Neurochemistry International 131 (2019) 104530

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/neuint

Anti-hyperalgesic effect of (-)-α-bisabolol and (-)-α-bisabolol/β-Cyclodextrin complex in a chronic inflammatory pain model is associated with reduced reactive gliosis and cytokine modulation

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ARTICLE INFO

Keywords:
-)-α-bisabolol
Neuropathic pain
Terpene
Natural product

ABSTRACT

Chronic pain is a continuous or recurring pain which exceeds the normal course of recovery to an injury or disease. According to the origin of the chronic pain, it can be classified as inflammatory or neuropathic. This study aimed to evaluate the antinociceptive and anti-inflammatory effect of (-)-α-bisabolol (BIS) alone and complexed with β-cyclodextrin (βCD) in preclinical models of chronic pain. Chronic pain was induced by Freund's Complete Adjuvant (FCA) or partial lesion of the sciatic nerve (PLSN). Swiss mice were treated with BIS, BIS-βCD (50 mg/kg, p.o) or vehicle (control) and mechanical hyperalgesia, thermal hyperalgesia, muscle strength and motor coordination were evaluated. In addition, levels of TNF-α and IL-10 and expression of the ionized calcium-binding adapter protein (IBA-1) were assessed in the spinal cord of the mice. The complexation efficiency of BIS in βCD was evaluated by High-Performance Liquid Chromatography. BIS and BIS-βCD reduced ($p < 0.001$) mechanical and thermal hyperalgesia. No alterations were found in force and motor coordination. In addition, BIS and BIS-βCD inhibited ($p < 0.05$) TNF-α production in the spinal cord and stimulated ($p < 0.05$) the release of IL-10 in the spinal cord in PLSN-mice. Further, BIS and BIS-βCD reduced IBA-1 immunostaining. Therefore, BIS and BIS-βCD attenuated hyperalgesia, deregulated cytokine release and inhibited IBA-1 expression in the spinal cord in the PLSN model. Moreover, our results show that the complexation of BIS in βCD reduced the therapeutic dose of BIS. We conclude that BIS is a promising molecule for the treatment of chronic pain.

1. Introduction

Neuropathic pain (NP) is defined by the International Association for the Study of Pain (IASP) as "pain arising from an injury or disease affecting the somatosensory system" (Jensen et al., 2011). The prevalence of NP globally in the general population is 6.9–10% (Van Hecke et al., 2014) and studies have proposed that the significant increase in the incidence of NP over recent decades is related to the development of better methods to identify such pain (Haanpää et al., 2009; Freynhagen et al., 2006). Most of the current treatments (myorelaxant drugs, antidepressants, anticonvulsants or strong opioids) usually used for NP share a modest efficacy or safety, causing severe side effects (Bouhassira and Attal, 2016; Finnerup et al., 2016; McDermott et al., 2006; O'Connor, 2009; Wan et al., 2016). Neuropathic pain is a public health problem and an area in which there is a need to find improved therapeutic options (Dutra et al., 2016).

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https://doi.org/10.1016/j.neuint.2019.104530
Received 2 July 2019; Received in revised form 10 August 2019; Accepted 12 August 2019
Available online 16 August 2019

In this search for new therapeutic agents, one promising area is natural products (NPs). According to the National Medicinal Plant Board (NMPB) (Shakya, 2016) natural products represent more than 50% of approved drugs over the last three decades. In addition, natural products, such as terpenes and essential oils, have been shown to be a promising source of bioactive molecules for novel drugs to treat neuropathic pain symptoms. One promising terpene is (-)-α-bisabolol (BIS), a natural monocyclic sesquiterpene alcohol, isolated from the essential oil of a variety of plants, mainly chamomile (*Matricaria chamomille*) (Braga et al., 2009; Melo et al., 2017; Rocha et al., 2011). Studies have reported that it has several pharmacological activities such as anti-inflammatory (Rocha et al., 2011), gastroprotective (Bezerra et al., 2009), antioxidant (Agatonovic-Kustrin et al., 2015; Meeran et al., 2018) and antinociceptive (Leite et al., 2012; Rocha et al., 2011; Melo et al., 2019) properties.

BIS is found in the formulation of 35 pharmaceutical products, including dermatological and cosmetic formulations such as hand- and body-lotions, deodorants, lipsticks, sun-care and after-sun products, baby care products and sport creams (Gomes-Carneiro et al., 2005; Kamatou and Viljoen, 2010). It is classified by the US Food and Drug Administration as a substance that is Generally Regarded as Safe (GRAS) due to its low toxicity (Kamatou and Viljoen, 2010).

Despite the pharmacological potential of BIS, terpenes have physical-chemical properties that may be problematic for oral administration, mainly their low water solubility, slow dissolution rate and low bioavailability (Lima et al., 2016; Quintans et al., 2013). These products have, therefore, been incorporated into cyclodextrins (CDs) to improve drug solubility and facilitate drug delivery. Cyclodextrins are therapeutic systems which have the purpose of improving a drug's stability and modulating its kinetic release and absorption. (Janes et al., 2001). They are macrocyclic oligosaccharides consisting of glucose units linked together by glycosidic bonds, produced in a synthetic or natural way, and totally or partially encapsulating solid or liquid compounds. Among them, the most commonly used CD in products is β-cyclodextrin (Lima et al., 2016; Szente et al., 2016).

