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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 $\mu mol \cdot L^{-1}$. 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 $\mu mol \cdot L^{-1}$ or hydrocortisone (Hyd) 900 $mg \cdot L^{-1}$. Both Que and Hyd scavenged the active oxyradicals *in vitro*. The Che was decreased by 75.7 % (Que 4 $\mu mol \cdot L^{-1}$) and 79.0 % (Hyd 900 $mg \cdot L^{-1}$) 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 (♂, $n = 13$) weighing 221 ± s 12 g were provided by the Animal Breeding Center, Suzhou Medical College.

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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 $mmol \cdot L^{-1}$ 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 № 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 $mmol \cdot L^{-1}$ solution in phosphate buffer 50 $mmol \cdot L^{-1}$ (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 $mg \cdot kg^{-1}$) 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 × g for 10 min, and platelet-poor plasma by centrifugation at 1 500 × g for 10 min. The platelet count of PRP samples was adjusted to $3 \times 10^{11} \cdot L^{-1}$.

Assay of platelet aggregation Platelet aggregation was determined by Born's method[5] with modifications. PRP (200 μL) was transfered into a cuvette and prewarmed at 37 ℃ for 3 min before Que or Hyd was added. ADP (200 $\mu mol \cdot L^{-1}$) 2 μL or plus Xan (1 $mmol \cdot L^{-1}$)/XO (500 $IU \cdot L^{-1}$) 10 μL/5 μL 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 $mmol \cdot L^{-1}$) 800 μL and XO (50 $IU \cdot L^{-1}$) 50 μL were mixed. The oxygen free radicals reacted with luminol (2 $mmol \cdot L^{-1}$, 50 μL) producing a blue fluorescence (λ = 425 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 $\mu mol \cdot L^{-1}$), 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 % Me_2SO + ADP, $^{f}P < 0.01$ *vs* 1 % Me_2SO + Xan/XO. $^{h}P < 0.05$, $^{i}P < 0.01$ *vs* 1 % Me_2SO + Xan/XO + ADP, $^{j}P > 0.05$, $^{l}P < 0.01$ *vs* saline + ADP. $^{o}P < 0.01$ *vs* saline + Xan/XO. $^{r}P < 0.01$ *vs* saline + Xan/XO + ADP.

	Rate of maximal aggregation/ %	Inhibitory rate of aggregation/ %
1 % Me_2SO + ADP	38 ± 8	
1 % Me_2SO + Xan/XO	9 ± 5	
1 % Me_2SO + Xan/XO + ADP	59 ± 18^{bf}	
Que 325 $\mu mol \cdot L^{-1}$ + ADP	35 ± 11^{a}	7.9
Que 650 $\mu mol \cdot L^{-1}$ + ADP	26 ± 10^{b}	31.5
Que 325 $\mu mol \cdot L^{-1}$ + Xan/XO + ADP	45 ± 9^{h}	23.7
Que 650 $\mu mol \cdot L^{-1}$ + Xan/XO + ADP	26 ± 11^{i}	55.9
Saline + ADP	29 ± 8	
Saline + Xan/XO	7 ± 4	
Saline + Xan/XO + ADP	70 ± 13^{lo}	
Hyd 450 $mg \cdot L^{-1}$ + ADP	25 ± 6^{j}	13.8
Hyd 900 $mg \cdot L^{-1}$ + ADP	21 ± 7^{j}	27.6
Hyd 900 $mg \cdot L^{-1}$ + Xan/XO + ADP	18 ± 6^{r}	74.3

Anti-aggregation effect of Que Que 325 $\mu mol \cdot L^{-1}$ and 650 $\mu mol \cdot L^{-1}$ inhibited the platelet aggregation induced by ADP, the IRA being 7.9 % and 31.5 %, respectively. Que 650 $\mu mol \cdot L^{-1}$ 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) $\mu mol \cdot L^{-1}$ (Tab 2).

Hyd effect on the enhancement Hyd 450 - 900 $mg \cdot L^{-1}$ yielded no significant influence on platelet aggregation mediated by low concentration of ADP ($P > 0.05$), while Hyd 900 $mg \cdot L^{-1}$ abolished the enhancement induced by oxyradicals ($P < 0.01$) (Tab 1).

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 ± 18^{a}	
Que 1.0 $\mu mol \cdot L^{-1}$	116 ± 18^{d}	14.7
2.0 $\mu mol \cdot L^{-1}$	99 ± 13^{f}	27.2
3.0 $\mu mol \cdot L^{-1}$	61 ± 10^{f}	55.2
4.0 $\mu mol \cdot L^{-1}$	33 ± 8^{f}	75.7
Hyd 300 $mg \cdot L^{-1}$	61 ± 11^{c}	52.3
600 $mg \cdot L^{-1}$	30 ± 9^{c}	75.8
900 $mg \cdot L^{-1}$	26 ± 8^{c}	79.0

Scavenging effect of Hyd on oxyradicals When Hyd concentration increased, the intensity of Che gradually decreased. The inhibitory effect at 900 $mg \cdot L^{-1}$ was not different from that at 600 $mg \cdot L^{-1}$ (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 remarkedly 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

PLA_2[6], as TXA_2, a metabolism product of membrane phospholipids, can be generated, and it was a potent vasoconstrictor and platelet aggregator. Hyd could reduce TXA_2 formation by inhibiting PLA_2 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].

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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

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

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