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Effect of quercetin on activities of protein kinase C and tyrosine protein kinase from HL-60 cells

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KEY WORDS HL-60 cells; quercetin; protein kinase C; tyrosine; protein kinases; enzyme inhibitors; cultured tumor cells

AIM: To study the effect of quercetin (Que) on the activities of cytosol and membrane protein kinase C (PKC) and tyrosine protein kinase (TPK) from HL-60 cells *in vitro*. **METHODS**: The number of viable cells was counted by a trypan blue dye exclusion test. PKC activiy was assayed by incubating PKC with histone ⅢS and [γ-^{32}P]ATP. TPK activity was assayed by incubating TPK with poly glutamate·tyrosine (4:1). **RESULTS**: Que inhibited the proliferation of HL-60 cells in a concentration-dependent manner, its IC_{50} was 29 (22 - 37) $\mu mol \cdot L^{-1}$ after 48-h treatment; Que strongly inhibited the activity of cytosol PKC and membrane TPK with IC_{50} 31 (20 - 48) $\mu mol \cdot L^{-1}$, 24 (13 - 45) $\mu mol \cdot L^{-1}$, respectively, but did not affect membrane PKC and cytosol TPK from HL-60 cells *in vitro*. **CONCLUSION**: The inhibitory effect of Que on the growth of tumor cells is related to its inhibitory effects on PKC and/or TPK.

Quercetin (Que) inhibited the growth of cancer cell lines[1-5]. The antiproliferative activity of this compound might be mediated by the type II estrogen binding sites (type II EBS)[3], and/or by the down-regulation of mutant p53[4], and/or by the induction of apoptosis[5], and/or by the effect on cell cycle[6].

Protein kinase C (PKC) and tyrosine protein kinase (TPK) play important roles in the growth and proliferation of cells and development of cancer[7-9]. Que inhibited PKC and TPK[10-12], but there was no direct evidence to show that such inhibitory effect was related to its inhibitory effects on the growths of tumor cells. In this paper, we examined the effects of Que on the proliferation of HL-60 cells, and on the activities of cytosol and membrane PKC and TPK from HL-60 cells *in vitro*.

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MATERIALS AND METHODS

Que, egtazic acid, diolein, histone ⅢS, phosphatidylserine (PS), leupeptin, $NaVO_3$, poly glutamate·tyrosine (4:1) and ATP were purchased from Sigma. RPMI 1640 was obtained from Gibco. Triton X-100 was purchased from Farco. DTT and edetic acid were obtained from Serva. pNPP and PMSF were purchased from Shanghai Institute of Biochemistry. Fetal bovine serum was obtained from Sijqing Institute of Biomaterials, Hangzhou, China. [γ-^{32}P]ATP was purchased from Beijing Yahui Biomedical Technology Co Ltd. All other chemicals were of reagent grade.

Cell culture HL-60, a human promyelocytic leukemia cell line, maintained in our laboratory, was cultured in RPMI 1640 medium supplemented with 10 % heat-inactivated fetal bovine serum in 5 % CO_2 atmosphere.

Growth inhibition of cells Cells were seeded 5×10^7 cells·L^{-1} medium in 60-mm diameter dishes. Que, dissolved in Me_2SO (this volume of Me_2SO added to control dishes had no measurable effects on HL-60 cells) was added. After 48-h treatment, the number of viable cells was counted by a trypan blue dye exclusion test.

PKC assay The method[13] was modified by us[12].

TPK assay The method[14] was modified by us[12].

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

RESULTS

Proliferation of HL-60 cells A concentration-dependent inhibition was induced by Que 20 - 80 $\mu mol \cdot L^{-1}$ after 48-h treatment. Its 50 % inhibitory concentration (IC_{50}) was 29 (22 - 37) $\mu mol \cdot L^{-1}$ (Tab 1).

Activities of cytosol and membrane PKC from HL-60 cells Que inhibited the activities of cytosol PKC from HL-60 cells with IC_{50} of 31 (20 - 48) $\mu mol \cdot L^{-1}$. When Que was at 120 $\mu mol \cdot L^{-1}$, the activity of cytosol PKC was 11 % of the control. But Que did not affect the activity of membrane

Tab 1. Effects of Que on proliferation of HL-60 cells after 48-h treatment. $\bar{x} \pm s$.

Quercetin/ $\mu mol \cdot L^{-1}$	$10^{-3} \times$ Viable cells	Inhibition rate/%
0	209 ± 9	0.0
10	152 ± 14	27.3
20	125 ± 5	40.1
40	98 ± 6	53.0
80	46 ± 7	78.2

The experiment was repeated 4 times.

PKC from HL-60 cells (Tab 2).

