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Anti-tumor effects of propranolol: Adjuvant activity on a transplanted murine breast cancer model

Somayeh Ashrafi[a], Reza Shapouri[b], Ahmad Shirkhani[c], Mehdi Mahdavi[c,d,*]

[a] Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Islamic Azad University, Pharmaceutical Sciences Branch, (IAUPS), Tehran, Iran
[b] Biology Research Center Zanjan Branch, Islamic Azad University, Zanjan, Iran
[c] Department of Immunology, Pasteur Institute of Iran, Tehran, Iran
[d] Recombinant Vaccine Research Center, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Propranolol (Pro), a non-specific β-adrenergic blocking drug, competitively prevents the binding of catecholamines to receptors and suppresses cancer cells. The anti-tumor activity of propranolol has been proved in different kinds of cancers. In this study, we assessed the adjuvant activity of propranolol combined with a tumor vaccine model on the immunological parameters of breast tumor-bearing mice. Breast tumor pieces were implanted into the flank of inbred BALB/C female mice from stock mice. Tumor-bearing mice were treated with tumor antigen lysate vaccine and propranolol/Vaccine (Pro/Vac) combination (as treatment groups), propranolol and PBS (as control groups) for 5 consecutive days, every 12 h. Moreover, all experimental groups received vaccine for three times with one-week interval via s.c injection. After immunization courses, spleens of tumor-bearing mice were removed and dissected, cell suspension was stimulated in vitro, and the cytokine levels in supernatant of splenocytes were measured via commercial ELISA kits. Compared with the vaccine group, immunization with tumor lysate in combination with propranolol significantly increased IL-2, IL-4, IL-12, IL-17, and IFN-γ cytokines. Considering the suppression of tumor growth, propranolol seems to be a potent immunomodulator capable of inducing cellular immune responses against breast cancer.

1. Introduction

Breast cancer is the most prevalent diagnosed cancer in the world, accounting for a leading cause of death in women in developed and developing countries [1]. According to the statistics provided by American Cancer Society, about 3.1 million women were detected with a history of breast cancer in 2017 in the USA. Overall, about 40,610 women were projected to die from breast cancer in the USA; in addition, it is estimated that about 30% of cancers diagnosed in women will be breast cancers [2].

There is studies showing that Beta-adrenoceptor (β-AR) agonists, such as epinephrine (EPI), norepinephrine (NE), and stress hormones that are locally released by sympathetic nervous system (SNS) terminals, could recruit and stimulate endothelial cells to proliferate and migrate, thereby starting the crucial step of tumor expansion [3]. These catecholamines inhibit apoptosis and upregulate vascular endothelial growth factor (VEGF) and MMP expression levels, resulting in angiogenesis and metastasis effects in some kinds of tumor cells in vitro such as breast cancer which can be blocked by propranolol [4]. Propranolol, a non-selective beta-adrenergic receptor blocking agent, is used to treat high blood pressure (hypertension) [5] and severe infant's hemangioma [6]. Propranolol, in competition with adrenergic receptor-stimulating agents (EPI, NE), inhibits the catecholamine's-induced cancer [7]. Studies showed that stimulation of beta adrenergic receptors (β-ARs), especially β2-ARs, on the surface of antigen presenting cells and T lymphocytes are responsible for its bioactivity [8–10]. Additionally, propranolol suppresses Treg, increases DC function in the induction of T cells and cellular immune responses, and stimulates DCs/Macrophages to produce the IL-12 cytokine that shifts the immune response toward the Th1 pattern [11–13]. A number of in vitro, in vivo and preclinical experiments have approved anti-tumoral, anti-metastatic and anti-angiogenic features of propranolol in most common malignancies [14,15]. Moreover, a variety of clinical studies have reported that propranolol decreases the mortality rate of breast cancer and improves survival [16–18].

It has been shown that chronic inflammation is the major stimulator factor of cancer progression and one of the decisive factors in tumor microenvironment [19]. Tumor microenvironment, via production of

* Corresponding author at: Departments of Immunology, Pasteur Institute of Iran, 69 Pasteur Ave, Kargar Ave, P.O. Box: 1316943551, Tehran, Iran.
E-mail address: M_Mahdavi@pasteur.ac.ir (M. Mahdavi).

