PREPARATION, CHARACTERIZATION, PHARMACOKINETICS AND ANTI-OSTEOPOROSIS ACTIVITY EVALUATIONS OF ICARITIN NANOSUSPENSION

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碩士學位論文

淫羊藿苷元納米混懸液的製備、表徵、藥動學和抗骨質疏鬆活性的評價

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摘 要

本研究利用反溶劑沉澱法製備淫羊藿苷元（Icaritin，ICT）納米混懸液以提高其溶出度及口服生物利用度。首先選擇不同種類的穩定劑製備了 ICT 納米混懸液，利用羥丙基甲基纖維素（HPMC）製備出的納米混懸液粒徑小且分佈均勻、短期穩定性好。通過單因素實驗考察不同型號的 HPMC、ICT 丙酮溶液濃度以及超聲時間對粒徑的影響。在以上影響因素為最優的情況下，利用星點設計-效應面優化法考察穩定劑濃度和注入 ICT 丙酮溶液的體積對粒徑的影響。獲得最優處方及工藝如下：將 0.16 mL 的 ICT 丙酮溶液（10 mg/mL）在超聲條件下注入到 0.02%（w/v）HPMC E3 溶液中並繼續超聲 10 sec。最優處方的粒徑、多分散係數及 zeta 電位分別為：（216.6±12.4）nm，0.124±0.013，（-13.8±1.4）mV。利用透射電鏡表徵納米混懸液的形態，並利用掃描電鏡表徵原藥及納米混懸液凍幹後的形態，發現納米混懸液與納米混懸液凍幹後的顆粒形態為棒狀。通過 X 光衍射掃描和差示掃描量熱分析發現，製備成納米混懸液後，ICT 原藥的晶體峰位置不變但強度下降，提示結晶度比原晶體下降。溶出實驗顯示，ICT 在納米混懸液中 15 min 內完全溶出，比原晶體的溶出速度更快也更完全。大鼠口服 ICT 納米混懸液和原藥粗混懸液後，納米混懸液的 AUC_{0-36h} 和 C_{max} 比原藥粗混懸液顯著提高，分別約為原藥粗混懸液的 2.0 倍和 4.7 倍。而 T_{max} 較原藥粗混懸液降低，說明納米混懸液可以在體內血漿中更快速達峰。

研究 ICT 對體外培養 MC3T3-E1 細胞系增殖和分化的影響。ICT 在 10^{-9}-10^{-6} M 摩爾濃度下對 MC3T3-E1 增殖無影響。ICT 表現出明顯的促進分化的作用（10^{-6} M 摩爾濃度下的鹼性磷酸酶活性是空白對照組的 1.75 倍）。利用血清藥理學方法評價 ICT 納米混懸液和原藥粗混懸液對成骨細胞增殖和分化的作用。ICT 納米混懸液和原藥粗混懸液分別口服給予大鼠後，於 10 min、1.5 h 和 24 h 時采血。血清中 ICT 總濃度經 β-葡萄糖醛酸酯酶/硫酸酯酶酶解後進行檢測，並將血清加入到 MC3T3-E1 細胞培養體系中。口服 ICT 納米混懸液後 10 min 和 1.5 h 含藥血清和空白對照組相比，細胞存活率分別提高至（120.1±9.0）%和（116.6±9.9）%，24 h 含藥血清無作用。而口服原藥粗混懸液 10 min、1.5 h 和 24 h 含藥血清對細胞存活率沒有顯著提高。口服納米混懸液後，10 min 和 1.5 h 含藥血清均可提高鹼性磷酸酶活性為空白對照組的 1.41 倍，表現出顯著的促進鹼性磷酸酶活

性的作用（p<0.05），24 h 含藥血清對鹼性磷酸酶活性無顯著性影響。而口服原藥粗混懸液 10 min、1.5 h 和 24 h 含藥血清沒有表現出顯著的促進鹼性磷酸酶活性的作用（p>0.05）。上述結果提示，納米混懸液對成骨細胞的增殖和分化的作用均有促進作用，其效果優於原藥粗混懸液。

本研究證明，ICT 納米混懸液可以提高體外溶出度和口服生物利用度，同時提高藥物的達峰速度。ICT 具有促進成骨細胞分化的作用，而血清藥理學結果顯示，ICT 的代謝產物可以有效促進成骨細胞增殖與分化。ICT 納米混懸液是一種有潛力被開發成抗骨質疏鬆的口服納米製劑。

