 or SEQ ID NO 46 to 81 so that TRVP1 expression is inhibited.

In a final aspect, the invention relates to a method of treatment of a disease characterised by increased expression and/or activity of TRPV1, comprising administering siNA to inhibit expression of TRPV1 gene in a patient wherein the disease condition is selected from the group comprising an abnormal eye condition, such as altered sensitivity of the cornea following refractive surgery, use of contact lenses, dry eyes, diabetic retinopathy, and other eye pathologies, as well as a hair follicle abnormal condition such as alopecia.

The terms "treating" or "treatment" as used herein describe the management or care of a patient for the purposes of combating disease, and includes the administration of the active agent to asymptomatic individuals, for example to prevent the onset of the symptoms or complications, i.e. prophylaxis.

The invention also relates to a pharmaceutical composition comprising the siNA compound as described herein.

- Design of siNAs.

A short fragment of the target gene sequence (e.g., 19-40 nucleotides in length) is chosen as the target sequence of the siNA of the invention. In one embodiment, the siNA is a siRNA. In such embodiments, the short fragment of target gene sequence is a fragment of the target gene mRNA. In preferred embodiments, the criteria for choosing a sequence fragment from the target gene mRNA to be a candidate siRNA molecule include 1) a sequence from the target gene mRNA that is at least 50-100 nucleotides from the 5' or 3' end of the

native mRNA molecule, 2) a sequence from the target gene mRNA that has a G/C content of between 30% and 70%, most preferably around 50%, 3) a sequence from the target gene mRNA that does not contain repetitive sequences (e.g., AAA, CCC, GGG, TTT, AAAA, CCCC, GGGG, TTTT), 4) a sequence from the target gene mRNA that is accessible in the mRNA, and 5) a sequence from the target gene mRNA that is unique to the target gene. The sequence fragment from the target gene mRNA may meet one or more of the criteria identified supra. In embodiments where a fragment of the target gene mRNA meets less than all of the criteria identified supra, the native sequence may be altered such that the siRNA conforms with more of the criteria than does the fragment of the target gene mRNA. In preferred embodiments, the siRNA has a G/C/ content below 60% and/or lacks repetitive sequences.

Practically, the selected gene is introduced as a nucleotide sequence in a prediction program that takes into account all the variables described above for the design of optimal oligonucleotides. This program scans any mRNA nucleotide sequence for regions susceptible to be targeted by siRNAs. The output of this analysis is a score of possible siRNA oligonucleotides. The highest scores are used to design double stranded RNA oligonucleotides (typically 21 bp long, although other lengths are also possible) that are typically made by chemical synthesis.

In addition to siNA which is complementary to the mRNA target region, degenerate siNA sequences may be used according to the invention to target homologous regions. WO2005/045037 describes the design of siNA molecules to target such homologous sequences, for example by incorporating non-canonical base pairs, for example mismatches and/or wobble base pairs, that can provide additional target sequences. In instances where mismatches are identified, non-canonical base pairs (for example, mismatches and/or wobble bases) can be used to generate siNA molecules that target more than one gene sequence. In a non-limiting example, non-canonical base pairs such as UU and CC base pairs are used to generate siNA molecules that are capable of targeting sequences for differing targets that share sequence homology.

In preferred embodiments, siNA molecules of the invention target a sequence selected from SEQ ID NOS: 1-81 (Figure 2).

Preferred siNA molecules of the invention are double stranded. In one embodiment, double stranded siNA molecules comprise blunt ends. In another embodiment, double stranded siNA molecules comprise overhanging nucleotides (e.g., 1-5 nucleotide overhangs, preferably 2 nucleotide overhangs). In a specific embodiment, the overhanging nucleotides are 3' overhangs. In another specific embodiment, the overhanging nucleotides are 5' overhangs. Any type of nucleotide can be a part of the overhang. In one embodiment, the overhanging nucleotide or nucleotides are ribonucleic acids. In another embodiment, the overhanging nucleotide or nucleotides are deoxyribonucleic acids. In a preferred embodiment, the overhanging nucleotide or nucleotides are thymidine nucleotides. In another embodiment, the overhanging nucleotide or nucleotides are modified or non-classical nucleotides. The overhanging nucleotide or nucleotides may have non-classical internucleotide bonds (e.g., other than phosphodiester bond).

In preferred embodiments, siNA molecules of the invention comprise nucleotide sequences selected from SEQ ID NOS: 82-162 (Figure 3). In another preferred embodiment, dsRNA compositions of the invention are any of SEQ ID NOS: 82-162. The invention also encompasses siNAs that are 40 nucleotides or less and comprise a nucleotide sequence of any of SEQ ID NOS: 82-162 as well as dsRNA compositions that are 40 nucleotides or less and comprise a nucleotide sequence of any of SEQ ID NOS: 82-162 hybridized to its complement. In one embodiment, the siNA is 21-30 nucleotides and comprises any one of SEQ ID NOS: 82-162.

- Synthesis of siNAs.

siNAs according to the invention can be synthesized by any method known in the art. RNAs are preferably chemically synthesized using appropriately protected ribonucleoside phosphoramidites and a conventional DNA/RNA synthesizer. Additionally, siRNAs can be obtained from commercial RNA oligo

synthesis suppliers, including, but not limited to, Proligo (Hamburg, Germany), Dharmacon Research (Lafayette, CO, USA), Glen Research (Sterling, VA, USA), ChemGenes (Ashland, MA, USA), and Cruachem (Glasgow, UK), Qiagen (Germany), Ambion (USA) and Invitrogen (Scotland). Alternatively, siNA molecules of the invention can be expressed in cells by transfecting the cells with vectors containing the reverse compliment siNA sequence under the control of a promoter. Once expressed, the siNA can be isolated from the cell using techniques well known in the art.

In embodiments where the siRNA is a dsRNA, an annealing step is necessary if single-stranded RNA molecules are obtained. Briefly, combine 30 μ l of each RNA oligo 50 μ M solution in 100 mM potassium acetate, 30 mM HEPES-KOH pH 7.4, 2 mM magnesium acetate. The solution is then incubated for 1 minute at 90 °C, centrifuged for 15 seconds, and incubated for 1 hour at 37 °C.

In embodiments where the siRNA is a short hairpin RNA (shRNA); the two strands of the siRNA molecule may be connected by a linker region (e.g., a nucleotide linker or a non-nucleotide linker).

- Chemical modification of siNAs.

The siNAs of the invention may contain one or more modified nucleotides and/or non-phosphodiester linkages. Chemical modifications well known in the art are capable of increasing stability, availability, and/or cell uptake of the siNA. The skilled person will be aware of other types of chemical modification which may be incorporated into RNA molecules (see International Publications WO03/070744 and WO2005/045037 for an overview of types of modifications). In one embodiment, modifications can be used to provide improved resistance to degradation or improved uptake. Examples of such modifications include phosphorothioate internucleotide linkages, 2'-O-methyl ribonucleotides (especially on the sense strand of a double stranded siRNA), 2'-deoxy-fluoro ribonucleotides, 2'-deoxy ribonucleotides, "universal base" nucleotides, 5-C-methyl nucleotides, and inverted deoxyabasic residue incorporation (see generally GB2406568).

In another embodiment, modifications can be used to enhance the stability of the siRNA or to increase targeting efficiency. Modifications include chemical cross linking between the two complementary strands of an siRNA, chemical modification of a 3' or 5' terminus of a strand of an siRNA, sugar modifications, nucleobase modifications and/or backbone modifications, 2'-fluoro modified ribonucleotides and 2'-deoxy ribonucleotides (see generally International Publication WO2004/029212).

In another embodiment, modifications can be used to increased or decreased affinity for the complementary nucleotides in the target mRNA and/or in the complementary siNA strand (see generally International Publication WO2005/044976). For example, an unmodified pyrimidine nucleotide can be substituted for a 2-thio, 5-alkynyl, 5-methyl, or 5-propynyl pyrimidine. Additionally, an unmodified purine can be substituted with a 7-deza, 7-alkyl, or 7-alkenyl purine.

