苦参碱对脂多糖/D-氨基半乳糖诱导的肝炎及离体巨噬细胞释放肿瘤坏死因子的影响

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关键词 苦参碱；中毒性肝炎；氨基半乳糖；脂多糖；肿瘤坏死因子；腹腔巨噬细胞

目的：研究苦参碱（Mat）对脂多糖（lipopolysaccharides，LPS）诱导的 D-氨基半乳糖（D-GalN）致敏小鼠致死性肝炎以及腹腔巨噬细胞（PMØ）释放肿瘤坏死因子（TNF）的影响 **方法**：小鼠 ip Mat 10，50 mg·kg^{-1}，bid×3 d，然后 ip LPS（1 μg·kg^{-1}）和 D-GalN（800 mg·kg^{-1}），通过病理组织学观察及测定血清丙氨酸转氨酶（ALT）活性来评估肝损伤。小鼠 PMØ 培养上清中的 TNF 活性以杀伤 L929 细胞的结晶紫染色法测定 **结果**：Mat 降低了 LPS/D-GalN 引起的血清 ALT 活性升高及小鼠对 LPS/D-GalN 致死毒性的敏感性并抑制 LPS 诱导的小鼠 PMØ 释放 TNF **结论**：Mat 防治 LPS/D-GalN 引起的致死性肝炎，并抑制 LPS 诱导的 TNF 释放

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Effects of tyrphostins on activity of casein kinase Ⅱ from rat liver

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KEY WORDS tyrphostins; caseins; protein kinases; liver; enzyme inhibitors

AIM: To investigate the effects of tyrphostins, (AG213, AG1394, AG114, AG1109, AG555) on the activity of casein kinase (CK) Ⅱ. **METHODS**: CK Ⅱ was partially purified from rat livers by sequential DE52 and heparin-Sepharose chromatography. CK Ⅱ activity was assayed by incubating CK Ⅱ with dephosphorylated casein and [γ-^{32}P]ATP. **RESULTS**: AG213 inhibited the activity of CK Ⅱ with IC_{50} 44.7 μmol·L^{-1} (41.5 - 47.9 μmol·L^{-1}), and AG1394 (144 μmol·L^{-1}) strongly inhibited the activity of CK Ⅱ with an inhibitory ratio of 89 %. AG114 (174 μmol·L^{-1}) and AG1109 (126 μmol·L^{-1}) had inhibitory effects on the activity of CK Ⅱ ($P<0.01$). AG555 (136 μmol·L^{-1}) had little effect on CK Ⅱ activity. **CONCLUSION**: Some tyrphostins are potent inhibitors of CK Ⅱ.

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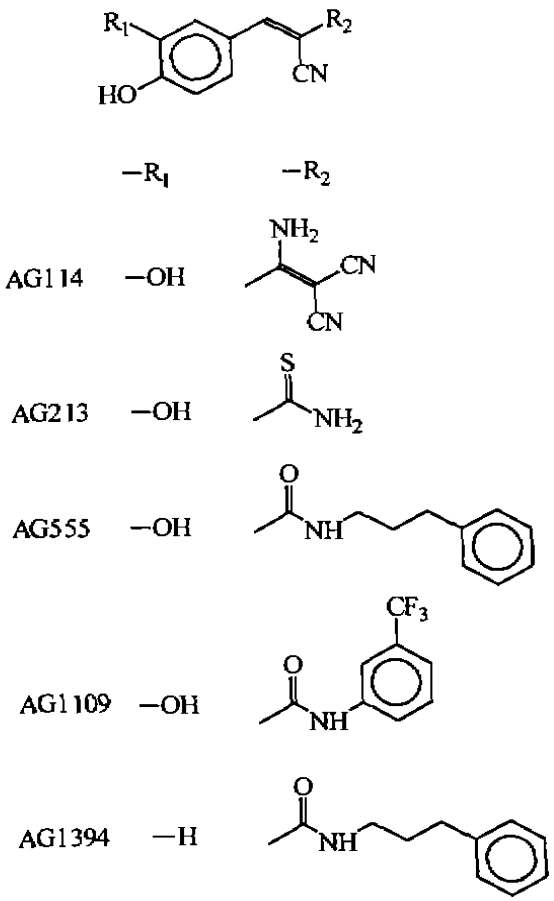
Casein kinase (CK) Ⅱ is a ubiquitous protein serine/threonine kinase in the cytosol, nucleus, and membranes of eukaryotic cells. CK Ⅱ purified from various tissues is usually a tetrameric complex with an $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, or $\alpha'_2\beta_2$ structure [1,2]. CK Ⅱ may play an important role in cell proliferation[3-6]. For example, CK Ⅱ phosphorylates a number of nuclear proteins including *Fox*, *Myb*, *Myc*, p53 tumor suppressor protein, and SV40 large T antigen. These proteins are implicated in oncogenic transformation and cell proliferation.

Tyrphostins (AG213, AG114, AG555, AG1394, and AG1109), a series of synthetic chemicals, are inhibitors of tyrosine kinase[7], but the efficacy of AG213 in inhibiting EGF-induced [^{3}H] thymidine uptake in A431 cells does not correlate with its tyrosine kinase inhibitory activity[8]. Their effects on CK Ⅱ are unknown. In the present study, the effects of tyrphostins on the activity of CK Ⅱ from rat liver were investigated.

