Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Metabolism of *Epimedium*-derived Flavonoid Glycosides in Intestinal Flora of Rabbits and Its Inhibition by Gluconolactone

YAO Zhi-Hong1, 2*, LIU Ming-Yan1Δ, DAI Yi1, 2, ZHANG Yi1, QIN Zi-Fei1, TU Feng-Juan3, YAO Xin-Sheng1, 2, 3

1College of Pharmacy, Jinan University, Guangzhou 510632, China; 2Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Guangzhou 510632, China; 3College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China

Available online 20 Nov. 2011

[ABSTRACT] AIM: The metabolism of *Epimedium*-derived flavonoid glycosides (EF, with icariin as the main component) in rabbit intestinal flora and its inhibition by gluconolactone were investigated in this paper to help reveal the metabolic pathway of EF in rabbits and to identify the *in vivo* bioactive components of EF in the prevention of steroid-associated osteonecrosis. METHODS: EF were incubated at 37 °C anaerobically with rabbit intestinal flora, and then water-saturated ethyl acetate was used for sample extraction at different time points. Furthermore, gluconolactone was added at different concentrations (8, 12 and 16 mg·mL⁻¹) to study its inhibition of the metabolism of EF in rabbit intestinal flora. The separation was performed on a ODS column by gradient elution with acetonitrile-water (including 0.1% formic acid respectively) as mobile phase at detection wavelength of 335 nm. RESULTS: EF were metabolized to icariside II in 2 h, and then to icaritin when incubated for 8 h; gluconolactone showed the inhibition of EF metabolism in rabbit intestinal flora in a concentration-dependent manner. CONCLUSION: EF were found to be metabolized rapidly by hydrolysis of rabbit intestinal flora to produce icariside II and icaritin; and the total inhibition was achieved by gluconolactone at a concentration of 16 mg·mL⁻¹.

[KEY WORDS] Epimedium-derived flavonoid glycosides; Prevention of steroid-associated osteonecrosis; Metabolism in rabbit intestinal flora; Gluconolactone; Inhibition

[CLC Number] R965 [Document code] A [Article ID] 1672-3651(2011)06-0461-05

1 Introduction

Traditionally, *Herba Epimedii* has been used as Chinese herbal medicine for invigorating the kidney, strengthening sinew and bone, dispelling wind and eliminating dampness [1]. Modern pharmacological research indicated that icariin was beneficial for vasodilatation [2], immunoregulation [3] and anti-osteoporosis [4]. Recent studies also showed that oral administration of *Epimedium*-derived flavonoid glycosides (EF, with icariin as the main component) could exert beneficial effect on the prevention of steroid-associated osteonecrosis in rabbit model with inhibition of both intravascular-thrombosis and extravascular-lipid-deposition [5]. Further studies *in vitro* showed that icaritin, but not EF, displayed direct effects on the protection of vein endothelial cell and inhibition of lipid deposition in a dose-dependent manner [6]. These suggested that EF might be used as prodrug and exert effects by being metabolized to their aglycone with *in vivo* bioactivity of preventing anti-steroid-associated osteonecrosis.

Since intestinal flora can hydrolyze glycoside drugs [7-8], and the metabolism of orally administered glycoside drugs in intestinal flora may affect their bioavailability and *in vivo* bioactivities, it is necessary to study the metabolism of EF in intestinal flora. Some investigation on the metabolism of
icariin in intestinal flora of rats \cite{9} and humans \cite{10} had been carried out. However, the metabolism of EF in rabbit intestinal flora is still difficult to be estimated because of the differences of metabolic abilities of intestinal flora among different animal species. Therefore, this paper marks the first time that the metabolism of EF in intestinal flora of rabbits is studied.

Glucuronolactone is a type of specific inhibitor of \(\beta\)-glycosidase, which has been extensively used for limiting the hydrolysis of flavonoid glycosides \cite{11}. Therefore, the inhibition of the metabolism of EF in rabbit intestinal flora by glucuronolactone at different concentrations was also investigated in this paper.

The present work might help to reveal the metabolic pathway of EF in rabbits as well as the \textit{in vivo} bioactive components of EF in the prevention of steroid-associated osteonecrosis.

