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Effect of semi-synthesized quercetin water-soluble derivatives on recombinant human phosphatidylinositol 3-kinase p110β catalytic subunit[1]

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KEY WORDS flavones; quercetin; phosphatidylinositols; recombinant proteins

ABSTRACT

AIM: To study the effect of semi-synthesized quercetin water-soluble derivativessodium quercetin-7-sulfate (SQMS) and disodium quercetin-7,4′-disulfate (SQDS) on recombinant human phosphatidylinositol 3-kinase (PI3-K) p110β catalytic subunit. **METHODS**: Recombinant human PI3-K p110β catalytic subunit was expressed by gene engineering. PI3-K was assayed by incubating recombinant PI3-K p110β with phosphatidylinositol-4, 5-bisphosphate and [γ-^{32}P] ATP; the ^{32}P-radiolabeled lipids were extracted with chloroform and methanol, assessed by thin layer chromatography and visualized by autoradiography. **RESULTS**: Wortmannin, a specific inhibitor of PI3-K, showed inhibition on recombinant PI3-K p110β catalytic subunit in a concentration-dependent manner (2.5 - 20 nmol/L); SQMS and SQDS showed inhibition on recombinant PI3-K p110β catalytic subunit in a concentration-dependent manner (2.5 - 20 μmol/L). **CONCLUSION**: Semi-synthesized quercetin water-soluble derivatives were a type of inhibitors of PI3-K. The recombinant PI3-K p110β catalytic subunit might be used as a molecular target for simpler filtrating and development of more effective inhibitors of PI3-K.

INTRODUCTION

Flavonoids are polyphenolic compounds naturally present in plants. They exhibit a variety of effects including inhibition of malignant cell growth. Several recent studies have demonstrated that flavonoids may be potent inhibitors of several kinases involved in signal transduction and cell transformation, mainly protein kinase C and tyrosine kinase[1]. Therefore, naturally occurring flavonoids have been proposed to exert biological effects on cells through inhibition of these different key enzymes. For these reasons, they may be considered as potential compounds for selectively blocking signal transduction pathways and for designing more potent analogues for use in proliferative disease therapy. But as most of flavonoids are not soluble in water, its biological utilizations *in vivo* are restricted. Theoretically, flavonoids is transformed as water-soluble derivatives while maintaining its active hydroxyl residues, and the derivatives could be developed as a anti-tumor agents. In this view, using quercetin (3,3′,4′,5,7-pentahydroxyflavone) as a parent molecular, semi-synthesized quercetin water-soluble derivatives were synthesized chemically. Structures of semi-synthesized quercetin water-soluble derivatives were determined as sodium quercetin-7-sulfate (SQMS) and disodium quercetin-7, 4′-disulfate (SQDS) by FAB-MS, ^{1}H-NMR, and ^{13}C-NMR[2-4]. The anti-tumor and anti platelet actions of semi-synthesized quercetin water-soluble derivatives were studied[5-7], but their molecular mechanism were still unclear. In this study, we described the effect of semi-synthesized quercetin water-soluble derivatives on recombinant human phosphatidylinositol 3-kinase (PI3-K) p110β catalytic subunit, which is another important enzyme involved in signal transduction and cell transformation.

MATERIALS AND METHODS

Chemicals and drugs Wortmannin, quercetin, egtazic acid, phosphatidylinositol-4, 5-bisphosphate (PIP_2) and HEPES (Sigma). [γ-^{32}P]ATP (Yahui

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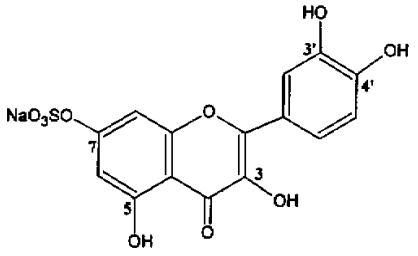
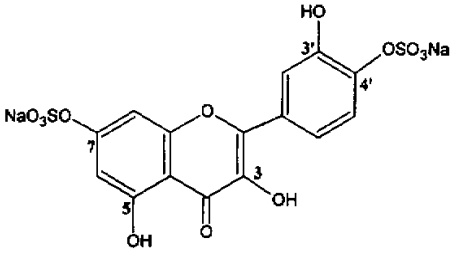


Sodium quercetin-7-sulfate (SQMS)



Disodium quercetin-7,4'-disulfate (SQDS)

Biomedical Technology Co Ltd, Beijing). All other chemicals were of analytical grade.