Therefore, given the limitations in respect of effective and safe drugs for the treatment of neuropathic pain; and considering that BIS has several promising pharmacological properties to alleviate the symptoms characteristic of this condition, this study aimed to evaluate the antinociceptive and anti-inflammatory effect of BIS alone and its β-cyclodextrin (BIS-βCD) inclusion complex in preclinical models of neuropathic pain.

2. Material and methods

2.1. Drugs and chemicals

Those used were: (-)-α-bisabolol (≥95% purity, Sigma/EUA), β-cyclodextrin (98% purity, Sigma/EUA), eBioscience mouse TNF-α and IL-10 Elisa Ready-SET-Go!® Rabbit Anti-IBA1 were obtained from WAKO, Anti-rabbit Alexa 488 was purchased from Life Technologies (USA).

2.2. Characterization of the β-cyclodextrin inclusion complex

2.2.1. Entrapment efficiency (EE %)

EE was performed by means of High-Performance Liquid Chromatography – Diode Array Detector (HPLC–DAD). For the chromatographic analysis, a standard solution (100 μg/mL) of BIS ($C_{15}H_{26}O$; ≥ 95.0% Sigma-Aldrich) was prepared dissolved in acetonitrile (HPLC – grade, Panreac, Darmstadt, Germany) which was immersed in an ultrasonic bath for 30 min. Before the HPLC injection, the sample was filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane filter. The HPLC analyses were performed using a high-performance liquid chromatography system that consisted of a degasser DGU-20A3, two LC-20AD pumps, a SIL-20A HT auto injector, a CTO-20A column oven, a SPDM20Avp photodiode array detector (DAD) and a CBM-20A system controller (Shimadzu Co., Kyoto, Japan). The chromatographic separation was performed using a Phenomenex Luna C18 analytical column 4.6 × 150 mm^2 (5 μm particle size). The injection volume was 20 μL and the mobile phase flow rate was 1.0 mL/min. The solvents used in the mobile phase were: ultrapure water (Milli-Q system, Millipore, Bedford, USA) and, acetonitrile. The elution profile was determined in isocratic mode (20/80 – A/B) performed over 10 min. The detector was set at 200 nm for acquiring the chromatogram. The data was obtained using the LC Solution software. To evaluate the EE%, 10 mg of the inclusion complex produced by the slurry complexation (SC) method in a 1:1 M ratio was dissolved in 10 mL of acetonitrile and left for 36 h after being well mixed (250 rpm) to allow enough time for all entrapped BIS to be in solution. After this procedure, the solution was centrifuged at 3500 rpm for 30 min to remove any βCD from the solution, leaving only the BIS. 2 mL of the supernatant was collected, filtered with a 0.45 μm membrane filter (PTFE) and analyzed by HPLC. The EE% was calculated as:

$$EE\% = \frac{amount\ of\ BIS\ \ entrapped}{initial\ BIS\ \ amount} * 100$$

where “amount of BIS entrapped” is the BIS amount present in the inclusion complex particles and “initial BIS amount” indicates the BIS amount initially used to prepare the inclusion complex with βCD.

3. Experimental procedures

3.1. Experimental animals

Male *Swiss* mice (30–40 g, 2–3 months of age), were randomly housed in appropriate cages at 22 ± 2 °C on a 12 h light/dark cycle (lights on 06:00–18:00 h) with free access to food (Purina®) and water. All experiments were carried out between 08:00 and 17:00 h in a quiet room. The Animal Research Ethics Committee of the Federal University of Sergipe approved the experimental protocols and procedures (CEPA/UFS 04/2017). During the experimental procedures every effort was made to minimize the number of animals used as well as any discomfort to them.

3.2. Experimental groups

The animals were divided into the following groups (n = 8/per group):

a. Negative control group: 0.2% Tween 80 in distilled water (0.1 mL/g; p.o. once a day);
b. Sham group: 0.2% Tween 80 in distilled water (0.1 mL/g; p.o. once a day); animals had no nerve damage or pain induction;
c. BIS group: 50 mg/kg BIS (0.1 mL/g; p.o. once a day);
d. BIS-βCD group: 50 mg/kg BIS-βCD (0.1 mL/g; p.o. once a day).

3.3. Induction of chronic inflammatory pain (Freund's Complete Adjuvant - CFA)

The animals were immobilized and injected (via i.pl.) with 25 μL of CFA (1 mg/mL of dry heat-killed *Mycobacterium tuberculosis* in paraffin oil and mannitol monooleate) (Quintão et al., 2008). Twenty-four hours after the CFA injection, the animals were treated daily. One hour after treatment administration, the animals were evaluated for mechanical hyperalgesia.

3.4. Induction of neuropathic pain - partial lesion of the sciatic nerve (PLSN)

Mice were anesthetized and a trichotomy was performed on the area to be operated. An incision was made starting below and medially to

the major trochanter of the femur and ending near the popliteal fossa at the level of the insertion of the hamstrings. The sciatic nerve was then exposed after the identification of the semitendinosus and rectus femoris muscles. With sterile silk suture Nº. 8.0, ligation was performed around 1/3 to 1/2 of the nerve diameter (Malmberg and Basbaum, 1998). The incision was repaired with two stitches with absorbable yarn (Ethicon, 6-0). Six days after the surgical procedure and 1 h after the treatment, the animals were evaluated for behavioral parameters: mechanical hyperalgesia, thermal hyperalgesia and muscle strength.

