Phytomedicine 21 (2014) 1627–1632

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Encapsulation and modified-release of thymol from oral microparticles as adjuvant or substitute to current medications

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ARTICLE INFO

Article history:
Received 7 May 2014
Received in revised form 17 June 2014
Accepted 29 July 2014

Keywords:
Thymol
Herbal drug encapsulation
Anti-infective formulations
Microparticles
Modified-release formulation
In vivo studies

ABSTRACT

The aim of this study was to encapsulate, thymol, in natural polymers in order to obtain (i) taste masking effect and, then, enhancing its palatability and (ii) two formulations for systemic and local delivery of herbal drug as adjuvants or substitutes to current medications to prevent and treat several human and animal diseases. Microspheres based on methylcellulose or hydroxypropyl methylcellulose phthalate (HPMCP) were prepared by spray drying technique. Microparticles were *in vitro* characterized in terms of yield of production, drug content and encapsulation efficiency, particle size, morphology and drug release. Both formulations were *in vivo* orally administered and pharmacokinetic analysis was carried out. The polymers used affect the release and, then, the pharmacokinetic profile of thymol. Encapsulation into methylcellulose microspheres leads to short half/life but bioavailability remarkably increases compared to the free thymol. In contrast, enteric formulation based on HPMCP shows very limited systemic absorption. These formulations could be proposed as alternative or adjuvants for controlling pathogen infections in human or animal. In particular, methylcellulose microspheres can be used for thymol systemic administration at low doses and HPMCP particles for local treatment of intestinal infections.

Introduction

Essential oils and their volatile constituents are used widely to prevent and treat human and animal diseases. Essential oils rich in phenolic constituents such as eugenol and thymol (*e.g. Thymus serpyllum or spathulifolius and Origanum vulgare* L.) possess an antioxidant activity against free radicals and other reactive oxygen species as well as against oxidation of low density lipoproteins (LDL), causative agents of cardiovascular diseases (Kulisic et al., 2004; Sokmen et al., 2004; Tepe et al., 2005). Recently, because of its inhibitory effect against various inflammatory cytokines, thymol is proposed as potential anti-inflammatory drug, especially in the case of lipopolysaccharide induced inflammation (Chauhan et al., 2014).

Thymol, and essential oils rich in thymol, have proven benefits in medical, food, agricultural, veterinarian and pest control applications because of their antibacterial and antifungal properties (Ulbricht, 2004; Sacchetti et al., 2005; Oussalah et al., 2006; Lazar-Baker et al., 2010). In fact, thymol inhibits Gram-positive and Gram-negative pathogenic bacteria; activity against some pathogenic bacterial strains such as *Escherichia coli*, *Salmonella enteritidis*, *Salmonella choleraesuis* and *Salmonella typhimurium*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus* is reported (Edris, 2007). Thymol, like other phenolic compounds, is hydrophobic and is likely to dissolve in the hydrophobic domain of the cytoplasmic membrane of bacterial cells, between the lipid acyl chains, thus, altering the fluidity and permeability of cell membranes (Trombetta et al., 2005). Due to this ability, many studies demonstrated an additively or moderate synergism of essential oil components in combination with antibiotics, indicating that they may offer possibilities for reducing antibiotic use (Hamoud et al., 2014; Langeveld et al., 2014).

Moreover, thymol interferes more than eugenol on the envelope of *Candida albicans* and thus its colonization and infectiousness (Braga et al., 2007) and reduces *in vitro* viability of the causative agent of cystic echinococcosis (Elissondo et al., 2013).

Unfortunately, while thymol provides beneficial therapeutic effects, it also provides the consumer with a flavour perception that can be described as unpleasant, harsh or medicinal in taste. Thus, taste-masked compositions would provide the consumer with a

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http://dx.doi.org/10.1016/j.phymed.2014.07.017
0944-7113/

product with pleasant, acceptable taste that increases the patient compliance towards this herbal drug.

Moreover, the use of natural essential oils or compounds extracted from essential oils as the main therapeutic agents still faces the problems of (i) the easiness of degradation or chemical reactivity, (ii) the limited water solubility of these materials which limits thymol administration and/or oral bioavailability (Shoji and Nakashima, 2004) and (iii) high volatile character (Wattanasatcha et al., 2012).

On the basis of these remarks, aim of this work was the encapsulation of thymol in microspheres based on natural polymers in order to obtain (i) taste masking effect and, then, enhancing its palatability and (ii) two formulations for systemic and local delivery herbal drug as adjuvants or substitutes to current anti-infective medications to prevent and treat human and animal infections. Furthermore, a simple preparative method is proposed for easy scale up for rapid industrial production.