2 Materials

2.1 Drugs and chemicals

Epimedium-derived flavonoid glycosides (purity of icariin \(\geq 83\%), epimedeside A \(\leq 1.65\%), hexandrase F< 0.91\%, epimedin A< 1.18\%, epimedin B< 1.52\%, epimedin C< 4.1\% and icariside II< 1.84\%) were provided by Beijing TongRenTang Health Pharmaceutical Co., Ltd. (Beijing, China). Icarin (purity\(\geq 98\%\)) and icariside II (purity\(\geq 98\%\)) were purchased from Shanghai Winherb Medical Science Co., Ltd. (Shanghai, China). Icariside I (purity\(\geq 98\%\)) was purchased from Tianjin Fjord Natural Product R&D Co., Ltd. (Tianjin, China). Desmethylicaritin (purity\(\geq 90\%\)) was self-made by our laboratory. Glucuronolactone (purity\(\geq 99\%\)) was purchased from Sigma (St. Louis, Mo, USA). Acetonitrile was of HPLC grade and purchased from Acros Organics (Morristown, NJ, USA). Acetonitrile (purity\(\geq 99\%\)) was self-made by our laboratory. Glucuronolactone (purity\(\geq 99\%\)) was purchased from Sigma (St. Louis, Mo, USA). Acetonitrile was of HPLC grade and purchased from Acros Organics (Morristown, NJ, USA). Acetonitrile (purity\(\geq 99\%\)) was self-made by our laboratory.

2.2 Equipments

An Agilent 1200 HPLC system consisted of a degasser (G1322A), a quaternary pump (G1311A), an autosampler (G1329A), a thermostat column compartment (G2316A) and a multiple wavelength detector (G1365D) were used for HPLC analysis of samples. XK-80A micro-vortex apparatus (Shanghai Huxi Analytical Instrument Factory, China) was used to vortex samples. A high-speed bench centrifuge (TGL-16G-A, Shanghai Anting Scientific Instrument Factory, China) was used to centrifuge samples. KQ3200E ultrasonic wave purifier (Kunshan Ultrasonic Instruments Co., Ltd.) was used to sonicate the intestinal incubation experiments. The intestinal incubation experiments were carried out in 2.5 L anaerobic incubation bags (MGC AnaeroPack-Aneaero, Mitsubishi Gas Chemical Co., Inc., Chiyoda-ku, Japan) and a 2.5 L anaerobic incubation pot (Labmed AG025, LABMED BIOTECH Co., Ltd., Guangzhou, China). LAIHENG L-128 Nitrogen blowing instrument (Beijing Laiheng Scientific Co., Ltd., Beijing, China) was used for sample condensation.

2.3 Animals

Four New-Zealand male rabbits with body weight of 3-4 kg were purchased from the Experimental Animal Center of Guangdong Province and kept in an animal room at constant temperature (23 \(\pm 2\) °C) and humidity (55\% \(\pm 10\%) with a 12 h of light per day and access to water and food ad libitum.

3 Methods

3.1 Preparation of anaerobic culture solutions \cite{12}

37.5 mL of A solution (0.78% \(\text{K}_2\text{HPO}_4\)), 37.5 mL of B solution (0.47% \(\text{KH}_2\text{PO}_4\), 1.18% \(\text{NaCl}\), 1.2\% \(\text{(NH}_4\text{)}_2\text{SO}_4\), 0.12\% \(\text{CaCl}_2\), 0.25\% \(\text{MgSO}_4\cdot\text{H}_2\text{O}\)), 50 mL of C solution (8\% \(\text{Na}_2\text{CO}_3\)), 0.5 g of L-cysteine, 2 mL of 25% L-ascorbic acid, 1 g of eurythrol, 1 g of tryptone and 1 g of nutrient agar were mixed together and diluted with distilled water to 1 L. Then the solution was adjusted to pH 7.5-8.0 with HCl (2 mol·L\(^{-1}\)).

3.2 Preparation of intestinal flora cultural solution \cite{13}

Fresh feces of rabbits were homogenized in normal saline solution at the ratio of 1 g to 4 mL immediately and then followed by filtration through gauze. 10 mL of the filtrate was mixed with 90 mL of anaerobic culture solutions to prepare 100 mL of intestinal flora cultural solution.

3.3 Preparation of EF standard solution

1 mg of EF was accurately weighed and first dissolved in 500 \(\mu\)L of methanol, then diluted with sufficient normal saline solution to create a final volume of 50 mL, yielding EF standard solution at a concentration of 20.0 \(\mu\)g·mL\(^{-1}\).

