Pharmacokinetic study of astragaloside IV, a saponin marker of Astragali Radix, and its intestinal bacterial metabolites in the rat

By

Ruina Zhou

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PHARMACOKINETIC STUDY OF ASTRAGALOSIDE IV, A SAPONIN MARKER OF ASTRAGALI RADIX, AND ITS INTESTINAL BACTERIAL METABOLITES IN THE RAT

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Ruina Zhou

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Approved by _____

Supervisor

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Post code: 457001
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碩士學位論文

中藥黃芪皂苷類成分黃芪甲苷及其腸道菌代謝產物的藥代動力學研究

研究生姓名：　周瑞娜

導　　師：　燕　茹 博士

專　　業：　中藥學

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澳門大學中華醫藥研究院

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University of Macau

Abstract

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by

Ruina Zhou

Supervisor: Dr. Ru Yan

Chinese Medicinal Sciences

Keywords: Astragali Radix; astragaloside IV; cycloastragenol; intestinal bacteria;; pharmacokinetics; metabolism; regio-selectivity.

As a traditional Chinese herbal medicine, Astragali Radix is widely used in clinic as well as function food nowadays in China and other Asian countries. Astragaloside IV (AIV), of which the pharmacology reports are commonly seen, is the most abundant saponin among all astragalosides, and chosen to be a marker compound in Astragali Radix. It is well accepted intestinal bacteria plays an important role in determining the absorption and systemic exposures of most saponin glycosides after oral intake. The aglycone of AIV, cycloastragenol, was reported with a moderate telomerase-increasing activity and showed perspective in the treatment of degenerating diseases and immunodeficiency diseases, which is consistent with the traditional use of Astragali Radix. For these reasons, we hypothesize that CA might be the active form after the oral administration of AIV. In this study, the intestinal bacterial metabolism of AIV and the *in vivo* pharmacokinetic profiles of AIV and its

intestinal bacterial metabolites including CA were characterized for the first time.

For investigation on intestinal metabolism and disposition of AIV and CA, intestinal bacteria metabolism model and *in vitro* hepatic subcellular fractions metabolism model were employed in the present study. When incubated anaerobically with rat intestinal bacteria, AIV generated five metabolites with three (monoglycosides brachyoside B and cyclogaleginoside B, the aglycone cycloastragenol (CA)) via stepwise deglycosylation and two from further isomerization (3-*epi*- cycloastragenol , CA-iso) and dehydrogenation (CA-2H) of CA. For the further *in vivo* study of AIV and CA, the plasma concentration of the parent compounds and according metabolites were determined on S.D. rat model. When AIV was orally administered at 40 mg/kg to the rat, the intestinal metabolites CA and CA-iso presented as main components in the plasma following AIV and their $AUC_{0-\infty}$ values were 88.60±9.66 (CA), 179.06± 28.53 (CA-iso) and 452.28 ± 43.33 nM·h (AIV). AIV kept intact in rat liver microsomes, while CA underwent a certain extent phase I metabolism and produced several products via oxidation. CA-2H was a predominant form in feces but not detected in urine and plasma. This agreed well with rapid hepatic metabolism of CA-2H to form CA and CA-iso and reversible conversions between CA-2H and CA/CA-iso by intestinal bacteria. Very interestingly, CA-iso was also detected in rat plasma after intravenous injection of CA (2.5 mg/kg). The AUC_{0-t} of CA-iso was 166.10±36.70 nM·h, accounting for about 24% of that of the parent compound (688.32±73.38 nM·h). When CA was dosed orally at 25 mg/kg, both CA and its isomer existed in rat plasma with AUC_{0-t} of 1241.17±141.64 nM·h and 628.55±84.88 nM·h respectively. The systemic exposure of CA-iso was about half (53%) that of CA. Contrasting to the results of hepatic metabolism *in vitro*, no other phase I or II metabolites were detected in the plasma after the intravenous or oral administration of CA, except tiny amount of CA-2H. Moreover, a positional preference of intestinal bacteria-mediated deglycosylation at the C-3 position rather than the C-6 position of saponin compounds was evidenced in a preliminary structure-metabolism relationship study with AIV and several other compounds.

Both *in vivo* and *in vitro* data obtained in the present study support a crucial role of gut bacterial conversion of AIV in traditional application of Astragali herb and warrant further investigational emphasis on AIV metabolites formed by gut bacteria.

