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Inhibition of 11β-hydroxysteroid dehydrogenase in guinea pig kidney by three bioflavonoids and their interactions with gossypol

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KEY WORDS drug interactions; flavones; gossypol; hydroxysteroid dehydrogenases; hypokalemia

ABSTRACT

AIM: To study the effects of some bioflavonoids on the gossypol-induced hypokalemia. **METHODS**: The 11β-hydroxysteroid dehydrogenase (11β-OHSD) protein was prepared from guinea pig kidney. The activity of 11β-OHSD with NAD as the coenzyme was measured by HPLC. The drug interaction was analysed by isobolographic method. **RESULTS**: The 11β-OHSD can be inhibited by some bioflavonoids. The IC_{50} (95 % confidence limits) values were: quercetin 164 (79-341) μmol/L, morin 913 (385 - 2173) μmol/L, and naringenin 2193 (1114-4315) μmol/L. When the 11β-OHSD was treated with quercetin, tangeretin, morin, naringenin plus gossypol, the combination index (CI) values were 0.92, 0.85, 0.98, and 1.01 respectively. **CONCLUSION**: The interaction of some bioflavonoids with gossypol might be one of the factors for gossypol-induced hypokalemia.

INTRODUCTION

The 11β-hydroxysteroid dehydrogenase (11β-OHSD, *EC* 1.1.1.146), a critical enzyme to control the level of glucocorticoid in kidney, was studied vastly in recent twenty years[1]. Its activity is lower in cases of apparent mineralocorticoid excess that the hypokalemia and paralysis is mainly clinical symptoms[2,3].

The antifertility activity of gossypol for male was first found by Chinese scientists[4]. It is not used in clinical because it induces hypokalemia in some subjects who take the gossypol as contraceptive for a long period of time[5,6]. It is proved that gossypol has a potent competitive inhibitory effect on the enzyme activity of 11β-OHSD[7].

Bioflavonoid is a large category of compounds and they are naturally present in the diet and medical plant widely. We know there are similar structures between gossypol and bioflavonoids, and the inhibitory activity of some bioflavonoids on 11β-OHSD *in vitro* was reported[8]. In this paper, we tried to determine whether there were interactions between these compounds and gossypol on the enzyme.

MATERIALS AND METHODS

Chemicals and solutions Gossypol acetate (purity >99 %) was a gift from Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Cortisol, cortisone, corticosterone, quercetin, morin, naringenin, and NAD were purchased from Sigma Co (USA), and tangeretin was a gift from Prof M REIDENBERG (Cornell University Medical College, USA). Gossypol acetate and bioflavonoids were dissolved in methanol. NAD (5 mmol) was dissolved in Tris-HCl buffer (0.1 mol/L, pH 8.0) before use.

Preparation of enzyme protein The kidneys were collected from 30 ♂ Hartley guinea pigs (Zhejiang Laboratory Animal Center, Certificate number 96001) weighing 300 - 350 g. The kidney cortex microsome was prepared[7].

Enzyme reaction and extraction of steroids Enzyme activity of 11β-OHSD was measured and steroid was extracted as described previously[9], except that in these experiments the compounds evaluated were added to the mixture before cortisol.

Steroid measurement by HPLC[9] A ZORBAX Bondclone 10 C18 column (250 mm × 4.6 mm,

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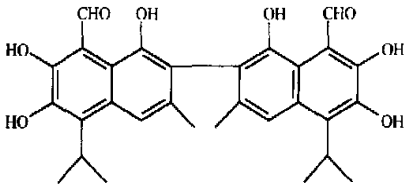
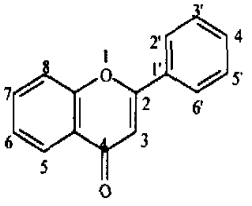
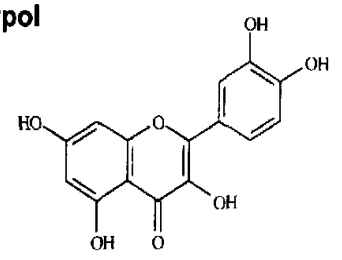