3.4 Pre-treatment of samples

The samples were pretreated by liquid-liquid extraction thrice. In each round, 1mL of water-saturated ethyl acetate was added into 0.5 mL of sample incubated anaerobically at 37 °C, and then vortexed for 1 min and centrifuged at 9 960 \(\text{r·min}^{-1}\) for 1 min. The supernatant obtained from the three rounds of liquid-liquid extraction was combined and dried under nitrogen stream at room temperature. The residue was re-dissolved in 150 \(\mu\)L of methanol by ultrasonic for 0.5 min and vortexed for 0.5 min. Then the supernatant was obtained by centrifuging at 9 960 \(\text{r·min}^{-1}\) for 1 min and transferred into a 400 \(\mu\)L borosilicate glass insert placed inside a sample vial for HPLC analysis.

3.5 HPLC conditions

Chromatographic separation was performed on a XB-C\(_{18}\) column (4.6 mm \(\times\) 250 mm i.d., 5 \(\mu\)m) coupled with a Phenomenex-C\(_{18}\) guard column (13 mm \(\times\) 4.6 mm i.d., 5 \(\mu\)m). Optimum separation was achieved with a binary mobile phase at a flow rate of 0.8 mL·min\(^{-1}\). The column temperature was held at 35 °C. The mobile phase consisted of acetonitrile (B)-water (A) (1/1) (including 0.1% formic acid respectively). The gradient program was as follows: 0–10 min 32%–80% B; 10–18 min 80%–100% B; 18–22 min 100% B; 22–23 min 100%–32% B; 23–28 min 32% B. The detection
wavelength and the reference wavelength were set at 335 nm and 400 nm, respectively. Finally, 10 μL of sample was injected automatically into the HPLC system.

3.6 Selection of mixed reference substances

As reported, the metabolites of Epimedium in vivo included icariin (parent drug), icariside I, icariside II, des-methylcaritin and icaritin \([14-15]\). Therefore, these five reference substances were selected and mixed to prepare the sample of mixed reference substances used for identification of the metabolites of EF in rabbit intestinal flora.

3.7 The study of EF metabolisms in rabbit intestinal flora

35 pieces of 2 mL of polypropylene centrifuge tubes were used, each of which was added 50 μL of EF standard solution (20.0 μg·mL\(^{-1}\)) as well as 500 μL of intestinal flora cultural solution and incubated at 37 °C anaerobically. Five tubes were taken out at each time point of 0, 0.5, 1, 2, 4, 8, and 12 h. Then the samples were pre-treated immediately referring to pre-treatment of samples in 3.4 immediately and analyzed as HPLC conditions in 3.5 in order to study the metabolism of EF in intestinal flora of rabbits.

In addition, EF were incubated in anaerobic culture solutions as negative control, and then subjected to the same procedures as for the rabbit intestinal flora described above to investigate whether EF were metabolized in anaerobic culture solutions.

3.8 The study of inhibition of the EF metabolism in rabbit intestinal flora by gluconolactone

Different amounts of gluconolactone were added into three portions of rabbit intestinal flora fluid to obtain three different concentrations: 8 mg·mL\(^{-1}\) as low concentration, 12 mg·mL\(^{-1}\) as intermediate concentration and 16 mg·mL\(^{-1}\) as high concentration. For each rabbit intestinal flora cultural solution at one of the three prepared gluconolactone concentrations, 35 pieces of 2 mL of polypropylene centrifuge tubes were used, each of which was added 50 μL of EF standard solution (20.0 μg·mL\(^{-1}\)) as well as 500 μL of correspondent rabbit intestinal flora cultural solution above and incubated at 37 °C anaerobically. Five tubes were taken out at each time point of 0, 0.5, 1, 2, 4, 8, and 12 h. Then the samples were pre-treated immediately as pre-treatment of samples in 3.4 immediately and analyzed as HPLC conditions in 3.5 in order to study the inhibition of the metabolism of EF in rabbit intestinal flora by gluconolactone.

4 Results

4.1 The metabolism of EF in rabbit intestinal flora

In the experiment, EF were shown to be metabolized by rabbit intestinal flora quickly, as was evidenced by the complete elimination of icariin (the main component of EF) as parent drug after incubation for 2 h, and a metabolite, icariside II reached the maximum peak area simultaneously and then decreased gradually with further incubation. After incubation for 8 h, another metabolite, icaritin was generated and its peak area could increase gradually upon longer incubation time. The mean profiles and corresponding chromatograms are shown in Figs. 1 and 2 respectively. The metabolism of EF in anaerobic culture solutions was also investigated as negative control and the results showed that EF could not be metabolized in anaerobic culture solutions.

