BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 May; 16 (3): 223—226

# Effects of quercetin on aggregation and intracellular free calcium of platelets

XIAO Dong, GU Zhen-Lun (*Department of Pharmacology, Suzhou Medical College, Suzhou 215007, China*) BAI Jian-Ping (*Department of Pharmacology, Datong Medical College, Datong 037008, China*) WANG Zhong (*Department of Pharmacology, the Chinese Academy of Medical Sciences, Beijing 100005, China*)

**AIM**: To study the effects of Que on the intraplatelet free calcium concentration and the effects of calcium on the inhibition of platelet aggregation by Que. **METHODS**: Using Quin-2 fluorescence technique. **RESULTS**: Que inhibited the platelet aggregation and the rise of $[Ca^{2+}]_i$ induced by thrombin in platelets. The values of $IC_{50}$ and 95 % confidence interval were 146.2 (92.4—231.3) and 78.5 (49.5 —124.4) $\mu mol \cdot L^{-1}$, respectively. The inhibitory effects of Que on platelet aggregation induced by thrombin were reduced by adding calcium to the medium, and Que had no effect on thrombin-induced internal $Ca^{2+}$ release from dense tubular system. **CONCLUSION**: The inhibitory effects of Que on aggregation and the rise of $[Ca^{2+}]_i$ in platelets was mainly due to an inhibition of $Ca^{2+}$ influx.

**KEY WORDS** quercetin; thrombin; platelet aggregation; calcium

Our previous studies showed that quercetin (Que) possessed an action on platelet aggregation in rats and in human platelets and on reperfusion-induced arrhythmias in rats[1—3]. An elevation of the cytosolic free calcium concentration was thought to the trigger for secretion, shape-change, and aggregation of platelets[4—6]. In the present work, we investigated the effects of Que on the intraplatelet free calcium concentration and the effects of calcium on the inhibition of platelet aggregation by Que to clarify the mechanism of the inhibitory actions of Que.

Received 1993-05-12 Accepted 1994-12-27

## MATERIALS AND METHODS

**Reagents** Que was from the Shanghai Second Chemical Reagent Factory (900905); Quin 2-AM, digitonin, epoprostenol, and thrombin were from Sigma. All other reagents were AR and dissolved triple distilled water.

**Preparation of platelets** Human blood was obtained from 56 healthy volunteers (M 30, F 26), aged 20—30 a. They had not been given antiplatelet drugs for at least 2 wk. Blood 10 mL was anticoagulated with acid citrate: dextrose (ACD) 1 : 6 (vol : vol). Platelets were obtained from platelet-rich plasma (PRP) in our laboratory[2], resuspended in a standard medium containing NaCl 145, KCl 5, $MgSO_4$ 1, glucose 10, HEPES 10 $mmol \cdot L^{-1}$, and maintained at 37 C, and pH 7.4. The washed platelet suspension was used for both the fluorescence and the aggregation studies. The platelet count was adjusted to $2 \times 10^{11} \cdot L^{-1}$.

**Aggregation experiments** Platelet aggregation was studied at 37 C using Born's method in a platelet aggregometer (TYXN-91, Shanghai)[7]. A final concentration of thrombin 500 $U \cdot L^{-1}$ was used in saline in a volume of 10 $\mu$L. Platelet aggregation was measured and the maximal deflection was obtained after 5 min of curve registration computed as a percentage of maximal aggregation.

**Measurement of cytosolic free $Ca^{2+}$** The measurement of $[Ca^{2+}]_i$ was performed using the fluorescent calcium indicator Quin-2[5]. Platelets were loaded with Quin 2-AM 15 $\mu mol \cdot L^{-1}$ and washed with $Ca^{2+}$-free HEPES buffer. Informations about the 3 types of passive $Ca^{2+}$ fluxes in human platelets were got by

Quin-2 techniques: (1) the basal $Ca^{2+}$, (2) the thrombin-induced $Ca^{2+}$ influx, and (3) the thrombin-induced internal $Ca^{2+}$ release. The latter 2 were quantitated by $Ca^{2+}$ containing medium (0.5 mmol·$L^{-1}$) and $Ca^{2+}$-depletion (adding egtazic acid 1 mmol·$L^{-1}$). Quin-2 fluorescence was measured at 37 C in a Hatachi-850 fluorescence spectrophotometer with 339 nm $\lambda_{ex}$ and 492 nm $\lambda_{em}$. $[Ca^{2+}]_i$ values were calculated [5].

