

Effect of quercetin on platelet aggregation induced by oxyradicals

XIE Mei-Lin, LU Qun, GU Zhen-Lun

(Department of Pharmacology, Suzhou Medical College, Suzhou 215007, China)

KEY WORDS platelet aggregation; adenosine diphosphate; free radicals; chemiluminescence; quercetin; hydrocortisone

AIM: To study the action of quercetin (Que) on inhibiting platelet aggregation. **METHODS:** Active oxygen free radicals produced by xanthine/xanthine oxidase (Xan/XO) reaction was used, platelet aggregation was determined by the turbidimetric method, and the Xan/XO oxyradicals generating reaction by luminol-dependent chemiluminescence (Che) method. **RESULTS:** Active oxygen free radicals enhanced the platelet aggregation induced by ADP $1.6 \mu\text{mol} \cdot \text{L}^{-1}$. The rate of maximal aggregation increased from 29 % - 38 % for ADP to 59 % - 70 % for ADP + Xan/XO. The enhancement was abolished by the treatment of platelet-rich plasma (PRP) with Que $650 \mu\text{mol} \cdot \text{L}^{-1}$ or hydrocortisone (Hyd) $900 \text{mg} \cdot \text{L}^{-1}$. Both Que and Hyd scavenged the active oxyradicals *in vitro*. The Che was decreased by 75.7 % (Que $4 \mu\text{mol} \cdot \text{L}^{-1}$) and 79.0 % (Hyd $900 \text{mg} \cdot \text{L}^{-1}$) as compared with control. **CONCLUSION:** Active oxygen free radicals participated in the platelet aggregation, and scavenging oxyradicals by Que was one of mechanisms of inhibiting platelet aggregation.

Oxygen free radicals produced by myocardial ischemia-reperfusion play an important role in platelet activation^[1] which can result in the platelet hyperaggregability^[2]. Quercetin (Que), one of the natural flavonoids, is known to be a good inhibitor of platelet aggregation^[3,4]. In this paper, the relationship between active oxyradicals and platelet aggregation was investigated *in vitro*, to provide a further understanding of the mechanism of action of Que on inhibiting platelet aggregation.

MATERIALS AND METHODS

Rats Wistar rats ($\hat{\sigma}$, $n = 13$) weighing $221 \pm s 12 \text{ g}$

were provided by the Animal Breeding Center, Suzhou Medical College.

Reagents Que (3, 3', 4', 5, 7-pentahydroxy-flavon; Fluka) was prepared as 0.01, 0.02, 0.03, 0.04, 2.5, and $5.0 \text{ mmol} \cdot \text{L}^{-1}$ solution in 1 % Me_2SO . Xanthine (Xan) was purchased from Sigma. Xanthine oxidase (XO) was supplied by Department of Biology, East China Normal University. Hydrocortisone (Hyd) injection was a product of Shanghai No 9 Pharmaceutical Factory (lot 940703). ADP was purchased from Shanghai Institute of Biochemistry, Chinese Academy of Sciences. Luminol (5-amino-2, 3-dihydro-1,4-phthalazinedione; Aldrich) was prepared as $2 \text{ mmol} \cdot \text{L}^{-1}$ solution in phosphate buffer $50 \text{ mmol} \cdot \text{L}^{-1}$ (pH 7.4) before use. Other were of AR.

Instruments Platelet aggregation and chemiluminescence (Che) were measured with TYNX-91 model aggregometer (Shanghai Institute of General Machinery and Electron Technology) and SHG-1 model lumiphotometer (Experimental Factory of Shanghai Measurement Administration), respectively.

Preparations of platelets Fresh blood was obtained from sodium pentobarbitone-anaesthetized (ip, $45 \text{ mg} \cdot \text{kg}^{-1}$) Wistar rat by abdominal aorta puncture. Whole blood was collected into 1:9 (vol: vol) of 3.8 % trisodium citrate. PRP was prepared by centrifugation at $250 \times g$ for 10 min, and platelet-poor plasma by centrifugation at $1500 \times g$ for 10 min. The platelet count of PRP samples was adjusted to $3 \times 10^{11} \cdot \text{L}^{-1}$.

