Gallic Acid Inhibits Murine Leukemia WEHI-3 Cells 

**In Vivo and Promotes Macrophage Phagocytosis**

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**Abstract.** Gallic acid is a polyhydroxyphenolic compound which can be found in various natural products. It is recognized to be an excellent free radical scavenger and has been shown to induce apoptosis in lung cancer and leukemia cells. No report has addressed whether gallic acid affects mouse leukemia cells in vivo. In this study, we examined the in vivo effects of gallic acid on leukemia WEHI-3 cells and on macrophage phagocytosis. Gallic acid caused a significant decrease in the weights of the spleens and livers from BALB/c mice. One of the major characteristic of WEHI-3 leukemia is the enlarged spleen in mice after i.p. injection of WEHI-3 cells. Gallic acid did not affect the percentages of CD3, CD11 and CD19 markers but decreased the percentage of Mac-3 in a high-dose (80 mg/kg) treatment while promoting Mac-3 levels in a low-dose (40 mg/kg) treatment. Gallic acid promoted the activity of macrophage phagocytosis in the white blood cells from peripheral blood mononuclear cells (PBMCs) at 40 and 80 mg/kg treatment doses, but decreased the macrophage phagocytosis in isolated peritoneal cells at the 80 mg/kg dose.

Leukemia is the thirteenth most common malignancy in Taiwan. About 2.1 persons per 100 thousand people die per year of leukemia, based on the reports from the "People Health Bureau of Taiwan". However, the best strategies for the treatment of human leukemia are not yet clear. Although many experiments have shown that increased consumption of a plant-based diet led to reduce the risk of cancer such as colon cancer (1, 2). No report has shown it can decrease leukemia in vivo. It is well documented that herbal-based dietary supplements contain a large array of phytochemicals which might mediate physiological functions related to cancer suppression in vivo, although the active ingredients remain to be identified.

Gallic acid (3,4,5-trihydroxybenzoic acid), a naturally occurring plant phenol produced from the hydrolysis of tannins, induced apoptosis in human lung cancer cells (3). Gallic acid has also been shown to have an antitumor effect on LL-2 lung cancer cells transplanted in mice (4). Gallic acid can be present as a free molecule or as part of the tannin molecule and can be taken orally from food and medicinal herbs. Gallic acid appears to be more safe for cancer patients compared with toxic chemotherapeutic agents. In vivo the anti-leukemia effects of oral administration of gallic acid have not been reported. In the present study, the effects of orally administered gallic acid were investigated on the growth of WEHI-3 mouse leukemia cells transplanted into mice.

**Materials and Methods**

**Materials and reagents.** Gallic acid and olive oil were obtained from Sigma Co. (St. Louis, MO, USA). RPMI-1640, fetal bovine serum, penicillin-streptomycin and glutamine were obtained from Gibco BRL (Grand Island, NY, USA).
Male BALB/c mice. Sixty male BALB/c mice of 22-28 g in weight at the age of 8 weeks were purchased from the Laboratory Animal Center, National Taiwan University College of Medicine (Taipei, Taiwan, ROC).

Murine WEHI-3 leukemia cells. The WEHI-3 murine myelomonocytic leukemia cell line was purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan, ROC). The cells were placed into 75-cm² tissue culture flasks and grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin (100 U/ml penicillin and 100 μg/ml streptomycin) and 1% glutamine at 37°C under a humidified 5% CO₂ atmosphere.

In vivo studies

Drug treatment. Sixty BALB/c mice were randomly divided into 5 groups of 12 animals. Group I and II were the control group and treated with olive oil alone (100 μl), respectively. Group III was injected with WEHI-3 only. Group IV and Group V were injected with WEHI-3 then treated with gallic acid (40 and 80 mg/kg respectively) in olive oil. All animals were given the above dose orally daily for up to 3 weeks (5).

Blood collection and immunofluorescence staining. Approximately 1 ml blood samples were collected from each animal from each group at the end of the treatment. Collected blood was immediately treated with ammonium chloride for lysing of the red blood cells followed by centrifugation for 15 minutes at 1500 rpm at 4°C. Isolated white blood cells were examined for cell markers (CD3, CD11b, CD19 and Mac-3) based on staining with anti-CD11b, CD3, CD19 and Mac-3 antibodies (PharMingen). These cells were then stained with the second fluorescent antibody for determining the cell marker levels by flow cytometry (FACS Calibur™, Becton Dickinson, NJ, USA) as described elsewhere (6, 7).

Liver and spleen samples. Each animal was weighed individually before blood was sampled. The liver and spleen samples were then obtained and weighed individually (5).

