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***In Vitro* characterization of Metabolism and Permeability of
Mulberroside A and Its Aglycone Oxyresveratrol**

By

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桑皮苷 A 及其苷元氧化白藜芦醇代謝和吸收 的體外研究

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Abstract

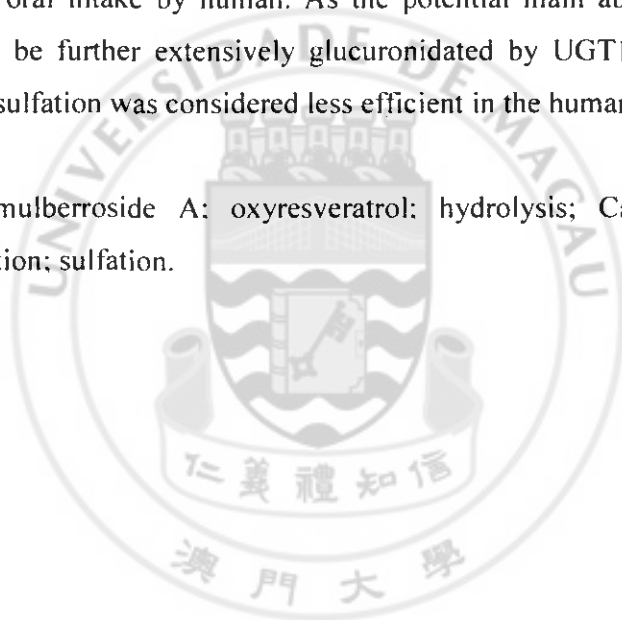
Background Cortex Mori, the root bark of *Morus alba* L., has been used extensively in traditional Chinese medicine as an antitussive and diuretic agent. Mulberroside A (Mula) is one of the major constituents in the water extract of the herb and showed various therapy-related effects. *In vivo* pharmacokinetic studies revealed an extremely low oral bioavailability (<1%) of Mula in the rat and oxyresveratrol (OXY) presented as a principal drug-related component in the plasma. However, its observed low oral bioavailability and the *in vivo* fate of OXY in human are not clearly understood. In the present study, we firstly examined the probable changes of Mula by performing the incubation of Mula together with intestinal bacteria *in vitro*, and compared the Mula biotransformation by intestinal bacteria from human with that from rat, and determined the intestinal bidirectional transport of Mula and the resultant oxyresveratrol (OXY) using *in vitro* Caco-2 monolayers. Moreover we investigated the metabolic stability of Mula and OXY in human liver for the first time, then focused on glucuronidation of OXY. Qualitative and quantitative analyses were performed by HPLC-DAD and HPLC-MS/MS.

Results When incubated anaerobically with intestinal bacteria, Mula decreased rapidly to generate two monoglucosides of OXY and OXY sequentially. Insignificant species difference was observed between human and rat. Transport study of Mula and resultant OXY revealed an ascending permeability order with deglycosylation. Moreover, Mula remained intact in human liver subcellular fractions within 1 h, while OXY underwent extensive glucuronidation (average velocity of OXY elimination: 7.24 $\mu\text{M}/\text{min}/\text{mg}$ protein), and moderate sulfation (average velocity of OXY elimination: 0.41 $\mu\text{M}/\text{min}/\text{mg}$ protein). HPLC-MS/MS analyses revealed 4 mono-glucuronidated (G1-G4) and 1 mono-sulfated (S1) metabolites of OXY. One glucuronidation metabolite named G4 was the primary glucuronidated metabolite with a transformation ratio (calculated based on 1:1 stoichiometric conversion) of 62.8%. And the kinetic studies showed that the formation of G4 followed substrate

inhibition kinetics with the apparent K_m value of $10.99 \mu\text{M}$. Further investigation of glucuronidation activity of recombinant UGT isozymes revealed that UGT1A9 exhibited the highest capacity of OXY glucuronidation, whereas UGT1A1 showed the highest affinity to OXY. UGT1A7, 1A8 and 1A10 also contributed but to a much less extent. No phase I reaction was observed for OXY. In addition, coincubation with propofol, a UGT1A9-specific inhibitor, could abolish G4 formation ($IC_{50} = 63.76 \mu\text{M}$).

Conclusions Mula might undergo a rapid hydrolysis by bacteria existing in the gut lumen after oral intake by human. As the potential main absorbed form of Mula, OXY might be further extensively glucuronidated by UGT1A9 when entering the liver. OXY sulfation was considered less efficient in the human liver.

