Inhibitory effect of compound 6F isolated from *Pteris semipinnata* L. on the activity of protein kinase C in HL-60 cells

HE Cheng-Wei[1], LIANG Nian-Ci[2*], MO Li-Er[3], LI Jin-Hua[1], ZHANG Xiao[1]

(1. *Institute of Biochemistry and Molecular Biology*, 2. *Guangdong Key Laboratory for Research and Development of Natural Drugs*, 3. *Department of Chemistry*, *Guangdong Medical College*, *Zhanjiang* 524023, *China*)

Abstract: **AIM** To investigate whether compound 6F isolated from *Pteris semipinnata* L inhibits the activity of protein kinase C(PKC) and whether the DNA fragment induction and cytotoxicity of 6F on HL-60 cells relate to PKC signaling pathway. **METHODS** HL-60 cells were used as *in vitro* model and its cytosol(soluble sample) and particle(insoluble sample including membrane system and nuclei) fractions obtained by ultracentrifugation were used as samples for PKC assay. PKC activity was measured by incorporation of [γ-^{32}P]ATP into exogenous substrate after stimulated by phosphatidylserine and diolein. Diphenylamine assay and MTT staining methods were applied for DNA fragmentation detection and cytotoxicity assay, respectively. **RESULTS** PKC activities in cytosol and particle fractions were inhibited by 6F in a dose-dependent manner($P < 0.05$ in the series of cytosol fraction, $P < 0.01$ in the series of particle fraction). Phorbol 12-myristate 13-acetate(PMA,65 nmol·L^{-1}) partially attenuated the DNA fragmentation induced by 6F in HL-60 cells, and protected cells against the cytotoxic effect of 6F ($P < 0.01$). The ability of reducing MTT to formazan in mitochondria of HL-60 cells was potentiated when the cells were treated with PMA alone at the same concentration for 24 h($P < 0.01$). **CONCLUSION** Compound 6F is an inhibitor for PKC. DNA fragment induction and cytotoxic effect of 6F may be partially through its inhibitory effect on PKC activity.

Received date: 2002-08-26 **Accepted date**: 2002-10-18

Foundation item: The project supported by National Natural Science Foundation of China(39870900); the Key Research Project Foundation of Guangdong Province(9622007-002); and the Key Subject Foundation of Guangdong Province(9306)

Biographies: HE Cheng-Wei(1972 –), male, native of Nankang, Jiangxi Province, Ph.D., main research fields are biochemical pharmacology, molecular biology of antitumor drugs, and experimental cancer gene therapy; Liang Nian-Ci(1940 –), male, native of Wuchuan, Guangdong Province, Professor of Biochemistry, main research fields are biochemical and molecular pharmacology, research and development of antitumor and anti-platelet drugs.

* Corresponding author. E-mail: ncliang @ gdmc.edu.cn Tel: 0759-2388501 Fax: 0759-2284104

Key words: *Pteris semipinnata* L.; protein kinase C; DNA fragmentation; cytotoxicity

CLC number: R979.1
Document code: A
Article ID: 1000-3002(2003)02-0081-06

Protein kinase C(PKC) is an isozyme family of serine-threonine protein kinases comprising at least 10 mammalian members. All PKC members contain conserved and variant amino acid sequences in both regulatory and catalytic subunits. The conserved domain in regulatory subunit contains cysteine-rich region that serves as the important regulating site. PKC plays a key role in mediating the signals of growth factors, neurotransmitters, and hormones that have been implicated in many cellular events such as proliferation, differentiation, immune response, carcinogenesis, and multi-drug resistance(MDR). A decrease in PKC activity suggests PKC act as an anti-oncogene, whereas an increase in PKC activity suggests an oncogenic role for PKC, and an increase in PKC activity correlates with increased drug resistance and metastatic potential[1]. PKC signaling is a rational target for the treatment of many diseases and PKC inhibitors are potential clinical drugs and useful tools for the study of mechanisms of this enzyme and related cellular events thereafter.

