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Enhancement of skin permeation of bufalin by limonene via reservoir type transdermal patch: Formulation design and biopharmaceutical evaluation

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ABSTRACT

A reservoir-type transdermal delivery system (TDS) of bufalin was designed and evaluated for various formulation variables like different penetration enhancers, formulation matrix, rate controlling membranes as well as biopharmaceutical characteristics. Hairless mouse skin was used in permeation experiments with Franz diffusion cells. In vitro skin permeation study showed that terpenes, especially D-limonene was the most effective enhancer when ethanol and PG were used as the vehicle with a synergistic effect. Among different rate controlling membranes, ethylene vinyl acetate (EVA) membrane containing 19% vinyl acetate demonstrated a more suitable release rate for bufalin than the other membranes. In vivo pharmacokinetic study of the bufalin patch in rat showed steady-state of bufalin from 3 h to 12 h. In vivo release rate and cumulative amount analyzed by deconvolution method demonstrated the sustained release of bufalin as long as the patch remained on the animal for at least 12 h. The MRT increased from 1 h of IV administration to 9 h of transdermal administration. In vitro permeation across mouse skin was found to have biphasic correlation with plasma AUC in the in vivo pharmacokinetic study. Current in vitro–in vivo correlation (IVIVC) enabled the prediction of pharmacokinetic profile of bufalin from in vitro permeation results. In conclusion, current reservoir transdermal patch containing 10% D-limonene as a permeation enhancer, 40% ethanol, 30% PG and 15% carbopol–water gel complex provided an improved sustained release of bufalin through transdermal administration. The bufalin patch was successfully applied to biopharmaceutical study in rats and demonstrated the feasibility of this transdermal formulation for future development and clinical trials.

1. Introduction

Bufalin is a major component of bufadienolides extracted from Chinese traditional medicine Chansu (toad venom). It is widely used as one of the key components in many traditional Chinese medicines, i.e., Liu Shen Wan (Hong et al., 1992) and Shexiang Baoxin Wan (Song et al., 2000). Bufalin exhibits potent anti-tumor activities against liver cancer (Zhang et al., 2012), lung cancer (Zhu et al., 2012), gastric cancer (Li et al., 2009), as well as prostate cancer (Yu et al., 2008). Bufalin has been reported to induce apoptosis in various cancer cells through both intrinsic and extrinsic pathways as elaborated in several publications (Hong and Choi, 2012; Yan et al., 2012; Zhu et al., 2012) and enhance the cytotoxicity of several anticancer drugs (Hashimoto et al., 1997). Because of dual effects of anti-cancer and pain relief activity, bufalin was approved as anticancer drugs in China and was used to treat late-stage of liver and lung cancers (Meng et al., 2009; Qin et al., 2008). The clinical study of bufalin was also conducted in the United States for the treatment of pancreatic cancer and showed potential therapeutic effect (Meng et al., 2009; Wang et al., 2011). Considering low survival rate of liver and lung cancer in late-stage, alternative medicines obtained from herb materials and animals may provide valuable benefits.

However, the clinical application of bufalin on cancer treatment was greatly limited by its unfavorable biopharmaceutical properties and cardiac toxicity. It has been reported to have poor solubility, short half-life and narrow therapeutic window upon intravenous administration (Yin et al., 2012). The short half-life limits its capability to maintain minimal plasma drug concentration to achieve therapeutical effect and demands multiple times administrations. Furthermore, bufalin belongs to toxic steroids, which produce digoxin-like cardiac toxicity (Wang et al., 1997). Bufalin has side effect of cardiac arrhythmia, breathlessness, convulsion and coma at high dosage, which may cause safety issues (Panesar, 1992).

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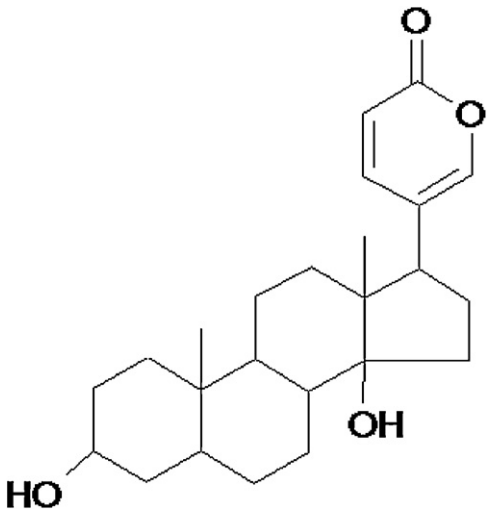

Fig. 1. The chemical structure of bufalin.

Currently developed formulation of bufalin include wheat germ agglutinin-grafted lipid nanoparticles (Liu et al., 2011), self-microemulsifing lipid-based formulation (Liu et al., 2010) and PEG–PLGA loaded nanoparticles (Yin et al., 2012). Although they have achieved some successes on improving solubility and overall bioavailability compared to traditional tablet and injections (Yang et al., 2008), the major issues including high dose derived cardiotoxicity and rapid decline of exposure still exist. Transdermal formulation was considered as an enabling strategy for this scenario by offering both controlled and sustained release patterns. A number of FDA approved transdermal products, like Transderm-Nitro™, Estraderm™, Duragesic™, D-TRANS™, Androderm™ and Catapres-TTS™ had demonstrated the successful application of transdermal administration.

