Phytomedicine 22 (2015) 1103–1111

Contents lists available at ScienceDirect

Phytomedicine

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

Enhanced oral bioavailability and anticancer activity of novel curcumin loaded mixed micelles in human lung cancer cells

Sharvil Patil[a,*], Bhavana Choudhary[a], Atul Rathore[b], Krishtey Roy[a], Kakasaheb Mahadik[b]

[a] Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Department of Pharmaceutics, Erandwane, Pune 411038, Maharashtra, India
[b] Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Department of Pharmaceutical Chemistry, Erandwane, Pune 411038, Maharashtra, India

ARTICLE INFO

Article history:
Received 11 October 2014
Revised 4 August 2015
Accepted 11 August 2015

Keywords:
Mixed micelles
Pluronic
Curcumin
Anticancer activity
Oral bioavailability

ABSTRACT

Background: Curcumin has a wide range of pharmacological activities including antioxidant, anti-inflammatory, antidiabetic, antibacterial, wound healing, antiatherosclerotic, hepatoprotective and anti-carcinogenic. However, its clinical applications are limited owing to its poor aqueous solubility, multidrug pump P-gp efflux, extensive *in vivo* metabolism and rapid elimination due to glucuronidation/sulfation.
Purpose: The objective of the current work was to prepare novel curcumin loaded mixed micelles (CUR-MM) of Pluronic F-127 (PF127) and Gelucire® 44/14 (GL44) in order to enhance its oral bioavailability and cytotoxicity in human lung cancer cell line A549.
Study design: 3^2 Factorial design was used to assess the effect of formulation variables for optimization of mixed micelle batch.
Methods: CUR-MM was prepared by a solvent evaporation method. The optimized CUR-MM was evaluated for size, entrapment efficiency (EE), *in vitro* curcumin release, cytotoxicity and oral bioavailability in rats.
Results: The average size of CUR-MM was found to be around 188 ± 3 nm with an EE of about 76.45 ± 1.18% w/w. *In vitro* dissolution profile of CUR-MM revealed controlled release of curcumin. Additionally, CUR-MM showed significant improvement in cytotoxic activity (3-folds) and oral bioavailability (around 55-folds) of curcumin as compared to curcumin alone. Such significant improvement in cytotoxic activity and oral bioavailability of curcumin when formulated into mixed micelles could be attributed to solubilization of hydrophobic curcumin into micelle core along with P-gp inhibition effect of both, PF127 and GL44.
Conclusion: Thus the present work propose the formulation of mixed micelles of PF127 and GL44 which can act as promising carrier systems for hydrophobic drugs such as curcumin with significant improvement in their oral bioavailability.

Introduction

Curcumin, a major active compound extracted from powdered dry rhizomes of *Curcuma longa* L. (Zingiberaceae) has been extensively studied for its wide range of pharmacological activities including antioxidant, anti-inflammatory, antidiabetic, antibacterial, wound healing, antiatherosclerotic, hepatoprotective and anti-carcinogenic (Bansal et al. 2011). Further, curcumin acts as chemosensitizer and radiosensitizer of tumors (Goel and Aggarwal 2010). Curcumin has shown cytotoxic activities in lung squamous cell carcinoma H520 and on-small cell lung cancer H460 cell lines (Sen et al. 2005; Chanvorachote et al. 2009). Various pharmacological activities associated with curcumin are believed to be due to its interference with diverse cell signaling pathways including cell cycle (cyclin-D1 and cyclin-E), apoptosis (activation of caspases and down-regulation of antiapoptotic gene products), proliferation (HER-2, EGFR, and AP-1), survival (PI3K/Akt pathway), invasion (MMP-9 and adhesion molecules), angiogenesis (VEGF), metastasis (CXCR-4) and inflammation (NFkB, TNF, IL-6, IL-1, COX-2, and 5-LOX). When tested in healthy human volunteers, curcumin was found to be safe at a high dose of about 12,000 mg/day (Lao et al. 2006).

Despite such a wide range of pharmacological activities, clinical applications of curcumin are limited owing to its poor aqueous solubility at acidic pH, instability at pH 7 and above, multidrug pump P-gp efflux, extensive *in vivo* metabolism and rapid elimination due

Abbreviations: PF127, pluronic F-127; PEO, poly(ethylene oxide); PPO, poly(propylene oxide); MDR, multidrug resistant; GL44, gelucire® 44/14;CUR-MM, curcumin loaded mixed micelles; EE, entrapment efficiency; B-MM, blank mixed micelles; rt, room temperature; CMC, critical micelle concentration; FTIR, Fourier transform- infrared spectroscopy; A-CUR, aqueous dispersion of curcumin; TEM, Transmission electron microscopy; SRB, sulforhodamine B; HLB, hydrophilic-lipophilic balance; PEG, polyethylene glycols;PA, peak area.

* Corresponding author. Tel.: +91 20 25437237; fax: +91 20 25439383.
E-mail address: sharvilpatil25@gmail.com (S. Patil).

http://dx.doi.org/10.1016/j.phymed.2015.08.006
0944-7113/

to glucuronidation/sulfation. It belongs to BCS class IV showing low aqueous solubility (log*P* 3.28) and low permeability (Xie et al. 2011). Oral bioavailability of curcumin is only 1% (Zhao et al. 2012). Thus it becomes difficult to achieve and maintain concentration of curcumin within therapeutic ranges upon its oral administration. Cancer leads to overexpression of P-gp which decreases therapeutic efficacy of anticancer drugs and in turn makes the task more difficult. Various formulations of curcumin have been prepared in order to enhance its bioavailability. However, they suffer from drawbacks such as low drug loading capacity, stability, drug leakage, etc.