Pteris semipinnata L. (PSL) is a Chinese traditional herb. Its ethanolic and water crude extracts showed obvious antitumor activity on hepatic carcinoma and sarcoma 180 in mice with low toxicity[2]. Several active compounds(*e.g.* 5F, 6F, A) were purified from PSL and showed strong cytotoxicity against various human tumor cell lines *in vitro*[3]. Compound 6F exhibits a stronger cytotoxic-

ity than that of 5F and A. All these compounds are diterpenoid that belong to the type of kaurane and possess α, β-methylene cyclopentanone moiety that can react with sulfhydryl in a Micheal addition. Our previous papers reported that compound 6F could strongly inhibit the activity of topoisomerase Ⅱ at a very low concentration (29 nmol·L^{-1}) and slightly inhibit topoisomerase Ⅰ and tyrosine protein kinase(TPK)[4]. The effect of compound 6F on the PKC activity and the effect of tumor-promoting diterpene phorbol 12-myristate 13-acetate(PMA) on the DNA fragment induction and cytotoxicity of 6F on HL-60 cells were studied. The mechanism of action was discussed.

1 MATERIALS AND METHODS

1.1 Drugs and chemicals

Compound 6F isolated from PSL was identified by the Laboratory of Phytochemistry of Guangdong Medical College. RPMI-1640 medium was obtained from Gibco. Fetal calf serum(FCS) was obtained from the Hangzhou Sijiqing Materials Company (Hangzhou, China). Phenylmethylsulfonyl fluoride(PMSF) was obtained from Merck. Histone Ⅲ-SS, diphenyl-amine, and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide(MTT) was purchased from Serva. [γ-^{32}P]-ATP was purchased from Beijing Yahui Biomedicine Engineering Co.(Beijing, China).

1.2 Cell culture

Human promyelocytic leukemia cell line HL-60, obtained from Shanghai Institute of Cell Biology, was cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FCS and antibiotics(penicillin 100 kU·L^{-1}, streptomycin 100 mg·L^{-1}) in 5% CO_2 atmosphere at 37℃.

1.3 Preparation of cell extracts

HL-60 cells 2×10^7 were homogenized by sonication(5 s with a Cole Parmer Ultrasonic Homogenizer 4710 Series at 70 W) in 1 mL buffer containing 20 mmol·L^{-1} Tris-HCl pH 7.4, 2 mmol·L^{-1} EGTA, 2 mmol·L^{-1} EDTA, 6 mmol·L^{-1} β-mercaptoethanol, 0.5 mmol·L^{-1} PMSF, and then centrifuged at 45 000×g for 2 h, yielding the cytosol fraction(supernatant) and particle fraction(pellet). The particle pellet was suspended in homogenization buffer plus 15 g·L^{-1} Nonidate P-40, sonicated for 10 s. The insoluble membrane and nuclei fraction (particle fraction) was separated from the pellet by centrifuged at 45 000×g for 2 h. The cell extract of cytosol or particle fractions were used as samples for the subsequent PKC assay. Protein concentration was measured by the method of protein-coomassie brilliant blue[5].