The data were expressed as $\bar{x} \pm s$. The significance was evaluated by group comparison of $t$ test for 2 values and by ANOVA for more than 2 values.

## RESULTS

### Effects of Que on platelet aggregation

Que caused a concentration dependent inhibition of platelet aggregation. The $IC_{50}$ and 95 % confidence interval was 146.2 (92.4 — 231.3) μmol·$L^{-1}$ ($r$=0.9645, Tab 1).

**Tab 1. Effect of quercetin on human platelet aggregation by thrombin (500 U·$L^{-1}$). $n$=6. $\bar{x} \pm s$. $^cP<0.01$ vs control.**

| Quercetin/ μmol·$L^{-1}$ | Aggregation/ % | Inhibition/ % |
|---|---|---|
| 0 | 82.3±3.7 | |
| 75 | 64.4±6.5$^c$ | 21.7 |
| 150 | 33.6±6.0$^c$ | 59.2 |
| 300 | 18.5±4.3$^c$ | 77.5 |
| 600 | 9.3±1.0$^c$ | 88.7 |

**Effect of $Ca^{2+}$ on inhibition of platelet aggregation by Que** Adding $CaCl_2$ (1 mmol·$L^{-1}$) to the platelet suspension, it showed neither leading to platelet aggregation nor increasing the aggregation of platelets induced 500 U·$L^{-1}$ thrombin ($P>0.05$). But the inhibitory effect of Que of thrombin (500 U·$L^{-1}$)-induced platelet aggregation was reduced ($P<0.01$, Tab 2).

**Effect of Que on $[Ca^{2+}]_i$ level in platelets** The basal level of $[Ca^{2+}]_i$ in resting platelets was 102±8 nmol·$L^{-1}$ ($n$=8), $CaCl_2$ 0.5 mmol·$L^{-1}$ and thrombin 500 U·$L^{-1}$ induced a rise in $[Ca^{2+}]_i$ from the basal level to 179±13 ($P<0.05$, $n=8$) and 933±66 nmol·$L^{-1}$ ($P<0.01$, $n$=8), respectively.

**Tab 2. Effect of calcium on inhibition of platelet aggregation by quercetin. $n=4$. $\bar{x} \pm s$. $^cP<0.01$ vs Que 0. $^dP>0.05$, $^fP<0.01$ vs $CaCl_2$ 0 and 1 mmol·$L^{-1}$ in platelets.**

| Quercetin/ μmol·$L^{-1}$ | Thrombin/ U·$mL^{-1}$ | Aggregation/% Free calcium | Aggregation/% $CaCl_2$/ 1 mmol·$L^{-1}$ |
|---|---|---|---|
| 0 | 0.5 | 85.9±6.9 | 92.7±4.9$^d$ |
| 600 | 0.5 | 9.8±1.5$^c$ | 57.6±2.7$^{cf}$ |

Adding egtazic acid 1 mmol·$L^{-1}$ to the platelet suspension decreased the cytoplasmic free $Ca^{2+}$ concentration from 102±8 to 74±9 nmol·$L^{-1}$ ($n$=8, $P<0.05$). At a concentration of thrombin (500 U·$L^{-1}$), the $Ca^{2+}$ released from dense tubular system caused an increase of $[Ca^{2+}]_i$ from 74±9 to 133±12 nmol·$L^{-1}$ ($P<0.05$, $n$=8). Que 25 — 400 μmol·$L^{-1}$ showed a significant inhibition of the $[Ca^{2+}]_i$ rise induced by thrombin in a dose-dependent manner, and Que 400 μmol·$L^{-1}$ decreased the response to thrombin to 83.8 % ($P<0.01$, $IC_{50}$ and 95 % confidence interval was 78.5 (49.5 — 124.4) μmol·$L^{-1}$, $r$=0.9967, Tab 3), but Que 400 μmol·$L^{-1}$ had

