Corneal antinociceptive effect of (-)-α-bisabolol

Gisele Façanha Diógenes Teixeira\textsuperscript{a,b}, Flávio Nogueira da Costa\textsuperscript{a} and Adriana Rolim Campos\textsuperscript{a} \textsuperscript{BD}

\textsuperscript{a}Experimental Biology Centre (Nubex), University of Fortaleza (Unifor), Ceará, Brazil; \textsuperscript{b}School of Medicine, Christus University Centre (Unichristus), Ceará, Brazil

\textbf{ABSTRACT}

\textbf{Context:} (-)-α-Bisabolol (BISA) is a sesquiterpene alcohol widely used as scent in cosmetic preparations, perfumes, shampoos, toilet soaps and other toiletries with potential for use in the pharmaceutical area.

\textbf{Objective:} To evaluate the corneal antinociceptive efficacy of BISA and to analyze the best solubilizing agent.

\textbf{Materials and methods:} Acute corneal nociception was induced by the local application of hypertonic saline (5 M NaCl; 20 \textmu L) to the corneal surface of Swiss mice (n = 8/group) 60 min after topical treatment with solutions or ointment containing BISA (50–200 mg/mL). The number of eye wipes performed with the ipsilateral forepaw was counted for a period of 30 s. Control groups (vehicles) were included.

\textbf{Results:} BISA (50, 100 or 200 mg/mL) solubilized with Tween 80 did not reduce the number of eye wipes. Animals treated with the ointment (BISA 50, 100 or 200 mg/mL; p < 0.001), as well the solution containing propylene glycol (BISA 100 mg/mL; p < 0.05), showed significant reduction in the number of nociceptive behaviours. Solutions containing propylene glycol and isopropyl myristate had no effects.

\textbf{Discussion and conclusion:} BISA possess corneal antinociceptive activity. Although the ointment presented antinociceptive effect, it is concluded that BISA when associated with propylene glycol has better potential for corneal nociceptive pain since it is more comfortable to use, leading to greater acceptance by patients.

\textbf{Introduction}

The topical drug application is preferably used in ocular disorders (Abdul Nasir et al. 2015). This form of delivery is widely used mainly because of the easiness of use and adherence to the treatment (Souza et al. 2014).

The biodistribution of drugs by this route is still a challenge, due to its low bioavailability (Abdul Nasir et al. 2013), which confers protection barriers mechanical (blinking, for example) and physiological (such as the corneal epithelium and the nasolacrimal system) that promote rapid removal of the substances from the ocular surface (Bucolo et al. 2012), which can make the absorption of the drug applied topically insufficient even when the focus of treatment is the anterior chamber (Souza et al. 2014).

The cornea has characteristics that make it an effective barrier to drug absorption: it has small size, and its relative impermeability is avascular (Das & Suresh 2010). On the corneal epithelium, the cells are lipophilic, which hinders the passage of hydrophilic substances. On the stromal layer of the cornea, there happens some difficulty of absorption of lipophilic molecules by their matrix cells consisting of hydrophilic cells (Souza et al. 2014).

For these reasons, high doses of drugs or frequent administrations are needed in order to achieve the desired result, which increases the risk of side effects (Cunha Junior et al. 2003). Aiming to have better absorption, the use of absorption facilitators (Furrer et al. 2002), microemulsions (Cunha Junior et al. 2003), liposomes (Abdul Nasir et al. 2013) or nanoparticles (Reus et al. 2009) are recommended. Systemic route can also be used, however, the drug distribution become impaired by the difficult passage through the blood-aqueous barrier and blood-retinal (Bucolo et al. 2012).

(-)-α-Bisabolol (BISA) is a sesquiterpene alcohol found in the essential oil of various plant species, among them \textit{Matricaria chamomilla} L. and \textit{Vanillosmopsis} are the important species (Kamatou & Viljoen 2010). This sesquiterpene is widely used as an additive in cosmetic products as anti-irritant creams, after sun lotions (Lee et al. 2010), astringent for the skin care, sunscreens and make-up (Spiegel 2012).