To the best of our knowledge, there is no transdermal formulation of bufalin that has been developed and evaluated. Bufalin is a small molecular compound (molecular weight = 386.5) with a suitable lipophilicity ($\log P = 2.78$). However, its poor aqueous solubility (33 μg/ml) may limit permeation force through dermis (Liu and Feng, 2008). The chemical structure of bufalin is shown in Fig. 1. For drugs with a short biological half-life and/or a narrow therapeutical range, transdermal administration is one of the attracting alternative approaches to decrease toxicity and prolong therapeutical action. Therefore, the skin has become an important route for drug delivery for topical, regional or systemic effects for the last two decade (Paudel et al., 2010). The major challenges of developing transdermal drug delivery lie on the poor permeation of API and its potential skin irritation. Using chemical enhancers is the most common strategy to improve the permeation of drugs through the skin barrier.

Therefore, the objective of this study can be divided into three aims, (1) develop a transdermal delivery system for bufalin administration, (2) evaluate its biopharmaceutical characteristics to ensure sustained release of bufalin in vivo, (3) perform in vitro–in vivo correlation and use in vitro data to predict and guide pharmacokinetics in future.

2. Materials and methods

2.1. Materials

Bufalin was extracted and purified in our lab from Traditional Chinese Medicine, Chansu. The purified bufalin powder had been identified by NMR and Mass Spectrometry and quantified by HPLC with a purity >98% in our previous study (Yang et al., 2008).

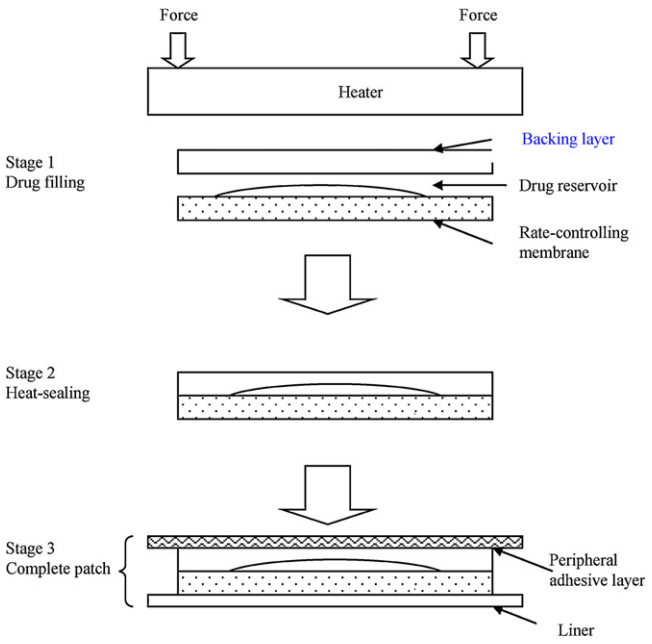

Fig. 2. The schematic preparation of reservoir-type bufalin patch.

3M™CoTran™ 9728, 3M™CoTran™ 9705, 3M™CoTran™ 9715 and other types of ethylene vinyl acetate (EVA) membrane with different vinyl acetate content 2%, 9%, 19%, 28% were obtained from 3M Pharmaceuticals (St. Paul, MN, USA). Diphenhydramine, oleic acid, lauric acid, sodium lauryl sulphate (SLS), azone, ethyl oleate, ethyl lauric acid, isopropyl myristate (IPM), Tween 80, N-methyl-2-pyrrolidone (NMP), ethanol, propylene glycol (PG), 1,8-cineole, D-limonene and L-menthol were purchased from Sigma (St. Louis, MO, USA). Carbopol 934 P (pharmaceutical grade) was purchased from Goodrich Co. Ltd., (Cleveland, OH, USA). All other chemicals were obtained from Shanghai Chemical Reagents Co. (Shanghai, China). Aluminum foil–polyethylene backing layer was purchased from Shanghai Package Material Company (Shanghai, China). Heat-sealing machine was purchased from Yiheng Inc. (Shanghai, China). Transdermal permeation diffusion instrument was purchased from Jiekai Inc. (Shanghai, China).

2.2. Preparation of transdermal delivery system (TDS)

Reservoir-type TDS was prepared with an active membrane release area of 15 cm^2. The schematic of TDS preparation in the present study is shown in Fig. 2.

2.2.1. Preparation of transdermal gel of bufalin

40 mg bufalin was firstly fully dissolved in 4 ml ethanol, and then 3 ml PG added. 1 ml D-limonene (10%, w/v of mixture) was added as a penetration enhancer to drug solutions, and then 0.1 ml Tween 80 added as a surfactant to form a uniform phase. 1 g carbopol used as gelling agent was swelled in 50 ml double distilled water (form 2% carbopol gel) overnight while pH value was adjusted to 7.4. 1.5 g carbopol–water gel (2%) was added to drug solutions (15%, w/v) and stirred well to form a uniform gel. The uniformity of drug content (4 mg/ml) was tested for all batches of gels by HPLC.

2.2.2. Preparation of reservoir transdermal patch

A procedure for drug-loaded-membrane laminate was divided into three main phases. Phase 1 involved filling of drug reservoir. 1 ml bufalin gel was squeezed to the EVA membrane from peristaltic pump and then the backing layer was used to cover the rate-controlling membrane on the top of the drug–gel complex. The