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THE INHIBITORY EFFECT OF EXTRACT OF CAMELLIA SINENSIS AND EXTRACT OF CAMELLIA PTILOPHYLLA CHANG ON DNA POLYMERASE OF EHRLICH ASCITES CARCINOMA CELLS*

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Objective: To detect the effect of extract of Camellia Sinensis (ECS) and extract of Camellia Ptilophylla Chang (ECPC) on DNA polymerase (Pol) of Ehrlich ascites tumor cells. Methods: Referring to the method of K.Ono, Pol was extracted from Ehrlich ascites tumor cells in mice. Pol α, β, and γ were separated by phosphocellulose column chromatography and were identified. The effect of ECPC and ECS on Pol was studied. Results: ECPC and ECS were shown to inhibit the activity of Pol α, β, and γ. IC_{50} values of ECS on Pol α, β, and γ were 10.2 μ g/ml, 9.9 μ g/ml and 28.9 μ g/ml respectively. IC_{50} values of ECPC on Pol α, Pol β and Pol γ were 5.6 μ g/ml, 15 μ g/ml and 14.7 μ g/ml respectively. The modes of inhibition of ECPC on Pol α, Pol β and Pol γ were noncompetitive with respect to template DNA. The Ki values of ECPC on Pol α, β, and γ were 2.68±0.12 μ g/ml, 2.24±0.12 μ g/ml, 2.56±0.18 μ g/ml. Conclusion: ECPC and ECS were shown to have inhibitory effect on DNA polymerase of tumor cells. The mode of inhibition of ECPC on Pol α, Pol β and Pol γ were noncompetitive with respect to template DNA.

Key words: Extract of camellia sinensis, Extract of camellia ptilophylla chang, Inhibition, Ehrlich ascites carcinoma, DNA polymerase

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DNA polymerase (Pol) is a key enzyme of DNA synthesis, it catalyzes dNTP polymerized into DNA, the 3'→5' exonuclease function of Pol ensures the authenticity of DNA sythesis, and it also relates to the repair of DNA[1-4]. Due to the close relationship between Pol and cell proliferation, Pol became target enzyme of some antitumor drugs.[5,6]

Our previous study had demonstrated the antitumor activity of extract of camellia sinensis and extract of camellia ptilophylla chang,[7] in this study, their effect on Pol of Ehrlich ascites carcinoma Cells was detected.

MATERIALS AND METHODS

Experimental Animals and Tumor Cell Line

KM mice were purchased from Department of Experimental Animals, First Military Medical University (Certificate No.94004 degree II). Ehrlich ascites carcinoma cell line (EAC) was routinely passed in our laboratory.

Drugs and Reagents

Extract of camellia sinensis (ECS) and extract of camellia ptilophylla chang (ECPC) were provided by Zhang Runmei and Zhang Hongda, Plant Research Institute, Department of Biology, Zhong Shan University. Activated calf thymus DNA, type XV (Act DNA)(Sigma Co, lot No 109F-6757); dATP, dCTP,

dGTP (Promega Co, lot No. 141401, 122302, 122401, respectively); [^{3}H] dTTP (Shanghai Institute of Nuclear Research, Chinese Academy of Sciences, lot No 911213, 814TBqmol^{-1}); Aphidicolin was produced by Sigma Co, lot No 31H4040. Phosphocellulose (Whatman Co). Acetylfibrocyn membrane(Siqing Biochemical Factory, Huangyan, Zhejiang).

Extraction, Isolation and Identification of Polα, Polβ, and Polγ

Referring to the method of Ono.K[8]. Ehrlich tumor cells were inoculated into body of mice, 7-9 days after, tumor cells were collected, washed. Following processes were performed at 0-4°C. 50ml of buffer A (50mmol.L^{-1} Tris-HCl, PH7.6, 1 mmol. L^{-1} DTT, 0.1 mmol.L^{-1} edetic acid, 100 mmol.L^{-1} KCl, 10% glycerol) was added to the EAC cells. The cells were ruptured by ultrasonic wave after mixed with buffer A homogeneously. The homogenate was centrifuged in high speed (27000g, 30min). The supernant contained crude enzyme of Pol.

The supernant was loaded onto phosphocellulose column, which was preequiliberated by buffer A for 24 hours, then the column was washed with buffer A to remove hybrid protein. The linear gradient isolation was conducted with buffer A 30ml (100 mmol. L^{-1} KCl) against buffer B (1000 mmol.L^{-1} KCl) 30ml. Different part of washing liquid was collected (1ml for one tube), the activity of enzyme was detected, and the curve of activity of enzyme was drawn.

Identification of Pol α, β, and γ: Comparing the curve of activity of enzyme with those demonstrated in literature, according to the sequence of washing out and the corresponding ion strength, as well as the reaction to Aphidicolin - specific Pol inhibitor, subunits of Pol were identified.

