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Effects of quercetin on production and release of endothelin and cGMP from cultured endothelial cells

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KEY WORDS quercetin; umbilical veins; vascular endothelium; cultured cells; thrombin; platelets; endothelins; cyclic GMP

AIM: To evaluate the effects of quercetin (Que) on the formation and release of endothelin (ET) and cyclic guanosine monophosphate (cGMP) by endothelial cells. **METHODS**: Radioimmunoassay (RIA) was used to assess the amounts of ET and cGMP produced by unstimulated human umbilical vein cells (HUVEC) or HUVEC stimulated with activated platelets and thrombin. **RESULTS**: Following the treatment of the cultured HUVEC with activated platelets, the amount of ET in the medium was increased, but no significant effect on cGMP within the cells was seen. Thrombin not only increased the amount of ET in the medium, but also raised the level of cGMP within the cells. Pretreatment of HUVEC with Que 0.5, 5, and 50 $\mu mol \cdot L^{-1}$, the release of ET by HUVEC stimulated with thrombin and thrombin-activated platelets was much inhibited. Que 0.5－50 $\mu mol \cdot L^{-1}$ markedly increased the amount of cGMP in HUVEC stimulated with thrombin and activated platelets. Que also inhibited the release of ET from unstimulated HUVEC, but increased the production of cGMP from unstimulated EC. **CONCLUSION**: Thrombin and activated platelets stimulated the release of ET from HUVEC and Que inhibited the release of ET and promoted the formation of cGMP from HUVEC.

Quercetin (Que) had strong antiplatelet effects[1-4], including the inhibition of platelet aggregation induced by adenosine diphosphate (ADP), thrombin, arachidonic acid, and the inhibition of production and release of thromboxane B_2 and 5-hydroxytryptamine by platelets[5]. It raised cAMP level, inhibited cGMP phosphodiesterase, and elevated cGMP level in some cells[6,7]. In the present work, we investigated the effects of Que on the production and release of cGMP and endothelin (ET) from cultured endothelial cells.

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MATERIALS AND METHODS

Chemicals Que was produced by Beijing Chemical Reagent Factory. RIA kits for ET and cGMP were produced by General Hospital of People's Liberation Army (Beijing, China) and Shanghai Medical University (Shanghai, China), respectively.

Cell culture Endothelial cells from human umbilical veins were cultured in DMEM (Sigma) supplemented with 20 % FBS, penicillin (1×10^5 $IU \cdot L^{-1}$), and streptomycin (100 $mg \cdot L^{-1}$) at 37 ℃ in 5 % CO_2 + 95 % air.

Platelet suspension Platelet pellet was obtained by spinning human blood from healthy donors (who were not taking drugs). The final pellet was resuspended with solution (KCl 2.6, NaCl 135, $MgCl_2 \cdot 6H_2O$ 1.0, and Glucose 5 $mmol \cdot L^{-1}$, FCS 0.25 %, pH 7.4) so as to adjust the platelet concentration to 2×10^{11}/L. The washed platelets were activated by incubating the platelets with thrombin (2000 $U \cdot L^{-1}$) at 37 ℃ for 5 min.

Protocol Cultured confluent human umbilical vein endothelial cells in plastic dishes were washed with HEPES buffer for 3 times. The confluent cells were incubated in DMEM without serum at 37 ℃. After preincubation for 10 min, thrombin-activated platelets (final concentration 2×10^{10}/L medium) and thrombin (2000 $U \cdot L^{-1}$) were added, and incubated at 37 ℃. In control experiments, HEPES buffer was used instead of platelet suspension. Que 0.5, 5, and 50 $\mu mol \cdot L^{-1}$ were added during the 10-min preincubation. The conditioned medium was collected at 4 and 24 h after incubation and centrifuged at $1500 \times g$ for 15 min and the supernatant fluid was used in the determination for ET. The cells were collected at 25 min and treated for the measurement of cGMP. ET and cGMP were determined by RIA[8,9].

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

RESULTS

Thrombin and thrombin-activated platelets

increased the release of ET from HUVEC at 4 and 24 h after coincubation ($P<0.01$, Tab 1).

Tab 1. Effects of Que on ET release from HUVEC unstimulated and stimulated by thrombin or thrombin-activated platelets. $n=6$ samples (each was pooled from 6 cultures and assayed in triplicate), $\bar{x}\pm s$. $^cP<0.01$ vs unstimulated; $^eP<0.05$, $^fP<0.01$ vs control.