In another embodiment, when the siNA is a double-stranded siRNA, the 3'-terminal nucleotide overhanging nucleotides are replaced by deoxyribonucleotides (see generally Elbashir et al., 2001, Genes Dev, 15:188).

- Formulations and routes of administration.

The siNA molecules of the invention and formulations or compositions thereof may be administered directly or topically (e. g., locally) to the organ of interest (for example, eye, skin, etc) as is generally known in the art. For example, administration may be intrarticular or intravenous. In a preferred embodiment, administration may be ocular., for example by means of eye drops.

For example, a siNA molecule can comprise a delivery vehicle, including liposomes, for administration to a subject. Carriers and diluents and their salts can be present in pharmaceutically acceptable formulations. Nucleic acid molecules can be administered to cells by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as biodegradable

polymers, hydrogels, cyclodextrins poly (lactic-co-glycolic) acid (PLGA) and PLGA microspheres, biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors. In another embodiment, the nucleic acid molecules of the invention can also be formulated or complexed with polyethyleneimine and derivatives thereof, such as polyethyleneimine-polyethyleneglycol-N-acetylgalactosamine (PEI-PEG-GAL) or polyethyleneimine- polyethyleneglycol-tri-N-acetylgalactosamine (PEI-PEG-triGAL) derivatives.

A siNA molecule of the invention may be complexed with membrane disruptive agents and/or a cationic lipid or helper lipid molecule.

Delivery systems which may be used with the invention include, for example, aqueous and non aqueous gels, creams, multiple emulsions, microemulsions, liposomes, ointments, aqueous and non aqueous solutions, lotions, aerosols, hydrocarbon bases and powders, and can contain excipients such as solubilizers, permeation enhancers (e. g., fatty acids, fatty acid esters, fatty alcohols and amino acids), and hydrophilic polymers (e. g. , polycarbophil and polyvinylpyrrolidone). In one embodiment, the pharmaceutically acceptable carrier is a liposome or a transdermal enhancer.

A pharmaceutical formulation of the invention is in a form suitable for administration, e.g., systemic or local administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art. For example, preservatives, stabilizers, dyes and flavouring agents can be provided. These include sodium benzoate, sorbic acid and esters of p-

hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize.

Generally, an amount between 0.1mg/kg and 100 mg/kg body weight/day of active ingredients is administered.

The formulations of the invention can be administered in unit dosage formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. Formulations can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavouring agents, colouring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets.

These excipients can be, for example, inert diluents; such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some

cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavouring agents can be added to provide palatable oral preparations.

These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavouring and colouring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavouring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavouring and colouring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension.

This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above.

A sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3- butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride

solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, e. g. , for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anaesthetics, preservatives and buffering agents can be dissolved in the vehicle.

It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the

overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

Alternatively, certain siNA molecules of the invention can be expressed within cells from eukaryotic promoters. Recombinant vectors capable of expressing the siNA molecules can be delivered and persist in target cells. Alternatively, vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siNA molecule expressing vectors can be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell.

Experimental Procedure

SiNA synthesis

An annealing step is necessary when working with single-stranded RNA molecules. It is critical that all handling steps be conducted under sterile, Rnase free conditions. To anneal the RNAs, the oligos must first be quantified by UV absorption at 260 nanometres (nm). The following protocol based on Elbashir et al. (2001) is then used for annealing:

- Separately aliquot and dilute each RNA oligo to a concentration of 50 μ M.
- Combine 30 μ l of each RNA oligo solution and 15 μ l of 5X annealing buffer. Final buffer concentration is: 100 mM potassium acetate, 30 mM HEPES-KOH pH 7.4, 2 mM magnesium acetate. Final volume is 75 μ l.
- Incubate the solution for 1 minute at 90 °C, centrifuge the tube for 15 seconds, let sit for 1 hour at 37 °C, then use at ambient temperature. The solution can be stored frozen at -20 °C and freeze-thawed up to 5 times. The final concentration of siRNA duplex is usually 20 μ M.

Alternatively, already annealed dsRNAs may be purchased from the suppliers.

Chemically modified nucleic acids may also be used. For example, an overview of the types of modification which may be used is given in WO03/070744. Particular attention is drawn to pages 11 to 21 of this publication. Other possible modifications are as described above. The skilled person will be aware of other types of chemical modification which may be incorporated into RNA molecules.

"In vitro" system

TRPV1 expression has been detected in cutaneous sensory nerve fibers, mast cells, epidermal keratinocytes, dermal blood vessels, the inner root sheet and the infundibulum of hair follicles, differentiated sebocytes, sweat gland ducts, and the secretory portion of eccrine sweat glands by immunoreactivity assays (Stander et al., 2004). Upon reverse transcriptase-polymerase chain reaction and Western blot analysis, TRPV1 has been detected in mast cells and keratinocytes from human skin (Stander et al., 2004). Recently, dendritic cells have also shown to express TRPV1 (Basu S & Srivastava P. Immunological role of neuronal receptor vanilloid receptor 1 expressed on dendritic cells. *Proc Natl Acad Sci U S A*. 2005 Apr 5;102(14):5120-5. Epub 2005 Mar 25.) and neuronal cell models have been developed with cells that express TRPV1 (Lilja J & Forsby A. Development of a sensory neuronal cell model for the estimation of mild eye irritation. *Altern Lab Anim*. 2004 Oct;32(4):339-43.).

Cell cultures expressing the target gene TRPV1 are used for a preliminary testing of the specificity of siRNA interference.

The cells are incubated with the corresponding siRNA duplexes, and analysis of the downregulation of expression of the target gene is carried out. For linking siRNA knockdown to specific phenotypes in cultured cells, it is necessary to demonstrate the decrease of the targeted protein or at least demonstrate the reduction of the targeted mRNA.

mRNA levels of the target gene can be quantitated by Real-time quantitative PCR (qRT-PCR). Further, the protein levels can be determined in a variety of ways well known in the art, such as Western blot analysis with specific antibodies to the target, which allow direct monitoring of the reduction of targeted protein.

Transfection of siRNA duplexes in cell cultures

Several examples of techniques well known in the art are as follows: We can perform a single transfection of siRNA duplex using a cationic lipid, such as RNAiFect™ Transfection Reagent (Qiagen) and Lipofectamine™ 2000 Reagent (Invitrogen) and assay for silencing 24, 48 and 72 hours after transfection.

A typical transfection protocol can be performed as follows: For one well of a 6-well plate, we transfect using 100nM as final concentration of siRNA. Following RNAiFect protocol, we seed, the day before transfection, $2-4 \times 10^5$ cells per well in 3ml of an appropriate growth medium, containing DMEM, 10% serum, antibiotics and glutamine, and incubate cells under normal growth conditions (37°C and 5% CO₂). On the day of transfection, cells have to be at 30-50% confluence. We dilute 15ul of 20uM siRNA duplex (corresponding to 100 nM final concentration) in 85ul of Buffer EC-R, to give a final volume of 100ul, and mix by vortexing. For complex formation, we add 19 ul of RNAiFect Transfection Reagent to the diluted siRNA and mix by pipetting or vortexing. After incubating the samples for 10-15 minutes at room temperature to allow formation of transfection complexes, we add the complexes drop-wise onto the cells with 2.9 ml of fresh growth medium low in antibiotics. After swirling the plates to ensure uniform distribution of the transfection complexes, we incubate the cells under their normal growth conditions. The day after, the complexes are removed and fresh and complete growth medium is added. To monitor gene silencing, cells are collected at 24, 48 and 72 hours post-transfection. The Lipofectamine 2000 Reagent protocol is quite similar. The day before transfection, we seed $2-4 \times 10^5$ cells per well in 3ml of an appropriate growth medium, containing DMEM, 10% serum, antibiotics and glutamine, and incubate cells under normal growth conditions (37°C and 5% CO₂). On the day of transfection, cells have to be at 30-50% confluence. We dilute 12.5ul of 20uM siRNA duplex (corresponding to 100 nM final concentration) in 250ul of DMEM, to give a final volume of 262.5ul, and mix. Also, 6ul of Lipofectamine 2000 is diluted in 250ul of DMEM and mixed. After a 5 minutes incubation at room temperature, the diluted oligomer and the diluted Lipofectamine are combined to allow complex formation during a 20 minutes incubation at room temperature. Afterwards, we add the complexes drop-wise onto the cells with 2 ml of fresh growth medium low in antibiotics and

mix gently by rocking the plate back and forth, to ensure uniform distribution of the transfection complexes. We incubate the cells under their normal growth conditions and the day after, the complexes are removed and fresh and complete growth medium is added. To monitor gene silencing, cells are collected at 24, 48 and 72 hours post-transfection.