關鍵字：淫羊藿苷元，納米混懸液，反溶劑沉澱法，超聲，溶出速度，口服生物利用度，骨質疏鬆

Abstract

In the present study, icaritin (ICT) nanosuspensions were prepared by antisolvent-precipitation method to enhance its dissolution rate and oral bioavailability. Firstly, different stabilizers were evaluated on their feasibility to form stable ICT nanosuspension. Hydroxypropyl methylcellulose (HPMC) was proved its superiority both in terms of particle size reduction and short-term physical stability. Three experimental parameters, viz., types of HPMCs, concentration of ICT in acetone and time length of ultrasonication were investigated on their impacts on particle size. Subsequently, central composite design was applied to evaluate the impacts of concentration of stabilizer solution and volume of ICT acetone solution injected on the particle size distribution of nanosuspension. The optimized formulation was determined as following: 0.16 mL of ICT acetone solution (10 mg/mL) injected into 2 mL of HPMC E3 solution (0.02% w/v) under ultrasonication for 10 seconds. The particle size, polydispersity index and zeta potential of nanosuspension were 216.6±12.4 nm, 0.124±0.013 and -13.8±1.4 mV, respectively. Nanosuspension was observed by transmission electron microscope (TEM) morphology. Raw ICT and freez-dried nanosuspension were also characterized by scanning electron microscope (SEM) morphology. Both of nanosuspension particles in the solution and in the freeze-dried form were rod shape. The X-ray diffraction (XRD) and differential scanning calorimetry (DSC) analysis showed that the characteristic peaks of ICT was maintained but with reduced peak intensity, indicating that the crystallinity of drug in nanosuspension was much lower than the raw material. Drug in nanosuspension completely dissolved within 15 min with much higher dissolution rate. After oral administration of ICT nanosuspensions and raw suspensions to rats, nanosuspension exhibited significant increased AUC_{0-36h} and C_{max} by 2.0 and 4.7 folds respectively than those of raw suspension. Meanwhile, the decreased T_{max} indicated that drug in nanosuspension could reach the peak concentration much faster than the raw suspension.

The effect of ICT on osteoblast proliferation and differentiation was examined in the culture system of MC3T3-E1 cells. ICT (10^{-9}-10^{-6} M) has no promoting proliferation effect. ICT showed statistically significant promoting differentiation effect (1.75 fold of the control group at 10^{-6} M). The serum pharmacological method was employed to investigate the effects of ICT nanosuspension and raw suspension on

proliferation and differentiation on MC3T3-E1 cell line. Two formulations, ICT nanosuspensions and raw suspensions, were orally administered to rats and blood samples were withdrawn at 10 min, 1.5 h and 24 h. The total concentrations of ICT in serum were determined after enzyme hydrolysis with β-glucuronidase/sulphatase. The serum was added into the culture system of MC3T3-E1 cells. When MC3T3-E1 cells were exposed to culture medium containing serum of 10 min and 1.5 h after administration with ICT nanosuspension, cell viability was significantly increased to (120.1±9.0) % and (116.6±9.9) % compared to the control group, respectively. The serum of 24 h after administration with ICT nanosuspension has no effect on proliferation. When MC3T3-E1 cells were exposed to culture medium containing serum of 10 min, 1.5 h and 24 h after administration with raw suspension, no effect on cell viability was observed. The culture medium containing serum of 10 min and 1.5 h after administration with ICT nanosuspension both induced increase of ALP activity (1.41-fold) compared to control group (p<0.05), serum of 24 h has no effect on ALP activity. However, the culture medium containing serum of 10 min, 1.5 h and 24 h after administration with raw suspension has no effect on ALP activity (p>0.05). These results suggested that ICT nanosuspension was superior to raw suspension on enhancing proliferation and differentiation of osteoblast.

In conclusion, the present study suggested that ICT nanosuspension increased the dissolution rate as well as the oral bioavailability of ICT. ICT has differentiation enhancing effect on osteoblasts. However, based on the result of experiment employed serum pharmacological method, the metabolites of ICT showed significant proliferation and differentiation promoting effect in vitro. ICT nanosuspension was very promising to be developed into a new formulation with anti-osteoprosis activity.

Keywords: Icaritin, Nanosuspension, Antisolvent-precipitation, Ultrasonication, Dissolution rate, Oral bioavailability, Osteoporosis

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縮寫	英文全稱	中文全稱
HPLC	High Performance Liquid Chromatography	高效液相色譜
ICT	Icaritin	淫羊藿苷元
HPMC	Hydroxypropyl methylcellulose	羥丙基甲基纖維素
F68	Poloxamer 188	泊洛沙姆 188
F127	Poloxamer 407	泊洛沙姆 407
PVP	Polyvinyl pyrrolidone	聚乙烯吡咯烷酮
PVA	Polyvinyl alcohol	聚乙烯醇
Tween 80	Polysorbate 80	吐溫 80
SDS	Sodium dodecyl sulfate	十二烷基磺酸鈉
DSC	Differential scanning calorimetry	差示掃描量熱法
TEM	Transmission electron microscope	透射電鏡
SEM	Scanning electron microscope	掃描電鏡
PXRD	X-Ray Diffraction	X 射線衍射
FTIR	Fourier transform infrared spectroscopy	傅裡葉變換紅外光譜
ALP	Alkaline phosphatase	鹼性磷酸酶