

Study on the Proapoptotic effect of Dehydrocorydaline and Celastrol

by

Xu, Zeng Tao



Institute of Chinese Medical Sciences

University of Macau



Study on the Apoptotic effect of Dehydrocorydaline and Celastrol

by

Xu, Zeng Tao

A thesis submitted in partial fulfillment of the
requirements for the degree of

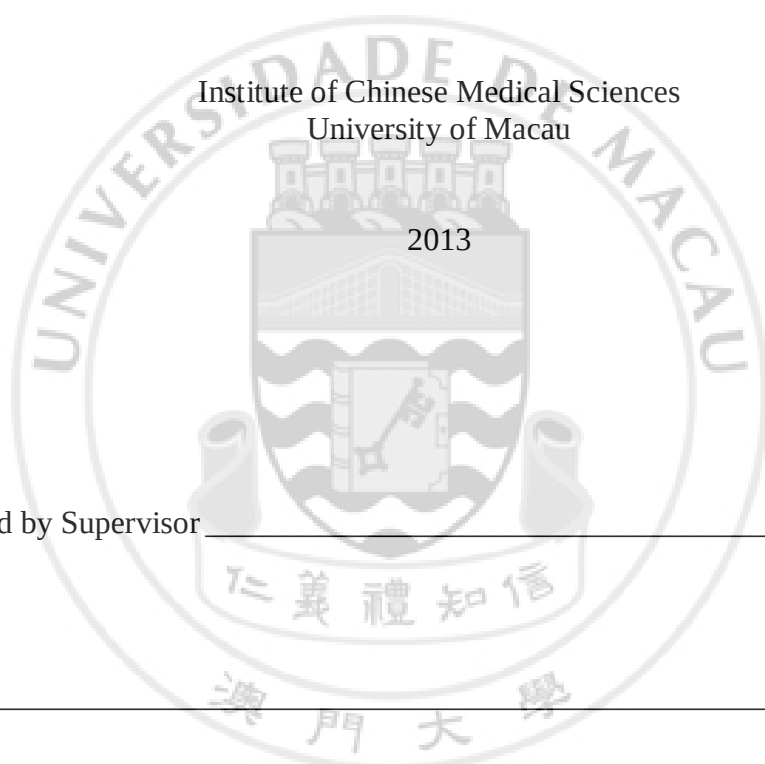
Master of Science

Institute of Chinese Medical Sciences
University of Macau

2013

Approved by Supervisor

Date



In presenting this thesis in partial fulfillment of the requirements for a Master's degree at the University of Macau, I agree that the Library and the Institute of Chinese Medical Sciences shall make its copies freely available for inspection. However, reproduction of this thesis for any purposes or by any means shall not be allowed without my written permission. Authorization is sought by contacting the author at

Address: Room 1602, Unit 1, Block 5, Qian Long Xue Fu Xiao Qu, Fu Zhou City,
P.R.China Post code: 350108

Telephone: +86-591-22861989

E-mail: xuzentao@Gmail.com



Signature _____

Date _____

碩士學位論文

去氫紫堇堍及雷公藤紅素促凋亡作用研究

研究生姓名： 徐曾濤

導 師： 王一濤教授

專 業： 中藥學

日 期： 2013.8



澳門大學中華醫藥研究院

目 錄

致謝	I
摘要	II
Abstract	IV
List of figures	VI
List of tabels	VII
縮略詞列表	iv
第一章 延胡索中去氫紫堇城誘導 MCF-7 細胞凋亡作用研究	1
1.1 研究概況	1
1.1.1 化學成份	1
1.1.2 藥理作用	1
1.1.3 臨床作用	3
1.1.4 研究目的及意義	3
1.2 實驗部份	4
1.2.1 試劑和儀器	4
1.2.2 實驗方法	5
1.2.3 結果	7
1.2.4 討論	12
1.2.5 結論	15
第二章 雷公藤紅素誘導人類風濕性關節炎纖維樣滑膜細胞凋亡的實驗研究 ...	17
2.1 研究概況	17
2.1.1 類風濕性關節炎	17
2.1.2 纖維樣滑膜細胞	18
2.1.3 雷公藤紅素	19
2.2 實驗部份	21
2.2.1 試劑和儀器	21
2.2.2 實驗方法	21
2.2.3 結果	24
2.2.4 討論	35
2.2.5 結論	37
References	38
Curriculum Vita	41

致 謝

首先感謝我的導師王一濤教授對我的關心和指導，王一濤教授以他豐富的人生閱歷和寶貴的經驗，指導我在學習中開拓思路，廣納百川，在人生的道路上積極進取，奮勇向前。王老師一絲不苟的作風，嚴謹求實的態度，踏踏實實的精神，不僅授我以文，而且教我做人，雖歷時四載，卻給以終生受益無窮之道。