Polymeric micelles have better stability compared to surfactant micelles, enhanced solubilizing power, longer circulating time due to outer hydrophilic shell, small size and targeting capability which make them an attractive delivery vehicle for a variety of drugs including biomolecules. Additionally, mixed micelles are preferred over single polymer micelles since they increase micellar stability and drug loading efficiency (Saxena and Delwar 2012).

Pluronic F-127 (PF127) is an amphiphilic, nonionic polymer consisting of hydrophilic poly(ethylene oxide) (PEO) and hydrophobic poly(propylene oxide) (PPO) blocks. PF127 copolymers are widely used in formulation due to their ability to form nanosized micelles (Dumortier et al. 2006). They are capable of sensitizing multidrug resistant (MDR) cancer cells. Additionally, PF127 shows P-gp inhibition effect by decreasing ATPase activity and thereby causing reduction in intracellular ATP. Many micellar preparations of pluronics containing biological active molecules have been reported (Butt et al. 2012; Chen et al. 2013). However, micelles formed by PF127 have poor stability and low drug entrapment efficiency (Butt et al. 2012). To overcome these drawbacks, mixed micelles of pluronics have been proposed for some formulations (Zhao et al. 2012).

It has been reported that encapsulation of drug in lipidic carrier alters its pharmacokinetic profile (Bansal et al. 2009). Gelucire® 44/14 (GL44) is a surface active lipid excipient belonging to generally regarded as safe category (Wehrung 2012). It is lauroyl polyoxylglycerides, consisting of mixtures of monoesters, diesters and triesters of glycerol along with monoesters and diesters of polyethylene glycols (PEG). It is widely used in the preparation of solid dispersions (Chauhan et al. 2005), SEDDS (Kallakunta et al. 2013), nanoparticles (Wehrung 2012) and has been reported to increase solubility of poorly water soluble drugs through micelle formation (Kawakami et al. 2004). Additionally, P-gp inhibition activity of GL44 has been well reported (Sachs-Barrable et al. 2007).

Thus the current work was planned with the objective of preparing curcumin loaded mixed micelles of PF127 and GL44 (CUR-MM) which may enhance oral bioavailability of curcumin and cytotoxicity in human lung cancer cell line A549. Curcumin has shown promising anticancer activity in human lung cancer cell line A549 with well-investigated biochemical factors. The effect of varied formulations (with different amounts of lipid and polymers) on micelle size and entrapment efficiency (EE) was analyzed by 3^2 factorial design.

Materials and methods

Materials

Curcumin containing ≥94% of curcuminoids and ≥80% of curcumin was purchased from Sigma-Aldrich (India). PF127 and GL44 were generous gifts from Alembic Pharmaceuticals (Mumbai, India) and Gattefossé S.A.S. (Saint Priest, France). Emodine was purchased from Chroma-standard Medical technology Co., Ltd. (Tianjing, China). All reagents and chemicals used in the study were of analytical grade.

Methods

Preparation of CUR-MM

Solvent evaporation method was used for the preparation of CUR-MM (Zhou et al. 2011). Different amounts of PF127and GL44 were dissolved in methanol (3 ml) separately along with curcumin (10 mg) and coded (1–9) accordingly (Table 1). Each solution was then added dropwise to double distilled water under high speed stirring which was continued till complete evaporation of methanol. The resultant dispersion was then filtered through 0.45 μm filter so as to remove unloaded curcumin. Blank mixed micelles (B-MM) devoid of curcumin were prepared using similar method. CUR-MM thus obtained were then evaluated for size, drug content and EE.

Optimization of CUR-MM by 3^2 factorial design

Amount of PF127 (X_1) and GL44 (X_2) were the primary independent variables which are believed to affect the dependent variables *viz.* micelle size (Y_1) and EE (Y_2) (Table 1). Factorial design has been reported to highlight the relationships between variables through minimum experimentation and reduced possibility of experimental error (Fisher 1926). The data obtained for all the batches were analyzed using STATISTICA software, version 12 in order to obtain Eq. (1).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (1)$$

where, Y is dependent variable, b_0 is mean response and b_1 and b_2 are corresponding coefficients of factors X_1 and X_2 respectively; X_1X_2 is interaction term representing changes in response when both factors are changed simultaneously. ${X_1}^2$ and ${X_2}^2$ are polynomial terms indicating nonlinearity in response. 3D surface was plotted for the understanding of the relationships among variables.

Preparation of aqueous dispersion of curcumin (A-CUR)

Curcumin (10 mg) was dispersed in distilled water (3 ml) using ultrasound probe sonicator (Vibra-Cell™, VCX500, Sonics & Materials, Inc. USA).

Table 1
The effect of varied formulations (with different amounts of lipid and polymers) on micelle size and EE of CUR-MM by 3^2 factorial design.

Batch	Amount of PF127 (X_1,mg)	Amount of GL44 (X_2,mg)	Micelle size (nm)	Percent EE of curcumin
1	−1 (150)	−1 (150)	210 ± 2	45.51 ± 1.31
2	−1	0 (175)	182 ± 4	71.04 ± 2.21
3	−1	+1 (200)	257 ± 3	60.11 ± 3.10
4	0 (200)	−1	231 ± 2	58.00 ± 2.45
5	0	0	188 ± 3	76.45 ± 1.18
6	0	+1	289 ± 5	69.70 ± 2.65
7	+1 (250)	−1	364 ± 3	35.02 ± 3.22
8	+1	0	312 ± 2	55.14 ± 2.14
9	+1	+1	415 ± 6	40.00 ± 2.24

Mean ± SD, $n = 3$.