

**Pharmacokinetic Characterization of Calycosin and
Calycosin-7-O- β -D-glucoside**

by

Ruan, Jianqing



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**Institute of Chinese Medical Sciences
University of Macau**

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University of Macau

Abstract

**PHARMACOKINETIC CHARACTERIZATION OF CALYCOSIN AND
CALYCOSIN-7-O- β -D-GLUCOSIDE**

by

Ruan, Jianqing

Supervisor: Dr. Yan, Ru

Chinese Medicinal Sciences

Keywords: calycosin; calycosin-7-O- β -D-glucoside; pharmacokinetics; Caco-2 cell monolayer; intestinal bacteria; absorption; metabolism; glucuronidation

Background: Calycosin and calycosin-7-O- β -D-glucoside (Cal-7-Glu) are two main bioactive flavonoid components of Radix Astragali, which has been widely utilized to reinforce vital energy, strengthen immune function and promote refreshment of flesh in China for centuries. Although pharmacological effects of calycosin and Cal-7-Glu have been reported, only a few studies reported pharmacokinetic properties of calycosin and Cal-7-Glu. A low oral bioavailability was evidenced and some metabolites, including calycosin and calycosin derivatives, were tentatively identified in rat plasma when Cal-7-Glu was taken orally, indicating formation and further biotransformation of calycosin which might play a role in *in vivo* actions of Cal-7-Glu. However, the information on pharmacokinetics of Cal-7-Glu and calycosin is very limited. There is no *in vivo* study of calycosin and mechanistic studies on absorption and metabolism of Cal-7-Glu and calycosin are lack. Therefore, in the present study, the *in vivo* fates of Cal-7-Glu and calycosin after oral intake were characterized in rats.

and the determinant factors were elaborated using *in vitro* models.

Methods:

For investigation on intestinal absorption, metabolism and disposition of calycosin and Cal-7-Glu, intestinal bacteria metabolism model, human Caco-2 cell monolayer model, and *in vitro* hepatic subcellular fractions metabolism model were employed in the present study. For the further *in vivo* study of calycosin and Cal-7-Glu, the plasma concentration of the parent compound and according metabolites were determined using male Sprague-Dawley rats.

Results: Cal-7-Glu was rapidly metabolized into calycosin, when Cal-7-Glu was incubated with rat intestinal bacteria, suggesting the low oral bioavailability of Cal-7-Glu might be due to the extensive metabolism in the intestinal tract, whereas no metabolism occurred when calycosin was incubated with rat intestinal bacteria. The transport study demonstrated that both Cal-7-Glu and calycosin exhibited high P_{app} value indicating their potential good absorption. In addition, calycosin underwent extensive metabolism including glucuronidation and sulfation in human liver microsome and S9 fractions, respectively. Glucuronidation was the most significant metabolism of calycosin with an intrinsic clearance of 480 $\mu\text{L}/\text{min}/\text{mg}$ protein. Further enzyme kinetics and reaction phenotyping studies using human recombinant glucuronosyltransferases (UGT) isozymes showed that UGT1A1 was the main isozyme for calycosin 7-O-glucuronide formation and 1A9 was the main isozyme responsible for calycosin 3'-O-glucuronide formation. *In vivo* study also demonstrated that when calycosin or Cal-7-Glu was orally taken by rats, both calycosin and Cal-7-Glu were not detectable in plasma and the main drug-related forms in the plasma were calycosin glucuronide and calycosin sulfate.

Conclusion: Cal-7-Glu underwent rapid and extensive hydrolysis by intestinal bacteria to produce calycosin. Both Cal-7-Glu and calycosin showed good permeability across Caco-2 cell monolayers. Thereafter, calycosin was subjected to

extensive hepatic glucuronidation which was mainly catalyzed by UGT1A1 and UGT1A9, and sulfation. When taken orally, calycosin and Cal-7-Glu per se were not detectable and glucuronidated and sulfated calycosin were main drug related forms in rat plasma in both cases.