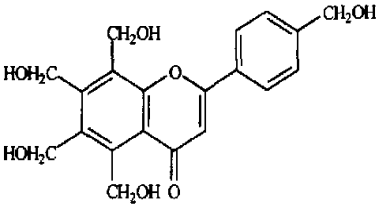
Gossypol



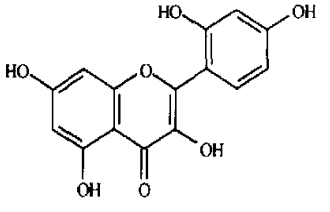
Flavone



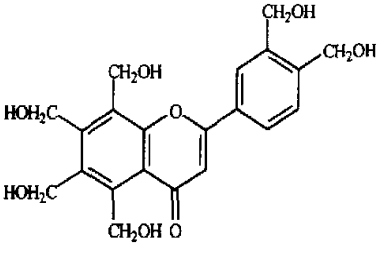
Quercetin



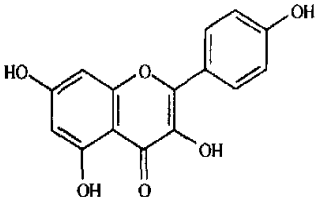
Tangeretin



Morin



Nobiletin



Naringenin

DuPont Co, USA) was used at 30 ℃ for all assays in a CTO-10A column oven. Components of the HPLC assembly (SHIMADZU Co, Japan) included a 7125 injector, a SPE-10A UV-vis detector, and LC-10AD liquid chromatograph pump. Output from the absorbance unit was recorded with a C-R 6A recorder. A wavelength of 245 nm and a maximal sensitivity of 0.005 absorbance units, full scale, were used. The mobile phase consisted of methanol-water (3∶2, v/v). The retention time (RT) for cortisol, cortisone, and corticosterone was 8.55, 7.24, and 13.20 min, respectively at a flow rate of 1.0 mL/min. The coefficient variation of cortisol assay was 0.79 % using 8 simultaneous replicate samples when cortisol 5.5 nmol was incubated with boiled microsome. The recovery rates by methylene chloride extraction were 102.5 %, 98.1 %, 99.3 %, and 96.1 % for cortisol and 117.3 %, 115.2 %, 117.7 %, and 102.8 % for cortisone when cortisol 1.10, 3.31, 5.52, and 11.03 μmol and cortisone 1.11, 3.33, 5.55, and 11.10 μmol were added into 40 μL microsome homogenate, respectively.

Analysis of data For determining the two-drug interaction of bioflavonoids with gossypol in inhibiting 11β-OHSD, the mixture of these compounds was made at a constant ratio of 3/4, 1/2, 1/4 of IC_{50} values of gossypol with 1/4, 1/2, 3/4 of IC_{50} values of

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bioflavonoids, respectively. Isobolographic method was used[10] and the Combination Index (CI) values was calculated based on the median-effect equation:

$$CI = [D]_1/[DX]_1 + [D]_2/[DX]_2$$

CI values of < 1, 1, and > 1 indicate synergism, additivity, and antagonism, respectively. $[DX]_1$ and $[DX]_2$ in the denominators are the concentrations (drug 1 and drug 2, respectively) that are required to produce X % effect alone[11,12].

RESULTS

Effects of bioflavonoids on 11β-OHSD *in vitro* The 11β-OHSD from guinea pig kidney cortex could be inhibited by tested compounds *in vitro* when NAD as the coenzyme and cortsol as the substratum. The IC_{50} values of gossypol, quercetin, morin, and naringenin alone were shown in Tab 1. The sequence for inhibiting 11β-OHSD in IC_{50} values was:

gossypol > quercetin > morin > naringenin

Interaction of bioflavonoids with gossypol in inhibiting 11β-OHSD *in vitro* The results of two-drug interaction of some bioflavonoids with gossypol were shown in Fig 1. The CI values of quercetin, tangeretin, morin, naringenin plus gossypol were 0.92, 0.85, 0.98, and 1.01 respectively. These results indicated that there were moderate or slight synergism or

Tab 1. Effects of some bioflavonoids and gossypol on 11β-OHSD.

Compounds	Concentration /μmol·L^{-1}	Inhibition/%	IC_{50}(95 % CL)/ μmol·L^{-1}
Gossypol	20.6	37.0 ± 0.3	
	41.3	51.3 ± 0.5	
	82.6	66.6 ± 0.7	38.2 (11.8 - 123.0)
Quercetin	84	27.4 ± 0.6	
	169	52.4 ± 1.0	
	338	74.0 ± 1.0	164 (79 - 341)
Morin	473	30.2 ± 0.6	
	945	53.1 ± 0.4	
	1891	70.0 ± 0.9	913 (385 - 2173)
Naringenin	1049	24.6 ± 0.7	
	2089	42.9 ± 0.5	
	4197	76.4 ± 0.7	2193 (1114 - 4315)