![Fig. 1](image)

**Fig. 1** Mean (SD) peak area-time curve of icariin and two metabolites icariside II and icaritin after incubation in rabbit intestinal flora \((n = 5 \text{ for each time point})\)

![Fig. 2](image)

**Fig. 2** The chromatograms of blank intestinal flora sample, EF sample incubated in intestinal flora of rabbits and mixed reference substances

**A:** blank intestinal flora sample; **B and C:** EF sample incubated in intestinal flora of rabbits for 0.5 and 12 h, respectively; **D:** mixed reference substances; 1: icariin; 2: icariside II; 3: icaritin

4.2 The inhibition of the metabolism of EF in rabbit intestinal flora by gluconolactone

The inhibition of the EF metabolism in rabbit intestinal flora with different concentrations of gluconolactone (8, 12, 16 mg·mL\(^{-1}\)) was performed. Gluconolactone showed this kind of inhibition at low concentration (8 mg·mL\(^{-1}\)) in rabbit intestinal flora, i.e., the amount of icariside II and icaritin decreased and the residue amount of icariin increased (Fig. 3A). The enhanced inhibition of gluconolactone was shown at the intermediate concentration (12 mg·mL\(^{-1}\)) in rabbit intestinal flora, i.e., icaritin formation was inhibited while the amount of icariside II decreased. And at each time point, the SD of icariside II was less than 1.0 (Fig. 3B). Gluconolactone showed complete inhibition at high concentration (16 mg·mL\(^{-1}\)) in rabbit intestinal flora; that is, neither metabolite (icariside II or icaritin) was formed nor icariin was metabolized. And at each time point, the SD of icariin was less than 2.0 (Fig. 3C).
YAO Zhi-Hong, et al. / Chinese Journal of Natural Medicines 2011, 9 (6): 461–465

**Fig. 3** Mean (SD) peak area-time curve of icariin and two metabolites icariside II and icaritin after incubation with different concentrations of gluconolactone in intestinal flora of rabbits ($n = 5$ for each time point)

A: low concentration ($8 \text{ mg·mL}^{-1}$) of gluconolactone in rabbit intestinal flora; B: intermediate concentration ($12 \text{ mg·mL}^{-1}$) of gluconolactone in rabbit intestinal flora; C: high concentration ($16 \text{ mg·mL}^{-1}$) of gluconolactone in rabbit intestinal flora.

In brief, at certain concentrations, gluconolactone showed inhibition of the metabolism of EF in rabbit intestinal flora in a concentration-dependent manner, and $16 \text{ mg·mL}^{-1}$ of gluconolactone could totally inhibit the metabolism of EF in rabbit intestinal flora.

### 5 Discussion

On the basis of the above results, in intestinal flora of rabbits, EF (with icariin as the main component) were thought to be metabolized by hydrolysis to first produce icariside II after removal of glucose, and then icariside II was metabolized to produce icaritin after further removal of rhamnose. Fig. 4 is the corresponding flow diagram.

For both the metabolism of EF in rabbit intestinal flora in our experiment and the metabolism of icariin in human intestinal flora reported [10], the same final metabolite, icaritin was produced. Considering that icaritin, but not EF, displayed direct protective effects dose-dependently on steroid-induced cell damage model for in vitro bioactivity evaluation [9], EF could reduce the risk of steroid-associated osteonecrosis in rabbit model [5], and *Epimedium* extract could reduce the probability of steroid-associated osteonecrosis for SARS patients in clinical evidence-based medicine [16-17].

**Fig. 4** The proposed metabolic pathway of EF (with icariin as the main component) in rabbit intestinal flora

icaritin might be implied as the exact in vivo bioactive component of EF in the prevention of steroid-associated osteonecrosis.

Gluconolactone was found to concentration-dependently inhibit the metabolism of EF in rabbit intestinal flora in the study. It showed that gluconolactone may be used as a potential tool medicine to investigate whether EF could reduce the risk of steroid-associated osteonecrosis in rabbit or not when its in vivo metabolism was inhibited by gluconolactone and accordingly confirm whether EF might act as a prodrug in the prevention of steroid-associated osteonecrosis.

In summary, some partial research has been carried out in our study for the aim of revealing the in vivo bioactive component of EF in the prevention of steroid-associated osteonecrosis; nevertheless, further investigation on the in vivo metabolism and bioactivity evaluation of EF using rabbit as model should be launched to help achieve the above objective.

### Acknowledgements

Thanks are given to Prof. SONG Li-Yan for her kind help in revising this paper.