Recently, Solovâstru et al. (2015) reported that a spray containing ozonated oil and BISA could be a new therapeutic option for the adjuvant treatment of venous ulcers. In another study, BISA had excellent results when applied in the form of shampoo after hair transplant and sensitive scalp. This is due to the fact that it has anti-inflammatory properties and the absence of potentially irritating ingredients (Schweiger et al. 2015).

Our group has demonstrated the topical anti-inflammatory effect of BISA in experimental models of ear oedema (Leite et al. 2011) and that BISA may attenuate nociceptive sensorimotor responses and central sensitization evoked by noxious orofacial stimuli (Melo et al. 2015). Moreover, the essential oil of \textit{Vanillosmopsis arborea} Baker rich in BISA presented antinociceptive topical effect when applied to the paw in mice and the eye (Leite et al. 2014).

This sesquiterpene is completely soluble in ethanol and isopropyl alcohol. To mix it with water, it is necessary to use...
solubilizing agents. This study aimed to evaluate the corneal antinociceptive efficacy of BISA and to analyze the best solubilizing agent.

Materials and methods

Obtaining of (-)-α-bisabolol

(-)-α-Bisabolol (purity ≥93%) was purchased from Sigma-Aldrich (St. Louis, MO).

Animals

Eighty-four Swiss albino mice (20–30 g) from the experimental animal facility at the Christus University Center and University of Fortaleza (UNIFOR) were kept in a controlled environment (circadian cycle, 22 °C) with free access to water and standard pellet diet (Purina, São Paulo, Brazil). The experimental protocols followed the ethical guidelines of CONCEA (Brazilian Council for the Control of Animal Experimentation) and were approved by the UNIFOR Animal Research Ethics Committee under entry number 004/2012.

Solubilizing agents

Solutions containing Tween 80 (BISA 50, 100 or 200 mg/mL), isopropyl myristate (BISA 100 mg/mL) and propylene glycol (BISA 100 mg/mL) were manufactured. Ointment [(liquid petrolatum (40%) and petrolatum (60%)] containing different concentrations of BISA (50, 100 or 200 mg/mL) was also developed.

Eye wiping test

Corneal nociception was induced in mice by instillation of one drop (20 μL) of hypertonic saline (5 M NaCl) on the corneal surface using a fine dropper (Farazifard et al. 2005). The number of eye wipes performed with the ipsilateral forepaw during the first 30 s was registered. Mice (n = 6/group) were pretreated topically (20 μL) with vehicles (controls), solutions or ointment before induction.

Statistical analysis

The results are presented as mean ± SEM of each group of six animals. The statistical analysis consisted of one-way analysis of variance (ANOVA), followed by the Tukey’s post hoc test for multiple comparisons. Student’s t test was used to determine the significance of the differences between two groups. The level of statistical significance was set at 5% (p < 0.05).

Results

BISA (50–200 mg/mL) solubilized with Tween 80 did not reduce the number of eye wipes (Table 1). Animals pretreated with the

| Table 1. Effect of BISA + Tween 80 on corneal nociception induced by NaCl 5M. |
|-----------------|-----------------|-----------------|
| Group           | BISA (mg/mL)    | Number of eye wipes (30 s) |
| Control         | –               | 12.33 ± 3.11     |
| Tween 80 solution | 50              | 9.91 ± 6.16      |
|                 | 100             | 10.17 ± 3.13     |
|                 | 200             | 10.58 ± 5.61     |

Data are expressed as mean ± SEM. ANOVA followed by Tukey test.

Discussion

The cornea is the most densely innervated tissue in the body. The majority (about 70%) of sensory afferent fibres are polymodal nociceptors activated by mechanical forces, exogenous chemical irritants, endogenously released chemical mediators and extreme temperatures (Belmonte et al. 2004). Pain management strategies include topical anaesthetics and non-steroidal anti-inflammatory with caution, since these drugs may be toxic to keratocytes (Moreira et al. 1999) and promote transient burning, stinging and conjunctival hyperaemia (Kim et al. 2010).

