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厦门大学

硕士学位论文

雷公藤的谱效关系研究

The spectrum effect relationship of *Tripterygium Wilfordii* Hook.f.

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摘 要

雷公藤的药理作用广泛，其中抗炎和免疫抑制作用尤其突出，临床多用于类风湿性关节炎、自身免疫疾病如肾病综合征等的治疗。但其不良反应不容忽视，雷公藤的不良反应涉及多器官、多系统且多数患者不能耐受。其不良反应发生的主要原因有雷公藤化学成分复杂且其药效、毒性物质基础尚不明确，缺乏科学、有效的质量评价和控制方法，导致不同厂家生产的雷公藤制剂疗效及毒副作用差异大，临床上缺乏使用指南等。因此，如何快速识别雷公藤中的药效成分和毒性成分，建立科学、有效的质量评价和控制方法，提高雷公藤制剂质量是促进雷公藤在临床安全使用的前提。虽然目前许多学者通过改良炮制方法、研究中药配伍和新剂型等以期得到雷公藤低毒高效制剂，但仍无法根本解决其毒性过大的问题。本课题以阐明雷公藤药效成分及毒性成分物质基础为出发点，通过体外药效和毒性研究，评价不同产地雷公藤的药效和毒性差异，并建立不同产地雷公藤的高效液相指纹图谱，利用统计学分析方法建立不同产地雷公藤成分与药效、毒性之间的关系，识别指纹图谱中的主要药效成分峰和毒性成分峰，从而为雷公藤制剂质量控制指标选择、质量控制方法的建立以及高效低毒制剂的研究提供指导和参考，以期解决雷公藤因其毒性限制而难以在临床广泛应用的问题。

本课题选取了产自福建、湖南、云南、广东、广西、安徽、江苏、湖北八个代表性产地的雷公藤的带皮根，提取纯化后采用高效液相色谱分析法建立指纹图谱，依指纹图谱及药效、毒性特性将其分为两大组，福建、湖南、云南为A组，该组峰号在前面缀以a，广东、广西、安徽、江苏、湖北为B组，该组峰号在前面缀以b。分别在组内选取共有峰，合并A、B组间相同峰后共得到39个色谱峰。以不同产地雷公藤对脂多糖(LPS)诱导的小鼠单核巨噬细胞系RAW264.7细胞分泌TNF-α和IL-6的半数抑制浓度IC_{50}为抗炎药效指标，利用偏最小二乘法建立谱效关系。结果以TNF-α为指标时筛选出主要药效峰为第a4、a5、a6、a7、a13、a14、a18、a20、a23、a24、a27、b2、b8、b9、b11、b17、b18号峰；以IL-6为指标时，筛选出主要药效峰为第a1、a3、a9、a12、a16、a17、a18、a21、a22、a24、a26、a28、a29、a31、b2、b9、b11号峰。

以不同产地雷公藤对脂多糖(LPS)和植物凝集素(PHA)共同诱导的人外周血单个核细胞(PBMC)分泌 IFN-γ和 IL-2 的半数抑制浓度 IC_{50} 为免疫抑制药效指标，利用偏最小二乘法建立谱效关系。结果以 IFN-γ为指标时筛选出主要药效峰为第 a2、a17、a18、a20、a23、a24、a27、a30、a31、b24 号峰；以 IL-2 为指标时，筛选出主要药效峰为第 a4、a5、a6、a7、a8、a10、a11、a13、a14、a15、a25、b11 号峰。

以不同产地雷公藤对大鼠肝细胞 BRL-3A、小鼠肾小球系膜细胞 GNM-SV40、大鼠心肌细胞 H9C2 的半数抑制浓度 IC_{50} 为雷公藤的肝、肾、心脏体外细胞毒性指标，利用偏最小二乘法建立谱效关系。由结果可知主要肾毒性峰为第 a1、a3、a12、a16、a17、a18、a19、a22、a24、a25、a26、a28、a29、a31、b24 号峰；主要心脏毒性峰为第 a2、a3、a9、a16、a17、a18、a21、a24、a26、a29、a30、a31、b14 号峰；主要肝毒性峰为第 a1、a3、a12、a16、a17、a18、a19、a22、a24、a25、a26、a28、a29、a31、b24 号峰。

比对 A、B 组共有峰的保留时间，同峰合并，得八个产地的有效有毒峰：a1、a2、a3、a9、a12、a14、a16、 a17、a18、a20、a21、a22、a24、a25、a26、a28、a29、a30、a31、b8、b9、b24 号峰；有效无毒峰：a4、a5、a6、a7、a8、a10、a11、a13、a15、a23、a27、b2、b11、b17、b18 号峰；无效有毒峰：a19、b14。