### References

[1] Zhonghuayaohai [M]. Harbin Publishing Company, 1998: 1714-1718.
[2] Wang M, Liu CM, Zhang JF. Effects of *Epimedium icariine* on rabbit and dog cerebral blood flow [J]. *J Shenyang Coll Pharm*, 1991, 8 (4): 272-276.
[3] He W, Sun H, Yang B, et al. Immunoregulatory effects of the Herba Epimediia glycoside icariin [J]. *Arzneimittel-Forsch*, 1995, 45 (8): 910-913.
[4] Zhai YK, Li ZF, Cheng GZ, et al. Current status of the anti-osteoporosis mechanism of icariin [J]. *Chin J Osteoporosis*, 2009, 15 (7): 543-545,531.
[5] Zhang G, Qin L, Sheng H, et al. Epimedium-derived phytoestrogen exert beneficial effect on preventing steroid-associated

---

**Fig. 3** Mean (SD) peak area-time curve of icariin and two metabolites icariside II and icaritin after incubation with different concentrations of gluconolactone in intestinal flora of rabbits ($n = 5$ for each time point)

A: low concentration ($8 \text{ mg·mL}^{-1}$) of gluconolactone in rabbit intestinal flora; B: intermediate concentration ($12 \text{ mg·mL}^{-1}$) of gluconolactone in rabbit intestinal flora; C: high concentration ($16 \text{ mg·mL}^{-1}$) of gluconolactone in rabbit intestinal flora.

In brief, at certain concentrations, gluconolactone showed inhibition of the metabolism of EF in rabbit intestinal flora in a concentration-dependent manner, and $16 \text{ mg·mL}^{-1}$ of gluconolactone could totally inhibit the metabolism of EF in rabbit intestinal flora.

### 5 Discussion

On the basis of the above results, in intestinal flora of rabbits, EF (with icariin as the main component) were thought to be metabolized by hydrolysis to first produce icariside II after removal of glucose, and then icariside II was metabolized to produce icaritin after further removal of rhamnose. Fig. 4 is the corresponding flow diagram.

For both the metabolism of EF in rabbit intestinal flora in our experiment and the metabolism of icariin in human intestinal flora reported [10], the same final metabolite, icaritin was produced. Considering that icaritin, but not EF, displayed direct protective effects dose-dependently on steroid-induced cell damage model for in vitro bioactivity evaluation [9], EF could reduce the risk of steroid-associated osteonecrosis in rabbit model [5], and *Epimedium* extract could reduce the probability of steroid-associated osteonecrosis for SARS patients in clinical evidence-based medicine [16-17].

**Fig. 4** The proposed metabolic pathway of EF (with icariin as the main component) in rabbit intestinal flora

icaritin might be implied as the exact in vivo bioactive component of EF in the prevention of steroid-associated osteonecrosis.

Gluconolactone was found to concentration-dependently inhibit the metabolism of EF in rabbit intestinal flora in the study. It showed that gluconolactone may be used as a potential tool medicine to investigate whether EF could reduce the risk of steroid-associated osteonecrosis in rabbit or not when its in vivo metabolism was inhibited by gluconolactone and accordingly confirm whether EF might act as a prodrug in the prevention of steroid-associated osteonecrosis.

In summary, some partial research has been carried out in our study for the aim of revealing the in vivo bioactive component of EF in the prevention of steroid-associated osteonecrosis; nevertheless, further investigation on the in vivo metabolism and bioactivity evaluation of EF using rabbit as model should be launched to help achieve the above objective.

### Acknowledgements

Thanks are given to Prof. SONG Li-Yan for her kind help in revising this paper.

### References

[1] Zhonghuayaohai [M]. Harbin Publishing Company, 1998: 1714-1718.
[2] Wang M, Liu CM, Zhang JF. Effects of *Epimedium icariine* on rabbit and dog cerebral blood flow [J]. *J Shenyang Coll Pharm*, 1991, 8 (4): 272-276.
[3] He W, Sun H, Yang B, et al. Immunoregulatory effects of the Herba Epimediia glycoside icariin [J]. *Arzneimittel-Forsch*, 1995, 45 (8): 910-913.
[4] Zhai YK, Li ZF, Cheng GZ, et al. Current status of the anti-osteoporosis mechanism of icariin [J]. *Chin J Osteoporosis*, 2009, 15 (7): 543-545,531.
[5] Zhang G, Qin L, Sheng H, et al. Epimedium-derived phytoestrogen exert beneficial effect on preventing steroid-associated
YAO Zhi-Hong, et al. /Chinese Journal of Natural Medicines 2011, 9 (6): 461–465