感謝李紹平教授、李銘源副教授、張慶文副教授、陆金建博士對我無私的關心和幫助，特別感謝陳修平博士，對我實驗和論文寫作過程中的指導和幫助，感謝實驗室技術員Leon、Sandy、Wing、Kio、Dorian以及Joanna對我實驗的支持和幫助，感謝藥理實驗組對我的鼓勵和協助，感謝中華醫藥研究院所有關心我的老師和同學！

最後，感謝我的家人對我默默的支援！



中文摘要

爲了探索延胡索抗腫瘤作用，爲其臨床應用提供科學依據。本課題對延胡索甲醇提取物及其中活性成份去氫紫堇堯對人乳腺癌細胞 MCF-7 的促凋亡作用進行研究。實驗方法包括 MTT、Hoechst 染色、細胞週期分析、DNA Ladder、JC-1 和 Western-blot 等。結果顯示延胡索甲醇提取物和去氫紫堇堯能夠抑制 MCF-7 細胞增殖，誘導凋亡發生。延胡索甲醇提取物誘導細胞發生 G2/M 期阻滯，並明顯降低細胞線粒體膜電位；去氫紫堇堯誘導細胞發生 G1 期阻滯，對細胞線粒體膜電位影響不大。去氫紫堇堯作用 72 小時後，細胞 DNA 結構被破壞，生成斷裂的 DNA 片段，去氫紫堇堯刺激 MCF-7 細胞表達凋亡相關蛋白 caspase-7,8 及 cleaved PARP，降低 Bcl-2/BAX，而對 caspase-9 表達沒有明顯影響。

論文的第二部分爲雷公藤紅素誘導人類風濕性關節炎纖維樣滑膜細胞凋亡及 DNA 損傷的研究，旨在爲雷公藤臨床治療類風濕性關節炎提供基礎理論依據。經前期篩選，本課題以雷公藤紅素爲實驗藥物，類風濕性關節炎特徵病變細胞——纖維樣滑膜細胞爲實驗對象，採用 MTT、LDH、DAPI 染色、細胞週期分析，Annexin-V/PI 雙染、彗星電泳、免疫螢光及 western-blot 等方法，對雷公藤紅素誘導纖維樣滑膜細胞凋亡及 DNA 損傷的作用進行研究。結果顯示，濃度爲 5 μ M 雷公藤紅素即能明顯抑制滑膜細胞的增殖。藥物處理後，滑膜細胞形態明顯改變，細胞核具有凋亡特徵，核偏向一極，染色質固縮，呈高亮狀態，通過 Annexin-V/PI 雙染實驗證實，細胞發生凋亡且主要為晚期凋亡。雷公藤紅素能夠誘導滑膜細胞發生 G2/M 期阻滯，並對滑膜細胞的 DNA 造成損傷且損傷程度與藥物濃度正相關，免疫螢光實驗顯示細胞核內標誌 DNA 損傷的特徵性蛋白 γ H2AX 逐漸聚集並且含量增加。根據實驗結果，我們推測，雷公藤紅素能夠誘導滑膜細胞 DNA 損傷，使滑膜細胞阻滯在 G2 期，G2 期 DNA 修復失敗的細胞退出細胞週期，進入凋亡程式，最終抑制滑膜細胞的增殖。

關鍵字：延胡索；MCF-7；雷公藤紅素；類風濕性關節炎；滑膜細胞；凋亡；DNA 損傷



Abstract

In order to elucidate the anti-tumor effect of *Corydalis yanhusuo* W.T.Wang, a study on apoptotic effect of a *corydalis yanhusuo* W.T.Wang extract and dehydrocorydaline, a pure compound isolated from *corydalis yanhusuo* W.T.Wang, was carried out on human breast cancer cells -- MCF-7. In this study, methods including MTT, JC-1, Hoechst, Cell cycle analysis, DNA Ladder and Western blot were employed. Results showed that both the extract and dehydrocorydaline could inhibit the proliferation and induce the apoptosis of MCF-7. The extract treatment would lead to cell cycle arrest at G2 phase and the significant decreased of $\Delta\psi_m$, while the dehydrocorydaline treatment would lead to cell cycle arrest at G1 phase and no significant change of $\Delta\psi_m$. DNA structure was destructed after treated by dehydrocorydaline for 72 h and DNA fragment could be observed in DNA Ladder. The expression of caspase-7,8 and cleaved PARP were increased and the ratio of bcl-2/bax was decreased after dehydrocorydaline treatment in MCF-7.