The development of an effective topical solution for the treatment of corneal disorders, which is able to achieve a therapeutic dose without the need for high concentrations, or frequent administration, is a challenge for biotechnology.

Analgesics currently available for the treatment of pain following ophthalmic surgery or injury are limited by transient effectiveness and undesirable or adverse side effects (Bates et al. 2010). Topical ophthalmic non-steroidal anti-inflammatory drug (NSAID) preparations have been used for a range of painful eye conditions (Smith & Goldman 2012). However, adverse events associated with ophthalmic NSAIDs include brief burning and stinging, hyperaemia of the conjunctiva, and contact dermatitis (Calder et al. 2005). A more serious complication involves the association of topical ophthalmic NSAIDs with indolent corneal ulceration and full-thickness corneal melts (Gaynes & Fiscella 2002).

Due to low toxicity, the Food and Drug Administration has classified BISA as ‘generally regarded as safe’ (GRAS), boosting its use as active ingredient in commercial products (Kamatou &

| Table 2. Effect of an ointment containing BISA on corneal nociception induced by NaCl 5M. |
|-----------------|-----------------|-----------------|
| Group           | BISA (mg/mL)    | Number of eye wipes (30 s) |
| Control         | –               | 8.90 ± 1.03     |
| Ointment        | 50              | 3.60 ± 0.65***  |
|                 | 100             | 4.20 ± 0.55***  |
|                 | 200             | 3.30 ± 0.55***  |

Data are expressed as mean ± SEM. ***p < 0.001 compared to control group. ANOVA followed by Tukey test.

| Table 3. Effect of BISA + propylene glycol in solution on corneal nociception induced by NaCl 5M. |
|-----------------|-----------------|-----------------|
| Group           | BISA (mg/mL)    | Number of eye wipes (30 s) |
| Control         | –               | 6.43 ± 2.12     |
| Polypropylene glycol solution | 100            | 0.42 ± 0.20*    |

Data are expressed as mean ± SEM. *p < 0.05 compared to control group. Student t-test.

| Table 4. Effect of BISA + isopropyl myristate in solution on corneal nociception induced by NaCl 5M. |
|-----------------|-----------------|-----------------|
| Group           | BISA (mg/mL)    | Number of eye wipes (30 s) |
| Control         | –               | 6.71 ± 1.01     |
| Isopropyl myristate | 100            | 5.71 ± 0.55     |

Data are expressed as mean ± SEM. Student t-test.
BISA decreased the number of eye wipes and this effect may be related 5-HT, z1, TRPV1 and central muscarinic receptors (Leite et al. 2014). Barreto et al. (2016) found the orofacial effect of BISA is associated with TNF-α but not with IL-1β. Others have reported the effect of BISA to be associated with the inhibitory activity of COX (Oritz et al. 2016) and antioxidant effects in encephalic tissue (Leite et al. 2016). Furthermore, the antinociceptive action of BISA is not linked to a central mechanism (Rocha et al. 2011) but is more likely due to anti-inflammatory properties.

Here, some solubilizing agents have been tested in order to verify their influence on the corneal antinociceptive effect of BISA. Tween 80 surfactant is a polyethylene sorbitan ester (polysorbate), non-ionic widely used commercially to solubilize oil into water (Narayanan 2008). Its action can be limited due to their high viscosity (Shaaban & Edris 2015) and probably is not suitable for use in formulating a topical solution containing BISA.

Isopropyl myristate is an emollient ester which provides spreadability without presenting irritating or sensitizing properties (Morselli et al. 2014). Although it shows good skin penetration (Pastore Jr & Araújo 2005), in our study, this ester abolished the antinociceptive effect of BISA.

Propylene glycol is a diol alcohol used in the pharmaceutical industry as a humectant and also in the preparation of plant extracts (Oliveira et al. 2014). It can form a film between BISA and water, increasing the elasticity and surface tension of the solution and can lead to the formation of a soluble solution, possibly allowing the antinociceptive effect of BISA (Shaaban & Edris 2015), since BISA, when in solution with propylene glycol, showed highly significant analgesic effect, reducing the nociceptive behaviour at 93.5%.