The efficiency of transfection may depend on the cell type, but also on the passage number and the confluency of the cells. The time and the manner of formation of siRNA-liposome complexes (e.g. inversion versus vortexing) are also critical. Low transfection efficiencies are the most frequent cause of unsuccessful silencing. Good transfection is a non-trivial issue and needs to be carefully examined for each new cell line to be used. Transfection efficiency may be tested transfecting reporter genes, for example a CMV-driven EGFP-expression plasmid (e.g. from Clontech) or a B-Gal expression plasmid, and then assessed by phase contrast and/or fluorescence microscopy the next day.

Testing of siRNA duplexes

Depending on the abundance and the life time (or turnover) of the targeted protein, a knock-down phenotype may become apparent after 1 to 3 days, or even later. In cases where no phenotype is observed, depletion of the protein may be observed by immunofluorescence or Western blotting.

After transfections, total RNA fractions extracted from cells are pre-treated with DNase I and used for reverse transcription using a random primer. PCR-amplification is carried out with a specific primer pair covering at least one exon-exon junction in order to control for amplification of pre-mRNAs. RT/PCR of a non-targeted mRNA is also needed as control. Effective depletion of the mRNA yet undetectable reduction of target protein may indicate that a large reservoir of stable protein may exist in the cell. Alternatively, Real-time PCR amplification can be used to test in a more precise way the mRNA decrease or disappearance. Real-time reverse-transcriptase (RT) PCR quantitates the initial amount of the template most specifically, sensitively and reproducibly. Real-time PCR monitors the fluorescence emitted during the reaction as an indicator of amplicon production during each PCR cycle, in a light cycler apparatus. This

signal increases in direct proportion to the amount of PCR product in a reaction. By recording the amount of fluorescence emission at each cycle, it is possible to monitor the PCR reaction during exponential phase where the first significant increase in the amount of PCR product correlates to the initial amount of target template.

To verify the interference pattern of TRPV1 gene in the cell cultures, qRT-PCR is performed according to the manufacturer protocol. For quantitative qRT-PCR, approximately 250-500 ng of total RNA are used for reverse transcription followed by PCR amplification with specific primers for TRPV1 in reaction mixture containing Master SYBR Green I. Basic PCR conditions comprise an initial step of 30 min at 91°C, followed by 40 cycles of 5 s at 95°C, 10 s at 62°C and 15 s at 72°C. Quantification of b-actin mRNA can be used as a control for data normalization. Relative gene expression comparisons work best when the gene expression of the chosen endogenous/internal control is more abundant and remains constant, in proportion to total RNA, among the samples. By using an invariant endogenous control as an active reference, quantitation of an mRNA target can be normalised for differences in the amount of total RNA added to each reaction. The amplification curves obtained with the light cycler are analyzed in combination with the control kit RNA, which targets in vitro transcribed cytokine RNA template, according to the manufacturer protocol. In order to assess the specificity of the amplified PCR product a melting curve analysis is performed. The resulting melting curves allow discrimination between primer-dimers and specific PCR product.

Animal studies

In vivo silencing effect of siNA molecules on TRPV1 expression levels can be tested on a guinea-pig cornea model such as the one described by Brock et al. Tetrodotoxin-resistant impulses in single nociceptor nerve terminals in guinea-pig cornea. J Physiol. 1998 Oct 1;512 (Pt 1):211-7.

The basic procedure consists on the instillation of the siNA molecule to be tested, contained in a small volume, on the guinea-pig corneal surface. Contralateral eyes are treated with the vehicle alone, and can be used as

controls in each experiment lest there is a sympathy phenomenon with the other eye. Multiple experiments in the same animal should be abolished.

Extracellular recording of electrical activity of sensory axons of siNA-treated or control guinea pig cornea can be carried out as described in Brock et al. in 1998 (see Brock JA, McLachlan EM, Belmonte C. Tetrodotoxin-resistant impulses in single nociceptor nerve terminals in guinea-pig cornea. *J Physiol.* 1998 Oct 1;512 (Pt 1):211-7). Basically, eyes from guinea-pigs (150-300 g, killed with 100 mg kg⁻¹ pentobarbitone I.P.) are mounted in a recording chamber and superfused with physiological saline of the following composition (mM): Na⁺, 151; K⁺, 4.7; Ca²⁺, 2; Mg²⁺, 1.2; Cl⁻, 144; H₂PO₃⁻, 1.3; HCO₃⁻, 16.3; and glucose, 9.8. This solution is gassed with 95 % O₂-5 % CO₂ (to pH 7.4) and maintained at 31-33°C. The optic nerve and associated ciliary nerves are drawn into a suction stimulating electrode. The stimulus parameters are modified as required throughout the experiment (pulse width, 0.1-0.5 ms, 5-30 V). A glass recording electrode (tip outer diameter, 50 µm) filled with physiological saline is applied to the surface of the corneal epithelium with slight suction. Electrical activity is recorded through an AC amplifier (Neurolog NL104, Digitimer Ltd, Welwyn Garden City, UK; gain, 2000; high pass filter set at 0.1 Hz) and the output digitized at 44 kHz and stored on magnetic tape using a PCM recorder (A. R. Vetter Co. Inc., Rebersburg, PA, USA). Recordings are only made from sites where the nerve impulses are readily distinguished from the noise (10 µV peak-to-peak when low pass filtered at 3-5 kHz). At many sites on the cornea, no evoked or spontaneous electrical activity is recorded or the signals are too small to be analysed. Internal perfusion of the recording electrode is achieved by inserting a fine plastic tube to within 200 µm of the electrode tip (see Brock & Cunnane, 1995, Effects of Ca²⁺ and K⁺ channel blockers on nerve impulses recorded from postganglionic sympathetic nerve terminals. *The Journal of Physiology* 489, 389-402). A MacLab data acquisition system (ADInstruments Pty Ltd, Castle Hill, NSW, Australia) is used to digitize (sampling frequencies, 10-20 kHz) electrophysiological signals previously recorded on tape. Prior to digitizing, the signals are filtered using a low pass filter (cut-off, 3-5 kHz). Subsequent analysis is made with the computer program Igor Pro (Wavemetrics, Lake Oswego, OR, USA). TRPV1 mRNA levels can be quantitated by Real-time quantitative PCR (qRT-PCR) while reduction in the protein levels can be directly monitored in a variety of ways well known in the art, such as Western blot analysis with specific antibodies to the target.

Downregulation of expression of TRPV1 by siNA in hair follicle can be monitored by means of the following representative models without excluding other animal models well known in the art:

- *In vivo* mouse hair follicle transfection. Under general anesthesia, dorsal skin of 50-day-old Balb/c mice (Charles River, Wilmington, MA) is clipped of hair and treated with a depilatory cream or a shaving machine (Neet; Premier Consumer Products, Inc., Englewood, NJ) for 5 min. At different time points after depilation, 50 µl of lipoplex containing 50 µg of lipid and different amounts of individual siRNAs together with 10 µg of pCMV-b-gal is applied topically to 1 cm² of dorsal skin in 5 µl aliquots using a micropipette over 90 min. DNA amounts are similar to previously published studies. Control skin is treated with a scrambled siRNA together with 10 µg naked plasmid or 50 µg of lipid alone. Transfected skin is harvested 24 or 48 h after transfection, and stained for b-gal activity.