MATERIALS AND METHODS

Heparin and phosphatidylserine (PS) (Sigma); ATP



Tyrphostins

(Boehringer Mannhein); Sepharose 4B (Pharmacia); CNBr (Fluka); DEAE-cellulose (DE52) (Whatman); Casein (ICN); tyrphostins (purity 99 %, derived from benzenemalonitrile, and synthesized by Prof A Gazit, Department of Organic Chemistry, The Hebrew University of Jerusalem, Israel). [γ-^{32}P]ATP (370 GBq·L^{-1}, > 185 PBq · moL^{-1}) was purchased from Yahui Biomedical Technology Co Ltd, Beijing. All other chemicals were AR.

Two SD rats (♀, 2-month-old, 250 g and 350 g, respectively) were provided by the Animal Center of Guangdong Medical College.

Heparin-Sepharose 4B was synthesized by the method[9].

Extraction and partial purification of CK Ⅱ Two rat livers were homogenized with 100 mL of buffer A (Tris 20, edetic acid 5, egtazic acid 1, β-mercaptoethanol 10, PMSF 1 mmol·L^{-1}, pH 7.2) in an ice bath. The homogenate was centrifuged at 20 000 × *g* at 4 ℃ for 10 min. The supernatant was loaded to 30 g of DE52 that had been equilibrated with buffer B (Tris 20, edetic acid 2, β-mercaptoethanol 5, PMSF 0.5 mmol·L^{-1}, pH 7.4) in a filter funnel. Having been washed with 500 mL of buffer B, the protein-bound DE52 was packed into a column (20 mm × 300 mm) and washed with buffer B containing NaCl 500 mmol·L^{-1}. The eluate from DE52 (160 mL) with CK Ⅱ activity was diluted 1:5 with buffer B, and then loaded on to a heparin-Sepharose 4B column (15 mm × 200 mm). After equilibrating with buffer B, the column was developed with a 200 mL linear increasing gradient of NaCl from 0 to 1 mol·L^{-1} in buffer B, and 6 mL fractions were collected, from which CK Ⅱ was eluted by buffer B containing NaCl 700 mmol·L^{-1}. The kinase from 2 chromatography steps purified over 104-fold, with a final recovery of 33 % of the starting activity of CK Ⅱ.

CK Ⅱ activity assay Partially dephosphorylated casein was prepared by incubating 5 g casein in 50 mL of Tris 50 mmol·L^{-1} (pH 9.5) at 100 ℃ for 10 min and dialyzing against buffer C (Tris 50, edetic acid 5 mmol·L^{-1}, pH 7.5). CK Ⅱ activity was assayed at 25 ℃ in a final volume of 100 μL with Tris-HCl 50, KCl 150, $MgCl_2$ 10 μmol·L^{-1}, [γ-^{32}P]ATP 50 μmol·L^{-1} (37 kBq), partially dephosphorylated casein 2 g·L^{-1}, and partially purified CK Ⅱ 2.8 μg (or CK Ⅱ eluate 10 μL) for 10 min. The reaction was terminated by spotting 90 μL on to 3 pieces of 15 mm-diameter Xinhua № 3 filter paper, and dropped into 10% trichloroacetic acid (TCA) containing ATP 1 mmol·L^{-1}. After the filter papers were washed thoroughly with TCA as above, the radioactivity was measured in a LS6000C (Beckman) scintillation counter.

Statistical significance was analyzed by *t* test.

RESULTS

Characterization of CK Ⅱ Partially dephosphorylated casein was efficiently phosphorylated by CK Ⅱ. However, at 0.5 g·L^{-1}, histone ⅢS was phosphorylated rather poorly, at 1.4 % of the rate of casein. Heparin (4 mg·L^{-1}) inhibited the activity of CK Ⅱ by 76 %, but Ca^{2+}, Ca^{2+}-phosphatidylserine, or cAMP had little effects on the activity of CK Ⅱ (Tab 1).

Effect of AG213 and analogues on CK Ⅱ activity AG213 inhibited the activity of CK Ⅱ with IC_{50} 44.7 μmol·L^{-1} (41.5 μmol·L^{-1}, 47.9 μmol·L^{-1}). At 200 μmol·L^{-1}, the inhibitory ratio of AG213 on the activity of CK Ⅱ was 92 % (Fig 1).

AG1394 (144 μmol·L^{-1}) strongly inhibited the activity of CK Ⅱ, with an inhibitory ratio of 89 %. AG114 (174 μmol·L^{-1}) and AG1109 (126 μmol·L^{-1}) had significant inhibitory effects on the

activity of CK Ⅱ ($P<0.01$). AG555 (136 μmol·L^{-1}) had little effect on CK Ⅱ activity ($P>0.05$) (Tab 2).

Tab 1. Characterization of CK Ⅱ from rat liver. $n=3$ wells for 1 homogenate (pooled from 2 rat livers), $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs control.