Assay of platelet aggregation Platelet aggregation was determined by Born's method^[5] with modifications. PRP ($200 \mu\text{L}$) was transferred into a cuvette and prewarmed at $37 \text{ }^\circ\text{C}$ for 3 min before Que or Hyd was added, ADP ($200 \mu\text{mol} \cdot \text{L}^{-1}$) $2 \mu\text{L}$ or plus Xan ($1 \text{ mmol} \cdot \text{L}^{-1}$)/XO ($500 \text{ IU} \cdot \text{L}^{-1}$) $10 \mu\text{L}/5 \mu\text{L}$ was added, and a 5-min aggregation curve was recorded for each cuvette. The inhibitory rate of aggregation (IRA) = (RA of control - RA after drug)/RA of control.

Generation and measurement of oxygen free radicals

Oxygen free radicals were produced when Xan ($0.05 \text{ mmol} \cdot \text{L}^{-1}$) $800 \mu\text{L}$ and XO ($50 \text{ IU} \cdot \text{L}^{-1}$) $50 \mu\text{L}$ were mixed. The oxygen free radicals reacted with luminol ($2 \text{ mmol} \cdot \text{L}^{-1}$, $50 \mu\text{L}$) producing a blue fluorescence ($\lambda = 425 \text{ nm}$).

Statistical analysis The *t*-test was used.

RESULTS

Oxyradicals enhanced platelet aggregation

Slight aggregation of platelet was triggered by low concentration of ADP (final concentration: $1.6 \mu\text{mol} \cdot \text{L}^{-1}$), the mean value obtained being 29 % - 38 %. As ADP and Xan/XO were simultaneously added, the RA increased to 59 % - 70 % ($P < 0.05$) (Tab 1).

Tab 1. Inhibition of oxyradicals-enhanced ADP-induced platelet aggregation by Que or Hyd. $n = 6 - 7$, $\bar{x} \pm s$.

^a $P > 0.05$, ^b $P < 0.05$ vs 1 % $\text{Me}_2\text{SO} + \text{ADP}$, ^c $P < 0.01$ vs 1 % $\text{Me}_2\text{SO} + \text{Xan/XO}$, ^d $P < 0.05$, ^e $P < 0.01$ vs 1 % $\text{Me}_2\text{SO} + \text{Xan/XO} + \text{ADP}$, ^f $P > 0.05$, ^g $P < 0.01$ vs saline + ADP, ^h $P < 0.01$ vs saline + Xan/XO, ⁱ $P < 0.01$ vs saline + Xan/XO + ADP.

	Rate of maximal aggregation/ %	Inhibitory rate of aggregation/ %
1 % $\text{Me}_2\text{SO} + \text{ADP}$	38 ± 8	
1 % $\text{Me}_2\text{SO} + \text{Xan/XO}$	9 ± 5	
1 % $\text{Me}_2\text{SO} + \text{Xan/XO} + \text{ADP}$	$59 \pm 18^{\text{af}}$	
Que $325 \mu\text{mol} \cdot \text{L}^{-1} + \text{ADP}$	$35 \pm 11^{\text{a}}$	7.9
Que $650 \mu\text{mol} \cdot \text{L}^{-1} + \text{ADP}$	$26 \pm 10^{\text{b}}$	31.5
Que $325 \mu\text{mol} \cdot \text{L}^{-1} + \text{Xan/XO} + \text{ADP}$	$45 \pm 9^{\text{h}}$	23.7
Que $650 \mu\text{mol} \cdot \text{L}^{-1} + \text{Xan/XO} + \text{ADP}$	$26 \pm 11^{\text{i}}$	55.9
Saline + ADP	29 ± 8	
Saline + Xan/XO	7 ± 4	
Saline + Xan/XO + ADP	$70 \pm 13^{\text{b}}$	
Hyd $450 \text{mg} \cdot \text{L}^{-1} + \text{ADP}$	$25 \pm 6^{\text{f}}$	13.8
Hyd $900 \text{mg} \cdot \text{L}^{-1} + \text{ADP}$	$21 \pm 7^{\text{f}}$	27.6
Hyd $900 \text{mg} \cdot \text{L}^{-1} + \text{Xan/XO} + \text{ADP}$	$18 \pm 6^{\text{f}}$	74.3