- Xenograft model. Four-week-old CB-17 Icr-scid/scid male mice (Charles River, Wilmington, MA) are maintained under pathogen-free conditions. Human fetal scalp (20-week gestation from Advanced Bioscience Resources, Alameda, CA), or human adult scalp from cosmetic surgery is grafted within 24 h of harvest. Grafting surgery is performed in a laminarflow hood using sterile procedures. Mice are anesthetized with ketamine–xylazine mixture, after which hair on the dorsum is clipped. Pieces of skin measuring 1x1 cm are grafted to a bed of similar size that had been prepared by removing mouse skin down to the fascia. Human skin grafts (usually two per mouse) are held in place with 6-0 nonabsorbable monofilament suture. The transplants are coated with petrolatum and covered with Tegadem™ (3M Health Care Ltd. (St. Paul, MN)), and sterile dressing. Bandages are removed after two to three weeks, and grafts are allowed to heal for an additional two to three weeks before proceeding with the experiments.

- Transfection of human xenografts *in vivo*. Before transfection, xenografts are depilated. Some grafts are also treated with 0.05% retinoic acid cream (Retin-A™,

Johnson & Johnson, Raritan, NJ) every other day for one week. On the day of transfection, mice are anesthetized and the xenografts are prehydrated with PBS for 15 min.. After 75 mg of pFx-1 lipid and 30 mg of pCMV-b-gal with or without individual siRNAs are mixed in OPTI-MEM, the mixture is pipetted topically in 5 ml aliquots (75 ml total) to the grafted skin every other day for three days. Higher doses of DNA and liposomes are used in human versus mouse transfections because of the thicker stratum corneum, which could potentially absorb the lipoplex and prevent adequate delivery to the follicle. Skin is harvested 48 h after transfection and processed for b-gal activity.

- Histochemical assays. Mouse and human tissue samples are fixed in freshly prepared 2% formaldehyde/0.2% glutaraldehyde in PBS at 4°C for 2–4 h, then washed in three changes of PBS at room temperature for 1 h. Fixed tissue is incubated at 37°C overnight in 1 mg ml⁻¹ X-gal in PBS. Then tissue is washed with PBS, fixed in formalin, and embedded in paraffin. Sections of 5 mm are counterstained with nuclear fast red. Fixed biopsies can also be stained with specific antibodies to test the inhibition of expression of TRPV1. Staining is performed as reported previously. Some biopsy slices are kept on RNA later (Ambion) and processed for RNA purification. Total RNA is analysed using specific probes/primers for specific interference after individual siRNA treatments.

EXAMPLES

Example 1

In order to determine whether the behavioral response to topical application of the TRPV1 agonist capsaicin was modified by pretreatment with siRNA prepared against the guinea pig TRPV1, experiments were carried out in adult, male guinea pigs.

1-Effect of topical capsaicin.

Two guinea pigs were treated with 10µL drop of a 0.1mM capsaicin solution in the right eye and 10µL of sterile isotonic saline in the left eye.

In the following 5 min following topical application the following parameters were measured:

- Blinking frequency
- Time of lid closure
- Scratching movements (with hind limb) and wiping movements (with the foreleg) directed to the treated eye.

After this 5 min period the following parameters were assessed:

- Conjunctival hyperemia and palpebral edema
- Tearing (with a Schirmer strip maintained 3 min in the subconjunctival sac).

Experiments were performed at 9:00am during 4 consecutive days. Both eyes were treated simultaneously with the corresponding solution, and parameters were measured simultaneously by an independent observer for each eye.

All the measured parameters were higher in the capsaicin-treated eye when compared with the control (saline-treated). The parameters that were most consistently altered were the number of scratching/wiping movements, hyperemia and lacrimation.

Figure 4 summarizes the results obtained in 2 animals. It is evident that capsaicin evoked scratching/wiping movements, conjunctival hyperemia and lacrimation that were absent in the saline-treated side. Also, no desensitization to repeated applications during successive days was observed.

2- Effect of oligonucleotide 3 (ON3), corresponding to SEQ ID NO 146 with dTdT overhangs in both 3' ends, on the capsaicin response.

The attenuating effect of ON3 on the behavioural response to topical capsaicin was initially explored in a group of 4 guinea pigs. Two doses of 15 μ L of a solution containing the ON3 was applied topically to the right eye (treated eye) at 9:00 am during the days 0, 1 and 2. At 3:00 pm during days 1,2,3,4,7,8 and 9, 10 μ of 0.1mM capsaicin solution were applied to both eyes and the

behavioral response to the drug measured in each eye, according to the methods described above.

As shown in figure 5, ON3 evoked a reduction of the behavioral response (number of scratching/wiping movements) to capsaicin application in comparison with untreated animals. This reduction was established gradually, starting on day 2 and augmenting in successive days. Seven days after treatment, differences in the response between ON3-treated and untreated eyes were still present. In contrast, tear secretion was not different between both eyes.

Figure 6 summarizes the average reduction in the number of scratching/wiping movements in all the studied animals, presented in absolute values (upper graph) and as percentage of reduction in the treated side from the control side, at the various days after treatment.

Example 2

Eyes from deeply anesthetized guinea pigs were dissected free from their orbits and mounted in a divided recording chamber. The eye was continuously perfused with physiological solution of the following composition (mM): Na⁺, 151; K⁺, 4.7; Ca²⁺, 2; Mg²⁺, 1.2; Cl⁻, 144.5; H₂PO₃⁻, 1.3; HCO₃⁻, 16.3; glucose, 7.8. This solution was gassed with carbogen (95% O₂, 25% CO₂) to pH 7.4 and maintained at approx 34 °C with a Peltier device. Activity of single corneal nerve fibers was recorded from the ciliary nerves at the back of the eye. The recording configuration is shown schematically in Figure 7.

Control eyes: Neural activity was recorded from 20 polymodal fibers obtained from 10 guinea pig eyes identified by their response to mechanical stimulation and by their response to a jet of gas containing CO₂ (98%). In each fiber a sequence of stimuli was applied: a drop of capsaicin 0.1mM followed by washing 30 sec later. Hot saline at 45°C during 1 min, Pulse of CO₂ applied during 30s. Between each stimulus, a pause of 5 min was allowed. Three of these sequences were applied per single fiber with a 15 min interval between

stimulation cycles. Quantification of the response was performed measuring the total number of impulses evoked by each stimulus during the stimulation period.

Treated eyes: In 5 guinea pigs, both eyes were treated with 15 μ L of a solution containing ON3 at 9:00am every day during three consecutive days. On the fourth day, both eyes were enucleated and studied 'in vitro' as described above.