1.4 Protein kinase C assay

Activity of protein kinase C was tested by measuring ^{32}P incorporation from [γ-^{32}P]ATP into histone Ⅲ-SS as described by Kikkawa, *et al*[6]. The catalytic reaction was performed in a total volume of 100 μL containing 20 mmol·L^{-1} Tris-HCl pH 7.4, 10 mmol·L^{-1} $MgCl_2$, 0.5 mmol·L^{-1} $CaCl_2$, 40 μg phosphatidylserine, 4 μg diolein, 20 μg histone Ⅲ-SS, 50 μmol·L^{-1} [γ-^{32}P]ATP (370 MBq·L^{-1}). The reaction was initiated by the addition of 20 μL cell extracts and the samples were incubated for 10 min at 32℃ in the reaction mixture, and then immediately spotted onto filter paper(Xinhua type Ⅲ). The papers were washed 7 times with 20% trichloroacetic acid containing 1 mmol·L^{-1} ATP, and then washed 1 time with acetone, dried in the air, and immersed in 3 mL scintillator containing 0.3 mmol·L^{-1} 1, 4-bis(5-phenyl-2-oxazolyl) benzene(POPOP) and 18 mmol·L^{-1} 2,5-diphenyloxazole(PPO). The radioactivity was measured by scintillation counter(Beckman). Basal PKC activity obtained with 0.5 mmol·L^{-1} EGTA in the absence of three elements: $CaCl_2$, phosphatidylserine and diolein, was subtracted for each experimental datum. PKC activity is defined as the amount of [γ-^{32}P]ATP (nmol) being transferred to histone Ⅲ-SS by the enzyme during 1 min by 1 g protein of cell extracts.

1.5 Quantification of DNA fragmentation

DNA fragmentation was determined using the diphenylamine assay[7]. 2×10^6 Cells were lysed in 500 μL lysis buffer(10 mmol·L^{-1} Tris-Cl pH 7.5, 1 mmol·L^{-1} EDTA, 2.5 g·L^{-1} Triton X-100). The

intact chromatin (pellet) was separated from DNA fragments (supernatant) by centrifugation for 10 min at 13 000 × *g*. The pellets were resuspended in 500 μL lysis buffer, and precipitated by adding 125 μL 50% trichloroacetic acid (TCA) at 4℃. Samples were centrifuged at 13 000 × *g* for 4 min. Two hundred microliters of 5% (*V*/*V*) TCA were added into the pellets, and incubated in 90℃ for 10 min. After centrifugation at 13 000 × *g* for 4 min, 100 μL supernatant was separated and 200 μL diphenylamine reagent (1.5 g diphenylamine, 1.5 mL oil of vitriol, 8 μg acetaldehyde, 100 mL acetic acid) was added. After incubated at 30℃ for 16 h, the absorbance was measured with Microplate reader (Bio-Rad, M_{450}) at 570 nm. The percentage of fragmented DNA was calculated as the ratio of the DNA content in the supernatant to that in the pellet. Quantification of fragmented DNA by this method was consistent with the DNA ladders electrophoresed on agarose gel[7].

1.6 Cytotoxicity assay

Cytotoxic effect of compound 6F was tested using MTT method. Briefly, 1×10^4 exponentially growing HL-60 cells in 90 μL complete medium were seeded in 96-well dish, then 10 μL of drugs were added. After incubation at 37℃ in humidified 5% CO_2 atmosphere for 24 h, 20 μL 12 mmol·L^{-1} MTT was added to each well and the plate was further incubated at 37℃ for 5 h. SDS (20% 150 μL) resolved in 50% dimethyl formamide was added thereafter to solubilize the formazan crystal. After 5 h incubation at 37℃, the absorbance was measured with Microplate reader (Bio-Rad, M_{450}) at 570 nm and referenced at 450 nm.

1.7 Results were presented as $\bar{x} \pm s$. Analysis of variance with one-way ANOVA was used to identify significant differences in multiple comparisons.

2 RESULTS

2.1 Inhibitory effect of compound 6F on protein kinase C activity in HL-60 cells

The crude extraction including various isoforms of PKC obtained from HL-60 cells by ultracentrifugation was used as sample to test the effect of compound 6F on this enzyme. PKC activities both in cytosol and particle fraction which included membrane system and nuclei were inhibited by compound 6F at the concentration of 0.1 μmol·L^{-1} (particle fraction) or 0.5 μmol·L^{-1} (cytosol fraction) ($P < 0.01$, $n = 3$). The inhibition effects of 6F on PKC were in a dose-dependent manner ($r = 0.781$, $P < 0.05$ in cytosol fraction; $r = 0.931$, $P < 0.01$ in particle fraction) (Tab 1).