澳門大學

摘要

中藥黃芪皂苷類成分黃芪甲苷及其腸道菌代謝產物的藥代動力學研究

周瑞娜

指導老師: 燕茹 博士

中藥學

關鍵字：黃芪，黃芪甲苷， 環黃芪醇，腸道細菌代謝，肝微粒體，脫氫，異構化，藥代動力學， 位點選擇性。

傳統中藥黃芪，具有具有補氣固表、利水退腫、托毒排膿、生肌等功，如今廣泛用於臨床和食品。黃芪的皂苷類成分，被確認為一類重要的活性物質， 黃芪甲苷（astragaloside IV, AIV）作為代表，更是成為了中國藥典規定的黃芪藥材的質控指標， 並同時在臨床上有多種應用。眾所周知，皂苷類成分口服後會經歷脫糖等腸道系統代謝反應，代謝產物往往能同時被吸收發揮效力。黃芪甲苷的苷元——環黃芪醇（cycloastragenol, CA），近年來被揭示出可以提高端粒酶活性，在促癒合、治療退行性疾病、抗衰老和免疫疾病方面具有廣闊前景，雖在藥材中含量極低，卻又與中藥黃芪的傳統應用相吻合。因此，環黃芪醇是否同時是黃芪甲苷及其他黃芪皂苷口服後在體內的起效物質需要驗證。同時環黃芪醇自身的藥代動力學也非常缺乏相關報導。本研究旨在通過結合體外模型和整體動物研究，揭示以黃芪甲苷為代表的黃芪皂苷類成分在體內的腸道代謝，原

藥和代謝產物在體內的藥代動力學行為和機制。

本研究利用體外腸道細菌孵育模型系統研究了黃芪甲苷的腸道細菌代謝路徑。黃芪甲苷與大鼠或人的腸道系統共孵育時，共檢測並鑒定出五種代謝產物，其中三個為黃芪甲苷脫糖分別脫糖的產物，兩個為環黃芪醇脫氫產物（CA-2H）或 3 位羥基差向異構化產物（CA-iso）。在此基礎上利用體外肝代謝模型推測原藥和各個腸道菌代謝產物在體內的代謝情況。在體外肝代謝研究中，AIV 未發現明顯的一相或二相反應，CA 則代謝生成大量氧化產物，CA-2H 可被代謝生成 CA 和 CA-iso。

SD 大鼠口服 AIV（40mg/kg）後，除原藥外血漿內同時檢到 CA 及 CA-iso，二者 AUC 分別為 88.60±9.66 nM·h 和 179.06±28.53nM·h, AUC 之和達到了原藥 AIV 的 AUC（452.28±43.33 nM·h）的 59%。尿液中只檢測到原藥 AIV，未檢測到其他各代謝產物。體外腸道細菌孵育模型中檢出的五個代謝產物在糞便裡均有檢出。

CA 單獨靜注 SD 大鼠（2.5mg/kg）後， CA-iso 作為主要的代謝產物出現在大鼠血漿中，其 AUC_{0-t}（166.10±36.70 nM·h）達到了原藥 CA 的 AUC_{0-t}（688.32 ±73.38 nM·h）的 24%。而大鼠口服 CA（25 mg/kg）後，血漿中主要的藥物存在形式依然為 CA（AUC_{0-t}=1241.17±141.64 nM·h）與 CA-iso(AUC_{0-t}=628.55±84.88 nM·h)。除 CA-iso 和 CA-2H（極少量）外，未有其他的一相和二相代謝產物在靜注組或口服組的血漿或尿液中檢出。

此外通過對體外人腸內菌系統的考察得到了孵育過程中細菌數量的變化趨勢，並證實了腸道細菌在對於四環三萜類皂苷成分的代謝上存在位點選擇性。

通過本研究可以證實，腸道細菌代謝在黃芪皂苷的口服藥動學中發揮了至關

重要的作用。腸道菌代謝產物與原藥一起進入體內歷經複雜的分佈代謝排泄過程，並共同構成體內的起效形式。而口服 CA 或 AIV 後，CA-iso 在體內較高的系統暴露量，則是由腸道細菌代謝，體內肝代謝，及 CA-iso 自身較差的腎排泄共同造成。本研究對黃芪皂苷的藥代動力學研究，生物標誌物的選擇，日後黃芪皂苷的藥理學研究方向和環黃芪醇的臨床應用均提供了指導和參考。

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LIST OF ABBREVIATIONS

AIV	astragaloside IV
AIV-xyl	brachyoside B
AIV-glc	cyclogaleginoside B
CA	cycloastragenol
CA-iso	isomer of CA, 3-*epi*-cycloastragenol;
CA-2H	dehydrogenated product of CA, 20R,24S-epoxy-6α,16β,25-trihydroxy-9,19-cycloartan-3-one
HPLC	High performance liquid chromatography
IS	Internal standard
RLM	Rat liver microsome
P_{app}	Apparent permeability coefficient