得到谱效、谱毒结合分析结果后，可以根据具体的药效目标对药效峰和毒性峰进行筛选和取舍，找到适宜目标药效的主要有效有毒峰、主要有效无毒峰、主要无效有毒峰，如在设计低毒高效部位制剂时，可以考虑剔除或控制主要有效有毒峰对应成分的含量，剔除无效有毒峰对应成分，保留有效无毒峰对应成分。本课题组后续将根据本研究结果对雷公藤提取后纯化并进行分段，并利用液相-质谱联用仪对主要有效有毒峰、有效无毒峰、无效有毒峰进行分析，以明确其化学物质基础。从而指导更为安全的雷公藤低毒高效制剂的研发以及雷公藤制剂质量标准及质量控制方法的提高，最终扩大雷公藤制剂的临床应用并降低临床应用中的不良反应，也为类似雷公藤的毒性中药的研究提供思路和方法学参考。

关键词：雷公藤；谱效结合；偏最小二乘法

Abstract

Tripterygium wilfordii shows a wide range of pharmacological effects, especially anti-inflammatory effect and immuno-suppressive effect. It is commonly used in the treatment of rheumatoid arthritis, auto-immune diseases such as nephrotic syndrome . But at the same time, the toxicity of *Tripterygium wilfordii* can not be ignored. The adverse reactions involve many organs and systems, and they are intolerant to most patients. The reason for the high incidence of adverse reactions including the complexity of the compositions of *Tripterygium wilfordii*, and their mechanisms of effect and toxicity are not clear. There also lack a scientific and effective method to evaluate and control the quality of *Tripterygium wilfordii* preparations. The effects of a single preparation made by different manufacturers can be of great difference. And clinically, there is no unified use guide. Therefore,distinguishing the effective components and toxic components, establishing a scientific and effective method to evaluate and control the quality of *Tripterygium wilfordii* preparations, then formulating a safe as well as reliable clinical use guide become the premise of the safe use of *Tripterygium wilfordii*. Currently, there are a lot of researches on its attenuated synergies, but they can hardly solve this problem radically. This issue evaluates the *in vitro* efficacy and toxicity of *Tripterygium wilfordii* grown from different places, and establishes their fingerprints by HPLC. Then analyzes the spectrum-effect relationship by statistical method and screen the effective peaks and toxic peaks. Therefore, the result can guide the preparation of *Tripterygium wilfordii* preparations with low toxicity and high effect, and establish a scientific and effective method to evaluate and control the quality of *Tripterygium wilfordii* preparations. Finally, free the toxic restriction of *Tripterygium wilfordii* and widen its usage.

This issue selects 8 batches of *Tripterygium wilfordii* growing in Fujian, Hunan, Yunnan, Guangdong, Guangxi, Anhui, Jiangsu and Hubei. After extraction and purification, the fingerprints of them are established by HPLC. According to the characteristics of fingerprint, efficacy and toxicity, they are divided into two groups, group A including Fujian, Hunan, Yunnan, the common peaks in this group are named after a. Group B including Guangdong, Guangxi, Anhui, Jiangsu, Hubei, the common peaks in this group are named after b. Select common peaks within each group

respectively, after merger same peaks in A and B there are totally 39 chromatographic peaks.

The IC_{50} values of 8 batches of *Tripterygium wilfordii* on the secretion of TNF-α and IL-6from mouse macrophage RAW264.7 cells which stimulated by LPS are used to investigate its anti-inflammatory effect of *Tripterygium wilfordii*. And then they are used to investigate the spectrum anti-inflammatory effect relationship of *Tripterygium wilfordii* with the data of the common peaks in fingerprints by partial least squares.With TNF-α as the index, No.a4, a5, a6, a7, a13, a14, a18, a20, a23, a24, a27, b2, b8, b9, b11, b17, b18 peaks are the main efficacy peaks; with IL-6 as the index, No.a1, a3, a9, a12, a16, a17, a18, a21, a22, a24, a26, a28, a29, a31, b2, b9, b11 peaks are the main efficacy peaks.

The IC_{50} values of 8 batches of *Tripterygium wilfordii* on the secretion of IFN-γ and IL-2 from human peripheral blood mononuclear cells(PBMC) which stimulated by LPS and PHA are used to investigate its immuno-suppressive effect. And then they are used to investigate the spectrum immuno-suppressive effect relationship of *Tripterygium wilfordii* with the data of the common peaks in fingerprints by partial least squares. With IFN-γ as the index, No.a2, a17, a18, a20, a23, a24, a27, a30, a31, b24 peaks are the main efficacy peaks; with IL-2 as the index, No.a4, a5, a6, a7, a8, a10, a11, a13, a14, a15, a25, b11 peaks are the primary efficacy peaks.

The IC_{50} values of 8 batches of *Tripterygium wilfordii* on the growth of rat liver cells BRL-3A, mouse mesangial cells GNM-SV40, and rat myocardial cells H9C2 are used to evaluate the toxicological effect of *Tripterygium wilfordii* on liver, kidney and heart respectively. And then they are used to investigate the spectrum toxicological effect relationship of *Tripterygium wilfordii* with the data of the common peaks in fingerprints by partial least squares. On the liver toxicity:No.a1, a3, a12, a16, a17, a18, a19, a22, a24, a25, a26, a28, a29, a31, b24 peaks are the primary toxic peaks; on the renal toxicity: No. a1, a3, a12, a16, a17, a18, a19, a22, a24, a25, a26, a28, a29, a31, b24 peaks are the primary efficacy peaks; on the cardiac toxicity: No.a2, a3, a9, a16, a17, a18, a21, a24, a26, a29, a30, a31, b14 peaks are the primary

efficacy peaks.

