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Nanocapsules improve indole-3-carbinol photostability and prolong its antinociceptive action in acute pain animal models

Mailine Gehrcke[a], Marcel Henrique Marcondes Sari[b], Luana Mota Ferreira[a], Allanna Valentini Barbieri[a], Laura Minussi Giuliani[a], Vinicius Costa Prado[b], Jessica Mendes Nadal[c], Paulo Vitor Farago[c], Cristina Wayne Nogueira[b], Letícia Cruz[a,*]

[a] Programa de Pós-graduação em Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil
[b] Programa de Pós-graduação em Bioquímica Toxicológica, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, RS, Brazil
[c] Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Estadual de Ponta Grossa, PR, Brazil

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ABSTRACT

This study aimed the development of nanocapsules (NCs) for oral indole-3-carbinol (I3C) administration and evaluation of antinociceptive potential of this compound in its two forms, free and nanoencapsulated, using acute pain models. NCs showed adequate physicochemical characteristics and protected the I3C against UVC radiation exposure. It was observed no chemical bond between I3C and polymer by FTIR. Besides, X-ray and DSC analysis suggested that I3C was molecularly dispersed in NCs. The dialysis bag technique showed that almost 100% of the compound was released from NCs at 360 min. Mathematical modeling demonstrated that this release occurred in two rates, with an initial burst effect followed by a slower release of I3C. Regarding the *in vivo* analysis, time-response curve showed that both forms of I3C caused an inhibition in inflammatory phase of nociception induced by formalin and increased the latency response in hot plate test. Interestingly, NCs were able to prolong the I3C effect in both tests. Furthermore, in dose-response curve, only I3C in its nanoencapsulated form presented effect on inflammatory phase of the formalin test. In conclusion, NCs to I3C incorporation presented adequate nanometric characteristics and prolonged its antinociceptive action in acute pain models tested.

1. Introduction

Inflammation and pain are related processes that share the same effectors and mediators. Pro-inflammatory molecules such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β) and prostaglandin E_2 (PGE_2) can act on peripheral terminals of neurons, called nociceptors, facilitating their activation and increasing pain sensitivity (Gudes et al., 2015; St-Jacques and Ma, 2014). In this sense, it is common in the clinical practice the anti-inflammatory drugs usage for pain treatment. However, these drugs present several adverse effects, which increase with their long-term administration (Süleyman et al., 2007). Therefore, the researches toward the development of novel pharmacological alternatives to treat and control pain have received special attention by the scientific community.

Indole-3-carbinol (I3C) is a phytochemical compound produced from myrosinase-catalyzed hydrolysis of glucobrassicin when vegetables of the Brassica genus are macerated, cut or cooked (Aggarwal and Ichikawa, 2005). Recently, some studies reported the multiple pharmacological actions exerted by I3C using different experimental animal models. Among its properties, the anti-inflammatory effect and the molecular targets concerning its mechanism of action were already described (El-Naga et al., 2014; Song et al., 2015). It seems that an interplay among the inhibition of cyclooxygenase-2 (COX-2) and lipoxigenase enzymes expression (Song et al., 2015), as well as the reduction in pro-inflammatory cytokine synthesis and release, such as IL-1β, IL-6 and TNF-α, and the suppression of tissue immune cells infiltration contributes to I3C anti-inflammatory effect (El-Naga et al., 2014; Jiang et al., 2013). Besides, studies showed that I3C presents little or no toxic effects (Reed et al., 2005). Thus, in view of such actions, it is relevant to study this phytochemical for the inflammatory pain treatment.

However, despite its pharmacological potential, I3C is light sensitive and unstable to temperature variations and acidic environment (Grose and Bjeldanes, 1992; Luo et al., 2013). Thereby, new strategies to overcome these limitations are required. Polymeric nanocapsules (NCs) are attractive colloidal systems to develop formulations

* Corresponding author at: Departamento de Farmácia Industrial, Universidade Federal de Santa Maria, Santa Maria 97105-900, Brazil.
E-mail address: leticiacruz@smail.ufsm.br (L. Cruz).

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containing labile substances. By definition, NCs are vesicular systems composed by a core, generally oily, surrounded by a polymeric wall (Mora-Huertas et al., 2010). These carriers can present several advantages such as enhancement of dissolution process, therapeutic index increased, controlled delivery and protection from photo and chemical degradation (Dong et al., 2016; Mora-Huertas et al., 2010). Moreover, it is interesting to highlight that the oil core composition can add some pharmacological actions or provides protection for the encapsulated substance and due to this fact the development of NC using natural oils as core is considerably growing (Santos et al., 2014; Venturini et al., 2015, 2016).

Concerning the natural source of oil cores, the rose hip oil (RHO), obtained from the seeds of *Rosa aff. rubiginosa*, is widely used in medical treatments for skin regenerating process. The phytochemical composition analysis demonstrated that the RHO is rich in retinoic acid, tannins, flavonoids, carotenoids and unsaturated and poly-unsaturated fatty acids, which can give to these oil antioxidant properties (Aladedunye et al., 2014; Franco et al., 2007). In a previous study, we developed poly(ε-caprolactone) NCs loaded with I3C using RHO as oil core, which improved the photostability, radical scavenging and antitumor effects of the compound (Gehrcke et al., 2017).

Thus, in order to further evaluate the I3C nanoencapsulation benefits, the aim of this study was the development and physicochemical characterization of RHO-based NCs containing I3C as a photostable strategy for oral administration of this compound using Eudragit® RS 100 as polymer wall. Moreover, *in vivo* studies were carried out to investigate a new pharmacological property for this phytochemical, the antinociceptive action, as well as the influence of the nanoencapsulation process on this effect.