Nine fibers identified as polymodal nociceptor fibers were identified in these eyes. The same stimulation protocol used for control eyes, namely capsaicin, heat and CO₂ stimuli applied sequentially with a 5 min pause between stimuli, repeated three times per fibers. Quantification of the response to each of these stimuli showed that the number of impulses evoked was significantly lower in ON3 treated animals. Reduction of the response in comparison with control eyes was: 60% for capsaicin, 56% for heat and 40% for acidic stimulation.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. Use of a pharmaceutically effective dose of siNA directed against transient receptor potential vanilloid-1 (TRPV1) in the preparation of a medicament for use in the treatment of an eye condition characterised by increased expression and/or activity of TRPV1, wherein said medicament is formulated for administration topically to the corneal surface.
2. The use of claim 1, wherein the medicament is formulated for administration as eye drops.
3. The use of claim 1 or claim 2, wherein the eye condition is discomfort or altered sensitivity of the cornea following refractive surgery, use of contact lenses, dry eyes, diabetic retinopathy.
4. The use of any one of claims 1 to 3, wherein the siNA is siRNA.
5. The use of claim 4, wherein the siRNA is dsRNA or shRNA.
6. The use of any one of claims 1 to 4, wherein the siNA comprises a modified oligonucleotide wherein said modification comprises phosphorothioate internucleotide linkage, a 2'-O-methyl ribonucleotide, a 2'-deoxy-fluoro ribonucleotide, a 2'-deoxy ribonucleotide, a "universal base" nucleotide, a 5-C methyl nucleotide, an inverted deoxyabasic incorporation, a chemical cross linking between the two complementary strands of an siRNA, a chemical modification of a 3' or 5' terminus of a strand of an siRNA, a sugar modification, a nucleobase modification and/or a backbone modification, a 2'-fluoro modified ribonucleotide or a 2'-deoxy ribonucleotide, a substitution of an unmodified pyrimidine nucleotide with a 2-thio, 5-alkynyl, 5-methyl, or 5-propynyl pyrimidine or substitution of an unmodified purine with a 7-deza, 7-alkyl, or 7-alkenyl purine.
7. The use of any one of claims 1 to 6, wherein the medicament comprises a plurality of species of siNA.

8. The use of claim 7, wherein said plurality of species are targeted to the same mRNA species.
9. The use of any one of claims 1 to 8, wherein the siNA is targeted to a sequence which is one of SEQ ID NOS 1 to SEQ ID NOS 81.
10. The use of any one of claims 1 to 8, wherein the siNA is targeted to a sequence comprising a sequence which is one of SEQ ID NOS 1 to SEQ ID NOS 81.
11. The use of any one of claims 1 to 10, wherein the siNA comprises a nucleotide sequence which is one of SEQ ID NOS 82 to SEQ ID NOS 162.
12. The use of claim 11, wherein siNA molecule contains 3' overhangs.
13. siNA directed against TRPV1 for use in the treatment of an eye condition characterised by increased expression and/or activity of TRPV1, wherein said siNA is formulated for administration topically to the corneal surface.
14. A siNA compound targeted to TRPV1 comprising a nucleotide sequence complementary to the nucleotide sequence of SEQ ID NO: 65.
15. A siNA compound according to claim 14, comprising the nucleotide sequence of SEQ ID NO: 146.
16. A siNA compound according to claim 15, comprising SEQ ID NO 146 with overhangs at both 3' ends wherein both overhangs are dTdT.
17. The siNA compound as defined in any one of claims 14 to 16, wherein the siNA is siRNA.
18. The siNA compound of claim 17, wherein the siRNA is dsRNA or shRNA.
19. The siNA compound according to any one of claims 14 to 18, wherein the siNA comprises a modified oligonucleotide wherein said modification is a phosphorothioate internucleotide linkage, a 2'-O-methyl ribonucleotide, a 2'-deoxy-fluoro ribonucleotide, a 2'-deoxy ribonucleotide, a "universal base" nucleotide, a 5-C methyl nucleotide, an

inverted deoxyabasic incorporation, a chemical cross linking between the two complementary strands of an siRNA, a chemical modification of a 3' or 5' terminus of a strand of an siRNA, a sugar modification, a nucleobase modification and/or backbone modification, a 2'-fluoro modified ribonucleotide or a 2'-deoxy ribonucleotide, a substitution of an unmodified pyrimidine nucleotide with a 2-thio, 5-alkynyl, 5-methyl, or 5-propynyl pyrimidine or substitution of an unmodified purine with a 7-deza, 7-alkyl, or 7-alkenyl purine.

20. A commercial package comprising a siNA compound as defined in any one of claims 14 to 19, together with instructions for the use thereof to treat an eye condition characterised by increased expression and/or activity of TRPV1.

21. Use of a pharmaceutically effective dose of a siNA compound as defined in any one of claims 14 to 19 in the preparation of a medicament for use in a method of treatment of an eye condition characterised by increased expression and/or activity of TRPV1 wherein said medicament is formulated for administration topically to the corneal surface.

22. A siNA compound as defined in any one of claims 14 to 19, for use in the treatment of an eye condition characterised by increased expression and/or activity of TRPV1 wherein said siNA is formulated for administration topically to the corneal surface.

23. A pharmaceutical composition comprising a siNA compound as defined in any one of claims 14 to 19, together with a pharmaceutically acceptable diluent or carrier.

24. The pharmaceutical composition according to claim 23, for the treatment of an eye condition characterised by increased expression and/or activity of TRPV1, wherein the pharmaceutical composition is formulated for administration topically to the corneal surface.

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FIGURES

Figure 1

Accession number	Definition
NM_080704	Transient receptor potential cation channel, subfamily V, member 1 (TRPV1), transcript variant 1, mRNA
NM_018727	Transient receptor potential cation channel, subfamily V, member 1 (TRPV1), transcript variant 2, mRNA
NM_080706	Transient receptor potential cation channel, subfamily V, member 1 (TRPV1), transcript variant 3, mRNA
NM_080705	Transient receptor potential cation channel, subfamily V, member 1 (TRPV1), transcript variant 4, mRNA

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Figure 2

Transient receptor potential cation channel, subfamily V, member 1 (TRPV1)	
SEQ ID 1	GAAATGGAGCAGCACAGAC
SEQ ID 2	ATGGAGCAGCACAGACTTG
SEQ ID 3	TGGAGCAGCACAGACTTGG
SEQ ID 4	AAGGACACCTGCCCAGACC
SEQ ID 5	GACCCTCAGGCTCTATGAT
SEQ ID 6	GCCGTTGCTCAGAATAACT
SEQ ID 7	TAAGTGCCAGGATCTGGAG
SEQ ID 8	CTGCCAGGATCTGGAGAGC
SEQ ID 9	GAGCAAGAAGCACCTACA
SEQ ID 10	GAAGCACCTCACAGACAAC
SEQ ID 11	GCACCTCACAGACAACGAG
SEQ ID 12	CGAGTTCAAAGACCCTGAG
SEQ ID 13	AGACCCTGAGACAGGGAAG
SEQ ID 14	GACCCTGAGACAGGGAAGA
SEQ ID 15	GACCTGTCTGCTGAAAGCC
SEQ ID 16	AGCCATGCTCAACCTGCAT
SEQ ID 17	GCCATGCTCAACCTGCATG
SEQ ID 18	CCTGCATGACGGACAGAAC
SEQ ID 19	ACGGACAGCCTGAAGGAGC
SEQ ID 20	CGGACAGCCTGAAGGAGCT
SEQ ID 21	GGAGCTTGTCAACGCCAGC
SEQ ID 22	GAAAACCAAAGGGCGGCCT
SEQ ID 23	AACCAAAGGGCGGCCTGGA
SEQ ID 24	ACCAAAGGGCGGCCTGGAT
SEQ ID 25	CCAAAGGGCGGCCTGGATT
SEQ ID 26	AGGGCGGCCTGGATTCTAC
SEQ ID 27	GGGCGGCCTGGATTCTACT
SEQ ID 28	CCAGCTGGGCATCGTGAAG
SEQ ID 29	GTTCCTGCTGCAGAACTCC
SEQ ID 30	CACGGCCGACAACACGAAG
SEQ ID 31	CACGAAGTTTGTGACGAGC
SEQ ID 32	GTTTGTGACGAGCATGTAC
SEQ ID 33	TGAGATTCTGATCCTGGGG
SEQ ID 34	ACTGCACCCGACGCTGAAG
SEQ ID 35	GCTGGAGGAGCTCACCAAC
SEQ ID 36	CAAGAAGGGAATGACGCCG
SEQ ID 37	GAAGGGAATGACGCCGCTG
SEQ ID 38	GATCGGGGTCTTGGCCTAT
SEQ ID 39	GTTCAACCGAGTGGGCCTAC
SEQ ID 40	GAAGTCCGGTGGTGGAGGTG
SEQ ID 41	CTCGGTGCTGGAGGTGATC
SEQ ID 42	TCGCCACGACATGCTCTTG
SEQ ID 43	CCGACTCCTGCAGGACAAG
SEQ ID 44	GTGGGACAGATTGCTCAAG
SEQ ID 45	GCGCATCTTCTACTTCAAC