3.5. Behavioral assessment

3.5.1. Mechanical hyperalgesia

The hyperalgesia was evaluated using an electronic analgesimeter (Von Frey, Model EFF 301, Insight®, Ribeirão Preto-SP, Brazil), adapted with a polypropylene tip (Cunha et al., 2004). This test consisted of evoking a flexion reflex of the hind paw with a hand force transducer. Before the PLSN, the animals had their paw withdrawal limit evaluated to record the baseline value. After the surgical procedure, another evaluation was performed to assess the hyperalgesic state of the animals. The evaluations took place daily, 1 h after treatment of the animal groups.

3.5.2. Thermal hyperalgesia - cold allodynia

Cold allodynia was evaluated using a modification of the acetone drop method described previously (Yoon et al., 1994). The test consisted of the application (with an automatic pipette) of 20 μL of acetone to the plantar surface of the right hind paw. The number of flinches (nociceptive behavior) performed by the animals in the first minute after application of acetone was observed.

3.5.3. Muscle strength

Hind paw and forepaw grip force were tested as previously described (Burnes et al., 2008) using a grip strength meter (Model EF 305, Insight Ltda., Ribeirão Preto, Brazil). The mice were placed on the grid of the apparatus and pulled by the tail to measure the force of adhesion of the front legs. The animals were evaluated three times and the mean was calculated to obtain the absolute force (g).

3.5.4. Motor coordination

The possible motor deficits associated with the administration of the substances were evaluated using a rota-rod apparatus. Specifically, the animals were placed on the rotating rod at a constant speed of 5–7 rpm for the first 120 s and then for a further 30 s at 9 rpm. The time (seconds) they remained on the bar for up to 180 s was recorded (Sluka and Wright, 2001).

3.6. Enzyme-linked Immunosorbent assay

On the third and seventeenth days after PLSN, the animals (n = 6–8) were sacrificed and the sciatic nerve and spinal cord (L4-L6) were dissected. Samples were homogenized in 1x PBS with protease inhibitor, centrifuged, their supernatant removed, and the protein was dosed by the Bradford method. The ELISA protocols for TNF-α and IL-10 cytokines were performed according to the manufacturer's instructions (eBioscience®) and analyzed with an Asys Expert plus, Biochrom® microplate reader.

3.7. Immunofluorescence

Mice (n = 6) were anesthetized using ketamine/xylazine and were perfused through the left cardiac ventricle with 0.01 M phosphate buffered saline solution, followed by 4% paraformaldehyde in 0.1 M PBS, pH 7.4. After perfusion, the spinal cord was removed, post-fixed in the same fixative solution for 2 h and cryoprotected by immersing in 30% sucrose solution in PBS at 4 °C. The spinal cord was then frozen and stored in a freezer (−80 °C) for later analysis. Serial coronal sections (30 μm) of spinal cord were obtained using a cryostat at −20 °C (Leica). Some sections were processed for immunofluorescence using IBA1. The free-floating sections were preincubated in 2% bovine serum albumin (BSA) diluted in PBS, containing 0.3% Triton X-100 (PBS-Triton X-100 0.3%) for 30 min. Immunofluorescence for IBA1 was carried out after 48 h incubation at 4 °C with rabbit monoclonal anti-IBA1 antibody diluted 1:1000 in BSA 1% PBS-Triton X-100 0.3%. The negative control was performed omitting the primary antibodies. After five washes in PBS, tissue sections were incubated with anti-rabbit Alexa 488, diluted 1:2000 in PBS-Triton X-100 0.3% for 2 h at room temperature. After five washes in PBS, sections were incubated with DAPI (0,0025 M) diluted in PBS-Triton X-100 (0.3%) and then the slices were transferred to gelatinized slides, mounted with glycerol-DABCO solution, covered with coverslips and sealed with nail polish. The images were obtained with a microscope and analyzed with Image J software.

3.8. Statistical analysis

Results were expressed as mean ± S.E.M. The differences between groups were analyzed using one-way or two-way ANOVA, followed by the Tukey test or Bonferroni test, respectively. Values of $p < 0.05$ were considered statistically significant. All statistical analyzes were performed using GraphPad Prism 5.0 software (GraphPad Prism Software Inc., San Diego, CA, USA).

4. Results

Fig. 1 represents the HPLC–DAD chromatographic profile of BIS (200 nm). The BIS peak showed excellent chromatographic resolution and a retention time of 5.3 min. The calibration curve generated the equation $y = 62102x + 41960$ and the R^2 value obtained from the data of six concentrations was 0.999, verifying the linearity of the equation in the concentration range (5–100 μg/mL). HPLC was used to determine the EE% of BIS in the inclusion complexes with βCD. EE% is a quantitative parameter used to calculate the amount of active compound entrapped in an inclusion complex. The inclusion complex produced by the SC method in a 1:1 M ratio showed an EE equal to 50% ± 0.19. Taking into account the molecular weight of βCD (1134.98) and BIS (284.7) in the molar ratio 1:1, the calculated dose of BIS present in the BIS-βCD solution complex is 10-fold smaller than in the BIS solution.

Injection of CFA caused mechanical hyperalgesia in the ipsilateral hind paw when compared to the sham group on all the evaluated days. The administration of BIS and BIS-βCD resulted in a significant increase ($F(3,312) = 857.58$, $p < 0.0001$) in the mechanical withdrawal threshold compared to the control group (Fig. 2A), persisting for every evaluation day. This is shown by the area under the curve (AUC) for all treatment days ($F(3,24) = 282.3$, $p < 0.0001$) (Fig. 2B).