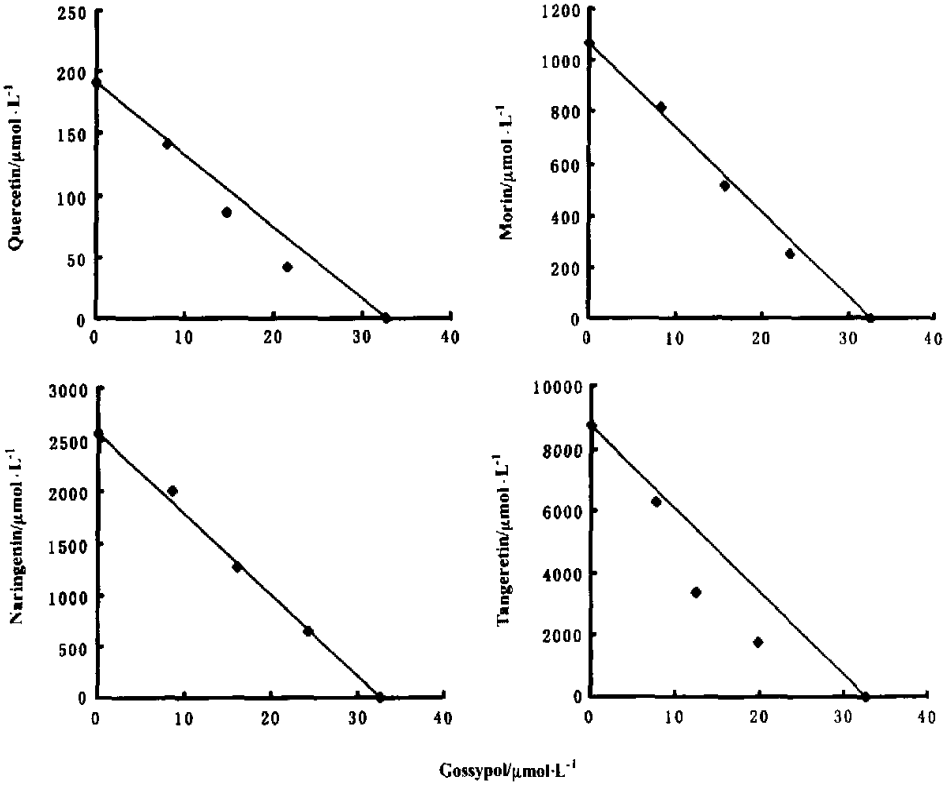

Fig 1. Isobologram for the interaction of some bioflavonoids with gossypol.

additive effects between gossypol and three bioflavonoids.

DISCUSSION

The previously studies have demonstrated that the 11β-OHSD can be inhibited by gossypol *in vivo* and *in vitro*[7], the NAD-dependent 11β-OHSD that is localized in the distal renal tubules is a more critical physiologic mechanism in regulating the renal glucocorticoid levels[9], and the gossypol caused the lower dosage of corticosteroid to elicit higher K^+/Na^+ ratio in 24-h urine and lower serum potassium level[13], and hypothesis remained that inhibition of NAD-dependent 11β-OHSD (11β-OHSD2) might be one of the mechanism of gossypol-induced hypokalemia.

The bioflavonoids widely existing in diet and medical plant is a large catalog of compounds. The previous and present study demonstrated that the 11β-OHSD can be inhibited by some bioflavonoids, and now we find there is synergism or additive effect when the enzyme was treated by these compounds with gossypol. From these results, we could ascribed the hypokalemia induced by gossypol to the bioflavonoid partially, and the interaction of some bioflavonoids with gossypol may be one of the factors for gossypol-induced hypokalemia. Whether variation in intake of dietary inhibitors of 11β-OHSD such as bioflavonoid in different geographic locations accounts for variation incident of gossypol-induced hypokalemia remains to be determined.

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三种生物黄酮类化合物及其与棉酚相互作用抑制豚鼠肾脏 11β-羟甾脱氢酶

R96 A

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关键词　药物相互作用；黄酮类；棉酚；羟甾类脱氢酶类；低钾血症

目的：探讨三种生物黄酮类化合物在棉酚诱发低血钾中的作用. **方法**：从豚鼠肾脏制备 11β-羟甾脱氢酶，NAD 为辅酶，加入待测化合物，37 ℃反应一小时后提取甾体，反相高压液相测定酶活性，热辐射图像法分析药物相互作用. **结果**：所试生物黄酮类化合物均能抑制 11β-羟甾脱氢酶，其 IC_{50}（95 % 可信限）分别为：槲皮素 164（79 – 341）μmol/L，桑色素

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913 (385 – 2173) μmol/L, 柑桔素 2193 (1114 – 4315) μmol/L. 当 11β-羟甾脱氢酶分别用槲皮素, 红桔素, 桑色素, 柑桔素加上棉酚处理时, 其 CI 值分别为: 0.92、0.85、0.98 和 1.01. **结论**: 三种生物黄酮类化合物与棉酚在抑制 11β-羟甾脱氢酶中具有协同或相加作用.

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