Meanwhile, as the second part of this thesis, a study on effect of proapoptotic and DNA damage of celastrol was carried out in rheumatoid arthritis fibroblast-like synovial cells, which might play a key role in pathological development of rheumatoid arthritis. Methods including MTT, DAPI staining, Cell cycle analysis, Annexin-V/PI, comet assay, immuno- fluorescence and Western blot were employed. Results showed that the proliferation of fibroblast-like synovial cells was suppressed and the cell morphology was changed with the occurrence of condensed chromatin, shrunken, crimped and condensed gray-blue nuclei after treatment. In treatment group, the percentage of annexin V-FITC binding cells significantly increased *versus* control group. Celastrol could induced the cell cycle arrest at G1 phase and DNA damage in fibroblast-like synovial cells. In addition, the expression of γ H2AX, a marker of DNA damage, was increased. In summary, Celastrol induced the DNA

damage of FLS, which lead to G2 phase arrest, and the process of apoptosis was initiated in FLS who failed in DNA repair and retreated the cell cycle, and the proliferation of FLS was suppressed.

Key words: Corydalis yanhusuo W.T.Wang; MCF-7; Celastrol; rheumatoid arthritis; fibroblast-like synovial cells; apoptosis; DNA damage



LIST OF FIGURES

Figure 1.1 After treatment for 24 h, MCF-7 proliferation was inhibit in a dose dependent manner.	8
Figure 1.2 Dehydrocorydaline induced apoptosis.....	9
Figure 1.3 Dehydrocorydaline unchanged the $\Delta\Psi_m$ of MCF-7.....	9
Figure 1.4 DNA fragment induced by dehydrocorydaline	10
Figure 1.5 Dehydrocorydaline increased the sub-G1 region	11
Fig.1.6 Effect of Dehydrocorydaline on protein expression of Bax, Bcl-2, caspase-7, and cleaved caspase-8, caspase-9, PARP.	12
Figure 2.1 Chemical structure of celastrol.....	19
Figure 2.2 After treatment for 24 h, FLS-RA proliferation was inhibit in a dose dependent manner.....	25
Figure 2.3 After treatment for 24 h, release ratio of FLS-RA was affected by different concentrations of celastrol in a dose dependent manner.....	26
Figure 2.4 The morphological changes of FLS-RA	27
Figure 2.5 DAPI staining on FLS-RA.....	28
Figure 2.6 Cell cycle analysis. cell cycle distribution of FLS-RA (A) and the column plot of G2/G1 ratio (B).	29
Figure 2.7 Rate of apoptotic cells after 24 h treatment	30
Figure 2.8 DNA damage effect of celastrol on FLS-RA.....	31
Figure 2.9 Immunofluoresence staining of γ H2AX on FLS-RA	32
Figure 2.10 Expression of apoptosis related proteins.....	33
Figure 2.11 Expression of DNA damage and cell cycle related proteins	34

LIST OF TABELS

Table 1.1 Dehydrocorydaline effect on viability of MCF-7.....	7
Table 1.2 Expression of apoptosis related proteins	11
Table 2.1 Celastrol effect on viability of FLS-RA	25
Table 2.2 Release ratio in different concentrations of celastrol.....	26
Table 2.3 Cell cycle distribution after treatment for 24 h	28
Table 2.4 Apoptotic cells after treatment for 24 h.....	30
Table 2.5 Expression of apoptosis related proteins	32
Table 2.6 Expression of DNA damage and cell cycle related proteins	34



LIST OF ABBREVIATIONS

RA	rheumatoid arthritis
FLS	fibroblast-like synoviocytes
LDH	Lactic Dehydrogenase
PBS	Phosphate Buffer Solution
DMSO	Dimethyl sulfoxide
SDS-PAGE	PolyAcrylamide Gel Electrophoresis
Acr/Bis	acrylamide-bisacrylamide
APS	ammonium persulfate
SDS	sodium dodecyl sulfate
TEMED	N,N,N',N'-Tetramethylethylenediamine
PI	Polyisocyanate
PS	phosphatidylserine
NAD	nicotinamide adenine dinucleotide,
PARP	poly ADP-ribose poly-merase
EDTA	Ethylene Diamine Tetraacetic Acid
Tris	2-Amino-2-(hydroxymethyl)-1,3-propane-diol
SLS	Sodium lauryl ether sulfate
PI-3K	phosphatidylinositol 3-kinase
SCGE	single cell gel electrophoresis assay
MRF	maturation promoting factor
RA	rheumatoid arthritis
FLS	fibroblast-like synoviocytes
LDH	Lactic Dehydrogenase
PBS	Phosphate Buffer Solution
DMSO	Dimethyl sulfoxide
SDS-PAGE	PolyAcrylamide Gel Electrophoresis
Acr/Bis	acrylamide-bisacrylamide
APS	ammonium persulfate