**Tab 3. Effect of quercetin on $[Ca^{2+}]_i$ of platelet activated by thrombin (500 U·$L^{-1}$). $n$=8. $\bar{x} \pm s$. $^dP>0.05$, $^cP<0.01$ vs saline.**

| Quercetin/ μmol·$L^{-1}$ | $[Ca^{2+}]_i$/ nmol·$L^{-1}$ | Inhibition/ % |
|---|---|---|
| 0 | 933±66 | |
| 12.5 | 910±62$^d$ | 2.5 |
| 25.0 | 714±62$^c$ | 23.5 |
| 50.0 | 592±49$^c$ | 36.5 |
| 100.0 | 431±75$^c$ | 53.9 |
| 200.0 | 246±65$^c$ | 73.6 |
| 400.0 | 151±26$^c$ | 83.8 |

no effect on thrombin-induced internal $Ca^{2+}$ release from dense tubular system ($[Ca^{2+}]_i$ was 127±13 nmol·$L^{-1}$, $P>0.05$ *vs* saline).

## DISCUSSION

The results showed that Que inhibited platelet aggregation induced by thrombin in a dose-dependent manner, but the inhibitory effect was reduced by adding $CaCl_2$ to the medium. It suggested that the effects of Que on platelet function are possibly related to calcium.

The present study applied Quin-2 indicator methods to the quantitative study of 3 types of passive $Ca^{2+}$ movement in platelets. Que inhibited $Ca^{2+}$ influx in thrombin-activated platelets, but had no effect on thrombin-induced $Ca^{2+}$ release from dense tubular system. Calcium influx is a majar pathway for elevating $[Ca^{2+}]_i$ by thrombin. $IP_3$ may be a messenger for intracellular $Ca^{2+}$ release[6,8-9]. These results indicated that, like verapamil, Que does not affect $IP_3$ directly. Its effect may be mediated through the $Ca^{2+}$ channel and postaglandin-related reactions.

Our results showed that $CaCl_2$ 0.5 mmol·$L^{-1}$ produced a rise in $[Ca^{2+}]_i$ in platelets, but no aggregation ($CaCl_2$ 1 mmol·$L^{-1}$), and this indicated that the threshold for the aggregometer responses was relatively high. It has been generally accepted that $[Ca^{2+}]_i$, the $K_m$ value for platelet aggregation is 400 nmol·$L^{-1}$[6], but $CaCl_2$ 0.5 mmol·$L^{-1}$ only increased the $[Ca^{2+}]_i$ to 179 nmol·$L^{-1}$, which was not sufficient to induce aggregometric responses. These results accorded with previous observations[5,6].

The $IC_{50}$ of Que for the inhibition of aggregation is 146.2 μmol·$L^{-1}$, which is higher than the $IC_{50}$ of Que for the inhibition of the thrombin-induced $Ca^{2+}$ rise (78.5 μmol·$L^{-1}$). The $IC_{50}$ for aggregation is higher than that for $Ca^{2+}$ rise because the drug does not inhibit internal $Ca^{2+}$ release. This suggested that the inhibitory effect of Que on the platelet aggregation is mainly due to the inhibition of the $Ca^{2+}$ influx. This observation could be explained from other complex effects of Que in platelets, such as the inhibition of cyclic AMP phosphodiesterase[10], inhibition of $TXA_2$[3,11].

## REFERENCES


1 Gu ZL, Xie ML, Qian ZN. Effects of quercetin on chemiluminescence of human platelets induced by arachidonic acid *in vitro*.
Acta Pharmacol Sin 1993; **14**: 263-5.

2 Gu ZL, Qian ZN, Xiao D, Zhou YH, Qin ZH, Wang ZY. Inhibitory effects of quercetin on platelet and its mechanism. Acta Acad Med Suzhou 1991; **11**: 262-5.

3 Xiao D, Gu ZL, Qian ZN. Effects of quercetin on platelet and reperfusion-induced arrhythmias in rats.
Acta Pharmacol Sin 1993; **14**: 505-8.

4 Kimura Y, Okuda H. Inhibitory effects of soluble elastin on intraplatelet free calcium concentration.
Thromb Res 1988; **52**: 61-4.

5 Rink TJ, Smith SW, Tsien RY. Cytoplasmic free $Ca^{2+}$ in human platelets: $Ca^{2+}$ thresholds and Ca-independent activation for shape-change and secretion.
FEBS Lett 1982; **148**: 21-6.

6 Jy W, Haynes DH. Thrombin-induced calcium movements in platelet activation.
Biochim Biophys Acta 1987; **929**: 88-102.

7 Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal.
Nature 1962; **194**: 927-9.

