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Effects produced by Royal Jelly on haematopoiesis: relation with host resistance against Ehrlich ascites tumour challenge

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Abstract

Royal jelly (RJ) was shown to exhibit immunomodulatory properties, although its biological activity is still unclear. In order to elucidate the mechanism whereby RJ activates the immunological system, we examined the role of this substance on the haematopoietic response of Ehrlich ascites tumour (EAT)-bearing mice. Our results demonstrated that RJ prevented the myelosupression induced by the temporal evolution of the tumour and abrogated the splenic haematopoiesis observed in EAT-bearing mice. The stimulating effect of RJ was also observed in vitro on the multipotent bone marrow stem cells, evaluated by the long-term bone marrow cultures (LTBMCs). The study of survival clearly showed the antitumour activity of RJ. Treatment was given prophylactically for 20 days and therapeutically for 3, 8 and 13 days. Except for the treatment with the lower dose of 500 mg/kg, given for 23 days, all the other dose schedules were able to prolong survival. A more effective antitumoural response was observed with the more prolonged treatment regimen. In this regard, the administration of RJ for 33 days produced the highest protection reaching an extension of survival at about 38%, 71% and 85% for the doses of 500, 1000 and 1500 mg/kg, respectively, whereas with the 23 and 28 days treatment schedules, survival increased at a rate of 19% and 23%, respectively, and comparable results were found among the effective doses of RJ. Increased survival rate might be related to the decreased Prostaglandin E_2 (PGE_2) levels observed in EAT-bearing mice after RJ treatment. These results point to RJ as a promising modifier of biological response leading to myeloprotection and antitumour activity.

Keywords: Royal Jelly; Erhlich ascites tumour; Haematopoiesis; GM-CFU; LTBMC

1. Introduction

Several types of immunopotentiators have been developed recently and are being studied for possible use in the treatment of patients suffering from

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malignant diseases [1–3]. The increased interest in new approaches to the immunotherapy of cancer and a considerable demand for therapeutic agents which can modulate the several forms of immunodeficiency has encouraged studies on the immunomodulatory mechanism of natural and synthetic substances [4–7].

Royal jelly (RJ), a food produced by the hypopharingeal and mandibular glands of the worker honey bees (*Apis mellifera* Linné) contains many important compounds with biological activity, such as free amino acids, proteins, sugars, fatty acids (mainly 10-hydroxy-2-decenoic acid; 10-HDA), minerals (mainly iron and calcium) and vitamins (mainly thiamine, niacin, riboflavin) [8–12]. Several works in the literature demonstrate the immunomodulatory properties of RJ [13–15]. According to [13] RJ stimulates antibody production and proliferation of immunocompetent cells in mice and depresses humoral immune functions in rats. RJ is also effective against the hematopoietic dysfunction observed in X-irradiated mice, promoting macrophage activity and hematopoietic stem cell proliferation [16]. Moreover, this substance was reported to suppress allergic reactions in association with the restoration of macrophage function and the improvement of Th1/Th2 cell responses in mice [17].

Tumour growth initiates a myriad of functional and phenotypic changes in macrophages and T-cells in association with alterations in cytokine synthesis and responsiveness [18]. According to DeGowin et al. [19] extramedullary tumours produce many substances, possibly colony stimulating factor (CSF), that selectively inhibit marrow stromal cells colony growth in vitro [19]. Several types of tumour also express receptors for granulocyte–macrophage colony stimulating factors or produce and use granulocyte–macrophage colony stimulating factors as an autocrine growth factor [20–22].

Ehrlich ascites tumour (EAT) cells grow rapidly in almost any mouse strain [23] inducing profound haematopoietic and immune dysfunction. They produce either ascitic or solid tumours, killing their host even when given in extremely small doses. EAT produces impairment in the number of granulocyte–macrophage colonies, associated with splenic haematopoiesis [5,6,24], and induction of natural suppressor cells [25]. Phagocytes, particularly macrophages and neutrophils, play a vital role in both the innate and the acquired immunity, exerting a key role in host defense against various infectious agents and tumour growth [26–28]. One of the most important characteristics of these cells, which is necessary for effective cell-mediated immune responses and non-specific inflammatory reactions, is their capacity to migrate into inflammatory sites [29]. Using animal models, studies have shown that a variety of tumour cells can produce factors which impair inflammatory responses, thus allowing tumour growth in vivo. Alternatively, the tumour cells can stimulate macrophage suppressor activities in host cells [30–34]. In addition to the deficient accumulation of phagocytes at the inflammatory sites, several other cellular parameters have been shown to be altered in the tumour-bearing state, in particular, the formation of granulocyte–macrophage colonies in response to CSF [35,36].

Based on these findings, we designed the present study to explore the prophylactic–therapeutic effects of RJ on the growth and differentiation of bone marrow and spleen granulocyte–macrophage progenitor cells in the Ehrlich ascites tumour bearing-mice. The antitumour effect of RJ, measured by host survival was also studied.

2. Material and methods

2.1. Mice

Male BALB/c mice, 8–10 weeks old inbred in our laboratory were matched for body weight before use. The animals were housed 10 per cage and were allowed access to laboratory chow and water ad libitum. All mice were raised under specific pathogen-free conditions and were maintained in conventional animal rooms before use. Animal experiments were done in accordance with institutional protocols of the Institutional Animal Care and Use Committee.

2.2. Tumour model

Erhlich ascites tumour was maintained in BALB/c mice by serial transplantation. Tumour cell suspensions were prepared in balanced salt solution at pH 7.4 to final concentrations 6×10^6 viable cells/animal. In all experimental protocols described,