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Transdermal delivery of the in situ hydrogels of curcumin and its inclusion complexes of hydroxypropyl-β-cyclodextrin for melanoma treatment

Yunbo Sun [a,1], Lina Du [a,1], Yangpu Liu [a], Xin Li [b], Miao Li [a], Yiguang Jin [a,*], Xiaohong Qian [a]

[a] Beijing Institute of Radiation Medicine, Beijing 100850, China
[b] Chinese PLA General Hospital, Beijing 100853, China

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ABSTRACT

Curcumin (Cur) is a hydrophobic polyphenol with diverse pharmacological effects, especially for cancer treatment. However, its weak water solubility and stability was the major obstacle for the formulation research of Cur. The complexation of Cur and hydroxypropyl-β-cyclodextrin (HP-β-CD) was done by grinding. The increasing solubility of Cur was achieved due to complexation and the photochemical stability of Cur was improved. The inclusion of Cur could happen when two ends of Cur were embedded into the cavity of the HP-β-CD rings. The in situ hydrogels (ISGs) of Cur and its inclusion complexes were prepared using poloxamers 407 and 188 as the matrix. The extent of drug's in vitro release from the ISGs depended on the dissolution of drugs. Both of the ISGs had transdermal effect and cytotoxicity on B16-F10 cells. However, the effects of the ISGs containing Cur inclusion complexes were much higher than those of Cur ISGs because of the improved Cur solubility in the former. The cytotoxicity of Cur on melanoma cells was related to blocking of cellular proliferation in the G_2/M stage followed by cellular apoptosis. The ISGs of Cur inclusion complexes are a promising formulation for melanoma treatment.

1. Introduction

Transdermal drug delivery systems (TDDSs) could not only act on the topical skin, but also deliver drugs to the blood circulation through skin. TDDS offered many advantages over the conventional dosage forms that could enhance the patient compliance by virtue of low dose frequency, less adverse effects and noninvasive delivery of drugs.

Curcumin (Cur), [7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] a hydrophobic polyphenol, is a principal component and main colorant of turmeric (Fang et al., 2003) used for centuries in Asian countries as a spice and also as a herbal anti-inflammatory agent. Cur has proven to be assuring as it exhibits promotion of wound healing (Mohanty et al., 2012b), antimicrobial (Hegge et al., 2010), anti-inflammatory (Ammon and Wahl, 1991) and anti-cancer effects (Kunnumakkara et al., 2008). Its pharmacological safety, combined with its dose-dependent chemotherapeutic effect in several tumor-bearing animal models, makes it an ideal agent for the transdermal delivery in the treatment of melanoma. However, the solubility and stability property of Cur was disadvantageous for its formulation preparation. Cur dissolved in methanol, ethanol and propylene glycol, and slightly dissolved in water. Cur was not stable in neutral medium, and it could produce ferulic acid. In addition, the aqueous solubility of Cur is as low as 0.0004 mg/ml at pH 7.4 (Mohanty et al., 2012a). To address the problems, some of the novel Cur formulations were investigated, including nanocrystal (Rachmawati et al., 2013), solid lipid nanoparticles (Tiyaboonchai et al., 2007), transdermal film (Vidyalakshmi et al., 2004), microspheres (Kumar et al., 2002), nanospheres (Mukerjee and Vishwanatha, 2009), nanoemulsion (Wang et al., 2008), phospholipid complexes (Liu et al., 2006; Maiti et al., 2007), etc.

The incidence of melanoma is increasing worldwide, especially in USA. Despite early detection, appropriate surgical resection and adjuvant therapy, the number of patients dying from metastatic

* Corresponding author at: Beijing Institute of Radiation Medicine, Department of Pharmaceutical Sciences, 27 Taiping Road, Haidian District, Beijing 100850, China. Tel.: +86 10 68246767; fax: +86 10 68214653.
E-mail address: jinyg@bmi.ac.cn (Y. Jin).
[1] The authors contributed equally.

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disease continues to rise. According to WHO, approximately 80% of all skin cancer-related deaths are attributed to melanoma, although it comprised only 5% of all skin cancers. Despite extensive clinical research, the treatment options for metastatic disease were limited, with melanoma being considered as one of the most chemotherapy-resistant malignancies. Until recently, and over the past 30 years, only three drugs had gained FDA approval for the treatment of melanoma, namely dacarbazine, hydroxyurea, and interleukin-2 (IL-2) (Gogas et al., 2013).

In situ-forming hydrogels (ISGs) are liquid aqueous solutions before administration, but gels under physiological conditions. Gelation can occur in situ by ionic cross-linking or after a change in pH or temperature (Eve and Leroux, 2004). ISGs as one of the most optimal transdermal formulations offer several advantages, such as simple mixing, easy application, long adhesion time on the skin surface, and good permeation ability of therapeutic agents. Therefore, ISGs are the best choice to prepare Cur transdermal formulation for melanoma treatment.