The Detection of Activity of Pol

The reacting mixed solution for the detection of activity of Pol α, β, and γ was 50 μl respectively. The common componants were 0.08g.L^{-1} Act DNA, 10μmol.L^{-1} dATP, dCTP and dGTP, 10mmol.L^{-1}MgCl$_2$, 5 mmol.L^{-1} DTT, 0.25g.L^{-1}BSA, 0.227 μmol.L^{-1} [^{3}H]-dTTP. The solution also included 25 mmol. L^{-1} Tris-HCl, PH8.5 for detecting the activity of Pol α; included 50 mmol.L^{-1} Tris-HCl, PH 9.0 and 100 mmol.L^{-1} KCl for Pol β; and 50 mmol.L^{-1} Tris-HCl, PH 7.5, 100 mmol. L^{-1} KCl for Pol γ. The mixed solution was incubatied in water bath of 37°C, 20 μl of 0.2 mol. L^{-1} edetic acid was added to stop reaction. The 50 μl mixture was transfered onto acetylfibrocyn membrane after 30 minutes to gather the acid insoluble part which was washed with 5% trichloroacetlic acid thrice, 95% ethanol twice after the acetylfibrocyn membrane being desiccated. The activity of enzyme was detected by counting the quantity of [^{3}H]-dTTP incorporated into Act DNA within 1 hour at 37°C.

The Inhibitory Effect of ECPC, ECS on Polα , β, and γ[9]

The method was similar to that for detecting the activity of Pol, however, drug solution was added into every experimental tube and tridistillated water into control tube. The IC_{50}were calculated in IBM computer by weighed probit analysis (the programs of Medical Statistics, POMS-2.00).

The Mode of Inhibition of ECPC on Pol[10]

Three groups were divided, the concentration of ECPC was 0μg/ml, 2.5μg/ml, 3.7 μ g/ml respectively, the reaction speed was detected when the concentration of Act DNA in individual group was 10μg/ml, 20μg/ml, 40μg/ml, 80μg/ml, 160μg/ml, respectively. There were three parallel tubes at every concentration of Act DNA. The results were treated by double reciprocal. The curve of reaction velocity against concentration of primer-template were plotted, the reciprocal of velocity (1/v) as ordinate, the reciprocal of concentration of Act DNA (1/[Act DNA]) as abscissa. The conclusion was made according to the curves and the K_I value was counted.

The Counting of Ki Values

Described as the reference[10].

RESULTS

The Isolation and Identification of Pol α, β, and γ

There were three peaks of activity in curve of activity of enzyme. According the sequence of washing out and the correspondent ionic strength, F1, F2 and F3 were identified as Pol α, Polγ and Polβ. F1 was inhibited by aphidicolin, the specific inhibitor

of Pol, with the inhibitory rate of 54.9%. But F2 and F3 could not be inhibited by aphidicolin, which was conformable to literatures. (Figure 1)

The inhibition of ECS and ECPC on Pol α, β, and γ

The results is shown in Table 1 and Figure 2. The detection of IC_{50} : The IC_{50} of ECS on Pol α, β, and γ were respectively 10.2μg/ml, 9.9μg/ml, 28.9μg/ml. The IC_{50} of ECPC on Pol α, β, and γ were respectively 5.6μg/ml, 15.0μg/ml and 14.7 μg/ml.

The inhibitory mode of ECPC on Pol α, β, and γ

According to,[10] the 3 lines intersected at the same point on x-axis, it meant that the inhibitory mode of ECPC on Pol α, β, and γ was noncompetitive inhibition respectively with template DNA (Figure 3).

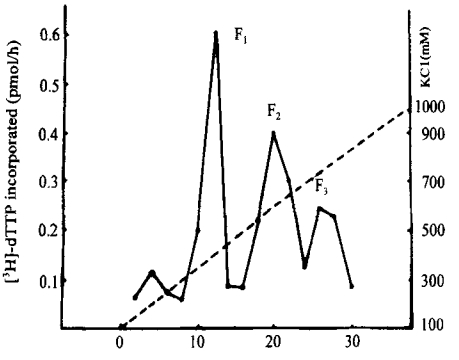

Fig 1. The peaks of activity of Pol extracted from EAC cells, separated by phosphocellulose column chromatography.