osteonecrosis in rabbits with inhibition of both thrombosis and lipid-deposition [J]. Bone, 2007, 40: 685-692.
[6] Zhang G, Wang XL, Sheng H, et al. Constitutional flavonoids derived from epimedium dose-dependently reduce incidence of steroid-associated osteonecrosis not via direct action by themselves on potential cellular targets [J]. PLoS ONE, 2009: 4.
[7] Kang B. Microecology [M]. Dalian Publishing Company, 1988: 95.
[8] Kobashi K. Metabolism of drug by intestinal bacteria [J]. Bifi-dobacteria Microflora, 2006, 11: 834-836.
[9] Liu TH, Wang Y, Wang BX, et al. Metabolism of icariin by intestinal bacteria. Part. I. the transformation of icariin by intestinal flora [J]. Chin Tradit Herb Drugs, 2000, 31 (11): 834-836.
[10] Liu Y, Hu M. Absorption and metabolism of flavonoids in the CACO-2 cell culture model and a perused rat intestinal model [J]. Drug Metab Dispos, 2002, 30 (4): 370-377.
[11] Chen HX, Huang JL, Li J. Characterization of metabolites of worenine in rat biological samples using liquid chromatography-tandem mass spectrometry [J]. J Pharm Biomed Anal, 2010, 51 (1): 236-243.
[12] Akao T, Che QM, Kyoichi K, et al. A purgative action of barbaloin is induced by Eubacterium sp. strain BAR, a human intestinal anaerobe, capable of transforming barbaloin to aloe-emodin anthrone [J]. Biol Pharm Bull, 1996, 19 (1): 136-138.
[13] Shen P, Wong SP, Yong EL. Sensitive and rapid method to quantify icarin and desmethylicaritin in human serum using gas chromatography–mass spectrometry [J]. J Chromatogr B, 2007, 857 (1): 47-52.
[14] Shen P, Shih SP, Li L, et al. Simple and sensitive liquid chromatography–tandem mass spectrometry assay for simultaneous measurement of five Epimedium prenylflavonoids in rat sera [J]. J Chromatogr B, 2009, 877 (1-2): 71-78.
[15] Griffith JF, Antonio GE, Kumta SM, et al. Osteonecrosis of hip and knee in patients with severe acute respiratory syndrome treated with steroids [J]. Radiology, 2009, 235 (1): 168-175.

淫羊藿总黄酮苷在家兔肠菌中的代谢及葡萄糖酸内酯对其肠菌代谢的抑制作用

姚志红1,2*, 刘明艳1△, 戴 毅1,2, 张 依1, 秦子飞1, 屠风娟3, 姚新生1,2,3

【摘 要】 目的: 考察淫羊藿总黄酮苷在家兔肠菌中的代谢以及葡萄糖酸内酯对肠菌代谢的抑制作用，旨在帮助揭示淫羊藿总黄酮苷在家兔体内的代谢途径和预防激素性骨坏死作用的物质基础。方法: 淫羊藿总黄酮苷与家兔肠菌液经37 °C厌氧温孵培养后，在不同时间点经水饱和的乙酸乙酯萃取，并对加入不同浓度 (8, 12和16 mg·mL−1) 的葡萄糖酸内酯对淫羊藿总黄酮苷经家兔肠菌代谢的抑制情况进行考察。采用 ODS 柱和乙腈-水 (各含0.1%的甲酸) 的流动相梯度洗脱，检测波长为335 nm。结果: 淫羊藿总黄酮苷在家兔肠菌液中温孵2 h时被代谢成淫羊藿次苷II，在温孵8 h时被代谢成淫羊藿苷。葡萄糖酸内酯能够浓度依赖性抑制家兔肠菌对淫羊藿总黄酮苷的代谢。结论: 淫羊藿总黄酮苷能被家兔肠菌迅速水解成淫羊藿次苷 II 和淫羊藿苷; 当葡萄糖酸内酯的浓度为16 mg·mL−1时，则能完全抑制家兔肠菌对淫羊藿总黄酮苷的代谢。

【关键词】 淫羊藿总黄酮苷; 抗骨坏死活性; 家兔肠菌代谢; 葡萄糖酸内酯; 抑制作用

【基金项目】 国家自然科学基金 NSFC-RGC (No. 30831160510)