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SEQ ID 46	CTTCCTGGTCTACTGCCTG
SEQ ID 47	GATGGAAAAAATTGGAGAC
SEQ ID 48	AAATTGGAGACTATTTCCG
SEQ ID 49	AATTGGAGACTATTTCCGA
SEQ ID 50	ATTGGAGACTATTTCCGAG
SEQ ID 51	TTGGAGACTATTTCCGAGT
SEQ ID 52	GACCCTGTTTGTGGACAGC
SEQ ID 53	GGAGTATGTGGCTTCCATG
SEQ ID 54	CATGCTCTACTACACCCGC
SEQ ID 55	GATGATCCTGAGAGACCTG
SEQ ID 56	GACGGGAAGAATGACTCCC
SEQ ID 57	GAATGACTCCCTGCCGTCT
SEQ ID 58	TGACTCCCTGCCGTCTGAG
SEQ ID 59	CAGCCTGTACTCCACCTGC
SEQ ID 60	GTTCACCATCGGCATGGGC
SEQ ID 61	CTATGACTTCAAGGCTGTC
SEQ ID 62	GGCTGTCTTCATCATCCTG
SEQ ID 63	TTCTCACCTACATCCTCCT
SEQ ID 64	CATGCTCATCGCCCTCATG
SEQ ID 65	CAAGATCGCACAGGAGAGC
SEQ ID 66	GATCGCACAGGAGAGCAAG
SEQ ID 67	GAACATCTGGAAGCTGCAG
SEQ ID 68	CATCTGGAAGCTGCAGAGA
SEQ ID 69	GCTGCAGAGAGCCATCACC
SEQ ID 70	GAGCTTCCTTAAGTGCATG
SEQ ID 71	GTGCATGAGGAAGGCCTTC
SEQ ID 72	CTGGACCACCTGGAACACC
SEQ ID 73	CACCAACGTGGGCATCATC
SEQ ID 74	CGTGGGCATCATCAACGAA
SEQ ID 75	CGAAGACCCGGGCAACTGT
SEQ ID 76	GCAGAGTTTCAGGCAGACA
SEQ ID 77	GAACTTTGCCCTGGTCCCC
SEQ ID 78	CTTTGCCCTGGTCCCCCTT
SEQ ID 79	GAGAGGCAAGTGCTCGAGA
SEQ ID 80	GTGCTCGAGATAGGCAGTC
SEQ ID 81	GTTTATCTGCGACAGTTTT

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Figure 3

Transient receptor potential cation channel, subfamily V, member 1 (TRPV1)			
SEQ ID 82	5'	GAAAU	3'
SEQ ID 83	3'	CUUU	5'
SEQ ID 84	5'	AUGG	3'
SEQ ID 85	3'	UACC	5'
SEQ ID 86	5'	UGG	3'
SEQ ID 87	3'	ACC	5'
SEQ ID 88	5'	AAG	3'
SEQ ID 89	3'	UUC	5'
SEQ ID 90	5'	GAC	3'
SEQ ID 91	3'	CUG	5'
SEQ ID 92	5'	GCC	3'
SEQ ID 93	3'	CGG	5'
SEQ ID 94	5'	UAA	3'
SEQ ID 95	3'	AU	5'
SEQ ID 96	5'	CUG	3'
SEQ ID 97	3'	GAC	5'
SEQ ID 98	5'	GAG	3'
SEQ ID 99	3'	CUC	5'
SEQ ID 100	5'	GA	3'
SEQ ID 101	3'	CU	5'
SEQ ID 102	5'	GC	3'
SEQ ID 103	3'	CG	5'

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SEQ ID 104	5' AACCAAAGGGCGGCCUGGA 3'
	3' UUGGUUCCCGCCGGACCU 5'
	5' ACCAAAGGGCGGCCUGGAU 3'
SEQ ID 105	
	3' UGGUUCCCGCCGGACCUA 5'
	5' CCAAAGGGCGGCCUGGAUU 3'
SEQ ID 106	
	3' GGUUUCCCGCCGGACCUAA 5'
	5' AGGGCGGCCUGGAUUCUAC 3'
SEQ ID 107	
	3' UCCCGCCGGACCUAAGAUG 5'
	5' GGGCGGCCUGGAUUCUACU 3'
SEQ ID 108	
	3' CCCGCCGGACCUAAGAUGA 5'
	5' CCAGCUGGGCAUCGUGAAG 3'
SEQ ID 109	
	3' GGUCGACCCGUAGCACUUC 5'
	5' GUUCCUGCUGCAGAACUCC 3'
SEQ ID 110	
	3' CAAGGACGACGUCUUGAGG 5'
	5' CACGGCCGACAACACGAAG 3'
SEQ ID 111	
	3' GUGCCGGCUGUUGUGCUUC 5'
	5' CACGAAGUUUGUGACGAGC 3'
SEQ ID 112	
	3' GUGCUUCAACACUGCUCG 5'
	5' GUUUGUGACGAGCAUGUAC 3'
SEQ ID 113	
	3' CAAACACUGCUCGUACAUG 5'
	5' UGAGAUUCUGAUCCUGGGG 3'
SEQ ID 114	
	3' ACUCUAAGACUAGGACCCC 5'
	5' ACUGCACCCGACGCUGAAG 3'
SEQ ID 115	
	3' UGACGUGGGCUGCGACUUC 5'
	5' GCUGGAGGAGCUCACCAAC 3'
SEQ ID 116	
	3' CGACCUCCUCGAGUGGUUG 5'
	5' CAAGAAGGGAAUGACGCCG 3'
SEQ ID 117	
	3' GUUCUCCCUUACUGCGGC 5'
	5' GAAGGGAAUGACGCCGUG 3'
SEQ ID 118	
	3' CUUCCCUUACUGCGGCGAC 5'
	5' GAUCGGGGUCUUGGCCUAU 3'
SEQ ID 119	
	3' CUAGCCCCAGAACCGGAUA 5'
	5' GUUCACCGAGUGGGCCUAC 3'
SEQ ID 120	
	3' CAAGUGGCUCACCCGGAUG 5'
	5' GAACUCGGUGCUGGAGGUG 3'
SEQ ID 121	
	3' CUUGAGCCACGACCUCCAC 5'
	5' CUCGGUGCUGGAGGUGAUC 3'
SEQ ID 122	
	3' GAGCCACGACCUCCACUAG 5'
	5' UCGCCACGACAUGCUCUUG 3'
SEQ ID 123	
	3' AGCGGUGCUGUACGAGAAC 5'
	5' CCGACUCCUGCAGGACAAG 3'
SEQ ID 124	
	3' GGCUGAGGACGUCCUGUUC 5'
	5' GUGGGACAGAUUCGUCAAG 3'
SEQ ID 125	
	3' CACCCUGUCUAAGCAGUUC 5'
	5' GCGCAUCUUCUACUUAAC 3'
SEQ ID 126	
	3' CGCGUAGAAGAUGAAGUUG 5'
	5' CUUCCUGGUCUACUGCCUG 3'
SEQ ID 127	
	3' GAAGGACCAGAUGACGGAC 5'

