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Evaluation of Manisa propolis effect on leukemia cell line by telomerase activity

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Abstract

Propolis is a resinous substance which is used by bees to repair and maintain their hives. It has more than 180 compounds including flavonoids, phenolic acids and its esters which have anti-inflammatory, antibacterial, antiviral, immunomodulatory, antioxidant and antiproliferative effects. Propolis is shown to inhibit cell division and protein synthesis. However the exact mechanism underlying antitumor effect is not clearly described. On the other hand progressive telomere shortening to a critical level results with senescence of normal cells by inducing apoptosis and telomerase prevents erosion of telomeres. In this study we aimed to evaluate hTERT ratios in propolis-treated T-cell acute lymphoblastic leukemia (CCFR-CEM) cell line. Cell counts and cell viability of propolis-treated and propolis-free T-cell acute lymphoblastic leukemia (CCFR-CEM) cell line were assessed by trypan blue dye exclusion test and MTT assay. The LightCycler instrument was used (online real-time PCR) for the quantification of hTERT in CCFR-CEM cell line. The hTERT ratio significantly decreased 60 and 93% after 24 and 72 h respectively compared to the initial value of the cells incubated with propolis. It had almost no cytotoxic effect and caused 30, 30, 22 and 12% decrease in cell counts after 24, 48, 72 and 96 h respectively which is statistically significant.

In conclusion propolis may show antitumor and apoptotic effect via inhibiting telomerase expression besides the mechanisms which have been described previously.

Keywords: Propolis; Antitumor; Leukemia; hTERT

1. Introduction

Propolis is a resinous material, which is a product of honeybees. It has been reported that propolis has anti-inflammatory, antibacterial, antiviral, immunomodulatory, antioxidant and antiproliferative effects [1–5]. Its chemical components and biological properties show variation in different geographical and botanical origin [6]. Flavonoids, phenolic compounds and wax are some of the well-known compounds of propolis [7]. It has been shown that caffeic acid phenethyl ester (CAPE) which is one of the components of propolis has an antitumor and antioxidant properties [8,9]. Cinnamic acid is also another phenolic compound which has antitumor activity [10]. Flavonoids, the major compounds exist in propolis, have anticancer activity as well as CAPE and cinnamic acid, and Yanagihara et al. have shown that this activity is due to inducing apoptosis in the target cells [11]. Inducing apoptosis is one of the mechanisms for several therapeutic agents as shown in propolis by a number of studies [12,13]. Chen et al. has demonstrated that propolin C inhibited the proliferation of human melanoma cells through inducing a cytotoxic effect and triggering apoptosis [14]. Aso et al. showed that propolis inhibited the growth of human leukemia cell line in a dose-dependent manner [13].

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On the other hand telomerase is an enzyme, which is necessary to synthesize new telomeres on human chromosomes and they do not have detectable activity in human somatic cells except germ cells and stem cells of renewable tissues [15]. However almost all human malignancies show telomerase activity [16]. Human telomerase reverse transcriptase (hTERT) is known to be catalytic subunit of telomerase and correlated with telomerase activity (TA) [17,18]. Therefore we investigated the effect of propolis on telomerase activity in acute lymphoblastic leukemia cell culture in this study.

2. Materials and methods

2.1. Chemicals

Ten grams of powered propolis which had been collected in Manisa city (Turkey) was dissolved in 50 mL of 60% ethanol. The ethanol extract was filtered and evaporated by waiting in 60 °C for 2 h. The reduced amount of solution was filled up to 50 mL by adding bidistilled-sterilized water to give a concentration of 300 mg/mL. Manisa propolis was diluted to a working concentration of 1/10 000 (0.03 μg/mL) which has an IC_{50} value for Manisa propolis in our study (data not presented).

2.2. Cell line and cell culture

T-cell acute lymphoblastic leukemia (CCFR-CEM) cell line was obtained from Memorial Sloan Kettering Oncology Center, New York. Cells were cultured in RPMI-1640 medium (Sigma Chemical Co., St. Louis, MO) supplemented with 10 000 U/mL penicillin, 10 mg/mL streptomycin, 2 mM L-glutamine and 10% heat-inactivated fetal bovine serum at 37 °C under a humidified 95% air 5% CO_2 atmosphere.

2.3. Cell proliferation experiments

Cells were incubated at a density of 6×10^6/mL in 1 mL of medium using 24-well and 96-well plates. Two wells for each period of incubation (0, 24, 48, 72 and 96 h) were prepared and the average cell count and cell viability were determined by trypan blue dye exclusion test and MTT assay.

2.4. Measurement of hTERT

A quantitative detection of hTERT mRNA was performed with the commercially available LightCycler Telo TAGGG hTERT Quantification Kit (Roche Applied Science, Mannheim, Germany) using the LightCycler instrument for real-time PCR. All subsequent quantification steps were carried out according to the manufacturer's instructions.

2.5. Statistical analysis

Student's *t*-test and non-linear regression analysis were performed using SPSS for windows.

3. Results

3.1. Cell viability

Cell counts of propolis-free and propolis-treated cell lines are shown in Table 1 and Fig. 1. Propolis caused a 30% decrease in cell viability after 24 and 48 h compared to the control cell culture (Fig. 2). Inhibitory effect of propolis on cell count decreased to 22 and 12% after 72 and 96 h ($t = 3.63$, $p = 0.022$).

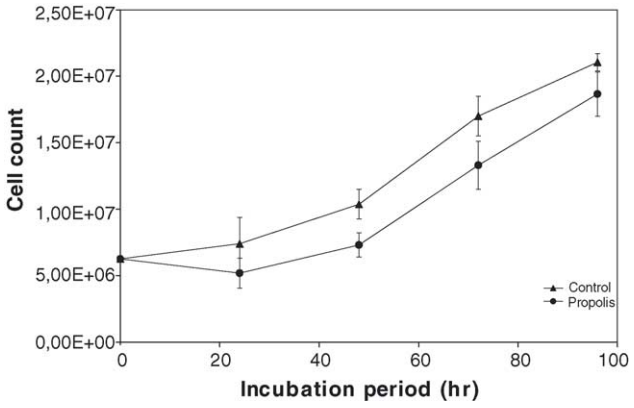

Fig. 1. Cell counts in propolis-treated and propolis-free cell cultures (working concentration of propolis: 0.03 μg/mL).

Table 1
Cell counts in propolis-treated and propolis-free cell cultures

Incubation period (h)	Control	Propolis[a]	Ratio	Control S.D.	Propolis S.D.
0	6.25E+06	6.25E+06	100.00		
24	7.41E+06	5.19E+06	70.04	1.98E+06	1.13E+06
48	1.04E+07	7.31E+06	70.48	1.11E+06	9.21E+05
72	1.70E+07	1.33E+07	78.31	1.49E+06	1.82E+06
96	2.11E+07	1.87E+07	88.63	6.57E+05	1.66E+06
Mean	1.24E+07[a]	1.01E+07[a]	81.49	1.31E+06	1.38E+06

[a] Working concentration: 0.03 μg/mL, $p = 0.022$.