ANTIINFLAMMATORY ACTIVITY OF THE CLOVE OIL-ISOLATED β-CARYOPHYLLENE CARRIED IN A SELF-EMULSIFYING SYSTEM

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Abstract

(-)-β-Caryophyllene is a sesquiterpene reported to present anti-inflammatory activity that can be obtained from asymmetric synthesis or, alternatively, from essential oils. Clove buds are rich in eugenol (70-90%), which is widely used in perfumes and flavorings, but also has a considerable amount of β-caryophyllene. Eugenol is extracted from clove oil by fractionated distillation, which also permits to separate a fraction exceptionally rich in βcaryophyllene. The lipophilic character of β-caryophyllene makes it difficult to interact with the gastrointestinal aqueous environment, but nano-emulsifying carriers have been shown to be very effective as systems for oral administration of active compounds with low water solubility. Therefore, they should improve the anti-inflammatory activity of the orally administered β-caryophyllene. To test this hypothesis, a lipid-based self-emulsifying drug delivery system (SNEDDS) containing clove oil-isolated β-caryophyllene was prepared and its acute anti-inflammatory effect when orally administered was evaluated by using the carrageenan-induced paw edema in rats. SNEDDS was characterized in relation to size and rheology (continuous flow and oscillatory). Cell viability and chemotaxis in vitro were additionally accessed with peritoneal leukocytes isolated from mice and oral bioavailability of the β-caryophyllene free and carried in SNEDDS was evaluated in rats.

Materials and methods

β-Caryophyllene from clove bud oil was purchased from Quinarí Fragrâncias Ltda (Ponta Grossa, PR, Brazil). The chemical characterization of βcaryophyllene was determined by gas chromatography-mass spectrometry (GCMS). SNEDDS was a system consisting of β-caryophyllene, cremophor (surfactant) and ethyl linoleate. The hydrodynamic size and polydispersion index of SNEDDS was evaluated by dynamic light scattering (DLS). The flow properties of SNEDDS were determined in tension rheometer on continuous mode and the up-ward flow curves were modeled using the Ostwald-de-Waele relationship to obtain the shear stress (τ), consistency index (k), shear rate (γ̇), and flow behavior index (n). The hysteresis area was calculated using specific software. In oscillatory mode, rheometry was performed at 25 and 37 °C to determine the elastic and viscous properties of SNEDDS. The storage modulus (G'), the loss modulus (G''), the dynamic viscosity (η') and the loss tangent (tan δ) were calculated using specific software. The carrageenan-induced paw edema 11 was carried out in Wistar rats, which orally (gavage) received free or formulated β-caryophyllene at the doses of 50, 100, 200 and 400 mg/Kg and 5 mg/Kg indomethacin. One hour after all animals intradermally received 200 ug carrageenan in a hind paw. The paw volume was monitored by plethysmography at 0, 1, 2 and 4 h after the injection. Chemiotaxis in vitro was carried out in Boyden chambers using leukocytes from the peritoneal cavity of mice with zymosan-induced peritonitis. The cells were incubated with free β-caryophyllene at the concentrations of 3, 10, 30 and 90 µg/mL for 30 min and fMLP was the chemoattractant. Cell viability was performed with free β-caryophyllene at the same concentrations by MTT. The oral bioavailability of β-caryophyllene was evaluated in fasted rats, which orally (gavage) received the free and formulated compound at the dose of 100mg/Kg. Plasma β-caryophyllene was quantified by GC-MS at specific times. Pharmacokinetic parameters were calculated using the program for Microsoft Excel Visual Basic for Applications (VBA), PKSolver®, using noncompartmental model analysis NCA extravascular. ANOVA ONE-WAY (and Neuman Keuls post hoc test) and student t test (p)

Results and discussion

Clove oil-isolated β-caryophyllene presented apurity level of 91% of purity level and humulene (8%) is also an important component of the product. A nano-structured SNEDDS was obtained and particle size was not modified as the system was progressively diluted in water or PBS, but in simulated intestinal fluid it increased by 32 and 83% when SNEDDS was concentrated by 1 and 10%, respectively (compared with 0.1%). The PDI was low. In relation to rheological properties, the increase in temperature significantly decreased the consistence index (K) by 37%, i.e., decreased the viscosity of SNEDDS. The flow behavior index (n) was minimally smaller than 1 and, then, exhibited a practically Newtonian behavior, however, the hysteresis area had highly positive values, characterizing SNEDDS as thixotropic, i.e., as the shear stress increases the viscosity decreases (a shear-thinning behavior). Regarding the oscillatory rheology, G' and G" presented dependence on oscillatory frequency, but not on temperature. The SNEDDS had G" modulus values lower than G' over the entire frequency range and consequently tan δ<1, shaping it into the viscoelastic systems, i.e., presenting predominantly elastic characteristics. Clove oil-isolated β-caryophyllene orally administered was effective in improving the carrageenan-induced paw edema in rats, but when compared to the free form, the higher effectivity of the compound carried in SNEDDS was found only at a low dose of 50 mg/Kg (**Fig1**). The latter shows that βcaryophyllene could be reducing the paw edema at low concentrations, which are achieved in the paw tissue when administered even in the free form at the doses of 100 or more mg/Kg. In fact, the fMLP-stimulated chemotaxis of leukocytes in vitro was considerably and equally inhibited by free βcaryophyllene at the concentrations of 3-90 µg/mL, a phenomenon that did not modified the cell viability and seems to be associated with downregulation of proinflammatory cytokines expression (**Fig2**). SNEDDS containing β-caryophyllene had decrease at Tmax and increase at T1/2, Cmax and AUC0–24. These values shows that the relative oral bioavailability of β-caryophyllene carried by SNEDDS is higher than that of the free compound. This effect may be due to the increase of β-caryophyllene in the intestinal aqueous environment since the surfactant it could be improving the contact of the compound with both the oil and the water. The above phenomenon could be additionally improved by the small size of the drops in the nanostructured SNEDDS.