澳門大學

摘要

毛蕊異黃酮及毛蕊異黃酮-7-氧- β -D-葡萄糖苷的藥代動力學研究

阮建清

指導老師：燕茹 博士

中藥學

關鍵詞：毛蕊異黃酮；毛蕊異黃酮-7-氧- β -D-葡萄糖苷；藥代動力學；Caco-2 單層細胞吸收模型；腸內菌；吸收；代謝；葡萄糖醛酸化

背景：黃芪作為中國著名的傳統中藥，用於補中益氣，提高免疫力，托瘡排膿有著悠久的歷史。毛蕊異黃酮及毛蕊異黃酮-7-氧- β -D-葡萄糖苷是黃芪中主要的生物活性黃酮成分，其多種藥理作用已有報導。但是有關於藥代動力學的研究報導較少。有研究發現毛蕊異黃酮-7-氧- β -D-葡萄糖苷的口服生物利用度非常低，且在大鼠體內檢測到了它的代謝產物。目前關於毛蕊異黃酮及毛蕊異黃酮-7-氧- β -D-葡萄糖苷的吸收、代謝的機理研究未見報導。本研究旨在通過結合體外模型和整體研究，揭示毛蕊異黃酮及毛蕊異黃酮-7-氧- β -D-葡萄糖苷經口服後的體內過程及其吸收、代謝、清除的分子機理。

方法：本研究利用腸內菌代謝模型、Caco-2 單層細胞模型和體外肝代謝模型探索毛蕊異黃酮及毛蕊異黃酮-7-氧- β -D-葡萄糖苷的吸收、代謝的機理。並且對

SD 大鼠血漿中的毛蕊異黃酮、毛蕊異黃酮-7-氧- β -D-葡萄糖苷的原藥以及代謝產物進行測定，研究其體內的存在形式。

結果：當毛蕊異黃酮-7-氧- β -D-葡萄糖苷與老鼠的腸內菌共同孵育時，毛蕊異黃酮-7-氧- β -D-葡萄糖苷被迅速代謝為毛蕊異黃酮，表明腸內菌代謝可能是毛蕊異黃酮-7-氧- β -D-葡萄糖苷口服生物利用度低的原因。毛蕊異黃酮-7-氧- β -D-葡萄糖苷、毛蕊異黃酮在 Caco-2 單層細胞模型均表現出較好的透過能力。另一方面，毛蕊異黃酮在肝微粒體和肝 S9 組分中會進一步發生較強的葡萄糖醛酸化和磺酸化，其中葡萄糖醛酸化最為強烈，固有清除率達到 480 $\mu\text{L}/\text{min}/\text{mg}$ 。進一步的酶動力學研究表明，葡萄糖醛酸基轉移酶 1A1 主要參與了毛蕊異黃酮在 7 位的葡萄糖醛酸化，而 1A9 主要介導了毛蕊異黃酮 3' 位的葡萄糖醛酸化。體內研究進一步證實，給予 SD 大鼠口服毛蕊異黃酮或毛蕊異黃酮-7-氧- β -D-葡萄糖苷後，血漿中均檢測不到原型藥物，而毛蕊異黃酮的葡萄糖醛酸化和磺酸化產物為主要檢出成分。

結論：毛蕊異黃酮-7-氧- β -D-葡萄糖苷在腸內菌群作用下迅速水解，代謝為毛蕊異黃酮。毛蕊異黃酮-7-氧- β -D-葡萄糖苷和毛蕊異黃酮在 Caco-2 細胞單層模型上均表現出較好的跨膜吸收能力。隨後，毛蕊異黃酮在肝臟中發生以葡萄糖醛酸化和磺酸化為主的 II 相代謝，參與葡萄糖醛酸化的主要酶亞型為 UGT1A9 和 1A1。當口服給予大鼠毛蕊異黃酮和毛蕊異黃酮-7-氧- β -D-葡萄糖苷時，在血漿中均檢測到毛蕊異黃酮葡萄糖醛酸及磺酸衍生物，而檢測不到原形藥物的存在。