2.2 Attenuation of PMA on the DNA fragmentation in HL-60 cells induced by compound 6F

The DNA fragmentation was 75.7% ± 1.4%

Tab 1. Effect of compound 6F on the activities of protein kinase C (PKC) extracted from HL-60 cells

Compound 6F /μmol·L^{-1}	PKC activity/nmol·min^{-1}·g^{-1} protein		Inhibitory rate/%	
	Cytosol	Particle	Cytosol	Particle
0	7.9 ± 0.8	7.7 ± 0.8	0	0
0.1	6.4 ± 0.3	4.8 ± 0.3**	20.0	37.7
0.5	1.3 ± 0.4**	4.1 ± 0.4**	83.5	46.8
2.5	1.6 ± 0.6**	3.4 ± 1.2**	79.7	55.8
12.5	2.5 ± 2.6**	2.8 ± 0.4**	68.4	63.6
62.5	1.6 ± 1.7**	1.4 ± 0.5**	79.7	81.8
312	0.9 ± 0.7**	1.1 ± 0.6**	88.6	85.7

The cytosol (soluble sample) and particle (insoluble sample including membrane system and nuclei) fraction of HL-60 cells were prepared by ultracentrifugation, and PKC activities in each fraction were assayed as described in Materials and Methods. $\bar{x} \pm s$, $n = 3$. ** $P < 0.01$, compared with the group of 0 μmol·L^{-1} 6F.

and 53.3% ± 3.1% in HL-60 cells treated with compound 6F (231 nmol·L^{-1}) alone or simultaneously combined with 65 nmol·L^{-1} PMA, respectively ($P < 0.01$, $n = 3$).

2.3 PMA protected HL-60 against the cytotoxicity of 6F

Compound 6F 116 nmol·L^{-1} showed obvious cytotoxicity on HL-60 cells which exhibited reduced magnitude of formazan formation measured by MTT method. This effect was attenuated by PMA at 65 nmol·L^{-1} ($P < 0.01$, Tab 2). The ability of reducing MTT to formazan in mitochondria of HL-60 cells was potentiated when treated the cells with PMA alone at the same concentration for 24 h ($P < 0.01$), *i.e.* viability of HL-60 were enhanced by PMA under this condition.

Tab 2. Viability of HL-60 cells treated with 6F alone or simultaneously combined with PMA for 24 h

Group	Cell viability ($A_{570\ nm}/A_{450\ nm}$)	Inhibitory rate/%
Control	0.58 ± 0.03	0
6F alone	0.36 ± 0.02	38 ± 4
6F + PMA	0.45 ± 0.02**	21 ± 3
PMA alone	0.66 ± 0.03##	− 14 ± 6

The cell viability was evaluated by measuring the absorbance of formazan on Microplate reader at 570 nm with reference at 450 nm as described in Materials and Methods. The concentrations of 6F and PMA were 116 nmol·L^{-1} and 65 nmol·L^{-1}, respectively. $\bar{x} \pm s$, $n = 3$. ** $P < 0.01$, compared with 6F alone; ## $P < 0.01$, compared with control.

3 DISCUSSION

This work revealed that compound 6F purified from *Pteris semipinnata* L. could efficiently inhibit PKC activities both in cytosol and particle fractions extracted from HL-60 cells in a dose-dependant manner. Cysteine-rich domains in conserved 1 (C1) and conserved 2 (C2) sequence act as a key regulatory site bound to the activator diacylglycerol as well as phorbol ester that account for cPKC and nPKC activities[8]. PKC expressed in HL-60 cells mainly is the classical isoforms (PKCα, PKCβ, and PKCγ). Each cysteine-rich domain is a 50- or 51-amino acid domain containing a zinc finger motif that has 6 cystein residues. The motif is duplicated in tandem in both cPKC and nPKC isozymes. Studies indicated that a single copy of motif is sufficient for the binding of phorbol ester. Site-directed mutagenesis revealed that five of the six cysteine residues involved in Zn^{2+} coordination are critical for the interaction of the protein with phorbol ester[9]. Cysteine-containing peptide substrate analogues such as *N*-biotinyl-Arg-Arg-Arg-Cys-Leu-Arg-Arg-Leu inactivated cPKC, nPKC and aPKC by forming disulfide-linked complexes with the active site cysteine-rich domain in the isozymes[10]. PKC isozymes also can be inactivated by oxidant-induced *S*-glutathiolation, *e.g.* disulfide linkage of endogenous glutathione(GSH) to PKC[11]. Structure-function relationship analysis[3] showed α, β-methylene cyclopentanone moiety(Fig 1) confers the cytotoxicity