Time	Que/ μmol·L⁻¹	Endothelin in medium/ng·L⁻¹ Unstimulated	TH-stimulated	APL-stimulated
4 h	0	39±8	136 ± 20^c	123 ± 14^c
	0.5	29±6	108±19	107±12
	5	7 ± 1^e	18 ± 5^f	37 ± 4^f
	50	3 ± 1^e	4 ± 1^f	6 ± 1^f
24 h	0	354±39	545 ± 28^c	529 ± 45^c
	0.5	290±24	441±60	282 ± 24^f
	5	183 ± 17^f	262 ± 47^f	84 ± 7^f
	50	66 ± 8^f	61 ± 8^f	39 ± 9^f

Thrombin raised the amount of cGMP within HUVEC at 25 min after coincubation ($P<0.01$, Tab 2), but the amount of cGMP showed no significant change in group treated with thrombin-stimulated platelets.

Tab 2. Effects of Que on cGMP production from HUVEC unstimulated and stimulated by thrombin or thrombin-activated platelets. $n=6$ samples (each was pooled from 6 cultures and assayed in triplicate), $\bar{x}\pm s$. $^cP<0.01$ vs unstimulated; $^fP<0.01$ vs control.

Que/ μmol·L⁻¹	Cyclic guanosine monophosphate/pmol·L⁻¹ Unstimulated	TH-stimulated	APL-stimulated
0	114±160	152 ± 3^c	113±9
5	159 ± 9^f	191±61	162±44
50	201 ± 12^f	248 ± 39^f	224 ± 67^f

Preincubation with HUVEC for 10 min, Que decreased the amounts of ET in the culture medium of HUVEC unstimulated and stimulated with thrombin or thrombin-activated platelets at 4 and 24 h (Tab 1).

Coincubation with HUVEC, Que 5 and 50 μmol·L⁻¹ stimulated the formation of cGMP within the cells at 25 min. Que also increased the amount of cGMP in the cells treated with thrombin and thrombin-activated platelets, following the preincubation (Tab 2). Que increased the formation of cGMP by HUVEC unstimulated and stimulated with thrombin or activated platelets.

DISCUSSION

The results showed that Que increased the formation of cGMP by HUVEC both unstimulated and stimulated with thrombin or thrombin-activated platelets. To some extent, the level of NO could be expressed as the amount of cGMP in EC[10]. So, we concluded that Que increased the formation of NO. NO is a kind of endothelium-derived relaxing factor (EDRF)[11]. NO reduced $[Ca^{2+}]_i$ in smooth muscle cells, resulting in vascular relaxation, by a cGMP-mediated way[12]. NO is also a kind of protective factor[13]. These suggested that Que could protect the endothelial cells, relax the vessel and then prevent the adhesion and aggregation of platelets and thrombosis, by stimulating the formation of NO.

In our present study, thrombin and thrombin-activated platelets increased ET release from cultured HUVEC, but Que remarkably inhibited the release of ET from HUVEC. ET is a potent and long-lasting vasoconstrictor, and ET release is mediated by Ca^{2+} and protein kinase C[14]. On the other hand, EDRF regulates the production of ET by a cGMP dependent mechanism. In our another experiment (data not shown), Que reduced $[Ca^{2+}]_i$ in endothelial cells. It was proposed that Que inhibited ET release by increasing cGMP and decreasing $[Ca^{2+}]_i$ in endothelial cells.

It is concluded from the present studies that Que has protective effect in endothelial cells and is benificial to the prevention of cardiocerebral vascular illness.

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槲皮素对培养内皮细胞产生和释放内皮素和环磷酸鸟苷的影响

R 963
R 977

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关键词　槲皮素；脐静脉；血管内皮；培养的细胞；凝血酶；血小板；内皮素；环鸟苷一磷酸

目的：研究槲皮素(Que)对不同状态下的内皮细胞形成和释放内皮素(ET)及环鸟苷一磷酸(cGMP)的影响　**方法**：采用放射免疫分析法测定内皮细胞培养液中ET量和内皮细胞内cGMP量　**结果**：凝血酶和活化血小板刺激后的内皮细胞培养液中ET量明显增加；同时，凝血酶作用的内皮细胞内cGMP量升高．Que 0.5，5，50 μmol·L^{-1}明显降低凝血酶和活化血小板作用的内皮细胞培养液中ET量，升高细胞内cGMP量．Que也降低正常内皮细胞培养液中ET量，增加细胞内cGMP量．**结论**：Que对这三种状态下的内皮细胞均抑制其释放ET，促进其形成cGMP

面向二十一世纪的新药研究与开发学术研修会

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