The ointment containing BISA presented antinociceptive effect at all concentrations tested. However, eye ointments have certain disadvantages as blurring of vision and sometimes have irritating effects, leading to lower acceptance by patients.

**Conclusions**

BISA possesses corneal antinociceptive activity. Although the ointment presented antinociceptive effect, it is concluded that BISA when associated with propylene glycol has better potential for corneal nociceptive pain since it is more comfortable to use, leading to greater acceptance by patients.

**Acknowledgements**

The authors are thankful to Capes, CNPq, Funcap for the fellowships and financial support and to Fundação Edson Queiroz for facilities.

**Disclosure statement**

The authors report no declarations of interest.

---

**ORCID**

Adriana Rolim Campos http://orcid.org/0000-0002-7355-9310

---

**References**

Abdul Nasir NA, Alyautdin RN, Agarwa R, Nukolova N, Cheknonin V, Mohd Ismail N. 2013. Ocular tissue distribution of topically applied PEGylated and non-PEGylated liposomes. Adv Mater Res. 832:1–8.

Abdul Nasir NA, Agarwal P, Agarwal R, Iezhitsa I, Alyautdin R, Nukolova NN, Cheknonin VP, Mohd Ismail N. 2015. Intraocular distribution of topically applied hydrophilic and lipophilic substances in rat eyes. Drugs Deliv. 14:1–7.

Barreto RS, et al. 2016. Evidence for the involvement of TNF-α and IL-1β in the antinociceptive and anti-inflammatory activity of Stachys lavandulifolia Vahl. (Lamiaceae) essential oil and (-)-α-bisabolol, its main compound, in mice. J Ethnopharmacol. 191:9–18.

Bates BD, Mitchell K, Keller JM, Chan CC, Swaim WD, Yaskovich R, Mannes AJ, Iadarola MJ. 2010. Prolonged analgesic response of cornea to topical resiniferatoxin, a potent TRPV1 agonist. Pain. 149:522–528.

Bhatia SP, McGinty D, Letizia CS, API AM. 2008. Fragrance material review on alpha-bisabolol. Food Chem Toxicol. 46 (Suppl 11):S72–S76.

Belmonte C, Acosta MC, Gallar J. 2004. Neural basis of sensation in intact and injured corneas. Exp Eye Res. 78:513–525.

Bucolo C, Drago F, Salomone S. 2012. Ocular drug delivery: a clue from nanotechnology. Front Pharmacol. 3:1–3.

Calder L, Balasubramanian S, Stiell I. 2005. Topical nonsteroidal anti-inflammatory drugs for oral orofacial pain. Cochrane Database Syst Rev. (2):CD003626.

Cunha Junior AS, Fialho SL, Carneiro LB, Ordonez E, Furrer P, Mayer JM, Plazonnet B, Gurny R. 2002. Ocular tolerance of absorptives as drug carriers for topical ocular administration. Arq Bras Oftalmol. 66:385–391.

Das S, Suresh PK. 2010. Drug delivery to eye: special reference to nanoparticles. Int J Drug Deliv. 2:12–21.

Farazifard R, Safarpour F, Sheblani V, Javan M. 2005. Eye-wiping test: a sensitive animal model for acute trigeminal pain studies. Brain Res Brain Res Protoc. 16:44–49.

Furrer P, Mayer JM, Plazennet B, Gurny R. 2002. Ocular tolerance of absorptives as drug carriers for topical ocular administration. Arq Bras Oftalmol. 66:385–391.

Kim SJ, Flach AJ, Jampol LM. 2010. Nonsteroidal anti-inflammatory drugs in ophthalmology. Surv Ophthalmol. 55:108–133.

Lee J, Jun H, Jung E, Ha J, Park D. 2010. Whitening effect of alpha-bisabolol in Asian women subjects. Int J Cosmet Sci. 32:299–303.

Leite GO, Leite LH, Sampaio Rde S, Araruna MK, de Menezes IR, da Costa MG, de Menezes IR, Campos AR. 2014. Topical antinociceptive effect of Vanillinosopsis arborea Baker on acute corneal pain in mice. Evid Based Complement Alternat Med. Article ID 708636.

