POTENTIATION OF BOANMYCIN ANTITUMOR ACTIVITY
BY CHEMOTACTIC PEPTIDE

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ABSTRACT

Objective: Chemotactic peptide may interfere with the process of tumor growth, invasion and metastasis by activating
and attracting leukocytes containing macrophages. fMLP (CHO-Met-Ile-Phe) is one of the chemotactic peptides. Boanmycin
(BAM), a single A6 component from the bleomycin complex, is effective against a panel of cancers in clinical trials. This
study was set to investigate the antitumor activity of BAM in combination with chemotactic peptide fMLP. Methods:
Cytotoxicity of BAM and fMLP to cancer cells was determined by MTT assay. Therapeutic effect was evaluated by using the
model of subcutaneously transplanted hepatoma 22 in mice. Results were judged as that a CDI less than 0.85 was considered
as synergism and one less than 0.75 as significant synergism. Results: BAM and fMLP showed no synergism in cytotoxicity
to cancer cells. In all in vivo experiments, fMLP was administered peritumorally at the dose of 1 mg/mouse; no significant
inhibition by fMLP alone on the growth of hepatoma 22 was found. Different settings of BAM and fMLP combination
included: (1) BAM, administered peritumorally×3, was started 24 h after tumor inoculation. BAM (0.5 mg/kg) alone and
BAM-fMLP combination inhibited the growth of hepatoma 22 by 26.6% and 64.7%, respectively (P<0.05, CDI=0.36) on day
13. (2) BAM, administered ip×3, was started 24 h after tumor inoculation. The growth of tumor in BAM (1 mg/kg) group was
faster than that in BAM-fMLP combination group. On day 14, BAM (1 mg/kg) alone and BAM-fMLP combination
suppressed the growth of tumor by 11% and 70.6%, respectively (P<0.05, CDI=0.42). (3) BAM, administered ip×3, was
started 96 h after tumor inoculation. The growth of tumor in BAM (1 mg/kg) group was faster than that in BAM-fMLP
combination group. On day 13, BAM (1 mg/kg) alone and BAM-fMLP combination suppressed tumor growth by 38.2% and
77.1%, respectively (P<0.05, CDI=0.51). As shown in all in vivo experimental settings, antitumor effect of BAM in
combination with fMLP was much more potent than that of BAM alone. Conclusion: This experiment shows that chemotactic
peptide fMLP may enhance the antitumor effect of BAM, which indicates that chemotactic modulation may play a positive
role in cancer chemotherapy.

Key words: Chemotactic peptide; fMLP; Boanmycin; Cancer chemotherapy

Boanmycin (BAM), a single A6 component of bleomycin, was isolated from streptomyces verticillus metabolites of our country[1]. Its phase III clinical trial

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has been finished. It inhibits the growth in subcutaneously transplanted human hepatoma Bel-7402, human colon carcinoma HT-29 and cecum carcinoma
Hce-8693 in nude mice[2,3]. It also inhibits liver metastasis of subcutaneous, intra-cecal wall, intra-hepatic and intra-splenic transplantable colon
carcinoma C26[4]. It is reported that BAM may activate macrophages[2].

Chemotactic peptide has influence on the process of tumor growth, invasion and metastasis[6-8]. Chemotactic
modulation may play important role in tumor therapy. Chemotactic peptide fMLP (CHO-Met-Ile-Phe, fMLP) is
one of the bacteria metabolites. It at lower concentration may cause leukocytes including macrophages to develop
chemotactic motion and at higher concentration may make leukocytes including macrophages develop cytotoxic action\(^\text{[9,10]}\). This study was aimed at investigating the antitumor action of BAM in combination with fMLP.

**MATERIALS AND METHODS**

**Reagents**

fMLP was purchased from Sigma Company. It was dissolved in 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, presented by prof. Xu hongzhang of Institute of Medicinal and Biotech, was dissolved in 0.9% NaCl before experiment started.

**Animals**

The animals used in the experiment were Kunming mice (Female, 18—22 g, Grade II, certificate No. SCXK II 00-0006, Experimental Animal Institute, Chinese Medical Academy).

**Tumor Cells**

Mouse hepatoma H22 cells were from our laboratory. Before formal experiment started, 0.2ml cell suspension (10\(^7\) cells) was injected into abdominal cavity of the mice. The cells was passed every 7 days and second passage was used for next experiment. In animal experiment, 0.2ml ascites was diluted with 0.9% NaCl physiological salt water to a cell density of 7.5x10\(^6\) cells/ml. At the time of experiment in vitro, ascites 0.2 ml was suspended with appropriate amount of RPMI-1640 medium and washed once by centrifugation at 400xg for 5 min. The pellet was diluted with RPMI-1640 medium supplemented with 10% inactivated fetal calf serum to a cell density of 1x10\(^5\) cells/ml for later use.

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

Mouse hepatoma H22 cell suspension (10\(^4\) cells/100 \(\mu\)l) 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 plate were incubated in 5% CO\(_2\) incubator at 37°C for 20 h. Drugs of various concentrations were added into each well and continued to incubate for 24 h. Stock MTT solution (2g/L) 50\(\mu\)l was added into each well and continued to incubated for 4 h in the same incubator. Supernatant from each well was drawn out carefully and 150\(\mu\)l DMSO was added. The plate was shaked strongly for 15 min. Absorbance was measured at 560 nm.

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 hepatoma H22 cells (1.5x10\(^6\) in 200\(\mu\)l) were injected s.c. into right lacteral chest wall near the axilla of each mouse. These animals were divided into vehicle control group, BAM group, BAM+fMLP group and fMLP group. BAM was administered ip or peritumorally and fMLP peritumorally once every 3 days for three times starting on day 1 or day 4. Tumor diameters were measured with a caliper. Tumor size was calculated by the formula \(a\times b^2/2\) , where \(a\) represents long diameter and \(b\) short diameter. Inhibitory rate was calculated as follows: inhibitory rate (%)=(C-T)/C\times 100%, where \(C\) represents tumor size of control group and \(T\) tumor size of treatment group.

**Data Analysis**

Drug combination synergism was evaluated as follows\(^\text{[11]}\): CDI=AB/AxB, AB was the ratio of the data of combination groups to that of control group and A or B was the ratio of the data of drug group to that of control group. CDI<0.85 represents synergism and CDI<0.75 significant synergism in the experiment. Data expressed as mean±standard deviation ( x±s). The significance of the results was determined by student’s \(t\) test (two tailed).

**RESULTS**

**Cytotoxicity of BAM in Combination with fMLP to Tumor Cells**

fMLP at the concentration of 10, 20 and 40 \(\mu\)g/ml showed no cytotoxicity to mouse hepatoma H22 cells. CDI of cytotoxicity of fMLP at the concentration of 10, 20 and 40 \(\mu\)g/ml in combination with BAM at the concentration of 4, 8 and 16\(\mu\)g/ml to mouse hepatoma H22 cells were all more than 0.85 (Table 1). This experiment indicated that fMLP-BAM combination had no synergism in cytotoxicity to mouse hepatoma H22 cells.

**The Effect of fMLP-BAM Combination Administered Peritumorally on Tumor Growth**

Mouse hepatoma H22 cells were injected s.c. into