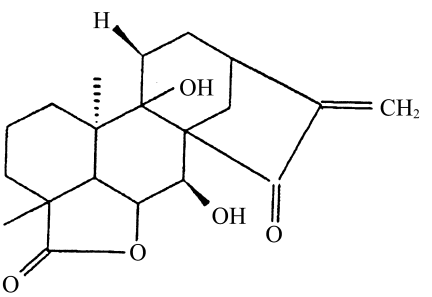

Fig 1. The structure of compound 6F.

of active compounds isolated from PSL. Saturation of the 16,17-double bond in the moiety resulted in lose of the activity. Since the sulfhydryl group plays a pivotal role in conforming a potential active PKC, the inhibitory effect of 6F on PKC activity was likely mediated through conjugate addition reaction between electrophilic group 16,17-double bond on 6F with nucleophilic group sulfhydryl on PKC (Fig 2).

The effective concentration of 6F on PKC was comparable with that of cytotoxicity and apoptosis induction on HL-60 cells[12], although 6F showed much more inhibitory effect on topoisomerase Ⅱ[4]. It was speculated that these activities of 6F on HL-60 cells were partially through inhibiting PKC activity. To further examine the role of PKC sig-

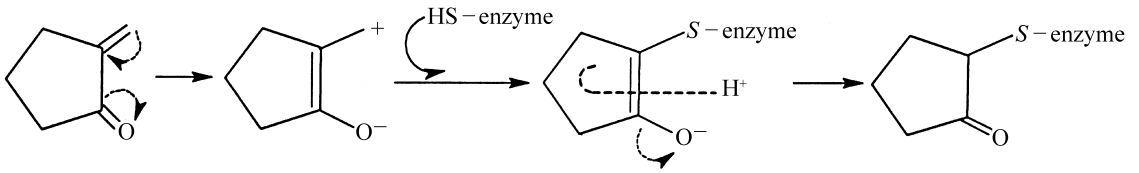

Fig 2. Schematic showing formation of 6F-PKC complex. Only the active moiety α,β-methylene cyclopentanone in 6F was shown here. The electrons were attracted by carbonyl and hydrogen bond that resulted in positive charged methylene. The electrophilic group methylene reacted with nucleophilic group sulfhydryl on PKC enzyme and a proton released.

