Antitumoral Effect of Bleomycin + Dolomite Combination Treatment, in Mice Bearing Ehrlich Ascites Carcinoma

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Dolomite, a mineral composed of magnesium and calcium carbonates, potentiates the antitumoral activity of bleomycin: While 40 days after inoculation, no mice survived the Ehrlich ascites tumor burden, 44% of them survived it after bleomycin treatment, and 63% after a simultaneous treatment of bleomycin and dolomite. The beneficial antitumor effect of dolomite is probably related to its high content (12.8%) of magnesium.

Introduction

Magnesium has been suggested as a one of the key elements in carcinogenesis, due to its involvement in immunologic mechanisms related to some neoplastic diseases [1, 2]. Low serum levels of magnesium elevate the concentration of arachidonic acid, and some of its metabolites, *e.g.* prostaglandines, inhibit early immunological reactions and cause excessive production of cachectine (TNF-α). Moreover, magnesium ions stimulate synthesis of cytotoxic antibodies, such as complement-mediated cytotoxicity (CMC) and antibody-dependent cell-mediated cytotoxicity (ADCC). Magnesium also activates alternative complement routes, thus increasing antitumoral effects. Non-immunogenic surveillance performed by NK cells also require Mg^{2+} ions. Magnesium-dependent tumor cytolysis requires T-lymphocytes, macrophages and neutrophils and forms activated species of oxygen [3, 4].

The antitumoral property of magnesium also reflect itself in an unbalanced lymphocyte formation: the number of CD4 lymphocytes decreases and that of CD8 increases [2]. Low magnesium serum levels also induce an oncogenic reaction, characterized by increase in inflammatory glycoproteins, leukotriene B4 and superoxide anions [5]. While in most reports magnesium is considered anticarcinogenic, like in cases of hemolymphoreticular carcinogenesis, in solid tumors it acts as a tumor-promoting factor. Elevation of magnesium levels in solid tumors is observed, when it is monitored with its simultaneous depletion in the surrounding healthy tissues. Magnesium is involved, therefore, in both carcinogenic and anti-carcinogenic activities [4, 6].

Our interest in exploring the possible potentiating effect of dolomite on antitumoral activity stems from the fact that while it is composed of carbonates of both magnesium and calcium, these two cations may have in some neoplastic diseases antagonistic activity. For instance, in chronic lymphoid leukemia and in some types of stomach cancer, an increase in magnesium serum levels is followed by a decrease in serum calcium levels [5, 7]. We decided to administer dolomite into Ehrlich ascitic mice simultaneously with bleomycin, an highly-effective agent against this tumor, acting through production of free radicals and damage to the DNA structure of neoplastic cells [8–11].

Materials and Methods

One hundred and ten BALB/c mice (55 males and 55 females), weighing 22–25 g each were used in this study. They were injected intraperitoneally (0.2 ml/mouse) with Ehrlich effusive carcinoma cells, pre-suspended in PBS to a concentration of 5×10^6 cells/1.0 ml and were divided into five groups of about the same size. Bleomycin (Nippon Kayaku Ltd., Tokyo, Japan) was administered intraperitoneally every other day, as a solution in saline (0.3 mg/0.2 ml/mouse), beginning with the second day and ending with the 30th day after inoculation. Dolomite (VIS Chemicals, Katowice, Poland) was mixed with the standard mice food (Centr. Lab. of Feeding, Motycz, Poland) at a concentration of 500 mg/kg, thus yielding a 24 h intake of about 3 mg per mouse. This feed was supplied freely throughout the study.

Each of the groups received one of the following treatments:

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(1) Bleomycin (15 IP injections, days 2–30) and dolomite (in food);
(2) Bleomycin only (15 IP injections, days 2–30);
(3) Dolomite 500 mg/kg in freely-supplied standardized mouse food;
(4) Saline (15 IP injections, days 2–30);
(5) Control group (no drugs).

Results and Discussion

Figure 1 summarizes the results obtained in all 5 groups. By day 40 after inoculation, not a single control mouse survived. This pertains to the mice that received no treatment (group 5) or a placebo injection at the same regimen as the treated groups (group 4), and were considered a negative control. Dolomite itself in food (group 3) did not improve the survival of the mice, and was considered as a positive control. Bleomycin treatment, 0.3 mg/mouse (15 treatments on alternate days 2–30) increased the survival of the tumor-bearing mice on day 40 to 44%, but a combined bleomycin + dolomite treatment potentiated the survival rate to 63%. These results are in line with previous findings from our laboratory, where bleomycin alone, in a similar regimen administered into Ehrlich ascites bearing mice yielded 40% survival on day 50 after inoculation [12].

However, an interesting finding was noticed in our study, on the 30th day after inoculation: while the survival rate on that day in the two untreated groups (4 and 5, negative controls) was less than 20%, both single treatment groups (bleomycin group 2 and dolomite group 3) survived 50–60%, while the potentiated effect of the combination therapy group (bleomycin + dolomite, group 1) survived 77%. The dolomite-only treatment (group 3) was effective until the 32nd day, when 60% of the tumor-bearing mice survived, but their death toll rose abruptly to 90% on day 33 (Fig. 1).

These data coincide with observations pertaining to the prophylactic capacity of magnesium in the early stages of carcinogenesis and in some hemolymphoreticular malignancies, where dolomite delayed and retarded the tumorogenic process [3, 10, 13]. Ehrlich ascites carcinoma belong to a group of malignancies that rapidly absorb magnesium from the tumor environment, with a simultaneous rise in glucose metabolism obtained *in vitro*, a rise that might slow down the rate of tumor growth [14]. The antitumoral activity of dolomite in our study may, therefore, be due to easy diffusion of magnesium ions from this large diffusable complex structure [15].

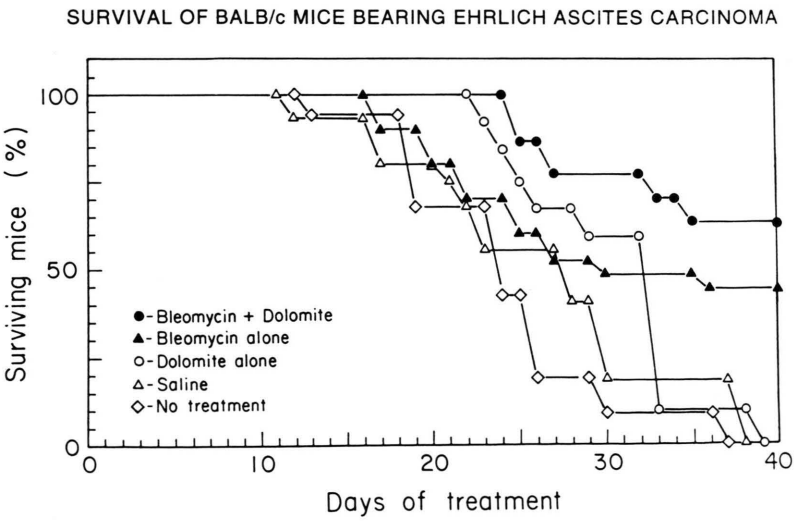

Fig. 1. Survival of BALB/c mice bearing Ehrlich ascites carcinoma after treatment with bleomycin, dolomite and their combination. Inoculation was on day "0", and the daily survival count continued until day "40".

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