Poloxamer block copolymers were one of the most important thermosensitive hydrogel materials. This copolymer consists of ethylene oxide (EO) and propylene oxide (PO) blocks arranged in a triblock structure EO_x–PO_y–EO_x. Poloxamer has been presented by FDA guide as an "inactive" ingredient for different types of preparations, such as intravenous injection, inhalation, oral solution, suspension, ophthalmic or topical formulation (Gilles et al., 2006). The thermo-responsive solution–gelation transformation is attributed to the interaction between the segments of the temperature-sensitive copolymers. Poloxamer 407 molecules in solutions could likely aggregate into micelles with the increase in temperature, resulting from dehydration of hydrophobic polypropylene oxide (PPO) blocks of the copolymer. The spherical micelles may have a hydrophobic PPO core and a hydrophilic shell of hydrated swollen polyethylene oxide (PEO) chains. The micelles would pack to form a hydrogel network. At the temperature lower than the sol–gel transition temperature ($T_{sol-gel}$), there was no intermolecular interaction between poloxamer molecules. As the temperature increased to $T_{sol-gel}$, hydrophilic PEO chain entanglement and hydrophobic PPO dehydration led to the formation of micelles. At the temperature higher than $T_{sol-gel}$, the outer PEO chain of each micelle was interacted due to hydrogen bonding, resulting in gel phenomena (Gaisford et al., 1998). The higher the concentration of the copolymer, the higher are the amounts of micelles and the easier is the formation of hydrogels. In general, poloxamer is the most common and appropriate excipient as the thermo-sensitive ISGs matrix.

In this study, the solubility and stability of Cur was improved by preparing cyclodextrin complexes. High transdermal efficiency and good melanoma therapy of the Cur-loaded ISGs were achieved. The physicochemical properties of the complexes, erosion of ISGs matrix, drug release, cytotoxicity and the inhibition mechanism on B16-F10 cells were also investigated.

2. Materials and methods

2.1. Materials

Cur was provided by Guangfu Fine Chemical Institute of Tianjin. Poloxamers 188 and 407 were purchased from BASF (Ludwigshafen, Germany). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) was supplied by Zhongqi Pharmaceutical Technology Co., Ltd., Shijiazhuang, China. Propidium iodide (PI) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Paclitaxel injection was supplied by Beijing Huasu Pharmaceutical Co., Ltd., Beijing, China. Pure water was used for all solutions and dilution. All other chemicals used were of analytical grade.

2.2. Preparation of inclusion complexes

The complexation of Cur/HP-β-CD (1:1, 1:2, 1:3, molar ratio, respectively) was prepared by the grinding method described previously (Mura et al., 1999). In detail, Cur and HP-β-CD (Fig. 1) with different molar ratios were grinded with 50% (v/v) ethanol for 0.5 h. The inclusion complexes were washed with methanol three times to remove free Cur and then dried at 50 °C to obtain a dry powder.

Inclusion complexes of Cur (20 mg) and HP-β-CD were dissolved in 50% ethanol (v/v) and filtered through a 0.45-μm filter membrane. Filtrated solution (0.1 ml) was metered to 10 ml with ethanol in a volume flask (10 ml). Then the Cur content was determined by high-performance liquid chromatography (HPLC). The inclusion efficiency (%) was calculated as the following formula.

$$\text{Inclusion efficiency}\,(\%) = \frac{(\text{Determined Cur contents})}{(\text{Theoretical Cur contents})} \times 100\%$$

The encapsulation of Cur in HP-β-CD was confirmed by differential scanning calorimeter (DSC) analysis. In order to verify encapsulation of Cur in HP-β-CD, DSC curves of four types of samples were obtained: (a) pure HP-β-CD, (b) pure Cur, (c) physical mixture of HP-β-CD/Cur, (d) inclusion complexes with HP-β-CD and Cur. A TA DSC (Q20, New Castle, DE, USA) was employed and air was used as the reference.

Cur (2 mg) and the inclusion complexes that contained Cur (2 mg) were weighed and placed in a 50-μl closed aluminum pan. The scans were conducted under nitrogen at a flow rate of 20 ml/min between 50 and 380 °C at 20 °C/min.

Cur and its inclusion complexes were analyzed by the Fourier transform infrared spectroscopy (FTIR, FTS-65A, Bio-Rad) in a region ranging from 400 to 4000 cm^{-1}. The samples (ca. 0.1 g) were mixed with KBr (0.1 g) and pressed to a tablet. The FTIR spectrum was then recorded.

2.3. HPLC analysis

HPLC experiments were performed on a Shimadzu 10Avp HPLC system (Japan) consisting of a LC-10Avp pump, an SPD-

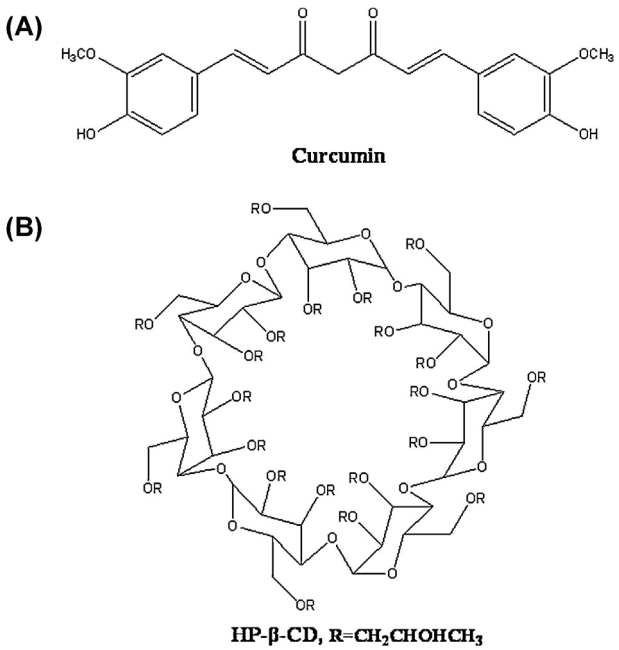

Fig. 1. Chemical structures of Cur (A) and HP-β-CD (B).