Tab.1 The inhibitory effect of extract of camellia sinensis and extract of camellia ptilophylla chang on DNA polymerase of Ehrlich ascites carcinoma cells

	The inhibitory rate (%)		
	Pol α	Pol β	Pol γ
ECS(μ g/ml)			
3.1	13.7± 1.3	39.6± 2.9	10.8± 2.0
9.3	54.8± 10.1	34.2± 16.3	14.7± 16.3
27.8	81.8± 1.1	73.5± 2.7	68.4± 5.3
83.3	94.2± 1.8	76.5± 8.5	77.8± 6.9
250.0	94.0± 1.7	88.7± 2.6	85.9± 0.9
ECPC(μ g/ml)			
3.1	28.8± 3.7	15.5± 13	22.5± 5.5
9.3	63.9± 4.4	53.4± 5.6	70.1± 7.8
27.8	85.5± 0.8	69.3± 9.3	70.9± 2.0
83.3	93.1± 0.1	81.1± 1.4	80.5± 3.2
250.0	93.9± 2.6	80.4± 0.8	76.4± 2.1

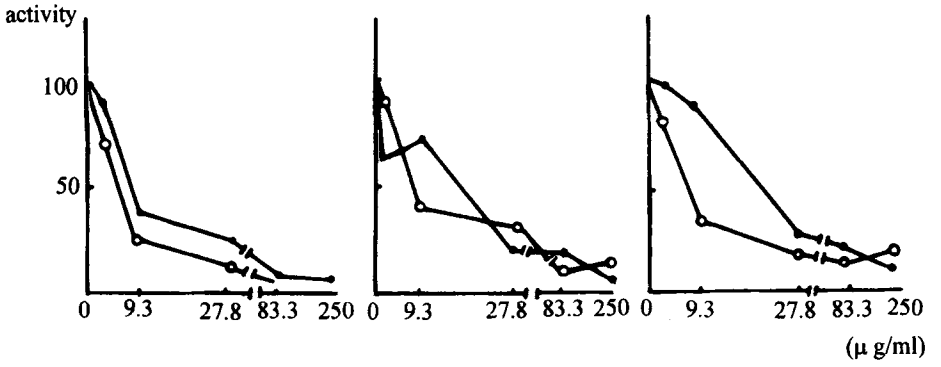

Fig 2. The effect of extract of camellia sinensis and extract of camellia ptilophylla chang on DNA polymerase α,β and γ (ECS • ECPC°)

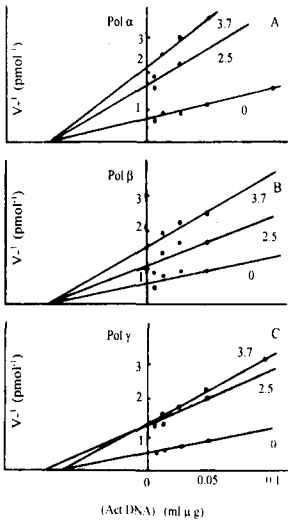

Fig 3. The mode of inhibition of extract of camellia ptilophylla chang on polymerase α, β and γ of EAC cells.

Ki value

The Ki values of ECPC on Pol α, β, and γ were respectively 2.68±0.12 μg/ml, 2.24±0.12 μg/ml, 2.56±0.18μg/ml.

DISCUSSION

At least 4 kinds Pol, Pol α, β, γ and δ were found in eukaryocytes. Pol α and Polδ were considered responsible to DNA replication, Pol γ related to replication of mitochondrial DNA. Any kind of Pol was inhibited, the proliferation and growth of cells would be affected in different degree.

In this paper, the phosphocellulose column chromotography was conducted to isolate Pol α, β, γ, combining the curve of enzyme activity and the use of specific inhibitor, three kinds of Pol were identified with well distinctiveness. About 30 compounds were studied using this method by us. It was shown that the compound, which inhibited Pol, would possess antitumor activity. This method may be as a complement of present screening method for antitumor drugs.[11]

It was shown that green tea suppressed cancer cells, but the report on inhibitory effect of ECS and ECPC on Pol was not found in literatures. The inhibitory effects of ECS and ECPC on Pol were compared with that of adriamycin using the IC_{50} as indicator, the potency were similar to adriamycin (the IC_{50} of adriamycin on Pol α, β and γ were detected simultaneously in our laboratory, they were 16.9,12.8 and 12.2 μg/ml correspondly), slightly high in ECPC. The study on mode of action showed that ECPC inhibited Pol α, β and γ by non-competitive inhibition on template DNA. It meant ECPC combined both of enzyme particles and reactive mediate products (E.S).

ECPC was a new wild tea discovered lately. Both of ECPC and ECS were belong to the same genus and family, but not same kind. ECS contained mainly alkali of coffee, but ECPC contained mainly alkali of cocoa. Catechol is the Physiological active substance of tea. The researches on ECS and ECPC conducted in our laboratory showed that both of them inhibited DNA topoisomerase II strongly, their inhibitory activity on tumor of mice had been proved[7]. In this study, the inhibitory effects of ECS and ECPC on DNA polymerase of tumor cells were proved, it may be one of mechanisms of antitumor action of ECS and ECPC. It was suggested that further investigation is valuable for developing this two extracts into anticancer drugs.

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