Refer to the retention time of common peaks in A, B group, and take the analysis results of the relationship between efficacy and toxicity together, we get the effective as well as toxic peaks are No.a1, a2, a3, a9, a12, a14, a16, a17, a18, a20, a21, a22, a24, a25, a26, a28, a29, a30, a31, b8, b9, b24 peaks; effective but non-toxic peaks are: No.a4, a5, a6, a7, a8, a10, a11, a13, a15, a23, a27, b2, b11, b17, b18 peaks; invalid as well as toxic peaks are No.a19, b14 peaks.

After getting the result of spectrum-effect and spectrum-toxicity relationship, we can select corresponding peaks according to the concrete target. Such as in the design of high-efficient and low-toxic preparation, we can consider to eliminate or control the content of ingredients which are effective as well as toxic, eliminate invalid but toxic ingredients, keep the effective with non-toxic components. Our research group will extract, purify and segment *Tripterygium wilfordii* according to this results, and use the liquid-mass spectrometry instrument to analyze these components, in order to make clear its chemical basis. Provide guidance for the research and development of *Tripterygium wilfordii* preparations with high-efficient and low-toxic. Help to enhance the quality standards and quality control methods of *Tripterygium wilfordii* preparations. Eventually expand the clinical application of *Tripterygium wilfordii* and reduce adverse reactions in clinical application, also provide references and methodology for similar traditional Chinese medicine research.

Key Words: *Tripterygium wilfordii*; spectrum-effect relationship; partial least squares

目 录

Table of Contents

缩 略 词

英文名称	中文名称
NF-κB	核因子-κB
IC_{50}	半数抑制浓度
PGE-2	前列腺素-E2
LPS	脂多糖
IL-1	白细胞介素-1
COX-2	环氧化物水解酶-2
TLR	Toll 样受体
ERK	细胞外调节蛋白激酶
IFN-γ	干扰素-γ
TNF-α	肿瘤坏死因子-α
PHA	植物血凝素
NO	一氧化氮
iNOS	诱导型一氧化氮合酶
HPLC	高效液相色谱法
PLSR	偏最小二乘回归法
β-actin	β-肌动蛋白
IL-2R	白介素-2 受体
ELISA	酶联免疫吸附法
JNK	c-Jun 氨基末端激酶
IκBα	核因子-κB 抑制因子-α
TRIF	Toll 样受体信号分子
MyD88	髓样分化因子 88
MAPK，p38	丝裂原活化蛋白激酶（家族成员 p38）

前 言

雷公藤为卫矛科雷公藤属植物雷公藤(*Tripterygium wilfordii* Hook.f.)的干燥根，在古籍《本草纲目拾遗》、《草药方》、《植物名实图考》等中均有记载，为著名的传统中药（TCM，Traditional Chinese Medicine）。“雷公”为神话中的司雷之神，雷公藤借以为名，以喻其暴烈之性。其药性凉，味苦涩、辛，有大毒，毒烈散泄，归肾、肝二经。其功效为祛风除湿，消肿止痛，活血通络，杀虫解毒。主要用于类风湿及风湿性关节炎、肾脏疾病、系统性红斑狼疮等炎症性和自身免疫性疾病的治疗。近十几年的研究发现雷公藤尚有抗癌、抗生育、抗神经系统退行性病变等药理作用。然而，雷公藤的毒性限制了其临床应用，2012 年 4 月，国家食品药品监督管理总局发布通报：雷公藤制剂有严重且高发的心、肝、肾、骨髓、血液及生殖系统毒性，呼吁广大医药工作者关注并开展相关减毒增效研究。雷公藤所含化学成分多达三百余种，这是其具有如此广泛药理作用和毒性的物质基础，但同时也给阐明其药效和毒性物质基础及作用机制带来了挑战。本课题旨在通过雷公藤药材提取物的“谱-效”关系和“谱-毒”关系研究，利用统计学分析方法确定雷公藤指纹图谱中的药效色谱峰和毒性色谱峰，为后续指导雷公藤制剂质量控制指标选择、质量控制方法建立以及减毒工艺研究提供研究基础，进而解决雷公藤受毒性限制而难以在临床广泛应用的问题。首先对雷公藤的研究进展进行综述。

1 雷公藤的主要化学成分

雷公藤所含化学成分多样复杂，包括二萜类、倍半萜类、三萜类、生物碱类、黄酮类、糖类、甾体类成分等，其中以二萜、三萜及生物碱成分为主。按母核类型分，倍半萜类主要有二氢沉香呋喃倍半萜；生物碱类主要有 wilfordate type，evoninate type, isoevoninate type, isowilfordate type；二萜类主要有三环氧型二萜、山海棠素型二萜、雷酚萜型二萜、山海棠酸型二萜、醌式二萜、贝壳杉烷型二萜；三萜类主要有齐墩果烷型三萜、乌苏烷型三萜、木栓烷型三萜等。