

Preparation and Anti-tumor Evaluation of Aloe-emodin Loaded Solid Lipid Nanoparticles

By

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Supervisor

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碩士學位論文

蘆薈大黃素固體脂質納米粒的製備與體外 抗腫瘤活性評價

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摘要

蘆薈大黃素固體脂質納米粒的製備與體外抗腫瘤活性 評價

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蘆薈大黃素是從百合科植物庫拉索蘆薈，蓼科植物大黃和豆科植物決明子等中藥中提取分離得到的一種蒽醌化合物，具有顯著的抗腫瘤活性和良好的抗氧化、抗菌、抗病毒作用。近年來，隨著其廣泛的藥理活性，尤其是抗腫瘤作用被不斷揭示，蘆薈大黃素受到科學家的普遍關注。但由於存在水溶性差，吸收利用率低，代謝消除迅速等缺點，蘆薈大黃素的口服生物利用度較低，很大程度上限制了其臨床運用。由於納米載藥系統在改善藥物生物利用度和降低不良反應方面具有獨特的優勢，因此，運用納米載藥系統是提高藥物的臨床適用性有效途徑之一。固體脂質納米粒作為近年來備受關注的納米載藥系統，可有效地提高藥物穩定性，控制藥物釋放，改善藥物生物利用度，具有良好的開發價值和廣泛的應用前景。因此，本研究希望通過製備蘆薈大黃素的固體脂質納米粒，提高其生物利用度，改善療效。

本研究首先建立了蘆薈大黃素的體外含量測定方法，並採用高壓乳勻法製備製備蘆薈大黃素固體脂質納米粒，分別從工藝參數和處方因素兩方面對蘆薈大黃素固體脂質納米粒的處方進行了優化，主要通過預實驗篩選了影響納米粒質量的主要因素，並運用單因素試驗考察了高壓均質條件，脂質材料種類，脂質濃度，乳化劑種類及用量對納米粒粒徑和穩定性的影響，確定最優處方。而後採用透析法研究了固體脂質納米粒的體外釋藥行為，並對其釋藥曲線進行擬合，探索其釋藥機理。最後使用細胞模型比較了原藥和固體脂質納米粒的攝取情況和抗腫瘤療效，對其進行藥理研究。

實驗結果表明，以乙腈-水（50:50, v/v）為流動相的高效液相色譜法，方法

簡單，重複性好，準確性高，符合體外藥物含量測量要求。在處方優化方面，以 10 mg/ml 的單硬脂酸甘油酯為脂質材料，占脂質比重 40% 的吐溫-80 為乳化劑，成功製備了蘆薈大黃素固體脂質納米粒。優化後的得到的納米粒平均粒徑為 78.9 nm，分佈均勻，包封率高達 97.7%，zeta 電位為 -42.8 mV，穩定性良好，達到了預期目標。納米粒的體外釋藥行為表明蘆薈大黃素納米粒具有緩釋作用，釋放曲線符合 Weibull 模型，藥物主要是依靠擴散作用從脂質骨架中釋放。納米粒的體外攝取研究顯示，MCF7 細胞對固體脂質納米粒中蘆薈大黃素的攝取量是原藥的 1.6 倍，很大程度上提高了藥物的利用率。此外，其抗腫瘤活性研究也表明將藥物包載於固體脂質納米粒中能有效提高其抗腫瘤活性，改善療效。

關於蘆薈大黃素固體脂質納米粒的研究目前國內外尚未見報道。本研究通過優化的處方成功製備了蘆薈大黃素的固體脂質納米粒，該納米粒具有良好的緩釋作用，並能增強藥物的抗腫瘤作用，為蘆薈大黃素的臨床應用研究奠定了基礎。

關鍵字：蘆薈大黃素；固體脂質納米粒；處方篩選；體外釋放；抗腫瘤作用

Abstract

Preparation and Anti-tumor Evaluation of Aloe-emodin loaded Solid Lipid Nanoparticles

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Aloe-emodin (AE), a natural biologically active anthraquinone, is mainly isolated from *Rheum palmatum* L., *Aloe barbadensis* Miller (Aloe vera) and *Cassia obtusifolia* L.. Recent studies have demonstrated that AE is a promising drug with various pharmacological effects, including anti-tumor, anti-oxidant, anti-bacterial and antiviral activities and so on. During the past years, with the developing elucidation of its antineoplastic principle and other pharmacological activity, AE has attracted much attention. Nevertheless, despite of the significant effects, it suffers much limitations such as poor aqueous solubility, bad absorption, rapid metabolism and elimination, which have greatly restricted its clinical application. Therefore, looking for effective methods to ameliorate these limitations is necessary for the sake of better efficacy. During the recent decades, Novel Drug Delivery Systems have presented superior advantages in improving drug's efficacy and patients' compliance. In particular, the mention and application of Solid Lipid Nanoparticles (SLNs), have absorbed intense concern. Taking the various advantages into consideration, SLNs is regarded as a promising drug carrier system. For instance, it could increase drug's stability, display sustained-release property and improve bioavailability. Therefore, we hope to prepare AE loaded SLNs, in an attempt to improve the bioavailability and efficacy of AE.

Determining method of AE content was first established and then High pressure homogenization (HPH) was used for preparation of AE-SLNs. Based on the results of

pre-formulation study, we investigated the influence of several factors on the particle size and stability of AE-SLNs, such as HPV condition, lipid materials, lipid concentration, emulsifiers, ratios of emulsifier to lipid, which cover both processing parameter and prescription factors. Besides, in vitro drug release profile was carried out and the release mechanism was studied as well. In addition, the cellular uptake and anti-tumor activity of SLNs was evaluated in comparison to free drug.

Ultimately, a convenient, accurate and repeatable HPLC method was established with a mobile phase of acetonitrile : water (50:50, v/v), which could meet the requirements of drug determination in vitro. On formulation optimization, glycerol monostearate and Tween 80 were selected as excipients. The AE-SLNs showed stable particle size at 78.9 nm, ideal drug entrapment efficiency (EE) of 97.7% and good stability with regard to zeta-potential as high as -42.8 mV. The in vitro release profile of AE-SLNs also revealed that it has sustained release function which may result from the drug diffusion from lipid matrix. Furthermore, the increased drug uptake and enhanced anti-tumor activity indicated that the preparation of AE-SLNs is an effective way for improving the bioavailability and efficacy of AE.

Nowadays, there has been no report about AE loaded SLNs throughout the world. In our study, AE-SLNs have been successfully prepared with optimized prescription. Based on the improved bioavailability and enhanced efficacy, the particles may have good prospects for further development.

Key words: Aloe-emodin; Solid lipid nanoparticles; formulation design; in vitro release; anti-tumor effect

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縮略詞列表

AE	Aloe-emodin	蘆薈大黃素
SLNs	Solid Lipid Nanoparticles	固體脂質納米粒
PDI	Polydispersion Index	多分散係數
FBS	Fetal Bovine Serum	胎牛血清
PBS	Phosphate Buffer Solution	磷酸緩衝液
DSC	Differential Scanning Calorimetry	差示掃描量熱分析
DAD	Diode Array Detector	二極體陣列檢測器
LOD	Limit of Detection	檢測限
LOQ	Limit of Quantification	定量限