Condition	CK Ⅱ activity/ dpm	%
Control	59 148 ± 472	100
+ histone (0.5 g·L^{-1}) but no casein	835 ± 80^c	1
+ egtazic acid (1 mmol·L^{-1})	59 554 ± 3 297^a	101
Ca^{2+} (0.5 mmol·L^{-1})	53 032 ± 1 540^b	90
+ Ca^{2+} (0.25 mmol·L^{-1}) and *phosphatidyserine* (50 μmol·L^{-1})	63 068 ± 3 803^a	107
+ cAMP (5 μmol·L^{-1})	61 362 ± 3 803^a	104
+ heparin (4 mg·L^{-1})	14 306 ± 1 958^c	24

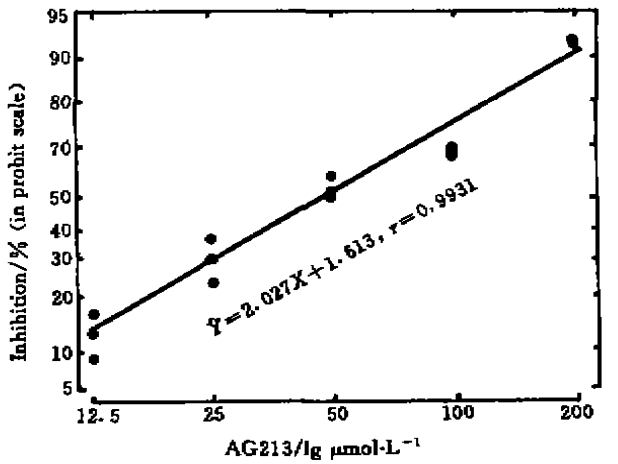


Fig 1. Effects of AG213 on activity of CK Ⅱ from rat liver. $n=3$ wells for 1 homogenate (pooled from 2 rat livers).

DISCUSSION

Tyrphostins are a series of synthetic chemicals, which have been proved to inhibit tyrosine kinase activity. In the present study, some of tyrphostins inhibited the activity of CK Ⅱ, suggesting that some tyrphostins might not be specific tyrosine kinase inhibitors.

Tab 2. Effects of the analogues of AG213 on the activity of CK Ⅱ from rat liver. $n=3$ wells for 1 homogenate (pooled from 2 rat livers), $\bar{x}\pm s$. $^aP>0.05$, $^cP<0.01$ vs control.

Addition	CK Ⅱ activity/ dpm	Inhibition/ %
Control	36 541 ± 75	-
AG555 (136 μmol·L^{-1})	36 374 ± 313^a	0.5
AG1394 (144 μmol·L^{-1})	4 036 ± 115^c	89.0
AG114 (174 μmol·L^{-1})	16 298 ± 1790^c	54.7
AG1109 (126 μmol·L^{-1})	26 287 ± 1242^c	29.2

The inhibitory effects of tyrphostins on CK Ⅱ are related to their structures. The only difference of the structure of AG555 from that of AG1394 is a hydroxyl, AG1394 strongly inhibited the activity of CK Ⅱ, but AG555 had little effect on the activity, suggesting that the modification in the structures of tyrphostins could develop more effective inhibitors of CK Ⅱ.

CK Ⅱ is a cyclic nucleotide and calcium-independent serine/threonine-specific protein kinase. The physiological substrates of CK Ⅱ include metabolic enzymes, cytoskeletal proteins, transcription factors, as well as products of several oncogenes and tumor suppressor[1,3-6]. CK Ⅱ activity is increased in neoplastically transformed cell lines[10], as well as in tumors[11]. CK Ⅱ was stimulated in response to various growth factors in cultured cells[12,13]. These findings suggest that CK Ⅱ may play an important role in cell proliferation. In this paper, our finding that some tyrphostins inhibited activity of CK Ⅱ, can explain why the inhibitory effects of some tyrphostins on [^{3}H] thymidine uptake do not correlate with their inhibition on tyrosine kinase.

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Tyrphostins 对大鼠肝酪蛋白激酶Ⅱ活性的影响

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关键词 tyrphostins; 酪蛋白类; 蛋白激酶类; 肝; 酶抑制剂

目的: 研究 Tyrphostins (AG123, AG1394, AG114, AG1109, AG555)对酪蛋白激酶Ⅱ (CK Ⅱ)活性的影响. **方法**: 依次采用 DEAE-纤维素和肝素-Sepharose 层析将大鼠肝 CK Ⅱ纯化了 104 倍, 通过将去磷酸化的酪蛋白和[γ-^{32}P]ATP 与 CK Ⅱ保温的方法测定 CK Ⅱ的活性. **结果**: AG213 对 CK Ⅱ有强烈的抑制作用(IC_{50} 44.7 μmol·L^{-1}[41.5 μmol·L^{-1}, 47.9 μmol·L^{-1}]), AG1394 (144 μmol·L^{-1})对 CK Ⅱ的抑制率为 89 %. AG114 和 AG1109 对 CK Ⅱ也有明显的抑制作用, 而 AG555 对 CK Ⅱ的活性没有影响. **结论**: 某些 tyrphostins 是 CK Ⅱ抑制剂.

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