Anti-aggregation effect of Que Que $325 \mu\text{mol} \cdot \text{L}^{-1}$ and $650 \mu\text{mol} \cdot \text{L}^{-1}$ inhibited the platelet aggregation induced by ADP, the IRA being 7.9 % and 31.5 %, respectively. Que $650 \mu\text{mol} \cdot \text{L}^{-1}$ abolished the oxyradicals-stimulated enhancement of platelet aggregation. the RA was reversible in all PRP samples (Tab 1)

Scavenging effect of Que on oxyradicals Que markedly inhibited the Che system, the IC_{50} being 2.8 (95 % confidence limits 1.5 - 4.2) $\mu\text{mol} \cdot \text{L}^{-1}$ (Tab 2).

Hyd effect on the enhancement Hyd 450 - 900 $\text{mg} \cdot \text{L}^{-1}$ yielded no significant influence on platelet aggregation mediated by low concentration

Tab 2. Effect of Que or Hyd on the Xan/XO luminol-dependent chemiluminescence (Che) system. $n = 6$, $\bar{x} \pm s$. ^a $P > 0.05$, ^c $P < 0.01$ vs phosphate buffer solution. ^d $P > 0.05$, ^f $P < 0.01$ vs 1 % Me_2SO .

	Intensity of Che/cp2s	IRA/ %
Phosphate buffer solution	128 ± 18	
1 % Me_2SO	$136 \pm 18^{\text{a}}$	
Que $1.0 \mu\text{mol} \cdot \text{L}^{-1}$	$116 \pm 18^{\text{d}}$	14.7
2.0 $\mu\text{mol} \cdot \text{L}^{-1}$	$99 \pm 13^{\text{f}}$	27.2
3.0 $\mu\text{mol} \cdot \text{L}^{-1}$	$61 \pm 10^{\text{f}}$	55.2
4.0 $\mu\text{mol} \cdot \text{L}^{-1}$	$33 \pm 8^{\text{f}}$	75.7
Hyd $300 \text{mg} \cdot \text{L}^{-1}$	$61 \pm 11^{\text{c}}$	52.3
600 $\text{mg} \cdot \text{L}^{-1}$	$30 \pm 9^{\text{c}}$	75.8
900 $\text{mg} \cdot \text{L}^{-1}$	$26 \pm 8^{\text{c}}$	79.0

of ADP ($P > 0.05$), while Hyd 900 $\text{mg} \cdot \text{L}^{-1}$ abolished the enhancement induced by oxyradicals ($P < 0.01$) (Tab 1).

Scavenging effect of Hyd on oxyradicals

When Hyd concentration increased, the intensity of Che gradually decreased. The inhibitory effect at 900 $\text{mg} \cdot \text{L}^{-1}$ was not different from that at 600 $\text{mg} \cdot \text{L}^{-1}$ (Tab 2). Hyd only scavenged 1 or 2 kinds of active oxygen free radicals caused by Xan/XO reaction system.

DISCUSSION

In this study, effect of active oxygen free radicals on platelet aggregation was observed *in vitro*. In case of PRP, if a low concentration of ADP was used as an inducer of platelet aggregation, active oxygen free radicals produced by Xan/XO reaction enhanced the platelet aggregation (Tab 1). The results suggested that active oxygen free radicals participated in the platelet aggregation.