Keywords mulberroside A; oxyresveratrol; hydrolysis; Caco-2 cell monolayer; glucuronidation; sulfation.



摘要

背景：桑白皮 (Cortex Mori) 系桑科植物桑 *Morus alba* L. 除去栓皮后的干燥根皮，具有泻肺平喘、利尿消肿功能，在中医临床上具有广泛的应用。桑皮苷 A 作为其水提物中的主要成分之一，表现出与桑白皮治疗作用相关的药理活性。体内研究表明大鼠口服桑皮苷 A 后，其生物利用度不到百分之一，并且在大鼠血浆、胆汁、尿液中氧化白藜芦醇及其二相结合产物为主要检出成分，提示氧化白藜芦醇可能是桑皮苷 A 在体内发挥作用的主要效应成分之一。然而，对于桑皮苷 A 进入体内后的转化过程及造成其极低口服生物利用度的原因目前尚无研究报导。本研究针对可能影响桑皮苷 A 体内过程的吸收和代谢环节进行研究，考察了大鼠和人肠内菌对桑皮苷 A 的转化，并利用 Caco-2 单层细胞模型比较桑皮苷 A 及其肠内菌水解主要产物氧化白藜芦醇双向透过特性，最后，采用亚细胞组分温孵体系研究了桑皮苷 A 和苷元氧化白藜芦醇在肝脏中的代谢稳定性，并对氧化白藜芦醇在肝脏的葡萄糖醛酸结合机理进行了深入研究。所有样品采用 HPLC-DAD 进行定量分析，并用 HPLC-MS/MS 对代谢产物进行定性分析。

结果：当与人或大鼠肠内菌共同孵育时，体系中桑皮苷 A 发生迅速消除，顺次产生三个代谢产物，其中两个是桑皮苷 A 分别脱去一个葡萄糖的代谢产物，另一个则是其苷元氧化白藜芦醇，为主要代谢产物；桑皮苷 A 在人和大鼠肠内菌群的转化没有明显种属差异。对比桑皮苷 A 和氧化白藜芦醇在 Caco-2 单层细胞模型上的透过性，发现氧化白藜芦醇具有更强的透过能力，其表观透过系数 P_{app} 值约为桑皮苷 A 的 10 倍；此外，桑皮苷 A 主要通过被动扩散方式跨膜转运，而氧化白藜芦醇的转运过程表现出外排现象，提示外排转运蛋白可能参与了氧化白藜芦醇的吸收过程。肝脏代谢稳定性的研究发现，桑皮苷 A 在培养的一小时内保持稳定，氧化白藜芦醇没有发生一相代谢，但发生了广泛的二项结合反应，包括显著的葡萄糖醛酸化（一小时内氧化白藜芦醇平均清除速率：7.24 $\mu\text{M}/\text{min}/\text{mg}$ protein）和相对低程度的磺酸化（一小时内氧化白藜芦醇平均清除速率：0.41 $\mu\text{M}/\text{min}/\text{mg}$ protein），生成四个单葡萄糖醛酸化产物（少量 G1, G2, G3, 主要 G4）

和一个单磺酸化产物。酶促反应动力学研究表明人肝微粒体催化氧化白藜芦醇生成 G4 的反应符合底物抑制反应类型(K_m : 10.99 μ M, K_i : 249.6 μ M, Relative V_{max} : 3383.3 \pm 161.0 peak area/min/mg protein)；进一步对人葡萄糖醛酸转移酶 (UGT) 亚型进行筛选, 发现人 UGT1A9 的 G4 生成活性最高, 而 1A1 的亲合力最强; UGT1A7 和 1A8 也具有一定催化 G4 产生的活性。UGT1A9 的特异抑制剂 propofol 能够阻断 G4 的产生(IC_{50} = 69.91 μ M), 而 UGT1A1 的特异性抑制剂 bilirubin 最多只能抑制约 20% 的 G4 产生。

结论: 以上结果提示, 桑皮苷 A 自身难以在肠道吸收, 且在肠内菌群迅速发生水解, 这是造成其低的口服生物利用度的主要原因。而作为可能真正吸收进入体内发挥药理作用的苷元氧化白藜芦醇, 进入肝脏后可能发生显著首过代谢, 进行广泛的二相结合, 尤其是葡萄糖醛酸化反应。

关键词: 桑皮苷 A; 氧化白藜芦醇; 水解; Caco-2 单层细胞膜模型; 葡萄糖醛酸化; 磺酸化。