Antitumoral Property of Ethanolic Extract of Propolis in Mice-Bearing Ehrlich Carcinoma, as Compared to Bleomycin

S. Scheller, W. Krol, J. Swiacik, S. Owczarek, J. Gabrys*, and J. Shani**

Department of Microbiology and *Department of Histology and Embryology, Silesian School of Medicine, 19 Karl Marksa Street, 41-808 Zabrze-Rokitnica, Poland, and **School of Pharmacy, University of Southern California, Los Angeles, CA 90033, U.S.A.

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Antitumoral effect of ethanolic extract of propolis (EEP) was demonstrated in mature mice-bearing Ehrlich carcinoma. Survival rate after EEP treatment was compared to that of bleomycin, given alone or in combination every two days for 36 days and followed up for 14 additional days. The survival rate at 50 days was 55% after EEP and 40% after bleomycin, while all the mice-treated with EEP + bleomycin combination demonstrated shorter survival than the controls. It is concluded that while the *in vivo* activity of bleomycin is reduced in the presence of cytochrome-C-reductase inhibitors (like some of the EEP components are), the antitumoral property of EEP in the tumored animal model studied is significant and lasting.

Introduction

Propolis is a natural resinous product of honey bees, used to strengthen and wax their nests. It is rich in free amino acids and flavonoids, and has antibacterial properties [1]. Its ethanolic extract exhibited marked antiprotozoan activity [2], it raised the mitotic index of cells cultivated *in vitro,* and intensified NADH2-reductase activity in such cells [3]. *In vivo* EEP demonstrated enhanced activity of the enzymes NADH2 and glucose-6-phosphatase in rats [4], accelerated the rate of ossification and stimulated regeneration of dental pulp [5]. While parenteral administration of EEP to rabbits does not induce *anti*-EEP antibody synthesis *in vivo* [1], it increases the number of cells synthesizing antibodies *in vitro* [6]. In aging subjects with impairment of immunological functions, application of crude propolis or EEP restored several of these functions [7]. Its immunostimulatory activity was demonstrated *in vitro:* EEP increased the cytotoxicity of NK cells (S. Scheller and W. Krol, unpublished), inhibited the development of HeLa (cervix) and KB (nasopharynx) carcinoma cells *in vitro* and exerted cytotoxic activity on Ehrlich carcinoma cells [8]. *In vivo* studies indicated that EEP stimulated the immune system in patients with prostate inflammation [9]. These properties led us to compare its antitumorogenic properties in mice-bearing Ehrlich carcinoma with bleomycin, which is highly effective in this tumor model.

Materials and Methods

Forty BALB/c mice (20 males and 20 females), weighing 22–25 g each were used for this study. They were injected IP with Ehrlich effusive carcinoma cells suspended in Hank's medium, 10^6 cells/0.3 ml per mouse, and were divided into five equal groups. Each group was treated IP with 0.5 ml of one of the following preparations, on every other day, for 36 days: EEP 0.25% in 1% DMSO; bleomycin (Nippon Kayaku Comp., Tokyo, Japan), 0.001% in 1% DMSO; EEP and bleomycin mixture (0.5 ml solution containing 0.25% dried EEP and 0.001% bleomycin) in 1% DMSO; 1% DMSO or saline. Survival was monitored daily at 10 a.m., and presented graphically as a function of time. DMSO (BDH Ltd., Poole, England), was diluted with physiological saline. Propolis was collected in the beehive of the University's farm. The procedure for preparing its ethanolic extract was described in a previous publication from this laboratory [10].

Results and Discussion

Fig. 1 illustrates the survival of each of the experimental groups, as recorded daily from day 0, through the end of the treatment period (day 36) up to day 50. Survival patterns after EEP and bleomycin were similar, with the effect of bleomycin starting to diminish on day 17, while EEP managed to negate mortality of the tumored mice up to day 25. Survival of the mice undergoing separate EEP or bleomycin treatments were 55% and 40% on day 50, respectively. Mortality rates of control mice reached 100% on day 40–42, while mortality of the EEP + bleomycin combination group was higher than the controls, and reached 100% on day 33. There is no significant dif-

Reprint requests to Prof. Dr. S. Scheller, Head, Department of Microbiology, Silesian School of Medicine, 19 Karl Marksa Street, 41-808 Zabrze-Rokitnica, Poland.