Activities of cytosol and membrane TPK from HL-60 cells Que inhibited the activity of membrane TPK from HL-60 cells with IC_{50} value of 24 (13 − 45) $\mu mol \cdot L^{-1}$. At 120 $\mu mol \cdot L^{-1}$, the inhibitory rate of Que on the TPK was 95 %. But Que had no effect on activity of cytosol TPK from HL-60 cells (Tab 2).

DISCUSSION

Que inhibits the growths of cancer cell lines[1-6]. Our result that Que strongly inhibits the growth of HL-60 cells, is consistent with the papers[1,2], confirms, and extends those previous reports[3-6].

The antiproliferative activity of Que might be related to type II EBS[3], mutant p53[4], apoptosis[5], and cell cycle[6]. There were many studies showing that Que could inhibit TPK and PKC[10,11]. It was well-known that PKC and TPK played important roles in the cell growth, especially in the proliferation of tumor cells[7-9]. In general, the activity of membrane TPK is much more higher than that of cytosol in normal or tumor cells, but PKC mainly exists in cytosol. In this paper, our results of the activities of cytosol and membrane TPK and PKC from HL-60 cells also proved this view. There are different forms of TPK and isozymes of PKC between membrane and cytosol[7-9]. The study showed that Que markedly inhibited the activity of cytosol PKC and membrane TPK from HL-60 cells *in vitro*. We suppose that some forms of TPK and isozymes of PKC be not sensitive to Que, and that the antiproliferative activity of Que on HL-60 cells may be directly related to its inhibitory effects on cytosol PKC and/or membrane TPK, because PKC and TPK have been implicated to play some roles in signal transduction of type II EBS[3] and apoptosis[15], and in the regulation of mutant p53 and cell cycle[7,8]. It provides another direct evidence that inhibitory effect of Que on PKC and/or TPK was one of important causes for its inhibitory effect on the growth of tumor cells.

In the moment, we have approached the mechanisms of inhibitory effect of Que on the proliferation of HL-60 cells, and made some studies on effect of Que on the cell cycle and phosphoinositides turnover (the results will be shown in other papers). It makes a foundation for further studies on Que and for its clinical applications.

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Tab 2. Effects of Que on the activity of cytosol PKC and TPK from HL-60 cells. $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.

Quercetin/ $\mu mol \cdot L^{-1}$	Protein kinase C/Bq		Tyrosine protein kinase/Bq	
	Cytosol	Membrane	Cytosol	Membrane
0	16.4 ± 1.2 (0 %)	2.48 ± 0.16	2.82 ± 0.14	5.88 ± 0.15 (0 %)
7.5	14.6 ± 1.1 (12 %)	2.48 ± 0.17	2.76 ± 0.14	4.5 ± 0.8 (7 %)
15	11.4 ± 0.4 (30 %)^b	2.3 ± 0.3	2.69 ± 0.10	3.60 ± 0.05 (39 %)^b
30	8.4 ± 0.5 (49 %)^c	2.2 ± 0.4	2.65 ± 0.18	2.19 ± 0.07 (64 %)^c
60	5.8 ± 0.5 (64 %)^c	2.2 ± 0.4	2.60 ± 0.14	1.0 ± 0.3 (82 %)^c
120	1.8 ± 0.6 (89 %)^c	2.10 ± 0.22	2.53 ± 0.14	0.28 ± 0.03 (95 %)^c

The experiments for PKC and TPK were repeated 3 and 4 times, respectively.

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槲皮素对 HL-60 细胞中蛋白激酶 C 和酪氨酸蛋白激酶活性的影响

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关键词 HL-60 细胞；槲皮素；蛋白激酶 C；酪氨酸；蛋白激酶类；酶抑制剂；培养的肿瘤细胞

目的：研究槲皮素(Que)对 HL-60 细胞中细胞溶质和胞膜蛋白激酶 C(PKC)、酪氨酸蛋白激酶(TPK)活性的影响. **方法**：活细胞数的计数用苔盼兰拒染法；用组蛋白ⅢS、[γ-^{32}P]ATP 与 PKC 酶液一起保温测定 PKC 活性；用聚谷氨酸·酪氨酸(4:1)多肽)、[γ-^{32}P]ATP 与 TPK 酶液一起保温测定 TPK 活性. **结果**：Que 对 HL-60 细胞的增殖有抑制作用，呈剂量依赖关系，处理 48 小时后，其 IC_{50} 为 29 (22 – 37) $\mu mol \cdot L^{-1}$；在体外，Que 能强烈抑制细胞溶质 PKC 和胞膜 TPK 活性，其 IC_{50} 分别为：31 (20 – 48) $\mu mol \cdot L^{-1}$，24 (13 – 45) $\mu mol \cdot L^{-1}$，但不影响胞膜 PKC 和细胞溶质 TPK 活性. **结论**：这为 Que 对癌细胞的生长有抑制作用与其抑制 PKC 和/或 TPK 有关提供了直接证据.