The acute and chronic effect of BIS and BIS-βCD on mechanical hyperalgesia induced by partial sciatic nerve injury is shown in Fig. 3. A significant decrease in the paw withdrawal threshold was observed in injured mice when compared to the sham group. This effect persisted through all days studied in the control group. In the acute evaluation, animals treated with BIS and BIS-βCD demonstrated a rapid and long-lasting increase in the paw withdrawal threshold (seven and 8 h, respectively) when compared to the control group ($F(3,242) = 356.52$, $p < 0.0001$) (Fig. 3A). This is shown by the area under the curve (AUC) ($F(3,22) = 266.5$, $p < 0.0001$) (Fig. 3B).

In addition, the chronic evaluation showed an anti-hyperalgesic effect of BIS and BIS-βCD ($F(3,284) = 259.46$, $p < 0.0001$) when compared to the control (Fig. 3C) resulting in a reduction of mechanical hyperalgesia throughout the evaluation period, as confirmed by the AUC ($F(3,22) = 88.80$, $p < 0.0001$) (Fig. 3B and D). No tolerance effects were observed in the BIS and BIS-βCD groups.

As shown in Fig. 4, the PLSN increased in the number of flinches in

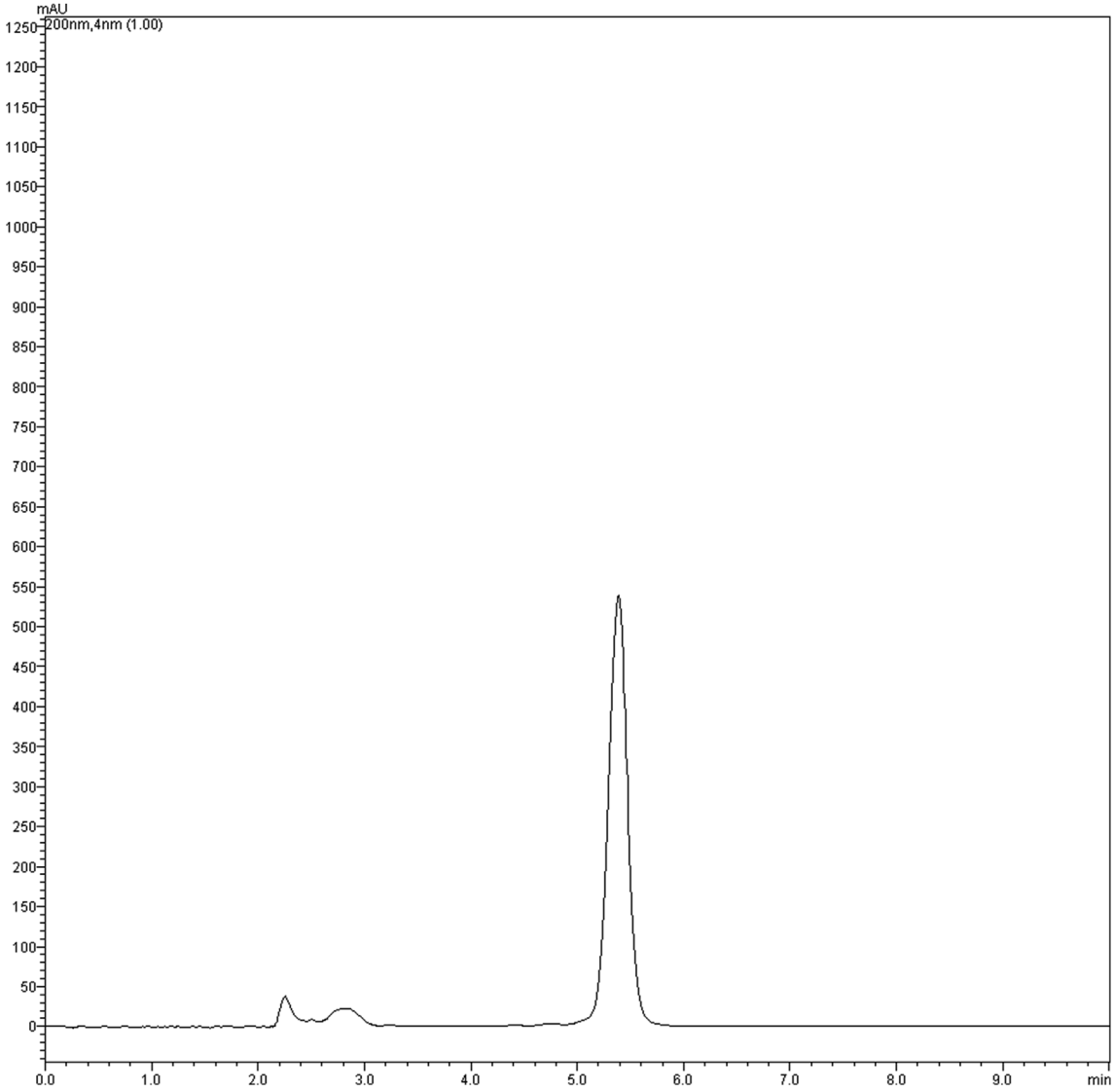

Fig. 1. Chromatographic profile of the (-)-α-bisabolol at 200 nm obtained by HPLC-DAD.

response to the cold stimulus. The administration of BIS and BIS-βCD caused an anti-allodynic effect evoked by acetone application on the left hind paw in animals with nerve lesion compared to the control and sham groups ($F(3,84) = 9,91$, $p < 0.0001$).

No change in muscle strength (Fig. 5A and B) or motor coordination in BIS and BIS-βCD treated mice (Fig. 5C) was found when compared to the control.