naling in the regulation of apoptosis in HL-60 cells induced by 6F, we investigated the effect of PMA, a PKC activator and tumor promoter, on DNA fragmentation by 6F. The results showed that DNA fragment induction was partially antagonized when HL-60 cells were treated with 6F plus PMA at 65 nmol·L^{-1} for 24 h. Long-term treatment(up to 12 h) with PMA down-regulated PKC activities in HL-60 cells preceded by a short period(within 30 min) of activation of PKC and followed by the differentiation of HL-60 cells to monocytes and macrophages. It was reported that differentiated HL-60 cells resisted various apoptosis-inducing stimuli and rapidly undergo apoptosis at the end stage of differentiation[13]. DNA fragmentation inhibition effect of PMA in this case might be associated with the down-regulation of PKC and subsequently the differentiation of the cells induced by PMA. This could also explain the protection effect of PMA against cytotoxicity of 6F on HL-60 cells since differentiated cells exhibited enhanced nitroblue tetrazolium(NBT)-reducing activity in mitochondria. Nevertheless, It's no doubt that the antagonistic action between 6F and PMA is originally due to the changing of PKC activity since the enzyme serves as the direct target for 6F and PMA. The detailed mechanisms by which PMA interferes apoptosis are still not well defined. However, several efforts have been made. ① PMA stabilized the intracellular calcium homeostasis, which was disturbed commonly in the apoptosis process[14]. ② PMA abrogated cytochrome C release, downregulation of IAP(inhibitor of apoptosis), and caspase 3 activation that were involved in the execution of apoptosis[15]. ③ Activated mitogen-activated protein kinases signaling pathway by PMA promoted the proliferation and survival of hematopoietic cells and protected the cells against undergoing apoptosis[16]. This paper revealed that DNA fragmentation in HL-60 cells induced by compound 6F was associated with PKC signal pathway. The exact mechanisms veiled on protecting effect of PMA on DNA fragment induction of 6F remain to be investigated.

Acknowledgments We thank Mrs. WANG Mei and Mr. XU Mei-Yi(Analytical Center of Guangdong Medical College) for expert technical assistance in measuring the radioactivity of ^{32}P by scintillation counter.

4 REFERENCES:

[1] Blobe GC, Obeid LM, Hannun YA. Regulation of protein kinase C and role in cancer biology[J]. *Cancer Metastasis Rev*, 1994, **13**(3－4):411－431.

[2] Cui L, Liang NC, Chen ZD, Song ZJ. Studies on the anticancer effect and acute toxicity of *Pteris semipinnata*[J]. *J Chin Med Mater*(中药材), 1996, **19**(1):29－32.

[3] Li JH, Liang NC, Mo LE, Zhang X, He CW. Comparison of the cytotoxicity of five constituents from *Pteris semipinnata* L. *in vitro* and the analysis of their structure-activity relationships[J]. *Acta Pharm Sin*(药学学报), 1998, **33**(9):641－644.

[4] Li JH, Liang NC, Mo LE, Zhang X, He CW. Effect of active compounds isolated from *Pteris semipinnata* L. on DNA topoisomerases and tyrosine protein kinase and expression of c-myc in lung adenocarcinoma cells[J]. *Chin J Cancer Res*(中国癌症研究), 2001, **13**(2):105－109.

[5] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding[J]. *Anal Biochem*, 1976, **72**:248－254.

[6] Kikkawa U, Takai Y, Minakuchi R, Inohara S, Nishizuka

Y. Calcium-activated, phospholipid-dependent protein kinase from rat brain. Subcellular distribution, purification, and properties[J]. *J Biol Chem*, 1982, 257(22):13341 – 13348.

[7] Bansal N, Houle AG, Melnykovych G. Dexamethasone-induced killing of neoplastic cells of lymphoid derivation: lack of early calcium involvement[J]. *J Cell Physiol*, 1990, **143**(1):105 – 109.

[8] Ichikawa S, Hatanaka H, Takeuchi Y, Ohno S, Inagaki F. Solution structure of cysteine-rich domain of protein kinase C alpha[J]. *J Biochem*(Tokyo), 1995, **117**(3):566 – 574.

[9] Kazanietz MG, Wang S, Milne GW, Lewin NE, Liu HL, Blumberg PM. Residues in the second cysteine-rich region of protein kinase C delta relevant to phorbol ester binding as revealed by site-directed mutagenesis[J]. *J Biol Chem*, 1995, **270**(37):21852 – 21859.

[10] Ward NE, Pierce DS, Stewart JR, O'Brian CA. A peptide substrate-based affinity label blocks protein kinase C-catalyzed ATP hydrolysis and peptide-substrate phosphorylation[J]. *Arch Biochem Biophys*, 1999, **365**(2):248 – 253.