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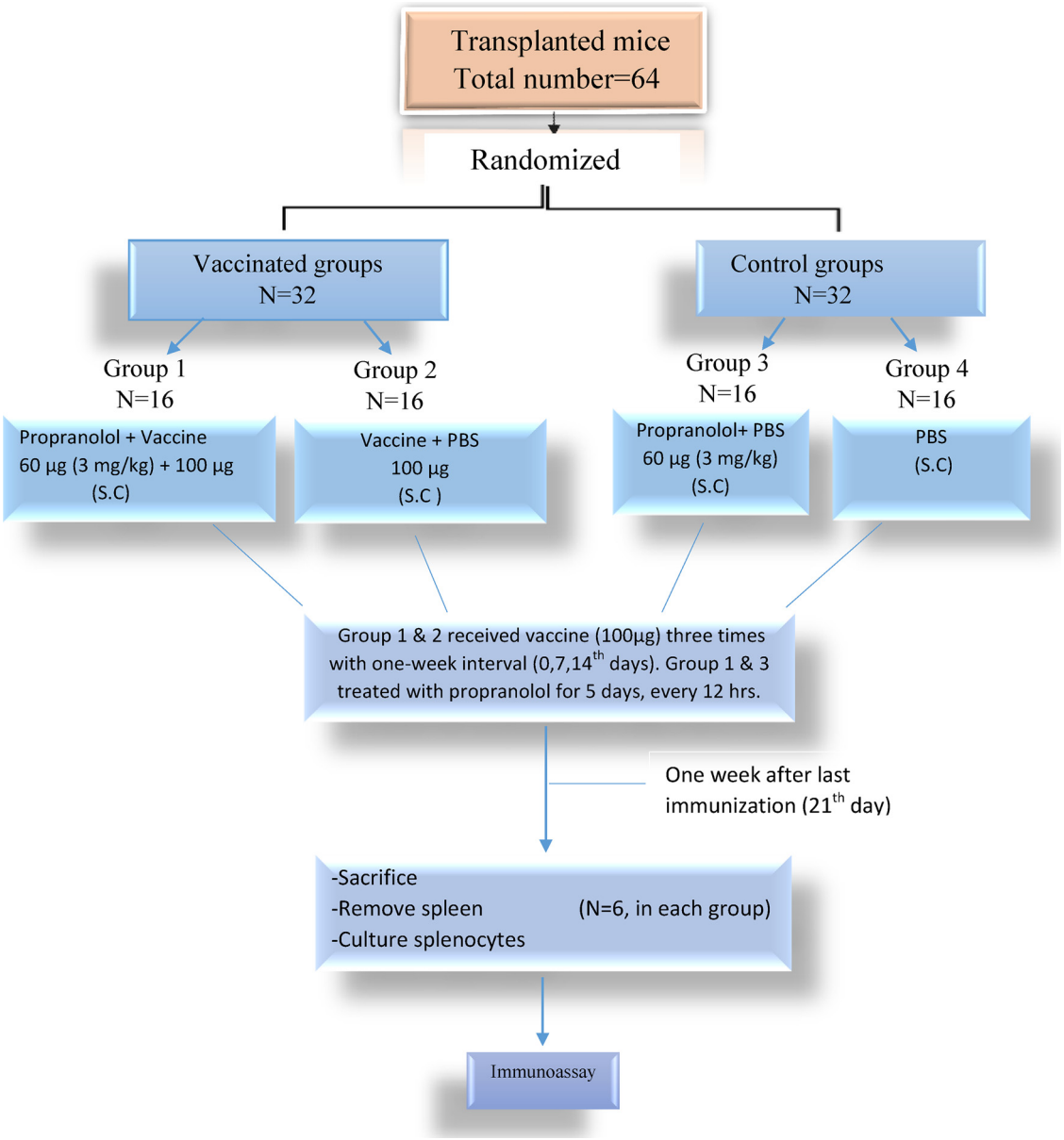

Fig. 1. Flow diagram of the methodology and study planning.

various pro-inflammatory cytokines activated by innate immune cells and inflammatory cells, contributes to tumor promotion and supports cancerous cell survival. Cytokines are key immune mediators that can function as potential therapeutic targets [20]. Cytokine-based cancer immunotherapy is a promising approach for cancer treatment and reveals a beneficial choice for molecular cancer diagnosis and cancer therapeutics.

The present study hypothesizes that administration of propranolol with vaccine (tumor lysate) to tumor-bearing mice in specified time intervals augments anti-tumor immune responses and simultaneously reduces tumor progression compared to those receiving mere vaccine. It also indicates that Pro/Vac combination inducing the production of cytokines (especially IL-17) creates an immuno-protective mechanism that leads to cancer control.

2. Materials and methods

2.1. 4T1 cell line propagation

Murine 4T1 cell line, purchased from National Cell Bank of Pasteur Institute of Iran, was cultured in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum (FBS), antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin), 4 mM L-glutamine,1 mM sodium pyruvate and 50 µm 2-ME. The cells were grown under standard conditions (in a cell culture incubator with 37 °C, 5% CO_2 and humidity 60%) and passaged at 80% confluency. To harvest, cells were detached from the flask by short incubation with Trypsin/EDTA (GIBCO, Germany) solution, then washed three times in cold PBS (PH = 7.4, centrifugation at 1200 rpm, 5 min), and resuspended in PBS (5×10^6 cells/ml) for further studies.

2.2. Vaccine preparation

4T1 cell suspension, with a density of 5×10^6 cell/ml in PBS containing 1 mM phenyl methane sulfonyl fluoride (PMSF), was prepared and 5 cycles of freeze/thaw were carried out in nitrogen/37 °C water bath. In the next step, cell suspension was sonicated for 6–7 cycles in 6 HZ, 0.5 amplitude. To achieve the sterile protein, the cells were centrifuged and supernatant was isolated and dialyzed versus PBS and filtered (0.2 µm). The protein concentration was determined using