In order to study the actions of oxyradicals on platelet aggregation, we examined the role of Hyd, an inhibitor of phospholipase A_2 (PLA_2). The results indicated that the Hyd remarkably both inhibited the luminol-dependent Che and abolished the oxyradical-enhanced ADP-induced platelet aggregation, whereas ADP-induced platelet aggregation was not inhibited by Hyd. From these results, we speculated that the enhancement of platelet aggregation induced by oxyradicals resulted from thromboxane A_2 (TXA_2) by its activating

PLA₂^[6], as TXA₂, a metabolism product of membrane phospholipids, can be generated, and it was a potent vasoconstrictor and platelet aggregator. Hyd could reduce TXA₂ formation by inhibiting PLA₂ and scavenging the active oxygen free radicals.

Que not only suppressed the platelet aggregation induced by ADP, but also abolished the oxyradical-enhanced ADP-induced platelet aggregation. On the other hand, Que obviously restrained the luminol-dependent Che as well, this indicated that it could scavenge the oxygen free radicals produced by Xan/XO system, namely, a scavenger of oxyradicals. These results confirmed that the scavenging oxyradicals by Que was one of action mechanism of inhibiting platelet aggregation, this conclusion accorded with previous studies^[7].

REFERENCES

- 1 Xiao D, Gu ZL, Qian ZN. Effects of quercetin on platelet and reperfusion-induced arrhythmias in rats. *Acta Pharmacol Sin* 1993; 14: 505 - 8.
- 2 Ohlstein EH, Storer B, Fujita T, Shebusku RJ. Tissue-type plasminogen activator and streptokinase induce platelet hyperaggregability in the rabbit. *Thromb Res* 1987; 46: 875 - 85.
- 3 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
- 4 Gu ZL, Qian ZN, Wang ZY, Xie ML, Xu X, Wang YQ, et al. Pharmacological studies on leaves of *Apocynum hendersonii* (Hook F) Woodson-their effect on aggregation of blood platelet

Chin Trad Patent Med 1989; 11 (11): 28 - 30.

- 5 Borr GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 1962; 194: 927 - 8.
- 6 Chen LY, Xu SH, Jin SM, Zhang SY, Jin L. Effect of snake venom antithrombus enzyme on the change of platelet induced by oxyradicals. *Basic Med Sci Ch* 1994; 14: 114 - 7.
- 7 Gu ZL, Xie ML, Qian ZN. Effect of quercetin on chemiluminescence of human platelets induced by arachidonic acid. *Acta Pharmacol Sin* 1993; 14: 263 - 5.

334-336

槲皮素对氧自由基诱发血小板聚集性变化的影响

谢梅林, 陆群, 顾振纶

(苏州医学院药理学教研室, 苏州 215007, 中国)

关键词 血小板聚集; 腺苷二磷酸; 自由基; 化学发光; 槲皮素; 氢化可的松

A 目的: 观察氧自由基对血小板聚集性的改变以及探讨槲皮素抑制血小板聚集可能性的作用机制. 方法: 利用黄嘌呤/黄嘌呤氧化酶体系产生的氧自由基, 分别按改良 Born's 法和化学发光法测定了血小板聚集性和氧自由基. 结果: 氧自由基能够加强低浓度 ADP (1.6 μmol·L⁻¹) 诱导的血小板聚集, 聚集率从 29 % - 38 % 增至 59 % - 70 %, 此加强作用可被槲皮素或氢化可的松所取消. 同时, 槲皮素 (4 μmol·L⁻¹) 和氢化可的松 (900 mg·L⁻¹) 在体外均可明显地清除氧自由基, 使发光强度分别下降 75.7 % 和 79.0 %. 结论: 氧自由基参与了血小板聚集过程, 槲皮素对氧自由基的清除作用是其抑制血小板聚集的又一作用机制

R285.5
R965