BIS and BIS-βCD reduced the TNF-α levels on both days evaluated -

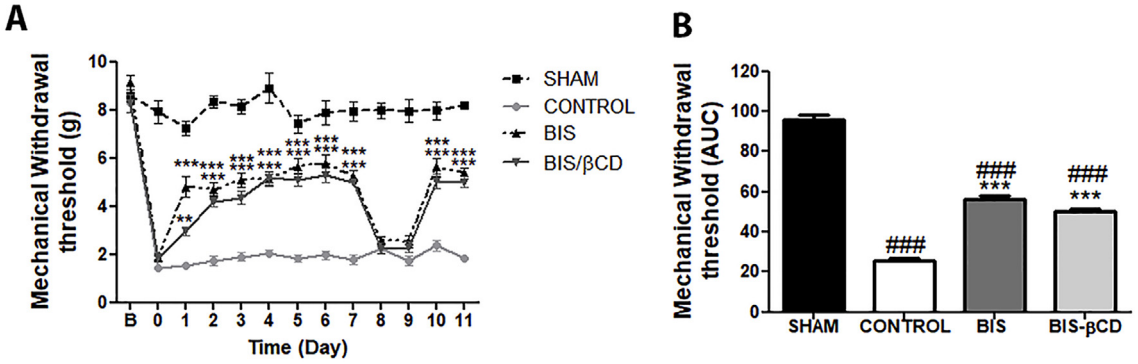

Fig. 2. Evaluation of the anti-inflammatory effect of BIS and BIS-βCD on the mechanical hyperalgesia induced by the chronic inflammatory pain model. **A.** Effect of chronic administration. **B.** Area under the curve of the chronic administration effect of BIS and BIS-βCD (50 mg/kg p.o.), vehicle (sham/control). Values expressed as mean ± S.E.M. (n = 8/group), ** $p < 0.01$ and *** $p < 0.001$ *vs* control; ### $p < 0.001$ *vs* Sham (Two-way Anova followed by the Bonferroni posttest in "A" and One-way Anova followed by Tukey posttest in "B").

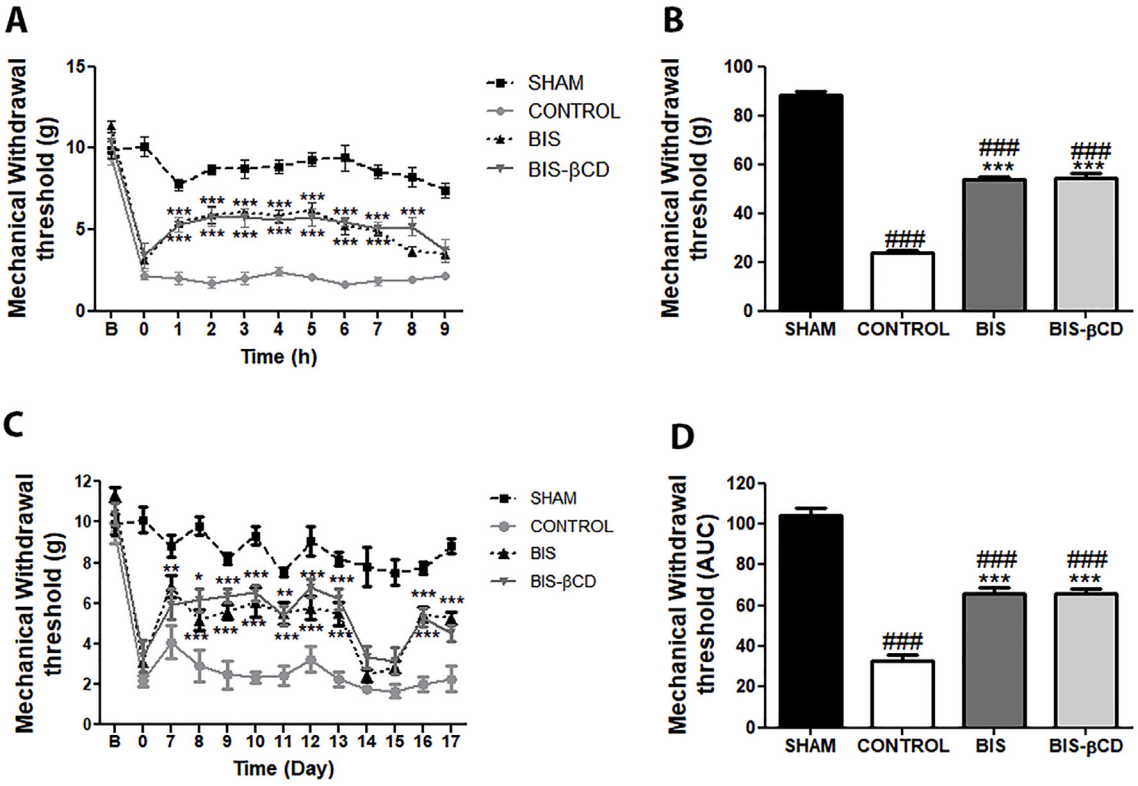

Fig. 3. Evaluation of the anti-hyperalgesic effect of BIS and BIS-βCD on mechanical hyperalgesia in a model of neuropathic pain induced by partial injury of the sciatic nerve **A**. Effect of acute administration. **B.** Area under the curve of the effect of acute administration. **C.** Effect of chronic administration. **D.** Area under the curve of the chronic administration. Values expressed as mean ± S.E.M. (n = 8/group), ***p < 0.001 *vs* control; ### p < 0.001 *vs* Sham (Two-way Anova followed by the Bonferroni posttest in "A" and "C"; One-way Anova followed by Tukey posttest in "B" and "D").