Leite GO, Dubois AF, Seeger RL, Boligon AA, Costa JGM, Lugokenski TH, Boligon AA, Costa JGM, Lugokenski TH, Boligon AA, Costa JGM. 2016. In vitro antinociceptive activity of Vanillinosopsis arborea Baker aqueous extracts, essential oil and isolated compound: (-)-α-bisabolol and α-bisabolol-rich oils. J Am Oil Chem Soc. 87:1–7.

Melo LT, Panchaligam V, Aivv-Arber L, Cherkas P, Campos AR, Sessle BJ. 2015. Effects of the natural substance (-)-α-bisabolol on trigeminal central sensitisation and sensorimotor behaviour induced by acute noxious orofacial stimuli. Neuroscience meeting planner. Washington (DC): Society for Neuroscience. Online.

Moreira LB, Kasetsuwan N, Sanchez D, Shah SS, LaBree L, McDonnell PJ. 1999. Toxicity of topical anesthetic agents to human keratocytes in vivo. J Cataract RefRACT Surg. 25:975–980.

Morselli LNS. 2014. Estudos de pré-formulação e desenvolvimento de cosméticos Dimora Del Sole. Araquari, Brasil: Universidade Estadual Paulista.

Narayanan V. 2008. Synthesis of mesoporous silica microsphere from dual surfactant. Mater Res. 11:443–446.

Oliveira JR, Fabrin TMC, Galbiati TA, Barboza LN, Furlanetto DO, Ferreira MF, de Oliveira JC, da Silva GR, Gasques LS. 2014. Interference check the surfactant. Mater Res. 11:443–446.

Ortiz MJ, Fernández-Martínez E, Soria-Jasso LE, Lucas-Gómez J, Villagómez-Ilbarra R, González-García MP, Castañeda-Hernández G, Salinas-Caballero M. 2016. Isolation, identification and molecular docking as cyclooxygenase inhibitors.
(COX) inhibitors of the main constituents of Matricaria chamomilla L. extract and its synergistic interaction with diclofenac on nociception and gastric damage in rats. Biomed Pharmacother. 78:248–256.

Pastore Jr, Araújo VF. 2006. Plantas da Amazônia para produção cosmética. Brasília, Brasil: Universidade de Brasília.

Reus M, Carmigna F, Senna E, Campos AM. 2009. Polymeric nanoparticles in topical ocular administration of drugs. Lat Am J Pharm. 28:125–132.

Rocha NF, Rios ER, Carvalho AM, Cerqueira GS, Lopes Ade A, Leal LK, Dias ML, de Sousa DP, de Sousa FC. 2011. Anti-nociceptive and anti-inflammatory activities of (-)-α-bisabolol in rodents. Naunyn Schmiedebergs Arch Pharmacol. 384:525–533.

Schweiger D, Schoelermann AM, Filbry A, Hamann T, Moser C, Rippke F. 2015. Highly efficient and compatible shampoo for use after hair transplant. Clin Cosmet Investig Dermatol. 8:355–360.

Shaaban HA, Edris HA. 2015. Factors affecting the phase behavior and antimicrobial activity of carvacrol microemulsions. J Oleo Sci. 64:393–404.

Smith HS, Goldman RD. 2012. Topical nonsteroidal anti-inflammatory drugs for corneal abrasions in children. Can Fam Physician. 58:748–749.

Solovástru LG, Stîncanu A, De Ascentii A, Capparé G, Mattana P, Vâja D. 2015. Randomized, controlled study of innovative spray formulation containing ozonated oil and α-bisabolol in the topical treatment of chronic venous leg ulcers. Adv Skin Wound Care. 28:406–409.

Souza JG, Dias K, Pereira TA, Bernardi DS, Lopez RF. 2014. Topical delivery of ocular therapeutics: carrier systems and physical methods. J Pharm Pharmacol. 66:507–530.

Spiegel M. 2012. Alpha-bisabolol – naturalness vs. sustainability? Sofw J. 138:1–8.