

Radio-adjuvant effects of ginsan on murine breast carcinoma xenografted model

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1. Introduction

In a number of studies, polysaccharide extracted from *Panax ginseng* C.A. Meyer, ginsan has been demonstrated to be a potent promising biological response modifier (BRM), including proliferation of lymphocyte, generation of lymphokine activated killer cells, and production of several cytokines [1-3]. Macrophages are the first line of defense to infections or pathogens in host innate immunity. In addition, it plays a prominent role as a professional antigen presenting cells to trigger cellular immunity. In the light of that, the current study was designed to evaluate whether ginsan exhibits anti-tumor effect as well as synergistic function with chemo- or radio-therapy.

2. Methods and Results

2.1 Isolation of peritoneal macrophage

Macrophages were isolated from peritoneal cavity by use of the 5ml syringe containing RPMI 1640 with 10% FBS and 0.5% penicilline-streptomycin. Peritoneal exudates cells were seeded on petri dishes and the macrophages were allowed to adhere for 2-3 h at 37°C under 5% CO₂ humidified atmosphere. After culture, non-adherent cells were removed and the macrophages were harvested by rinsing using a 10ml syringe. The viability of isolated peritoneal macrophages (PM) was assessed by trypan-blue exclusion.

2.2 Nitric oxide production

PM cells were incubated with or without ginsan for 48 h in 96-well microplate. After culture, 100 ul of each supernatant was taken and nitric oxide was measured using Griess reagents. PM incubated with ginsan significantly increased NO production in a concentration-dependent manner. We employed IFN- γ or LPS as a positive control, ginsan at the optimal dose exhibited the similar degree of NO production to IFN- γ . To confirm the nitric oxide production was accompanied by the induction of iNOS expression, we analyzed mRNA expression levels of iNOS. As shown in Figure 1, iNOS mRNA expression was also increased by ginsan at a dose-dependent. This result shows that ginsan can activate macrophages to produce reactive nitrogen intermediators.

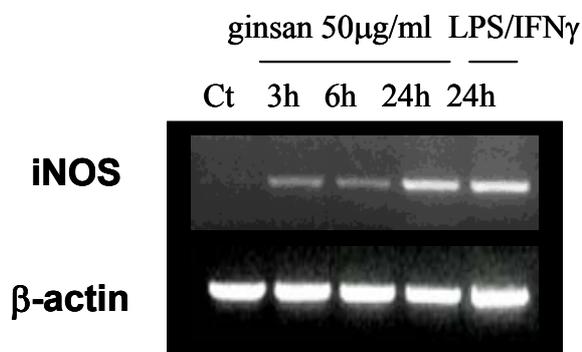


Figure 1. Effect of ginsan on iNOS mRNA expression.

2.3 The growth inhibition of tumor cells by PM cultured with ginsan.

A 4 h-[⁵¹Cr] release assay and 72 h-[³H] uptake assay were used to determine the cytotoxic activity of ginsan conferred by stimulated PM against Yac-1 tumor cells. The cytotoxic activity of PM incubated with 100ug/ml of ginsan was 1.5-fold higher than that untreated PM(Figure 2).

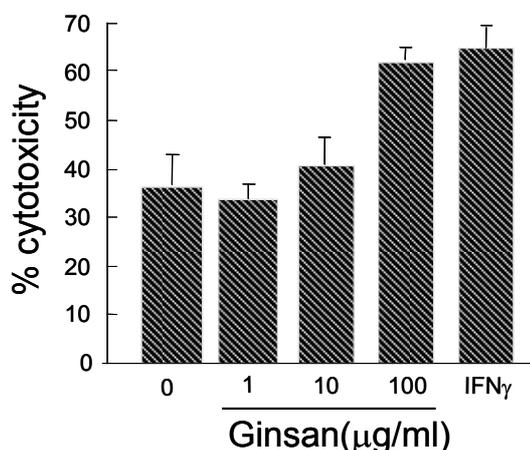


Figure 2. Effect of ginsan on cytotoxicity of macrophage.

2.4 Adjuvant effect of ginsan on tumor bearing mice with radiation.

Murine breast carcinoma EMT-6 cells (1×10^6 cells/mouse) were subcutaneously injected into right hind groin of BALB/c mice on day 0. After 7 days, the mice were each *i.p.* injected with 25 mg/kg and 100 mg/kg body weight of ginsan. At the same time, mice

were locally irradiated at a single dose of 8 Gy. Tumor dimensions were measured 2 and 3 times weekly with caliper, and the tumor volume was calculated by following formula: tumor volume = (longest diameter) x (shortest diameters)²/2. This tumor growth of 100 mg/kg ginsan treated mice was markedly decreased about 45% compared with that of control counterpart. Furthermore, the inhibition of tumor growth in 100 mg/kg of ginsan treated with radiation group was significantly higher than radiation alone treated group, even 40 % of the former group demonstrated totally regressed in this model, suggesting that a ginsan may be used as a possible adjuvant agent for radiotherapy.

3. Conclusion

Given the findings that ginsan can proliferate some immune cells drastically, we asked whether ginsan increases the survival of tumor cells concomitantly. To this end, we investigated that ginsan can inhibit the tumor growth combined with or without radiation on EMT-xenografted mice. Here, we showed that ginsan possesses a potent anti-tumor activity through macrophage activation include the production of nitric oxide and direct cytotoxicity. In addition, antitumor activity of ginsan was much greater than the radiation alone group, and may suggest synergistic effect of ginsan with radiation.

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