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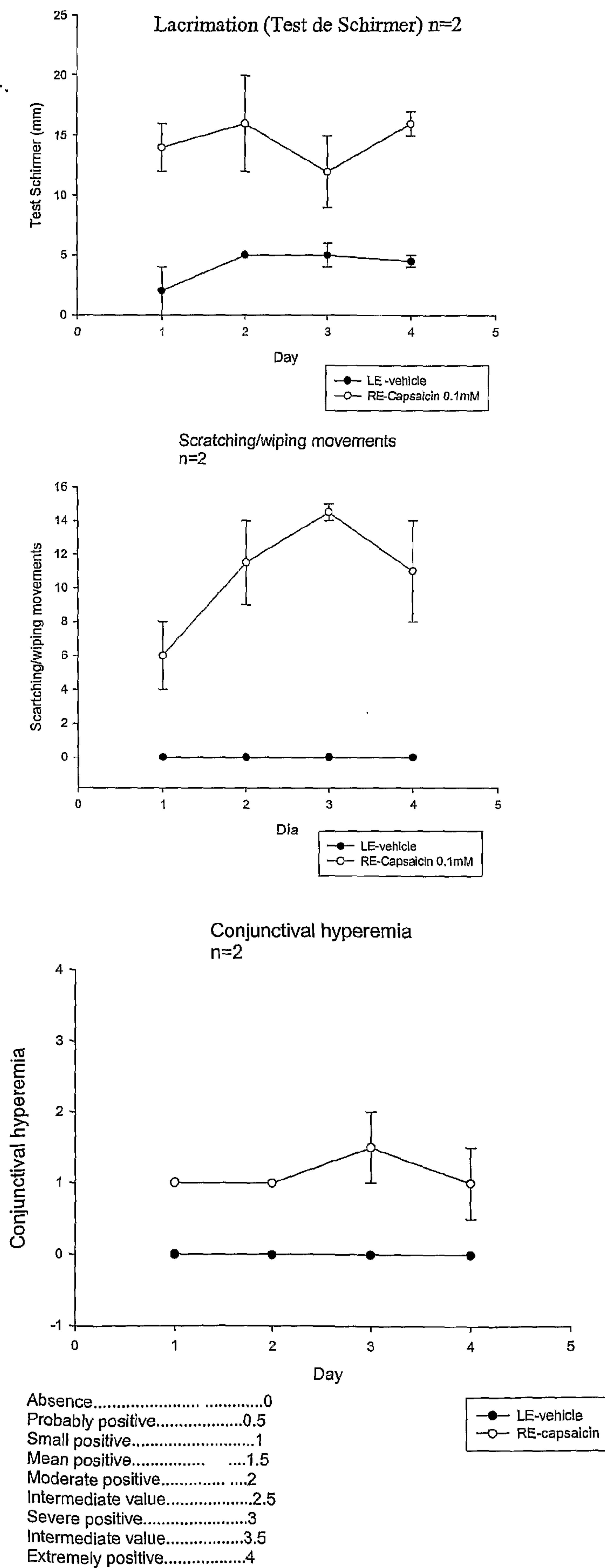
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	5'	AAUUGGAGACUAUUUCCGA	3'
SEQ ID 130			
	3'	UUAACCUCUGAUAAAGGCU	5'
	5'	AUUGGAGACUAUUUCCGAG	3'
SEQ ID 131			
	3'	UAACCUCUGAUAAAGGCUC	5'
	5'	UUGGAGACUAUUUCCGAGU	3'
SEQ ID 132			
	3'	AACCUCUGAUAAAGGCUCA	5'
	5'	GACCCUGUUUGUGGACAGC	3'
SEQ ID 133			
	3'	CUGGGACAAACACCUUGUCG	5'
	5'	GGAGUAUGUGGCUUCCAUG	3'
SEQ ID 134			
	3'	CCUCAUACACCGAAGGUAC	5'
	5'	CAUGCUCUACUACACCCGC	3'
SEQ ID 135			
	3'	GUACGAGAUGAUGUGGGCG	5'
	5'	GAUGAUCCUGAGAGACCUG	3'
SEQ ID 136			
	3'	CUACUAGGACUCUCUGGAC	5'
	5'	GACGGGAAGAAUGACUCCC	3'
SEQ ID 137			
	3'	CUGCCCUUCUACUGAGGG	5'
	5'	GAAUGACUCCCUGCCGUCU	3'
SEQ ID 138			
	3'	CUUACUGAGGGACGGCAGA	5'
	5'	UGACUCCCUGCCGUCUGAG	3'
SEQ ID 139			
	3'	ACUGAGGGACGGCAGACUC	5'
	5'	CAGCCUGUACUCCACCUGC	3'
SEQ ID 140			
	3'	GUCGGACAUGAGGUGGACG	5'
	5'	GUUCACCAUCGGCAUGGGC	3'
SEQ ID 141			
	3'	CAAGUGGUAGCCGUACCCG	5'
	5'	CUAUGACUUAAGGCUGUC	3'
SEQ ID 142			
	3'	GAUACUGAAGUUCGACAG	5'
	5'	GGCUGUCUUAUCAUCCUG	3'
SEQ ID 143			
	3'	CCGACAGAAGUAGUAGGAC	5'
	5'	UUCUCACCUACAUCUCCU	3'
SEQ ID 144			
	3'	AAGAGUGGAUGUAGGAGGA	5'
	5'	CAUGCUCUACGCCCUCUAG	3'
SEQ ID 145			
	3'	GUACGAGUAGCGGAGUAC	5'
	5'	CAAGAUCCGACAGGAGAGC	3'
SEQ ID 146			
	3'	GUUCUAGCGUGUCCUCUCG	5'
	5'	GAUCGCACAGGAGAGCAAG	3'
SEQ ID 147			
	3'	CUAGCGUGUCCUCUCGUUC	5'
	5'	GAACAUCUGGAAGCUGCAG	3'
SEQ ID 148			
	3'	CUUGUAGACCUUCGACGUC	5'
	5'	CAUCUGGAAGCUGCAGAGA	3'
SEQ ID 149			
	3'	GUAGACCUUCGACGUCUCU	5'
	5'	GCUGCAGAGAGCCAUCACC	3'
SEQ ID 150			
	3'	CGACGUCUCUCGGUAGUGG	5'
	5'	GAGCUUCCUUAAGUGCAUG	3'
SEQ ID 151			
	3'	CUCGAAGGAAUUCACGUAC	5'
SEQ ID 152			
	5'	GUGCAUGAGGAAGGCCUUC	3'

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	3'	CACGUACUCCUCCGGAAG	5'
	5'	CUGGACCACCUGGAACACC	3'
SEQ ID 153			
	3'	GACCUGGUGGACCUUGUGG	5'
	5'	CACCAACGUGGGCAUCAUC	3'
SEQ ID 154			
	3'	GUGGUUGCACCCGUAGUAG	5'
	5'	CGUGGGCAUCAUACGAA	3'
SEQ ID 155			
	3'	GCACCCGUAGUAGUUGCUU	5'
	5'	CGAAGACCCGGGCAACUGU	3'
SEQ ID 156			
	3'	GCUUCUGGGCCCGUUGACA	5'
	5'	GCAGAGUUUCAGGCAGACA	3'
SEQ ID 157			
	3'	CGUCUCAAAGUCCGUCUGU	5'
	5'	GAACUUUGCCCUGGUCCCC	3'
SEQ ID 158			
	3'	CUUGAAACGGGACCAGGGG	5'
	5'	CUUUGCCCUGGUCCCCCUU	3'
SEQ ID 159			
	3'	GAAACGGGACCAGGGGGAA	5'
	5'	GAGAGGCAAGUGCUCGAGA	3'
SEQ ID 160			
	3'	CUCUCCGUUCACGAGCUCU	5'
	5'	GUGCUCGAGAUAGGCAGUC	3'
SEQ ID 161			
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	5'	GUUUUAUCUGCGACAGUUUU	3'
SEQ ID 162			
	3'	CAAUAGACGCUGUCAAAA	5'