[11] Ward NE, Stewart JR, Ioannides CG, O'Brian CA. Oxidant-induced *S*-glutathiolation inactivates protein kinase C-alpha(PKC-alpha): a potential mechanism of PKC isozyme regulation[J]. *Biochemistry*, 2000, **39**(33):10319 – 10329.

[12] He CW, Liang NC, Mo LE, Zhang X, Li JH. Apoptosis in HL-60 cells induced by compound 6F isolated from *Pteris semipinnata* L.[J]. *Chin J Cancer Prevent Treat*(中国肿瘤防治杂志), 2002, **9**(1):11 – 14.

[13] McCarthy JV, Fernandes RS, Gotter TG. Increased resistance to apoptosis associated with HL-60 myeloid differentiation status[J]. *Anticancer Res*, 1994, **14**(5A):2063 – 2072.

[14] Zhu WH, Loh TT. Differential effects of phorbol ester on apoptosis in HL-60 promyelocytic leukemia cells[J]. *Biochem Pharmacol*, 1996, **51**(9):1229 – 1236.

[15] Kwon TK. Phorbol myristate acetate inhibits okadaic acid-induced apoptosis and downregulation of X-linked inhibitor of apoptosis in U937 cells[J]. *Biochem Biophys Res Commun*, 2001, **287**(1):135 – 141.

[16] Ramoneda BM, Perez-Tomas R. Activation of protein kinase C for protection of cells against apoptosis induced by the immunosuppressor prodigiosin[J]. *Biochem Pharmacol*, 2002, **63**(3):463 – 469.

半边旗提取物 6F 抑制 HL-60 细胞蛋白激酶 C 活性

何承伟[1], 梁念慈[2], 莫丽儿[3], 李金华[1], 张　晓[1]

(广东医学院 1. 生物化学与分子生物学研究所, 2. 广东省天然药物研究与开发实验室, 3. 化学教研室, 广东 湛江　524023)

摘要: 目的　探讨半边旗提取物 6F 的细胞毒作用及诱导 DNA 片段化与蛋白激酶 C(PKC)信号转导途径的关系,检测 6F 对 PKC 活性的影响。**方法**　受试对象为 HL-60 细胞,超速离心法获得的胞液(可溶部分)及颗粒(不可溶部分,包括细胞膜系统及胞核)部分用作 PKC 活性测定。经 0.4 g·L^{-1}磷脂酰丝氨酸, 0.04 g·L^{-1}甘油二油酸酯激动剂作用酶粗提物后,用液体闪烁计数仪计数[γ-^{32}P]ATP 参入外源底物的量以测定 PKC 活性。MTT 法测定 HL-60 细胞的活力,二苯胺法测定 6F 诱导 DNA 片段化程度。**结果**　在所测试的浓度范围内(0.5 ~ 312 μmol·L^{-1}),化合物 6F 显著抑制胞液及颗粒部分 PKC 活性,最大抑制率达 88.6%,呈浓度依赖关系(胞液部分 $r = 0.781$, $P < 0.05$, 颗粒部分 $r = 0.931$, $P < 0.01$)。6F 诱导 HL-60 细胞 DNA 片段化及对细胞的毒性作用可被具有致癌作用的 PKC 激活剂肉豆蔻酸酯(PMA,浓度为 65 nmol·L^{-1})拮抗,抑制率分别是 30%和 44%($P < 0.01$)。PMA 单独用使 HL-60 细胞线粒体将 MTT 还原为甲臜的能力增强 14%($P < 0.01$),即增强细胞活力。**结论**化合物 6F 是 PKC的抑制剂。6F 对 HL-60 细胞 DNA 片段化的诱导作用及其细胞毒作用至少可部分归因于其对 PKC 活性的抑制作用。

关键词: 半边旗; 蛋白激酶 C; DNA 片段化; 细胞毒作用

基金项目: 国家自然科学基金项目(39870900);广东省科委重大科技攻关项目(9622007-002); 广东省重点学科基金(9306)

(本文编辑　乔　虹)