Process Biochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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

Host–guest inclusion complexation of β-cyclodextrin and hecogenin acetate to enhance anti-hyperalgesic effect in an animal model of musculoskeletal pain

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ARTICLE INFO

Article history:
Received 22 June 2016
Received in revised form 12 August 2016
Accepted 19 August 2016
Available online xxx

Keywords:
Hecogenin
Cyclodextrin
Opioid
Substance P
Pain
Fibromyalgia

ABSTRACT

Hecogenin acetate (HA), a steroidal acetylated-sapogenin, has an analgesic profile already assigned, but its low water solubility and short half-life limit its use in chronic conditions. β-cyclodextrin (β-CD) can improve the chemical and pharmacological property of nonpolar compounds such as HA. Therefore, a HA complexed with β-CD (HA-CD) was prepared and characterized by thermal, morphological and spectroscopic analysis. A model of chronic musculoskeletal pain was induced by means of two injections of pH 4.0 saline (20 μL) into the left gastrocnemius 5 days apart was used. After confirming hyperalgesia, male mice were treated with HA, HA-CD (20 mg/kg; p.o.) or vehicle (saline 0.9%, p.o.). Motor coordination tests, substance P (SP) levels in a lumbosacral (L4-S2) spinal cord sample and a docking study were equally assessed to check for a possible action on the opioid receptors. Oral pretreatment with HA or HA-CD produced a significant antinociceptive ($p < 0.01$) profile and also decreased mechanical hyperalgesia, with HA-β-CD showing significantly better effects when compared to HA alone ($p < 0.05$). The interaction between HA and the opioid receptor (MU, Kappa, Delta) was corroborated by the docking study. Moreover, the SP level was reduced in a spinal cord sample. Our findings suggest that β-CD can improve the anti-hyperalgesic effect of HA in an animal model of musculoskeletal pain.

1. Introduction

Fibromyalgia (FM) is a disorder characterized by widespread musculoskeletal pain accompanied by fatigue and sleep, memory and mood disturbances. FM is present in as much as 2% to 8% of the population worldwide. It is characterized by widespread pain and is often accompanied by fatigue, memory problems, sleep disturbances and anxiety [1]. After osteoarthritis, FM is the second most common "rheumatic" disorder and it can develop at any age [2]. FM has been described as a 'dysfunctional pain',a type of chronic pain associated with a broad range of clinical disorders, including irritable bowel syndrome, interstitial cystitis as well as FM. Functional pain is emerging as a serious issue, due to the negative impact of inexplicable pain on quality of life, the lack of effective therapies and health care costs [3]. There is no generally accepted strategy for the pharmacological treatment of FM symptoms, mainly pain and fatigue. However, current pharmacotherapy has often been shown to be inefficient, unselective and to present side-effects, factors which have contributed to low adherence [1,3].

Abbreviations: FM, fibromyalgia; NP, natural product; HA, hecogenin acetate; CD, cyclodextrin; PM, physical mixture; SC, slurry complexation; SP, substance P.

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http://dx.doi.org/10.1016/j.procbio.2016.08.025
1359-5113/

Natural products (NPs) have emerged as sources of new compounds for drug development [4]. However, use of NPs has diminished over the past two decades, in part because of the technical hurdles involved in screening NPs in high-throughput assays against molecular targets [5]. Despite this, about 30–35% of medicines approved over the last two decades by the Food and Drug Administration (FDA) havestill originated from NPs [4,5]. Thus, the pharmaceutical industry consider NPs as an asset which can be used in the development of new products. Even with the current strategy of "Big Pharma" (the nickname given to the pharmaceutical industry) on "'drug repositioning strategy" (using current drugs for new indications), the study and discovery of new chemical entities (whether or not originating from NPs) for chronic pain (including "dysfunctional pain") remains one of the biggest challenges of modern medicine.

Hecogenin and diosgenin are steroidal sapogenins commonly found in the leaves of species from the *Agave* genus, including *Agave sisalana*, *Agave cantala* and *Agave aurea* [6]. Steroids, such as diosgenin and hecogenin, have been used by the pharmaceutical industry in the manufacture of oral contraceptives and other steroid hormones widely used in clinical treatments [7,8]. Moreover, hecogenin acetate (HA) is a steroidal acetylated-sapogenin that has important antioxidant, analgesic and anti-cancer properties [9,10]. Recently Gama et al., 2013 [9] and Quintans et al., 2014 [11] have suggested that HA, because of its analgesic properties, could play a role in pain-inhibitory mechanisms, probably through the opioid system. However, HA and other nonpolar compounds used in therapeutic applications (including to treat chronic pain) have several limitations such as poor water solubility, slow dissolution rates, chemical instability and a short half-life. Thus, in spite of the promising analgesic effect of HA, there are no studies using this steroidal acetylated-sapogenin in chronic conditions, probably due to the limitations previously described.

