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Improvement of anti-inflammatory and anti-angiogenic activity of berberine by novel rapid dissolving nanoemulsifying technique

Swati Pund*,[1], Ganesh Borade[1], Ganesh Rasve

Department of Pharmaceutics, STES's Sinhgad Institute of Pharmacy, Pune, India

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ABSTRACT

Berberine, an isoquinoline alkaloid, has wide biological and pharmacological actions. Despite the promising pharmacological effects and safety of berberine, poor oral absorption due to its extremely low aqueous solubility results in poor oral systemic bioavailability. This limits its clinical usage. This study describes the development and characterization of self-nanoemulsifying drug delivery system (SNEDDS) of berberine in liquid as well as solid form with improved solubility, dissolution and *in vivo* therapeutic efficacy. The SNEDDS of berberine were prepared using Acrysol K-150, Capmul MCM and polyethylene glycol 400. The formulations were characterized for various *in vitro* physicochemical characteristics. *In vivo* efficacy was evaluated in acetic acid induced inflammatory bowel model in rats. Anti-angiogenic activity of the developed SNEDDS of berberine was studied using chick chorioallantoic membrane assay. SNEDDS of berberine rapidly formed nanoemulsions with globule size of 17–45 nm. The *in vitro* rate and extent of release of berberine from SNEDDS was significantly higher than berberine alone. Chick chorioallantoic membrane assay revealed potent anti-angiogenic activity of SNEDDS of berberine. These studies demonstrate that the SNEDDS of berberine is a promising strategy for improving its therapeutic efficacy and have potential application in the treatment of chronic inflammatory conditions and cancer.

Introduction

Angiogenesis, formation of new blood vessels from a preexisting vasculature, is a complex process involving an extensive interaction between cells, the extracellular matrix, and soluble factors (Lee et al. 2010). Angiogenesis plays an important role in pathologic processes such as the growth and metastasis of tumours (Huang et al. 2006). The success of anti-angiogenic therapy for cancer treatment has led to the research for anti-angiogenic agents (He et al. 2009). The newly formed blood vessels promote cancer growth by supplying nutrients and oxygen and by removing waste products. Metastasis also depends on angiogenesis, as tumour cells are shed from primary tumour and grow at their target organs. Thus, anti-angiogenic activity is a promising move towards development of novel drugs to treat cancers and other diseases related to angiogenesis (He et al. 2009; Mathur et al. 2006).

There is now enough evidence that the chronic inflammatory process provides a microenvironment that includes up-regulation of inflammatory mediators. Inflammatory mediators suppress cell mediated immune responses and promote angiogenesis, facilitate tumour promotion and progression (Kundu and Surh 2008). Chronic inflammation causes the progression of the neoplastic process and therefore, disruption of inflammatory pathway, provides a promising opportunity for the treatment of cancer (Aggarwal et al. 2006). Traditional herbal medicines have long been recognized as a rich source for discovering new therapeutic agents. Several anti-inflammatory phytoconstituents have shown chemopreventive activities and can be used for prevention as well as treatment of cancer. In addition, bioactive phytoconstituents are safe and lack toxicity.

Berberine (BR) is an isoquinoline alkaloid of the protoberberine type, with a long history of medicinal use in traditional eastern medicine. BR is usually administered in a salt form for several clinical applications like anti-bacterial, anti-fungal, and anti-inflammatory, and has been used as a gastrointestinal remedy for thousands of years (Tan et al. 2011). BR also possesses anti-HIV, anti-fungal, cardioprotective, immunoregulative, anti-malarial, anti-inflammatory, anti-oxidant, cerebro-protective, anti-mutagenic, vasorelaxing, anxiolytic and analgesic activities (Zuo et al. 2006). However, due to its hydrophobic nature, poor stability and low bioavailability, the actual therapeutic application of BR is hampered for a long time. Poor water solubility of BR reflects in limited absorption in the gastrointestinal tract and subtherapeutic plasma concentrations. These issues have hindered the development of BR as a pharmaceutical formulation. Therefore, a

* Corresponding author at: Department of Pharmaceutics, STES's Sinhgad Institute of Pharmacy, Narhe (Ambegaon), Pune 411041, Maharashtra, India.
Tel.: +91 20 66831801; fax: +91 20 66831816.
E-mail address: swatipund@gmail.com (S. Pund).
[1] [1]These authors contributed equally to this work.

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novel delivery system to improve the solubility and bioavailability of BR is a matter of exigency (Tan et al. 2011; Zhu et al. 2013).

The use of nanotechnology in formulation enhances solubility and permeability of drugs with low solubility and poor permeability. It also improves the therapeutic activity by either active or passive targeting, while preventing physical and chemical degradation of the drug. Self micro/nanoemulsifying drug delivery systems (SM/NEDDS) are a current formulation strategy to enhance the oral bioavailability of poorly soluble drug. SM/NEDDS are isotropic preconcentrates of drug, oil, surfactant, cosolvent which readily generate ultrafine micro/nanoemulsions after dispersion in water with mild agitation. These systems avoid the dissolution step as observed for solid crystalline compounds. Furthermore, upon dispersion these systems form micro/nanosized globules of oil in which drug remains dissolved thus facilitating the absorption process (Gershanik and Benita 2000; Gursoy and Benita 2000; Pouton 1997, 2006).

In the present study, we combined the pharmacological benefits of BR with a lipid based nanoemulsifying delivery system to obtain more effective anti-inflammatory formulation having potential application in the treatment of chronic inflammation and cancer.