Microencapsulation provides an important tool for food, pharmaceutical, cosmetic and agrochemical industries, enabling protection and controlled release of several active agents. The encapsulation of essential oils in core–shell or matrix particles has been investigated for various reasons, *e.g.*, protection from oxidative decomposition and evaporation, odour masking or merely to act as support to ensure controlled release (Martins et al., 2014). Spray–drying is the most common and cheapest technique to produce microencapsulated materials. It results in powders with good quality, low water activity, easier handling and storage (Favaro-Trindade et al., 2010; Carneiro et al., 2013). Equipment is readily available and production costs are lower than most other methods (Gharsallaoui et al., 2007). In this work methylcellulose and hydroxylpropyl methylcellulose phthalate were chosen as polymers to produce microspheres. Methylcellulose swells in water and reproduces a clear to opalescent, viscous, colloidal solution (Kamel et al., 2008). Hydroxypropyl methylcellulose phthalate is a pH-sensitive polymer which is stable in acidic conditions of the stomach but degradable in enteric conditions. It is often used as an enteric drug carrier (Pal et al., 2009).

Materials and methods

Materials

Thymol (T) (purity ≥99.5%) and aniline (IS) were obtained from Sigma Aldrich (Italy). Methylcellulose (Methocel) (MC) and hydroxylpropyl methylcellulose phthalate NF (HPMCP) were purchased from BioChemika FlukaChemie (Buchs, Switzerland) and Eastman Chemical Company (Kingsport, Tennessee, USA), respectively. Dichloromethane (CH_2Cl_2, Riedel-de-Haen, Milan, Italy) and methanol (CH_3OH, Normapur p.a., BDH Prolabo, Paris, France) were of analytical grade. Ultra-pure water is prepared with the MilliQ R4 system Millipore (Milan, Italy).

Pre-formulation study

Determination of free thymol solubility in gastric and intestinal simulated fluid

The solubility of T was determined in two different pH values (1.2 and 6.8), representative of main sections of the gastrointestinal tract. Drug powder was added in excess in a flask, which contained 50 ml of the medium studied in each case. The flasks were placed in a thermostated water bath at 37.0 ± 0.5 °C, under magnetic stirring, for 24 h. Afterwards, 1 ml was withdrawn, filtered and injected in capillary electrophoresis–diode array detection system (Agilent Technologies).

An uncoated fused-silica capillary (50 cm × 50 μm ID) was used for the capillary electrophoresis separation. The running buffer consisted of 100 mM sodium phosphate pH 7.4. A separation voltage of 20 kV was applied. Samples were injected hydrodynamically with a pressure of 50 mbar for 20 s. The detection was made at 210 nm. Standard curves show linearity in the range 0.01–1 mg/ml with $r^2 = 0.999$.

Ex vivo permeation capability of free thymol through colonic pig mucosa

Experiments for testing the permeation capability of T to cross the intestinal mucosa were carried out by *ex vivo* permeation tests.

Experiments were performed using a new modified Franz diffusion system incorporating three in-line flow-through diffusion cells (Gavini et al., 2011). Each cell consisted of a donor compartment and a receptor compartment. The diffusion membrane was placed between the cell compartments; the diffusional area was 3.14 cm^2. The receptor solution was continuously stirred by means of a spinning bar magnet.

A fragment of colon was excised from intestine of pigs obtained from a local slaughterhouse, gently washed with PBS, stored in ice-cold PBS for transport to the laboratory, and finally deprived of the serosal mucosa. The mucosa was positioned to ensure that the mucosal portion was in contact with the microparticles and the muscular mucosa was in contact with the pH 6.8 buffer, in the receptor chamber thermostated at 37 °C. An exactly weighed amount of T powder (9 mg) was uniformly dispersed on the superior portion of the mucosa. The flux of liquid was set at 6.8 ml/min and saturated with 5% of CO_2.

The amount of buffer employed as acceptor medium was 250 ml. At selected time points (0–6 h), the amount of drug permeated was determined by capillary electrophoresis analysis. The acceptor medium withdrawn was refilled in order to maintain sink condition.

At the end of test, the surface of excised mucosa was washed with 10 ml of pH 6.8 buffer to remove T which eventually did not permeate; thus, the tissue was frozen at −20 °C before the T extraction process which was performed by placing the pieces of mucosa in 2 ml of methanol under constant stirring for 24 h at room temperature. Thereafter, the suspensions were stirred in vortex and centrifuged for 10 min at 3000 rpm; supernatant was analysed by capillary electrophoresis. Experiment was performed in triplicate.

Preparation of spray-dried microspheres

Two formulations containing T were produced using a co-current spray-dryer apparatus, Mini Spray Dryer, model B-191 (BüchiLabortechnik AG, Flawil, Switzerland).

Microspheres were prepared by spraying solutions obtained by dissolving T and MC (T–MC) or HPMCP (T–HPMCP) polymers in a methanol and dichloromethane mixture (50–50 v/v); concentrations of total solid in solutions were 2% and 5% (wt/v) for T–MC and T–HPMCP, respectively. The drug to polymer ratio was 1:2 (wt/wt).

The following conditions were used during spray drying: drying airflow, 31.3 m^3/h; spraying airflow, 500 l/h; solution feed rate, 2.9 ± 0.2 ml/min; nozzle size, 0.7 mm; the inlet temperature was established at 50 °C and the outlet temperature was 42–45 °C.

After production, microspheres were stored in desiccators at 20 °C before continuing with the experiments.

In vitro characterization of microparticles

Yield of production, drug content and encapsulation efficiency

Dried microspheres were accurately weighed, and considering the total amount of drug and polymers used for preparing the feed solution, the yield of production (YP) was calculated, as a