Cyclodextrins (CDs) have been widely used to prepare inclusion complexes with water-insoluble drugs to improve their stability and solubility, modify the release of the drugs and turn liquid substances into stable and free-flowing powders [12,13]. A recent study has shown the pharmacological benefits of the complexation with CDs with nonpolar natural products, such as terpenes and essential oils [13–18]. This pharmacological development, with natural nonpolar compounds being complexed in CDs, has been interestingly demonstrated in experimental models for the treatment of non-inflammatory chronic pain such as FM [14,17,19–21] and cancer pain [15]. CDs have been shown to be able to improve the efficacy and safety of analgesic compounds, with the widespread use of at least 38 drugs in the present pharmaceutical market [17,22,23].

Despite the central analgesic effect described above for HA, its poor solubility in water and short half-life have been limiting factors to the pharmacological use of HA; Therefore, we sought to assess whether a complex containing HA and β-cyclodextrin (HA-CD) could improve the anti-hyperalgesic effect of HA in a non-inflammatory animal model of musculoskeletal pain (considered to be a suitable animal model for FM) [21,24,25], and to evaluate its effectiveness by examining its effect on substance P (SP) in the spinal cord, and its influence on the opioid system; we also undertooka physical-chemical characterization of the HA-CD complex.

2. Materials and methods

2.1. Drugs and reagents

β-Cyclodextrin (≥97% purity), hecogenin acetate (≥90% purity), naloxone, morphine and chloride acid were purchased from Sigma-Aldrich (USA). Acetic acid was purchased from Vetec (Brazil).

2.2. Preparation of samples

The inclusion complexes were prepared by slurry complexation (SC) and compared with physical mixture (PM) as described by Guimarães et al., 2015 [15]. These samples were prepared based on HA's molecular weight (472,66 $g mol^{-1}$) and β-CD's molecular weight (1134,98 $g mol^{-1}$), with a molar ratio of 1:1. The PM was prepared by adding HA to an agate mortar containing β-CD under manual stirring, and was then stored in sealed glass vials. The SC was performed by adding 20 mL water to a beaker containing HA and β-CD, under magnetic stirring at 150 rpm for 36 h continuously (Quimis Q 261A21, Brazil). The material was then dried in a desiccator and removed by manual trituration as described in a paste complexation method.

2.3. Thermal analysis

Samples of HA, β-CD, PM and SC were subjected to differential scanning calorimetry (DSC) using a Shimadzu DSC-60 at a heating rate of 10 °C min^{-1}. The curves were obtained in the temperature range between 25 and 500 °C under a dynamic nitrogen atmosphere (100 mLmin^{-1}) using an aluminum capsule containing 2 mg of the sample. The equipment was calibrated or verified before testing in the temperature axis using the indium metal standard. TG/DTG curves were obtained with a Shimadzu TGA-60 thermobalance in the temperature range of 25–900 °C, in platinum crucibles containing approximately 3 mg of samples, under a dynamic nitrogen atmosphere (100 mLmin^{-1}) and a heating rate of 10 °C min^{-1}. The TG/DTG was verified with calcium oxalate monohydrate, according to the ASTM standard.

2.4. Fourier transform infrared spectroscopy (FT-IR)

The absorption spectra in the infrared region of HA, β-CD, PM and SC were obtained in the spectral range 4000–500 cm^{-1} in KBr pellets using a Fourier Transform Spectrometer model IRTracer, Shimadzu-100, at room temperature.

2.5. Scanning electron microscopy (SEM)

HA, β-CD, PM and SC were mounted on aluminum stubs, coated with a thin layer of gold and visualized with a scanning electron microscope (JSM-6390-LV JEOL) at an accelerated voltage of 12 kV.

2.6. Animals

For this study, we used male Swiss mice (31–39 g), about 2–3 months of age, obtained from the Animal Facilities of the Federal University of Sergipe (UFS) in all experimental protocols. The animals were randomly housed in appropriate cages at 21 ± 2 °C on a 12 h light/dark cycle (lights on from 6:00 a.m. to 6:00 p.m.) with free access to food (Purina®, Brazil) and water. Before the experiments, the mice were acclimatized to the laboratory for at least 1.5 h. The animals were used only once in each pharmacological protocol to avoid unnecessary suffering. Experiments were carried out between 9:00 a.m. and 3:00 p.m. in a quiet room. All experiments involving the behavioral analysis were carried out by the same visual observer and in a double-blind manner. Experimental protocols were approved by the Animal Care and Use Committee at the UFS (CEPA/UFS # 04/12).

2.7. Acetic acid-induced abdominal writhes

Abdominal writhes consisted of a contraction of the abdominal muscle together with a stretching of the hind limbs, induced by intraperitoneal (i.p.) injection in mice of acetic acid (0.65% solution,

Please cite this article in press as: J.S.S. Quintans, et al., Host–guest inclusion complexation of β-cyclodextrin and hecogenin acetate to enhance anti-hyperalgesic effect in an animal model of musculoskeletal pain, Process Biochem (2016), http://dx.doi.org/10.1016/j.procbio.2016.08.025