目的：观察卡托普利(Cap)对原癌基因 c-*myc* 在自发性高血压大鼠(SHR)左心室心肌不同细胞类型的表达的影响，探讨 Cap 抑制左心室肥大的分子机制．**方法**：SHR 宫内期口服给药(100 mg·kg^{-1}·d^{-1})，雄性 SHR 16 周龄时测定收缩压、左室重与体重比．左心室 c-*myc* mRNA，Ang Ⅱ及 c-*myc* 癌蛋白表达量分别用 RNA 印迹、免疫组化及蛋白质印迹测定．**结果**：Cap 明显降低 SHR 大鼠的收缩压，抑制左心室肥大及 c-*myc* 表达，心肌局部 Ang Ⅱ主要产生于心肌细胞，c-*myc* 则主要源于成纤维细胞．**结论**：Cap 通过抑制心肌细胞的 Ang Ⅱ和成纤维细胞的 c-*myc* 表达而逆转左心室肥大．

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Effects of genistein on aggregation and cytosolic free calcium in pig platelets[1]

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KEY WORDS isoflavones; genistein; thrombin; platelet aggregation; calcium; protein-tyrosine kinase

AIM: To study the effects of genistein on aggregation and cytosolic free calcium concentration in platelets. **METHODS**: Using turbidimetry to analyse aggregation and using Fura-2 fluorescence technique to determine Ca^{2+} level. **RESULTS**: Genistein strongly inhibited the pig platelet aggregation induced by thrombin (250 U·L^{-1}). When genistein concentrations were 5 and 20 μmol·L^{-1}, the inhibition rates on the aggregation were 52 % and 73 %, respectively. Genistein inhibited the rise of cytosolic free calcium concentration in platelets stimulated by thrombin (500 U·L^{-1}) in the presence of extracellular Ca^{2+} 1 mmol·L^{-1}. When genistein concentrations were 10, 20, 40, and 80 μmol·L^{-1}, the inhibition rates were 24 %, 40 %, 63 %, and 65 %, respectively, but no effect on thrombin-induced internal Ca^{2+} release from dense tubular system. **CONCLUSION**: Genistein is a potential anti-platelet agent, mainly due to an inhibition of Ca^{2+} influx.

Genistein (4,5,7-trihydroxyisoflavone) is a specific inhibitor of tyrosine protein kinase (TPK)[1]. It inhibits platelet shape change and protein tyrosine phosphorylation in platelet[2]. In the present study, we investigated the effect of genistein on thrombin-induced pig platelet aggregation.

MATERIALS AND METHODS

Thrombin, egtazic acid, Fura 2-AM, Triton X-100, bovine serum albumin (BSA), RPMI-1640, and genistein were from Sigma. All other chemicals were AR.

Pig blood collected in plastic tubes and anticoagulated with 0.15 volume of ACD (trisodium citrate 86, glucose 111, citric acid 53 mmol·L^{-1}).

Platelet aggregation Pig blood was spun at 200 × *g* for 15 min, and the supernatant was then spun at 800 × *g* for 10 min. The cells were resupended at 2 × 10^{11} platelets·L^{-1} in Tyrode-HEPES buffer (NaCl 140, KCl 5, $MgSO_4$ 1, HEPES 10, glucose 10 mmol·L^{-1}, pH 7.4). Platelet aggregometry was carried by an SPA-4 aggregometer (Shanghai). The platelet suspensions (0.2 mL) were incubated with genistein for 2 min, and then stimulated with thrombin 250 U·L^{-1} for 3 min.

Cytosolic free calcium Suspensions of 2 × 10^{11} platelets·L^{-1} in an RPMI-1640 (pH 7.4) containing 0.2 % BSA and HEPES 10 mmol·L^{-1} were incubated with Fura 2-AM 2.5 μmol·L^{-1} at 25 ℃ for 45 min. The cell suspension was then spun at 800 × *g* for 10 min. The resuspension was of 3 × 10^{10} – 4 × 10^{10} platelets·L^{-1} in a Tyrode-HEPES buffer. Fluorescence

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(λ_{ex} 340 nm; λ_{em} 500 nm) was measured at 25 ℃ using LS50B Luminescence Spctrometer (Perkin Elmer). Maximal fluorescence (F_{max}) was obtained after addition of Triton X-100 (0.1 % final concentration) in the presence of $CaCl_2$ 1 mmol·L^{-1} within 1 h. Minimal fluorescence (F_{min}) was obtained after addition of egtazic acid (10 mmol·L^{-1} final concentration) within 1 h. Cytosotic free calcium concentration ($[Ca^{2+}]_i$) was calculated: $[Ca^{2+}]_i = K_d(F - F_{min})/(F_{max} - F)$, $K_d = 224$ nmol·L^{-1} [3].

Data were expressed as $\bar{x} \pm s$ and analyzed by t test.

RESULTS

Stimulation of platelets with thrombin (250 U·L^{-1}) resulted in the aggregation of 85 % ± 12 % of platelets. Genistein strongly inhibited the platelet aggretation induced by thrombin. When genistein concentrations were 5 and 20 μmol·L^{-1} the inhibition rates on aggregation were 52 % and 73 %, respectively. (Tab 1)

Tab 1. Effect of genistein on pig platelet aggregation induced by thrombin 250 U·L^{-1}. $n = 4$ pigs. $^cP < 0.01$ *vs* control.