Prokaryotic expression of recombinant human PI3-K p110β catalytic subunit Prokaryotic expression of recombinant human PI3-K p110β catalytic subunit was performed as described by Liu *et al* in another paper[8].

PI3-K assay PI3-K activity was measured using enzyme and sonicated PIP_2 (250 μmol/L) as substrate in 100 μL containing HEPES 40 mmol/L, pH 7.5, NaCl 50 mmol/L, $MgCl_2$ 4 mmol/L, egtazic acid 1 mmol/L, ATP 50 μmol/L (74 kBq [γ-^{32}P] ATP). Incubation was carried out at 30 ℃ for 15 min. Reactions were stopped with 1.5 mL of chloroform : methanol (2 : 1, v/v), and 1.5 mL of HCl 3.1 mol/L and 1 mL of chloroform were added. After extensive vibration and centrifugation at 4000 × *g* for 10 min, the organic phase was removed and washed twice with 1 mL of NaCl 0.75 mol/L containing chloroform : methanol : HCl 0.1 mol/L (3 : 48 : 47, v : v : v), and the organic phase was dried under air or N_2. The lipids were redissolved in 20 μL of chloroform : methanol (2 : 1, v/v) and assessed by thin layer chromatography (TLC). The ^{32}P-radiolabeled lipids were visualized by autoradiography.

RESULTS

Effect of wortmannin on recombinant PI3-K p110β catalytic subunit Wortmannin, a specific inhibitor of PI3-K, showed inhibition on recombinant PI3-K p110β catalytic subunit in a concentration-dependent manner (2.5 – 20 nmol/L) (Fig 1).

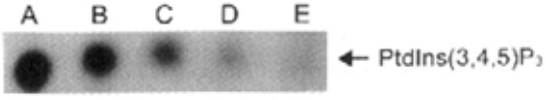


Fig 1. Effect of wortmannin on recombinant PI3-K p110β catalytic subunit. A: control; B: wortmannin 2.5 nmol/L; C: wortmannin 5 nmol/L; D: wortmannin 10 nmol/L; E: wortmannin 20 nmol/L.

Effects of SQMS and SQDS on recombinant PI3-K p110β catalytic subunit SQMS and SQDS showed inhibition on recombinant PI3-K p110β catalytic subunit in a concentration-dependent manner (2.5 – 20 μmol/L) (Fig 2, 3).

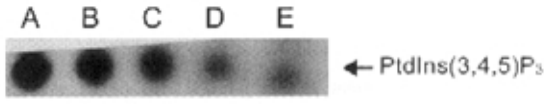


Fig 2. Effects of SQMS on recombinant PI3-K p110β catalytic subunit. A: control; B: SQMS 2.5 μmol/L; C: SQMS 5 μmol/L; D: SQMS 10 μmol/L; E: SQMS 20 μmol/L.

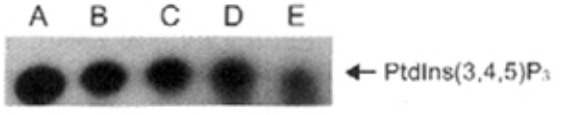


Fig 3. Effects of SQDS on recombinant PI3-K p110β catalytic subunit. A: control; B: SQDS 2.5 μmol/L; C: SQDS 5 μmol/L; D: SQDS 10 μmol/L; E: SQDS 20 μmol/L.