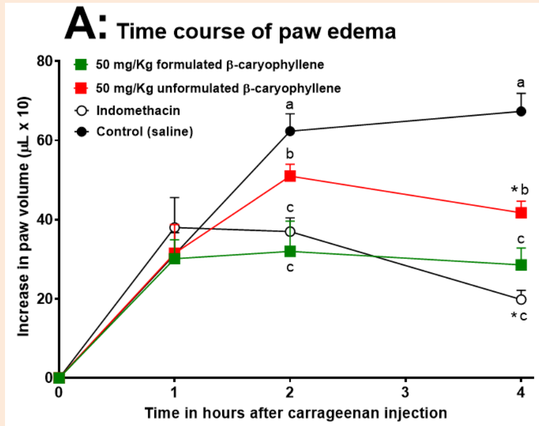


Fig1: Effects of β-caryophyllene free and carried in a lipid-based selfemulsifying drug delivery system (SEDDS) on the carrageenan-induced paw edema in rats.

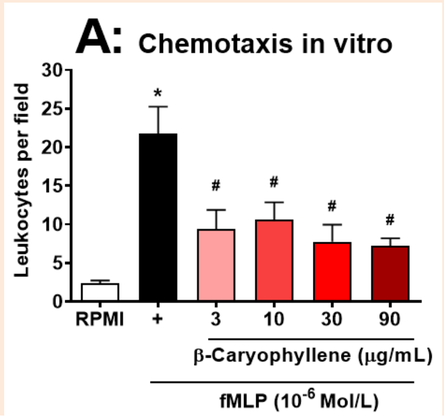
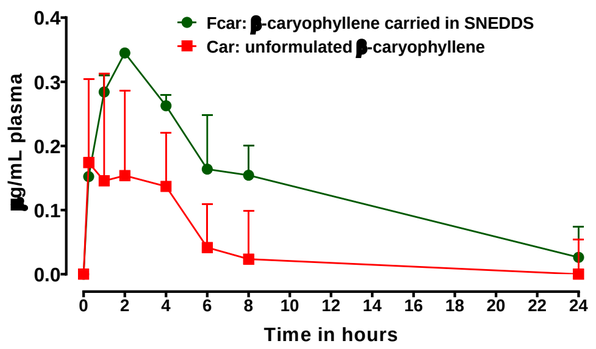


Fig2. Effects of unformulated β-caryophyllene on the leukocytes chemotaxis in vitro (A) and cell viability. Data are the mean ± SEM of 4 different replicates. Cytotoxicity is considered for cell viability lower than 75%. *p<0.05: for difference between RPMI and fMLP in absence of β-caryophyllene; #p<0.05: for difference from the positive control (+; fMLP).

Acknowledgements

We thank the funding agency CAPES and CNPq, for the support and incentive to research.



Time courses of β-caryophyllene concentration in the plasma of rats after orally administration (gavage) of unformulated (Car) and formulated (Fcar) β-caryophyllene at the dose of 100 mg/Kg. Blood sample were taken by tail incision immediately before (time 0) and at times 0.25, 1, 2, 3, 4, 6, 8 and 24 hours after β-caryophyllene administration. The plasma concentration of β-caryophyllene was quantified by GC-MS. The values are the mean ± SEM of 3 animals for each condition.

Conclusion

SNEDDS containing clove oil-isolated β-caryophyllene prepared in the present study was a pseudoplastic and viscoelastic nanostructured system with homogeneous sizes in the water, PBS and simulated intestinal fluid. βcaryophyllene orally administered was effective in improving the carrageenaninduced paw edema in rats, but when compared with the free form, the higher effectivity of the compound carried in SNEDDS was found only at low doses. This shows that β-caryophyllene can reduce the paw edema at low concentrations, which are achieved in the paw tissue even when administered in the free form at high doses. In fact, the leukocytes chemotaxis in vitro was equally inhibited by free β-caryophyllene at very low concentrations, a condition that did not modified the leukocytes viability. The comparison on pharmacokinetic parameters of free and formulated β-caryophyllene showed that when it is carried in SNEDDS its oral bioavailability is considerably improved. Therefore, SNEDDS proposed in the present study seems to be an appropriated carrier to increase the oral bioavailability of β-caryophyllene for clinical applications.

Recommendations

1 - Ames-Sibin AP, Barizão CL, Castro-Ghizoni CV, et al. B-Caryophyllene, the major constituent of copaiba oil, reduces systemic inflammation and oxidative stress in arthritic rats. J Cell Biochem. 2018, 119 10262–10277.