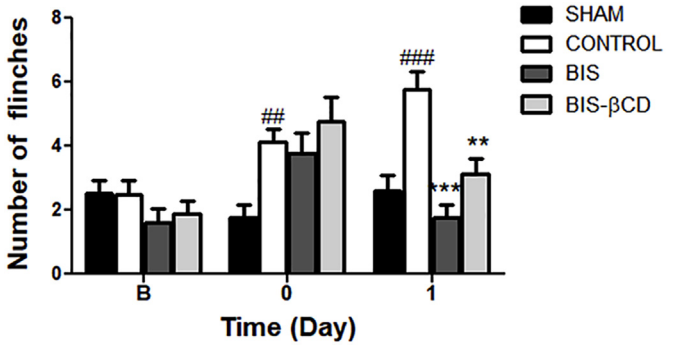

Fig. 4. Effect of acute administration of BIS and BIS-βCD (50 mg/kg; v.o.), vehicle (sham/control) on thermal hyperalgesia to cold stimulus in the neuropathic pain model induced by partial sciatic nerve injury. Values expressed as mean ± S.E.M. (n = 8/group). **p < 0.01 *vs* control, *** p < 0.001 *vs* control, ## p < 0.01 *vs* sham, ###p < 0.001 *vs* sham (Two-way Anova followed by the Bonferroni posttest).

three ($F(3,21) = 55.33$, $p < 0.0001$) and seventeen days ($F(3,19) = 13.37$, $p < 0.0001$) after PLSN. In addition, BIS and BIS-βCD stimulated IL-10 release in the spinal cord on the third ($F(3,53) = 9.407$, $p < 0.0001$), but not on the seventeenth day ($F(3,19) = 0.7114$, $p = 0.5571$) after PLSN (Fig. 6).

BIS and BIS-βCD attenuated the microgliosis induced by PLSN in the spinal cord (Fig. 7). Microglia cells on the ipsilateral side were heavily stained by Iba-1 immunostaining in the control animals, while BIS and BIS-βCD reduced IBA-1 immunostaining, evidence of reduced gliosis.

5. Discussion

In the present study, we evaluated the anti-inflammatory and antinociceptive effect of BIS and BIS-βCD in preclinical models of neuropathic pain. BIS is very lipophilic and easily oxidizes (Waleczek et al., 2003) and the products of its oxidation reduce its anti-inflammatory efficacy (Schilcher, 1987). The complexation of BIS with β-cyclodextrin renders this terpene less susceptible to oxidation (Thoss et al., 1994).

However, in pharmacological studies of a substance complexed with βCD it is important to know the amount of active principle incorporated in the βCD. Thus, we determined the EE% of BIS in an inclusion complex with βCD and found that there was a ten-fold decrease in the amount of BIS present in the BIS-βCD. It is interesting to note that no significant difference was observed between the free drug and the inclusion complex in the pharmacological assays, so the effective dose of BIS-βCD is much lower than pure BIS. Leite et al. (2018) similarly showed that the complexation of the essential oil of *Vanillosmopsis arborea* (98.35% of BIS) with βCD reduced the antinociceptive dose of the essential oil.

The increased pharmacological efficacy of BIS complexed with βCD probably decreases any adverse effects of the substance. Data in the literature suggest that βCD may improve the efficacy and bioavailability of analgesic and anti-inflammatory compounds (Brito et al., 2015) and can reduce the possibility of adverse events, as well as their potential toxicity.

The present research demonstrated that pretreatment with BIS and BIS-βCD reduced inflammatory and neuropathic pain by decreasing microglia activation and proinflammatory cytokine release. Moreover, this terpene upregulated anti-inflammatory cytokine levels.

CFA promoted mechanical and thermal hyperalgesia, producing a chronic inflammatory response. It is known that CFA increases TRPA1 expression in trkA-expressing DRG neurons (Obata et al., 2005) and