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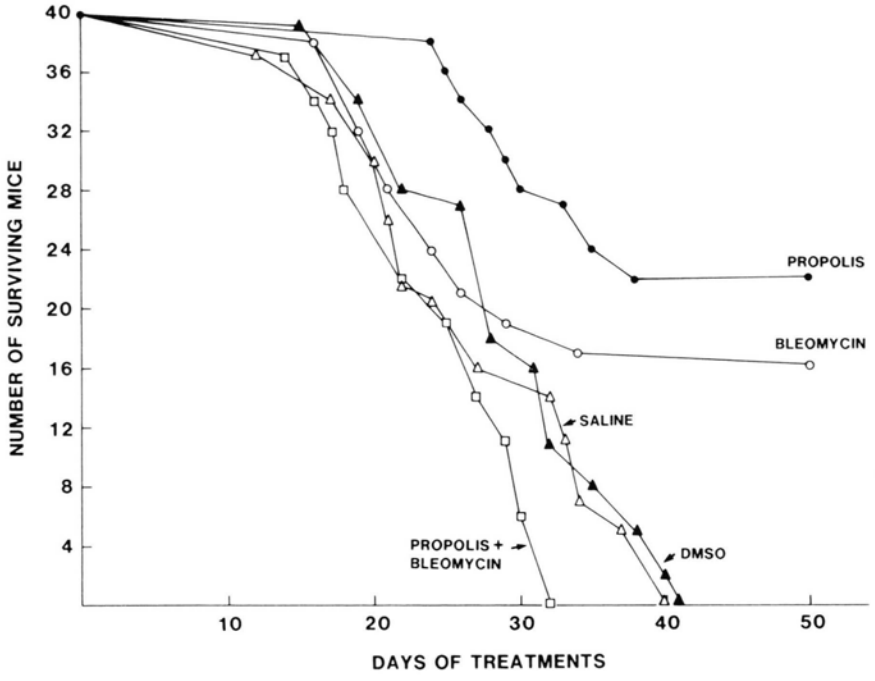

Fig. 1. The effect of ethanolic extract of propolis (EEP), as compared to bleomycin, alone and in combination, on survival rate of mice-bearing Ehrlich carcinoma (n = 40).

ference in mortality between male and female mice in any of the groups.

Antitumoral activity of EEP against Ehrlich carcinoma cells and the inhibitory effect of different extracts on development of HeLa and KB cells *in vitro* is suggested to be related to its content of flavonoids [8]. Flavonoids affect metabolic stages of Ehrlich carcinoma cells, *e.g.* inhibit the incorporation of thymidine, uridine and leucine into them which in turn lead to inhibition of DNA synthesis [11]. The inhibitory role played by flavonoids in the antineoplastic process, has also been confirmed in other experimental models. Flavonoids inhibit carcinogenesis induced by polycyclic aromatic hydrocarbons in cancer models [12]. The mechanisms of these activities are connected with the ability of the flavonoids to inhibit metabolic stimulation induced by such polycyclic aromatic hydrocarbons and by affecting the activity of some cell promoters [12].

Our previous *in vivo* observations suggest that the antitumoral activity of propolis extract is related to its immunostimulatory property [6]. Example of this activity is the enhanced antitumoral activity demonstrated by EEP on NK cells and Ehrlich carcinoma cells *in vitro*. The antagonistic effect of EEP + bleomycin combination is probably neutralizing the antioxidative property of the drug [13]. This property is antagonistic to the mode of action of bleomycin, as the latter is a glycopeptide antibiotic, which is activated by superoxide ion and by free radicals [14], and which *in vitro* exhibits reduced DNA degenerative activity in the presence of standard free radical scavangers [15]. Attempts to elucidate detailed mechanism of EEP activity on Ehrlich carcinoma and further studies on combined therapies using EEP and other antitumoral drugs, are currently being studied in our laboratory.

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