Materials and methods

Materials

BR was a generous gift from Indian Institute of Integrative Medicine (Jammu, India). Following excipients from respective sources were used as received. Capryol 90, Cremophor RH 40, Labrafac CC, Labrafil M1944CS, Labrasol, and Transcutol HP were gifted by Gattefosse India Pvt. Ltd. (Mumbai, India). Ethyl oleate, isopropyl myristate, olive oil, sesame oil, ethanol, polyethylene glycol 400 (PEG), propylene glycol, Tween 20, Tween 60 and Tween 80 were purchased from Loba Chemie Ltd. (Mumbai, India). Captex 200 and Capmul MCM were received as gift samples from Abitec Corporation, Mumbai, India. Acrysol K-150 and Neusilin US2 were gifted by Corel Pharma Ltd. (Ahmedabad, India) and Gangwal Chemicals Pvt. Ltd. (Mumbai, India) respectively.

Methods

Determination of solubility in various solvents

The solubility of BR in various oils/modified oils (Acrysol K150, Captex 200, ethyl oleate, isopropyl myristate, olive oil and sesame oil), surfactants (Capmul MCM, Capryol 90, Cremophor RH 40, Labrafac CC, Labrafil M1944CS, Labrasol, Tween 20, Tween 60 and Tween 80) and co-solvents (ethanol, polyethylene glycol 400, propylene glycol and Trancutol HP) was determined using shake flask method. An excess of BR (about 500 mg) was added to 1 ml of above mentioned vehicles and the resulting suspensions were shaken on a flask Shaker (Kytose EOS– 10 M, Electrolab) at room temperature for 3 days and centrifuged at 3000 rpm for 15 min (Spinwin MC 01, Tarson, Mumbai). The clear supernatant was analyzed for the content of BR by validated RP-HPLC method. Determinations were carried out in triplicate. The HPLC system consisted of Pump (Jasco PU-2080 plus, Intelligent LC pump, Japan) with a Interface (Jasco LC-Net II/ADC, Japan) connected to Detector (Jasco UV-2075 plus, Intelligent UV–vis detector, Japan). The chromatographic separation was performed using an isocratic elution. The mobile phase consisted of a mixture of acetonitrile, 0.05 M KH_2PO_4 and methanol (4:4:3) and delivered at a flow rate of 1 ml/min. The separation was carried out at 20 °C, on a reversed phase HiQ Sil C8 column (250 mm × 4.6 mm, 5 μm particle size). An injection volume of 20 μl was used. Detections were carried out at 343 nm.

Construction of pseudo-ternary phase diagram

The ternary phase diagrams were constructed by water titration method (Bachhav and Patravale 2009). From the results of solubility studies, Acrysol K-150, Capmul MCM and PEG 400 were selected as the oil, surfactant and co-solvent respectively. Distilled water was used as an aqueous phase for construction of phase diagrams. Surfactant and cosolvent were mixed in ratios 1:1, 1:2 and 2:1 (S_{mix}, w/w). Ternary mixtures with varying compositions of S_{mix}, and oil were prepared. Nine different combinations of oil and S_{mix}, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1, were made so that maximum ratios were covered to define the boundaries of phase formed in the phase diagrams. Homogeneous mixtures of oil and S_{mix}, at given volume ratio were prepared in dust-free glass vial. The preconcentrates were mixed on orbital shaker (Kytose EOS– 10 M, Electrolab) for 10 min. After equilibrium was reached, the mixtures were further titrated with aliquots of distilled water. During the titration, samples were stirred to ensure homogeneity and visually monitored against a dark background by illuminating the samples with white light. Slow titration with aqueous phase was done to each weight ratio of oil and S_{mix}. The formation of the nanoemulsion was visually observed as transparent or slightly bluish o/w nanoemulsion and marked on the pseudo-ternary phase diagram. Clear and isotropic samples were deemed to be within the nanoemulsion region. These titration results were then used to determine the boundaries of the emulsion regions corresponding to the selected optimum ratios of combination vehicles for developing phase diagram (Setthacheewakul et al. 2010). The tendency to spontaneously emulsify was also examined. After being equilibrated, the efficiency of self-emulsification, dispersibility, and appearance were graded based on rapidity in emulsification and the colour of the emulsion (Singh et al. 2008).

BR loaded SNEDDS

Based on solubility studies and the pseudo-ternary phase diagrams, SNEDDS composition was optimized composed and were composed of Acrysol K-150, Capmul MCM and PEG 400; 1:0.33:0.66. Calculated amount of BR was added in the oily phase; Acrysol K-150 in small increment with continuous stirring. The S_{mix} was prepared by mixing separately Capmul MCM and PEG 400 in 1:2 ratio and added to BR containing Acrysol K-150 with continuous stirring. The stirring was continued till the homogenous mixture was formed. BR loaded SNEDDS were further characterized for various physicochemical parameters.

Determination of globule size by photon cross correlation spectroscopy and Zeta potential

BR loaded SNEDDS (100 mg) were diluted to 100 ml with double distilled water, 0.1 N HCl and buffer pH 6.8. Visual observations were made immediately after dilution for assessment of self-emulsification efficiency, phase separation, and precipitation of drug. Each sample was placed in transparent polystyrene cuvette (path length = 1 cm) and placed in thermostatic sample chamber. Mean globule size and the polydispersity index of the resulting emulsions were determined by photon cross-correlation spectroscopy (Nanophox, Sympatec, Germany). Sample temperature was set at 25 °C and 3 runs of 60 s were performed. Detection was carried out at a scattering angle of 90°. From the resulting correlation curves, a 2nd order analysis was performed to calculate the mean globule size and standard deviation. The globule size distribution was expressed in terms of polydispersity index, which is a measure of the width of the globule size distribution. It is defined as:

$$\text{Polydispersity index} = \frac{\{D(v, 0.9) - D(v, 0.1)\}}{D(v, 0.5)}$$