Original Article

Synergistic Action of fMLP-boanmycin Combination on the Growth of Mouse Colon Carcinoma and Its Action Mechanisms

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ABSTRACT

Objective: The inhibitory action of fMLP-boanmycin (BAM) combination on the growth of mouse colon carcinoma and its action mechanisms were observed in order to provide experimental proof for probing novel regimen of chemotactic modulation in combination with chemotherapy in the treatment of cancer.

Methods: Cytotoxicity of BAM-fMLP combination to tumor cells was determined by MTT assay *in vitro*. Antitumor activity of BAM-fMLP combination was assessed in mice subcutaneously transplanted colon carcinoma 26. The amount of superoxide anion ($O_2^{\cdot -}$) released from fMLP stimulated macrophages was determined by NBT assay. The amount of nitric oxide (NO) was indirectly determined by Griess method.

Results: BAM-fMLP combination had no synergistic effect on tumor cells(CDI>0.85), but BAM at the doses of 10μg/ml, 30μg/ml and 100μg/ml in combination with fMLP at the concentration 20μg / ml exhibited synergistic effect on tumor cells in the presence of macrophages(CDI<0.75). fMLP inhibited the growth of colon carcinoma 26 by 50.0% when it at dose of 1 mg/mouse was administered peritumorally. BAM (1 mg/kg, intraperitoneally, three times) alone and BAM - fMLP combination inhibited the growth of colon carcinoma 26 by 38.6% and 78.4%, respectively (CDI=0.71) on day 12. The amount of $O_2^{\cdot -}$ released from fMLP 4.6×10^{-7} mol/L (0.2μg/ml) stimulated macrophages which were treated by BAM *in vitro* increased significantly(P<0.01). fMLP 2.3×10^{-6} mol/L (1μg/ml) could not stimulate macrophages to release NO, but may stimulate macrophages treated with BAM 10μg/ml and 100μg/ml to release NO significantly(P<0.01).

Conclusion: The inhibitory action of fMLP-boanmycin combination on the growth of mouse colon carcinoma have synergism, which may associate with the increase of $O_2^{\cdot -}$ and NO released by macrophages. Chemotactic modulation in combination with chemotherapy may be a novel regimen in the treatment of cancer.

Key words: Chemotactic peptide; fMLP; Boanmycin; Colon carcinoma

Chemotactic peptide can attract and activate leukocytes including macrophages, which can interfere the process of tumor growth, invasion and metastasis[1-3]. Bacterial chemotactic peptide fMLP (CHO-Met-Ile-Phe, fMLP) can induce the adhesion of leukocytes to endothelial cells, and promote them to migrate through endothelial cell space residing outside blood vessels[4]. It was reported that fMLP induced immune inflammation of mucus membrane when it was injected into mouse colon[5]. Zhang L et al[6] discovered that when fMLP was administered peritumorally in mice 48 h after tumor inoculation once every 2 days for 21 days, tumor growth delayed significantly. Ottonello L et al[7-8] reported that monoclonal antibody (Lym-1) identified antigen HLA-DR on B type lymphoma cells could inhibit the proliferation of tumor cells by activating peripheral blood neutraphils only when fMLP was applied at the same time.

Boanmycin (BAM), a single A6 component of bleomycin, was isolated from *streptomyces verticillus*

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metabolites of China. It can inhibit the growth of subcutaneously transplanted human hepatoma Bel-7402[9], human colon carcinoma HT-29 and cecum carcinoma Hce-8693[10] in nude mice. It can also inhibit liver metastasis of subcutaneous, intracecal wall, intra-hepatic and intra-splenic transplanted colon carcinoma 26[11]. It was reported that bleomycin at lower dose may restore the tumoricidal activity of macrophages from rats bearing KDH-8 hepatoma[12].

This paper was set to investigate the synergistic action of fMLP-BAM combination on the growth of mouse colon cancer and its action mechanisms in order to provide experimental proof for probing novel regimen of chemotactic modulation in combination with chemotherapy in the treatment of cancer.

MATERIALS AND METHODS

Reagents

fMLP was purchased from Sigma Company. It was dissolved with tiny amount of DMSO and then diluted with PBS 0.05 mol/L (pH 8.5) so that DMSO content was less than 5%. BAM, was dissolved with 0.9% NaCl before experiment.

Animals

The animals used in the experiment were Balb/C mice (Female, 18-22 g, Grade Ⅱ, certificate No. SCXK (Army) 2002-001, from Experimental Animal Institute, Chinese Military Medical Academy, Beijing, China)

Tumor cells

Mouse colon carcinoma 26 cell suspension of 0.2 ml (10^7 cells) stored in liquid nitrogen was placed in room temperature for some time, then centrifuged at 400×g for 5 min. The pellete was diluted with RPMI-1640 medium supplemented with 10% inactivated fetal calf serum, and then the dilution was transferred into culture flask and cultivated in 5% CO_2 incubator at 37°C for 3 days. Cells in the phase of exponential growth were collected and resuspended at 1×10^5 cells/ml for next use.

Macrophages

Peritoneal macrophages were collected from peritoneal lavage of Balb/c mice injected intra-peritoneally with 2 ml RPMI-1640 medium. The macrophage suspension was centrifuged at 400×g for 5 min, and then the macrophage pellet diluted with about 10 ml RPMI-1640 medium was transferred into culture dishes to cultivate for 2 h in 5% CO_2 incubator at 37°C. After the supernatant was dislodged, the cells were collected by scraping with scraper and cell suspension at $5\sim6\times10^5$ cells/ml in RPMI-1640 medium was prepared for next use.

Evaluation of Cytotoxicity of BAM in Combination with fMLP to Tumor Cells by MTT Assay

Mouse colon carcinoma 26 cell suspension (10^4 cells/100 μl) was seeded into each well of 96-well plate. Each well was further supplied with an equal volume of RPMI-1640 culture media. Vehicle control group, BAM group, fMLP group and BAM+ fMLP group were designed in the experiment. The plates were incubated in 5% CO_2 incubator at 37°C for 4 h after drugs of various concentrations were added into each well of drug groups. Stock MTT solution (2 g/L) of 50 μl was added into each well and the plates were continued to incubate for 4 h in the same incubator. Supernatant from each well was drawn out carefully and 150 μl DMSO was added. The plate was shaked strongly for 15 min. Absorbance was measured at 560 nm. To observe the cytotoxicity of the drugs to tumor cells in the presence of macrophages, 100 μl tumor cell suspension (1×10^4 cells) and 100 μl macrophage cell suspension (5×10^4 cells) were mixed, and then seeded into 96 well plate and cultivated for 4 h in 5% CO_2 incubator at 37°C. The inhibitory rate was calculated as follows:

Inhibitory rate=$[(A_0-A_1)/A_0] \times 100\%$,

A_0 represents total absorbance of tumor cell control,

A_1 represents the absorbance of drug treatment group.

Animal Experiment

Mouse colon carcinoma 26 cell suspension (10^6 cells) were injected subcutaneously into each mouse's right lateral chest wall near the axilla. The cells were passed every 13 days and the second passage was used for the experiment. At the time of animal experiment, mouse subcutaneous tumor was removed, weighed, cut up with eye scissors and transferred into homogenizer. After homogenization, the cell suspension was sedimented for 5 min and upper cell suspension was collected for next use.

The above cell suspension(0.2 ml) was injected subcutaneously into each mouse's right lateral chest wall near the axilla. Twenty four animals were averagely divided into vehicle control group, BAM