DISCUSSION

PI3-K are divided into three main classes. Class Ⅰa PI3-K has been described as two highly homologous heterodimers consisting of a regulatory subunit (p85α and β; M_r = 85 000) and a catalytic subunit (p110α, p110β, and p110δ; M_r = 110 000). The kinase domain of all the PI3-K are highly homologous and p110 possesses PI3-K activity. PI3-K activation leads to rapid and transient production of phosphatidylinositol 3, 4 bisphosphate [PtdIns (3, 4) P_2] and phosphatidylinositol 3, 4, 5 trisphosphate [PtdIns (3, 4, 5) P_3]. These novel

phosphoinositides have been proposed to act as second messengers and play an important role in mitogenesis. PI3-K has been shown to be an important effector of polyphosphoinositide pathway and a key enzyme involved in signal transduction and cell transformation. The development of PI3-K inhibitors as anti-tumor agents would be helpful in designing analogues that could be used for the treatment of proliferative diseases[9-13].

The recombinant plasmid containing human PI3-K p110β cDNA was transformed into *Escherichia coli* BL21 (DE3) and expressed significantly and specifically after induced by IPTG. SDS-PAGE analysis of the recombinant protein showed a new protein (M_r = 110 000) was expressed[8]. Our results showed that the recombinant human PI3-K p110β catalytic subunit was strongly inhibited by wortmannin at nanomolar concentrations, which was considered to be a specific inhibitor of PI3-K. This result demonstrated that the recombinant human PI3-K p110β catalytic subunit possessed biological activity of PI3-K. The inhibitory effect of flavonols on PI3-K activity was dependent on the number and the position of hydroxyl residues. The most potent inhibitors of PI3-K in flavonoids have 3′,4′ OH group on the B ring. The results showed that SQMS and SQDS had inhibitory effects on PI3-K, these and other evidence[5-7] indicate that the semi-synthesized quercetin water-soluble derivatives possess biological activity. The inhibitory effects of SQMS and SQDS on malignant cell growth and platelet aggregation were due to their blocking signal transduction pathway introduced by PI3-K. In the structure of sodium quercetin sulfates, SQMS has two active hydroxyl residues: 3′ and 4′ OH group on the B ring, but SQDS has only one, the 4′ OH group was esterized by acid. The biological activity of SQDS should be weaker than SQMS on PI3-K, our results supported this view as well.

In summary, this study demonstrated that SQMS and SQDS had inhibitory effects on PI3-K. This work primarily provided evidence that helped to clarify the molecular mechanism of SQMS and SQDS. Water-soluble quercetin derivatives will be developed as anti-tumor agents. The recombinant PI3-K p110β catalytic subunit might be used as a molecular target for simpler filtrating and development of more effective inhibitors of PI3-K.

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人工半合成槲皮素水溶性衍生物对重组人磷脂酰肌醇3-激酶 p110β 催化亚基的影响[1]

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关键词 黄酮类; 槲皮素; 磷脂酰肌醇类; 重组蛋白

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目的：研究人工半合成槲皮素水溶性衍生物—槲皮素-7-硫酸酯钠盐(SQMS)和槲皮素-7,4′-二硫酸酯二钠(SQDS)对重组人磷脂酰肌醇 3-激酶(PI3-K) p110β 催化亚基的影响. **方法**：利用基因工程的方法获得 PI3-K p110β 催化亚基. 用磷脂酰肌醇-4,5-二磷酸，[γ-^{32}P]ATP 与重组 PI3-K p110β 催化亚基一起保温的方法测定 PI3-K 的活性；^{32}P标记的磷脂用氯仿和甲醇抽提、板薄层层析和放射自显影来分析. **结果**：Wortmannin 是 PI3-K 特异的抑制剂，Wortmannin (2.5－20 nmol/L)对重组 PI3-K p110β 亚基有抑制作用；SQMS 和 SQDS (2.5－20 μmol/L)对重组人 PI3-K p110β 催化亚基有抑制作用. **结论**：人工半合成槲皮素水溶性衍生物是一种类型的 PI3-K 抑制剂. 重组人 PI3-K p110β 催化亚基可作为一种较为简便地筛选和开发有效的 PI3-K 抑制剂的分子靶点.

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