2. Materials and methods

2.1. Materials

Indole-3-carbinol (99% purity) and Span 80® (sorbitan monooleate) were obtained from Sigma Aldrich (São Paulo, Brazil). Eudragit® RS 100 (Röhm Pharma, Germany) was supplied by Almapal (SãoPaulo, Brazil). Rose hip oil was purchased from Pharma Nostra (Rio de Janeiro, Brazil) and Tween 80® (polysorbate 80) bought from Delaware (Porto Alegre, Brazil). HPLC-grade methanol and acetonitrile were acquired from Tedia (Rio de Janeiro, Brazil). All other solvents and reagents were analytical grade and used as received.

2.2. Preparation of nanocapsule suspensions

I3C-loaded NCs were prepared using interfacial deposition of preformed polymer method (Fessi et al., 1989). At 40 °C, Eudragit® RS 100 (0.1 g), Span 80® (0.077 g), RHO (330 μL), and I3C (0.005 g) were dissolved in acetone (27 mL) under moderate magnetic stirring. After 60 min, this organic phase was injected into 53 mL of an aqueous dispersion of Tween 80® (0.077 g), previously alkalized using 0.2 M NaOH to pH 9.5, under magnetic stirring at room temperature. After 10 min, the acetone and part of the water were eliminated by evaporation under reduced pressure to achieve a final volume of 10 mL, which corresponds to an I3C concentration of 0.5 mg/mL. Then, the formulations pH was adjusted to 8.5 using 0.2 M NaOH due to the compound instability in acid pH. This formulation was named NC-I3C. For comparison purposes, formulations without the drug were also prepared (NC-B).

In order to characterize these NCs by X-Ray powder diffraction (XRPD), Fourier-transformed infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC), NC-I3C and NC-B were freeze-dried using 10% lactose (w/v) as cryoprotectant. The lactose was added into NC suspensions and, subsequently, they were frozen for 24 h. After of this, the samples were subjected to dehydration in a freeze-dryer (LIOTOP L 101, Liobras, São Paulo, Brazil) at − 55 °C for 24 h. For providing a comparative characterization, a physical mixture (PM) of Eudragit® RS 100 and I3C at a 1:1 M proportion was also prepared by mortar and pestle mixing and lyophilized before analyses.

2.3. Physicochemical characterization of nanocapsule suspensions

2.3.1. Granulometric distributions, particle size and zeta potential analysis

Laser diffraction (Mastersizer® 3000E, Malvern Instruments, UK) was used to determine the NCs granulometric distribution profiles. For this, the formulations were directly inserted into the equipment sampling apparatus, without previous treatment, until laser obscuration reached 15%. Distilled water was used as dispersing phase. The refractive index of the dispersed phase was 1.31.

The NC-I3C and NC-B mean particle sizes and polydispersity index (PDI) were determined by photon correlation spectroscopy (PCS), after the samples dilution in ultrapure water (1:500) using a Zetasizer® Nano-ZS ZEN 3600 model (Malvern Instruments, UK). Using the same equipment, zeta potential was measured by microelectrophoresis, after NCs dilution in 10 mM NaCl (1:500).

2.3.2. Morphological analysis

For morphological analysis, the NC suspensions were placed on stubs, coated with gold, and analyzed using a field emission gun-scanning electron microscopy (FE-SEM) (MIRA3 LM, Tescan Orsay Holding, Czech Republic) at an accelerating voltage of 15.0 kV.

2.3.3. Drug content and encapsulation efficiency

The quantitative analyses were performed on a LC-10A HPLC system (Shimadzu, Japan) equipped with a SIL-20A HT valve sample automatic injector, an UV-VIS SPD-M20A detector, a LC-20AT pump, CBM-20A system controller, a guard column, and a RP C_{18} LiChrospher Phenomenex column (250 mm × 4.00 mm, 5 μm; 100 Å), which was kept at room temperature. The mobile phase consisted of water and acetonitrile (70:30, v/v) at isocratic flow rate (1.0 mL/min). The wavelength used for I3C detection was 288 nm and a sample volume of 20 μL was injected into the equipment. This HPLC method was previously validated and proved to be linear (range 3 to 15 μg/mL), specific and precise. The total I3C content in NC-I3C was quantified after dissolving the formulation (180 μL) in 10 mL of methanol followed by sonication for 30 min to extract the compound. Before injecting into the HPLC system, the samples were filtered in a 0.45 μm membrane.

The I3C encapsulation efficiency was estimated using the ultrafiltration/centrifugation technique. For this, 300 μL of NC-I3C were placed in a 10,000 MW centrifugal filter device (Amicon® Ultra, Millipore) and centrifuged at 2200 × *g* for 10 min. The ultrafiltrate was diluted 20 times and analyzed by HPLC method. The encapsulation efficiency (EE %) was calculated by the difference between I3C total concentration (NC-I3C) and free I3C concentration (ultrafiltrate).

2.4. X-ray powder diffraction

X-ray diffractograms of raw materials, freeze-dried NCs, and lyophilized PM were performed using a Shimadzu X-ray diffractometer (Shimadzu XRD-6000, Kyoto, Japan). The 2θ value was increased from 5° to 80° at a scan rate of 2°/min using a Cu-Kα source (λ = 1.5418 Å) at 40 kV and 40 mA.

2.5. Fourier-transformed infrared spectroscopy

Chemical interactions between I3C and polymer were investigated by Fourier-transformed infrared (FTIR). For this, the spectra of I3C, Eudragit® RS 100, lactose, freeze-dried NCs, and lyophilized PM were recorded from 4000 to 400 cm^{-1} using a Shimadzu IR Prestige-21 spectrophotometer (Kyoto, Japan) with KBr pellets with 32 scans and a resolution of 4 cm^{1}.