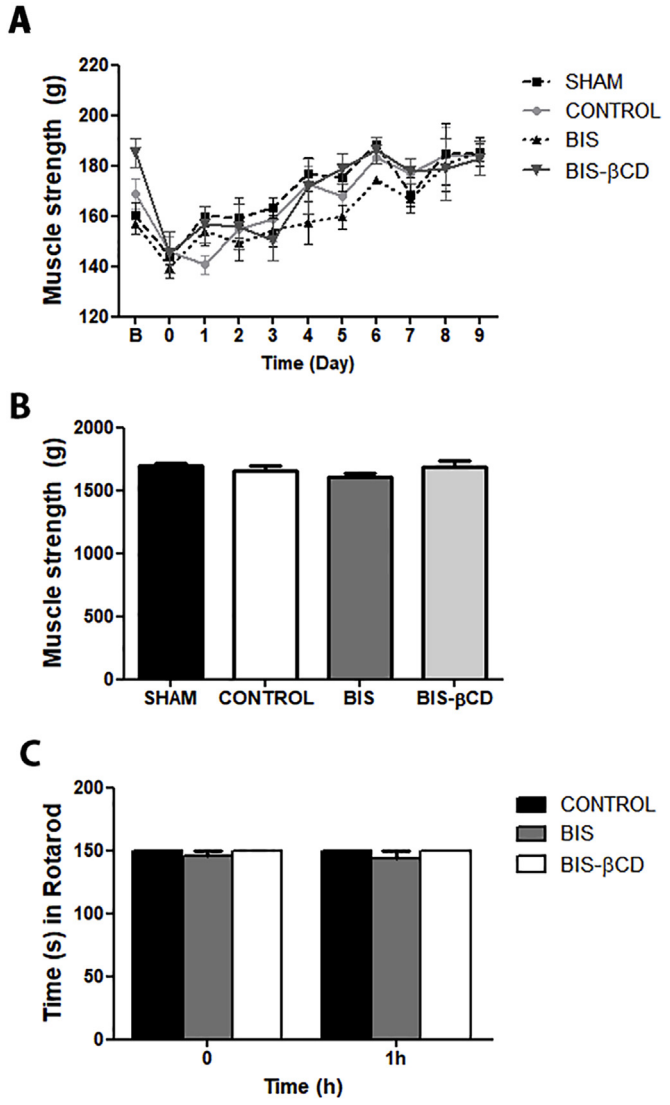

Fig. 5. A. Effect of BIS and BIS-βCD (50 mg/kg v.o.) on muscle strength in the neuropathic pain model induced by partial sciatic nerve injury. **B.** Area under the curve of the chronic administration effect of BIS and BIS-βCD (50 mg/kg; v.o.) on muscle strength. **C.** Effect of administration of BIS and BIS-βCD (50 mg/kg; v.o.) on motor coordination. Values expressed as mean ± S.E.M. (n = 8/group) (Two-way Anova followed by the Bonferroni posttest in "A"; One-way Anova followed by Tukey posttest in "B" and "C").

that BIS seems to be a TRPA1 antagonist (Melo et al., 2017). BIS and BIS-βCD reduced the hyperalgesia induced by CFA and this effect may be related to TRPA1 inhibition.

To better understand the anti-hyperalgesic effect of BIS and the role of inflammatory mediators in its pharmacological profile, we evaluated the activity of BIS and BIS-βCD in a neuropathic pain model induced by partial sciatic nerve injury (PLSN). BIS and BIS-βCD significantly reduced mechanical hyperalgesia and the allodynia induced by PLSN. In addition, the anti-hyperalgesic effect of BIS was associated with decreased TNF-α levels and upregulated IL-10 production in the spinal cord of mice.

Several studies have shown that neuroinflammation plays an essential role in the induction and maintenance of neuropathic pain (Austin and Moalem-Taylor, 2010; Clark et al., 2013; Thacker et al., 2007). In peripheral nerve damage, glial cells support neuroinflammation and are associated with the production and release of pro-inflammatory mediators, such as cytokine TNF-α (Kiguchi et al., 2010; Schäfers et al., 2003; Scholz and Woolf, 2007). TNF-α plays a pivotal role in neuropathic pain development because this inflammatory mediator can change several mechanisms of pain transmission.

El Karim et al. (2015) showed that TNF-α induced up-regulation of TRPA1 and according to Khalil et al. (2018) TRPA1 agonists stimulate the release of TNF-α. TRPA1 is a final common pathway for many chemically diverse pronociceptive agonists generated in various pathophysiological pain conditions and blockade of TRPA1 has effectively attenuated mechanical and cold pain hypersensitivity in various experimental models of pathophysiological pain, with only minor side effects (Koivisto et al., 2018). The reduction of TNF-α levels may be related to the inhibitory effect of BIS on the TRPA1 channel.

On the other hand, the anti-inflammatory cytokine IL-10 attenuates the inflammatory process and consequently reduces pain by counter-regulating the pro-inflammatory mediator release. IL-10 has been described as a cytokine that protects tissue from damage and it is associated with downregulation of the immune system and neuropathic pain relief (Meng et al., 2018; Ramesh et al., 2013).

BIS and BIS-βCD decreased the pro-inflammatory reaction in the spinal cord characterized by mitigation of microglia activation caused by sciatic nerve injury. It is thought that microgliosis can play a crucial role in the physiopathology of neuropathic pain (Gu et al., 2016; Scholz and Woolf, 2007), releasing several pronociceptive substances, which may be fundamental to the generation and maintenance of pain (Iyengar et al., 2017). The microglial response after a peripheral nerve injury is associated with several functions, such as: cellular migration, proliferation, cytokine release and may have a role in the resolution of neuroinflammation (Calvo and Bennett, 2012; Gu et al., 2016; Raghavendra and DeLeo, 2003). All these phenomena could contribute to the characteristics of neuropathic pain and its progression. The interruption of microglia response induced by BIS could, therefore, play an essential role in the decreased pain hypersensitivity observed in BIS-treated mice.

Cinnamaldehyde, a TRPA1 agonist, increases TNF-α secretion and suppresses IL-10 secretion (Billeter et al., 2015). In the same study, it was shown that TRPA1 antagonism promoted a substantial decrease in TNF-α levels, as well as increasing IL-10 secretion. We can assume that the decrease in TNF-α levels and the increase of IL-10 secretion presented here is related to the blockade of TRPA1 by BIS. In addition, BIS and BIS-βCD also reduced IBA-1, an important marker of microglia activation; Meotti et al. (2017) showed that the knock-down of the TRPA1 channel completely prevented IBA-1 immunostaining.

BIS and BIS-βCD produced no changes in muscle force and motor coordination (Leite et al., 2012, 2018). Thus the antihyperalgesic effect of BIS and BIS-βCD was not compromised by any muscular relaxation or motor impairment of the animals.

6. Conclusion

BIS and BIS-βCD have an anti-hyperalgesic effect in a chronic inflammatory pain model as well as in a neuropathic pain model. This effect is associated with the reduction of reactive gliosis, leading to modulation of the release of pro- and anti-inflammatory cytokines that may be related to TRPA1 antagonism. Thus, we conclude that BIS is a promising molecule for the treatment of chronic pain.