Quantification of phagocytic activity of macrophages. Phagocytosis was measured using the PHAGOTEST kit. Cells were isolated from peripheral blood mononuclear cells (PBMCs) and peritoneal. The isolated cells were incubated for 4 h at 37°C with opsonised...
fluorescein isothiocyanate-labelled *Escherichia coli* (20 μl), in compliance with the manufacturer’s instruction. The reaction was stopped by the addition of ice-cold quenching solution (100 μl). At the completion of phagocytosis, monocytes/macrophages were fixed, and DNA was stained according to the manufacturer’s instructions. Cell preparations were then analyzed by flow cytometry in a flow cytometer (FACSCalibur, Becton Dickinson). Fluorescence data were collected on 10,000 cells and analyzed using CELLQUEST software (8).

**Statistical analysis.** The results were expressed as mean±SD and the difference between groups was analyzed by Student’s-

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-test. *p*<0.05 was considered significant.

**Results**

**Leukemia in BALB/c mice.** Representative image of dissected animals after injection with WEHI-3 cells for 3 weeks with or without gallic acid treatment are shown in Figure 1.

**The effect of gallic acid on the total body, liver and spleen weights of BALB/c mice.** The body and tissue weights are presented in Figure 2. Gallic acid did not significantly affect the mean total body weight of the mice treated with WEHI-3 alone or with gallic acid (Figure 2A). The results also showed that gallic acid significantly reduced the weight of the spleens (Figure 2B) and livers (Figure 2C) as compared to mice treated with WEHI-3 alone, although not significantly so at 80 mg/kg gallic acid.

**The effect of gallic acid on white blood cell surface markers from BALB/c mice after injection with WEHI-3 cells.** The percentages of the cell markers in the white blood cells from BALB/c mice after treatment with gallic acid in olive oil are presented in Figure 3. The results indicated that gallic acid did not affect the levels of CD3 (Figure 3A), CD19 (Figure 3B) or CD11b (Figure 3C), but promoted the Mac-3 level at the 40 mg/kg dose rate (Figure 3D) while decreasing it at 80 mg/kg.

**The effect of gallic acid on macrophage phagocytosis in BALB/c mice after injection with WEHI-3 cells.** The results of phagocytosis of macrophages from PBMCs and peritoneal of BALB/c mice after treatment with gallic acid in olive oil are presented in Figure 4A and B. Gallic acid promoted macrophage phagocytosis in the PBMCs (Figure 4A) and the peritoneal cells (Figure 4B) at the 40 mg/kg dose, but the use of 80 mg/kg gallic acid led to a decrease in macrophage phagocytosis from peritoneal cells (Figure 4B) of BALB/c mice.

**Discussion**

Gallic acid plays an important role in the prevention of malignant transformation and cancer development. Gallic acid and its derivatives were shown to induce Ca\(^{2+}\)-dependent apoptosis in leukemia cells (9, 10). It was also reported that...
gallic acid inhibits superoxide dismutase during apoptosis in HL-60 leukemia cells (11). But there are no reports on the effects of gallic acid on the promotion of macrophage phagocytosis. Murine host systems have been used for experimental tumor therapy due to the low cost and the ease for the establishment of cancer production. Murine WEHI-3 leukemia cells show characteristics of myelomonocytic leukemia and were originally derived from the BALB/c mouse (12). Therefore, we used WEHI-3 cells for generating the leukemia model in vivo. WEHI-3 cells have also been used to induce leukemia in syngenic BALB/c mice to evaluate anti-leukemia effects of drugs (13). Chemotherapy agents such as ATRA, aclacinomycin A, interleukine-6 and G-CSF as well as the vitamin D3-induced in vitro differentiation of WEHI-3 cells in monocytic and granulocytic lineages, had been used in the WEHI-3 in vivo model for the study of anti-leukemia activity (12-15).

In the present study, gallic acid treatment reduced the weights of the livers and spleens. Gallic acid statistically increased the percentage of Mac-3 cell at 40 mg/kg but decreased it at 80 mg/kg treatment. However, there was no significant difference with regard to CD3, CD11b and CD19 positive cells treated or untreated WEHI-3 cells. In the gallic acid-treated groups, the activity of macrophage phagocytosis increased in cells isolated from PBMCs and peritoneal at both doses of gallic acid, except at a high dose in peritoneal cells. An important observation is that there was a significant difference in spleen weight between the control and gallic

Figure 3. The effect of gallic acid on cell markers of white blood cells from BALB/c mice. The animals were injected with WEHI-3 cells (1x10^5 cells/100 μl) in PBS for 3 weeks and treated with or without gallic acid for 3 weeks. Blood was collected from animals and was analyzed for cell markers by flow cytometry as described in Materials and Methods. Each point is the mean±S.D. of three experiments. *Significantly different at p<0.05.
acid-treated groups. Therefore, gallic acid inhibition of the spleen tumor may be associated with the increased macrophage phagocytosis activity.

In conclusion, gallic acid reduced the size and weight of the spleens of BALB/c mice after peritoneal injection with WEHI-3 cells which may have been through the promotion of macrophage phagocytosis.

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References


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