Genistein/μmol·L^{-1}	Maxinal aggregation/%	Inhibition/%
0	85 ± 12	
5	41 ± 5^c	52
20	23 ± 8^c	73

The fluorescence of platelets relatively stable in 1 h with no drug in the presence of extracellular Ca^{2+} 1 mmol·L^{-1} (Fig 1).

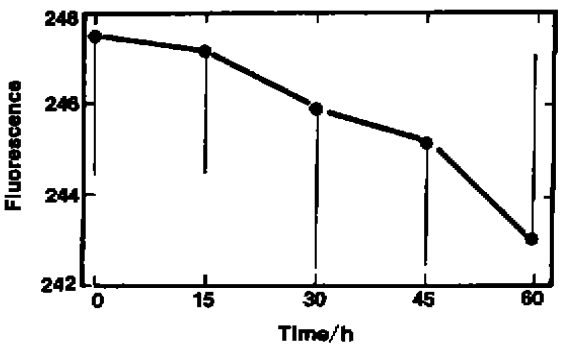

Fig 1. Fluorescence of platelets in the presence of extracellular Ca^{2+} 1 mmol·L^{-1}. $n = 4$ pigs. $\bar{x} \pm s$. $^aP > 0.05$ *vs* control.

Thrombin 500 U·L^{-1} stimulated the rise in $[Ca^{2+}]_i$ in the absence or presence of extracellular Ca^{2+} 1 mmol·L^{-1}. The effect was inhibited in the presence of extracellular Ca^{2+} 1 mmol·L^{-1} by genistein in a concentration-dependent manner. When genistein concentrations were 10, 20, 40, and 80 μmol·L^{-1}, the inhibition rates were 24 %, 40 %, 63 %, and 65 %, respectively (Tab 2), but genistein was not inhibited on internal Ca^{2+} release in the absence of extracellular Ca^{2+} (Tab 3).

Tab 2. Effect of genistein on $[Ca^{2+}]_i$ of platelet stimulated by thrombin 500 U·L^{-1} in the presence of extracellular calcium 1 mmol·L^{-1}. $n = 4$ pigs. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Genistein/μmol·L^{-1}	Thrombin/U·L^{-1}	$[Ca^{2+}]_i$/nmol·L^{-1}	Inhibition/%
0	0	163 ± 6	
0	500	472 ± 10	
10	500	361 ± 6^b	24
20	500	283 ± 9^c	40
40	500	173 ± 11^c	63
80	500	165 ± 7^c	65

Tab 3. Effect of genistein on $[Ca^{2+}]_i$ of platelet stimulated by thrombin 500 U·L^{-1} in the absence of extracellular calcium 1 mmol·L^{-1}. $n = 5$ pigs. $^aP > 0.05$ *vs* control.

Genistein/μmol·L^{-1}	Thrombin/U·L^{-1}	$[Ca^{2+}]_i$/nmol·L^{-1}
0	0	35 ± 5
0	500	67 ± 8
20	500	71 ± 4^a
40	500	59 ± 9^a
80	500	63 ± 11^a

DISCUSSION

The results demonstrated that genistein strongly inhibited thrombin-induced platelet aggregation. This evidence indicates that inhibitor of TPK is a type of anti-platelet agents.

Elevation of cytosolic free calcium concentration is thought to be important to many aspects of blood platelet function, including shape change, secretion and aggregation[4-6]. Genistein inhibited Ca^{2+} influx in thrombin-induced platelets, but had no effect on Ca^{2+} release from dense tubular system. Calcium influx is a major

pathway for elevating $[Ca^{2+}]_i$ by thrombin. IP_3 is a second messenger for intracellular Ca^{2+} release[7-9]. These results indicated that genistein did not affect IP_3 directly. The effect of genistein on cytosolic free calcium could be related to its inhibition on platelet aggregation. This suggests that the inhibitory effect of genistein on the platelet aggregation is mainly due to the inhibition of the Ca^{2+} influx.

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金雀异黄素对猪血小板聚集和胞浆游离钙的影响[1]

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关键词 异黄酮类; 金雀异黄素; 凝血酶; 血小板聚集; 钙; 蛋白质-酪氨酸激酶

目的: 研究金雀异黄素对猪血小板聚集和$[Ca^{2+}]_i$的影响. **方法**: 用比浊法检测血小板聚集和用Fura-2荧光法检测$[Ca^{2+}]_i$. **结果**: 金雀异黄素强烈地抑制凝血酶(250 $U \cdot L^{-1}$)诱导的猪血小板聚集, 当金雀异黄素的浓度为5和20 $\mu mol \cdot L^{-1}$时, 它对聚集的抑制率分别为52 %和73 %. 当血小板胞外存在Ca^{2+} 1 $mmol \cdot L^{-1}$时, 金雀异黄素抑制凝血酶(500 $U \cdot L^{-1}$)诱导的血小板$[Ca^{2+}]_i$的升高; 金雀异黄素对凝血酶诱导的钙释放无影响. **结论**: 金雀异黄素是潜在的抗血小板药物, 抑制钙内流导致抑制$[Ca^{2+}]_i$的升高, 这与其抑制血小板聚集有关.