Acknowledgements

This study was financed in part by the Conselho Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brasil (CNPq), the Fundação de Apoio à Pesquisa e a Inovação Tecnológica do Estado de Sergipe (Fapitec/SE) - Brasil, the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES - Finance Code 001), and the Financiadora de Estudos e Projetos - Brasil (FINEP), all from Brazil.

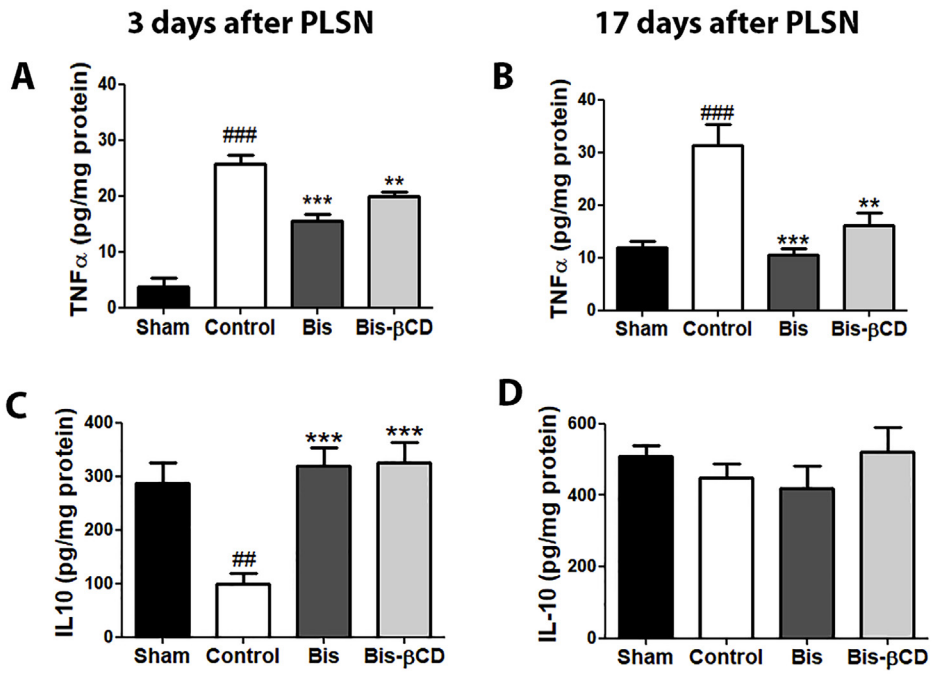

Fig. 6. Effect of BIS and BIS-βCD on cytokine modulation in the spinal cord in the neuropathic pain model induced by partial lesion of the sciatic nerve. **A.** Levels of TNF-α 3 days after PLSN **B.** Levels of TNF-α 17 days after PLSN, **C.** Levels of IL-10 3 days after PLSN **D.** Levels of IL-10 17 days after PLSN. Values expressed as mean ± S.E.M. (n = 8/group). ** p < 0.01 *** p < 0.001, when compared to control; ## p < 0.01, ### p < 0,001 compared to Sham (One-way ANOVA followed by Tukey's test).

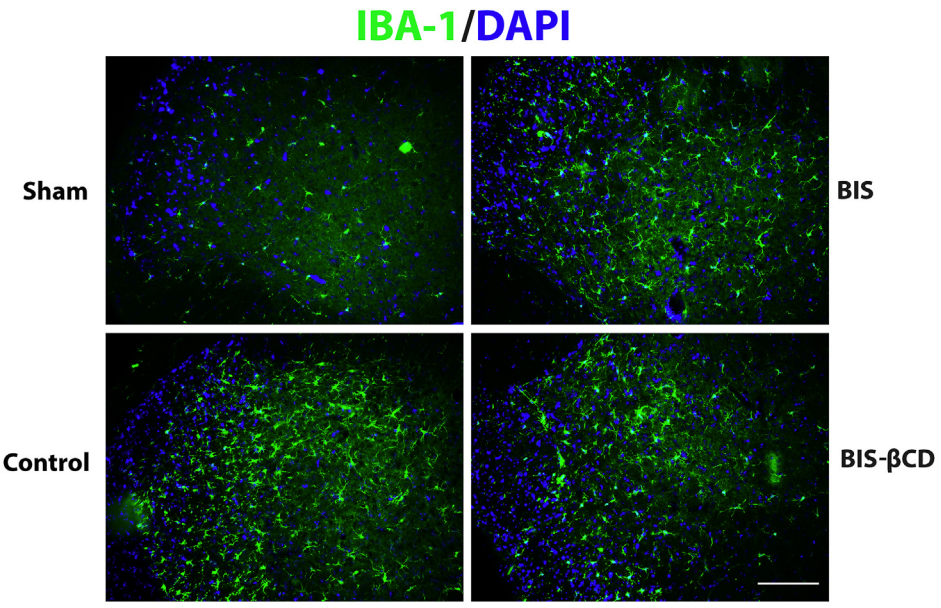

Fig. 7. Effect of BIS and BIS-βCD on microglia biomarker IBA1 modulation in the neuropathic pain model induced by partial lesion of the sciatic nerve. Immunofluorescence for IBA1 (green) and DAPI stained (blue). The panels were obtained by optical microscopy. Representative images of 6 animals. Bar scale 30 mm (magnification: 20).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2019.104530.

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