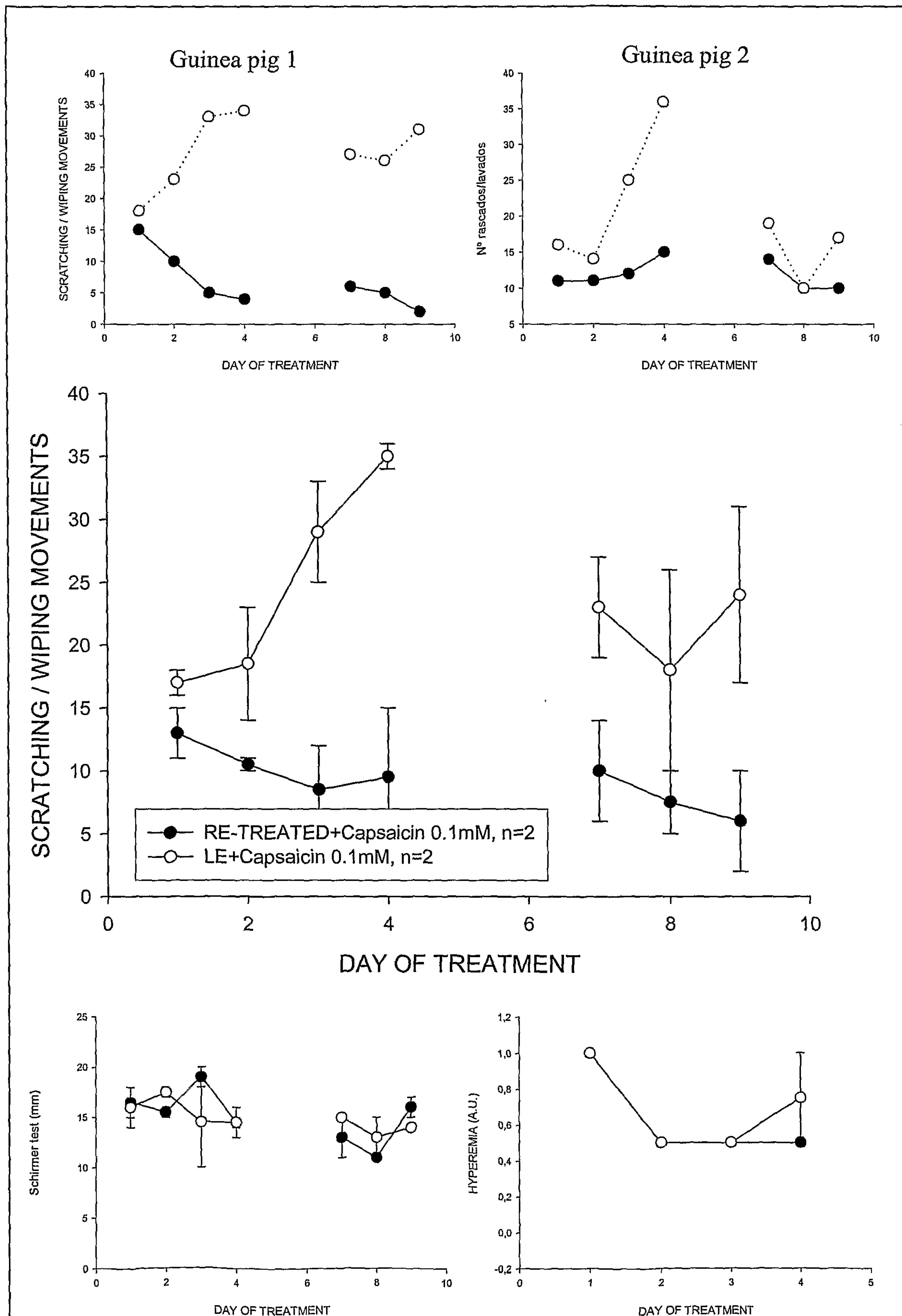
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Figure 4.



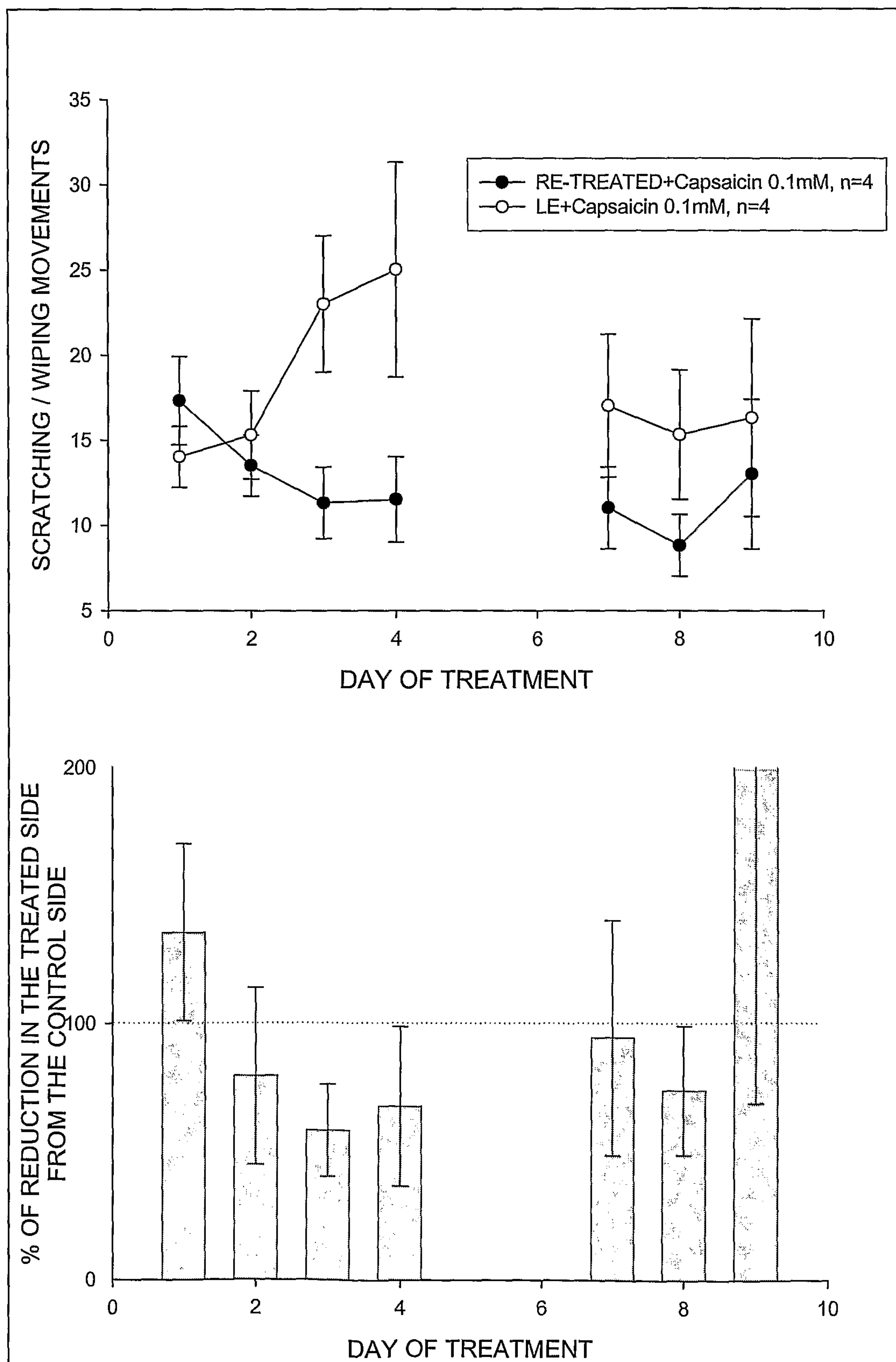
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Figure 5.



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Figure 6.



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Figure 7.

Stimulation:

- Mechanical: von Frey hairs
- Chemical: CO₂ pulse
- Thermal (heat): 45°C solution
- Capsaicin 0.1mM