8 Knezevic I, Dieter JP, Le Breton GC. Mechanism of inositol 1,4,5-trisphosphate-induced aggregation in saponin-permeabilized platelets.
J Pharmacol Exp Ther 1992; **260**: 947-55.

9 Pales J, Palacios-Araus L, Lopez A, Gual A. Effects of dihydropyridines and inorganic calcium blockers on aggregation and on intracellular free calcium in platelets Biochim Biophys Acta 1991; **1064**: 169-74.

10 Schaeffer P, Lugtner C, Follenius-Wund A, Gerard D, Stoclet JC. Comparative effects of calmodulin inhibitors on calmodulin's hydrophobic sites and on the activation of cyclic nucleotide phosphodiesterase by calmodulin.
Biochem Pharmacol 1987; **36**: 1989-96.

11 Corvazier E, Maclouf J. Interference of some flavonoids and non-steroidal anti-inflammatory drugs with oxidative metabolism of arachidonic acid by human platelets and neurrophils. Biochim Biophys Acta 1985; 835:315-21.

## 槲皮素对血小板聚集和胞浆游离钙的影响

肖 东，顾振纶（苏州医学院药理学教研室，苏州 215007，中国） 白建平（大同医学专科学校药理学教研室，大同037008，中国） 汪 钟（中国医学科学院药理学教研室，北京100005，中国）

**目的**：研究槲皮素对凝血酶诱导的血小板聚集和胞浆游离钙浓度的影响及钙对槲皮素的血小板聚集抑制效应的作用。**方法**：用荧光钙离子指示剂观察槲皮素对血小板胞浆游离钙的影响。**结果**：槲皮素明显抑制凝血酶诱导的血小板聚集和游离钙的升高。$IC_{50}$和95 %可信区间分别为146.2（92.4－231.3）和78.5（49.5－124.4）$\mu mol \cdot L^{-1}$。槲皮素对血小板的抑制作用可被钙翻转。槲皮素对凝血酶诱导的钙释放无影响。**结论**：抑制钙内流是槲皮素抑制血小板聚集和$[Ca^{2+}]_i$升高的机制。

**关键词** 槲皮素；凝血酶；血小板聚集；钙

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 May; 16 (3): 226－230

# Effects of propylene glycol mannate sulfate on thrombosis in abdominal aorta in rabbits

CHEN Xiao-Ming, PAN Jia-Qi, ZHANG Zhi-Nan
(*Division of Hematology, Peking Union Medical College Hospital, Beijing 100730, China*)

**AIM**: To study the effects of propylene glycol mannate sulfate (PGMS) on platelet adhesion, aggregation and thrombosis in abdominal sorta in rabbits. **METHODS**: Platelet adhesion assay was performed with a platelet-adhesion-meter. The platelet concentration was determined with an electronic particle counter, and the total radio-activity was determined wiht Clinigamma-1272. **RESULTS**: PGMS inhibited washed platelet aggregation induced by thrombin *in vitro*. The value of $IC_{50}$ was 0.9 $mg \cdot L^{-1}$. (95 % confidence limit = 4.1－13.7 $mg \cdot L^{-1}$). At 10, 30, and 60 min after iv PGMS 75 $mg \cdot kg^{-1}$, the inhibition rates of platelet adhesion were 90.4 %, 41.8 %, and 26.3 % and the inhibition rates of platelet aggregation induced by thrombin were 99 %, 122 %, and 110 %, respectively. It did not exhibit any noticeable effect on platelet aggregation induced by ADP and collagen at this dosage. After 1.5 h of iv PGMS 75 $mg \cdot kg^{-1}$ and total autologous $^{111}$In-platelets $3.3 \times 10^9$ the radioactivity and dry weight of the injured and uninjured segments were determined. The PGMS group showed a significantly lower radioactivity (1.1 ± 0.6 MBq), $^{111}$In-platelets (8.3 ± *s* $3.3 \times 10^8$) as welll as the radioactivity deposited $\cdot g^{-1}$ out of the total radioactivity infused % (0.24 ± 0.21) compared with the control group (3.7 ± 0.5 MBq, 24.6 ± $3.5 \times 10^8$, and 0.86 ± 0.25, respectively). **CONCLUSION**: PGMS prevented the platelet adhesion and aggregation at the injured arterial wall.

Received 1991-10-22 Accepted 1994-07-15

**KEY WORDS** propanediols; platelet aggre-