Recent Advances on the Nutritional Effects Associated with the Use of Garlic as a Supplement

Enhanced Immunocompetence by Garlic: Role in Bladder Cancer and Other Malignancies

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ABSTRACT Of the many beneficial actions of garlic, inhibition of the growth of cancer is perhaps the most remarkable. Our previous animal studies demonstrated that aged garlic extract was highly effective, and unlike the approved immunotherapy for human bladder cancer, bacillus Calmette-Guérin (BCG), garlic was effective when added to the diet. To elucidate the mechanism of this antitumor effect, the literature describing antitumor and immune-enhancing effects of garlic is reviewed. Garlic can detoxify carcinogens by stimulation of cytochrome P450 enzymes, antioxidant activity or sulfur compound binding. Studies demonstrate a direct toxic effect of garlic to sarcoma and gastric, colon, bladder and prostate cancer cells in tissue culture, but these effects cannot explain the inhibition of growth of transplanted cancer in animal models. The most likely explanation of this effect is immune stimulation. Comparison of the effects of garlic to BCG immunotherapy reveals many similarities. Both stimulate proliferation of lymphocytes and macrophage phagocytosis, induce the infiltration of macrophages and lymphocytes in transplanted tumors, induce splenic hypertrophy, stimulate release of interleukin-2, tumor necrosis factor-α and interferon-γ, enhance natural killer cell, killer cell and lymphokine-activated killer cell activity. These activities represent effective stimulation of the immune response. Studies suggest that garlic may be useful in preventing the suppression of immune response that is associated with increased risk of malignancy. Data suggest that maintenance of immune stimulation can significantly reduce the risk of cancer. Clinical trials should be initiated to test the hypothesis that the immune stimulation and other beneficial effects of garlic are able to reduce the incidence of cancer. J. Nutr. 131: 1067S–1070S, 2001.

KEY WORDS: • immunocompetence • garlic • cancer • bladder • mouse

Transitional cell carcinoma of the bladder is highly susceptible to immunotherapy and is one of a very few human malignancies for which immunotherapy is the current treatment of choice. Immunotherapy with bacillus Calmette-Guérin (BCG) is superior to chemotherapy in the treatment of carcinoma in situ of the bladder; unlike chemotherapy, it provides long-term protection from tumor recurrence and disease progression. Clinical efficacy in the treatment of bladder cancer has also been reported with other immunotherapies, including Keyhole limpet hemocyanin (KLH), interleukin-2 (IL-2), interferon-α (INF-α) and the interferon inducer, bropirimine.

Previous studies have demonstrated that BCG immunotherapy is superior to chemotherapy with thiopeta, doxorubicin or mitomycin in clinical trials, and laboratory studies have suggested the superiority of BCG over alternative immunotherapies. We were, therefore, surprised by the report of Lau et al. (1990) that intralesional aged garlic extract (AGE) was more effective than BCG in the treatment of transplanted transitional cell carcinoma in the mouse model. In an effort to develop improved treatments for bladder cancer, we evaluated AGE in the murine model. These results and the data that suggest that the antitumor activity of garlic may be related at least in part to immune stimulation will be reviewed.

Antitumor activity of garlic

The recorded use of garlic in the treatment of tumors dates all the way back to 1550 BC when ancient Egyptians administered it orally and topically; the modern era, however, begins in the 1950s when Weisberger and Pensky (1958) demonstrated in vitro and in vivo that thiosulfinate extracts of garlic inhibited the growth of malignant cells and prevented growth of sarcoma 180 ascites tumor. Since that time, garlic has been demonstrated in epidemiologic studies to be associated with a reduced risk of stomach cancer (You et al. 1989) and, in...
animal models, to have antitumor activity in sarcoma, mammary carcinoma, hepatoma, colon cancer, and squamous cell carcinoma of the skin and esophagus (Lau et al. 1990). These effects appear to be mediated by various mechanisms. Prevention of malignant transformation after the administration of chemical carcinogens may result from inhibition of the activation of procarcinogens by garlic’s effect on cytochrome P450 enzymes (Dion and Milner 1997), antioxidant activity, or detoxification by binding to sulfur compounds in garlic (Abdullah et al. 1988). Direct inhibition of cancer cell growth in tissue culture has been documented in sarcoma as well as gastric, colon, bladder and prostate carcinoma cell lines (Knowles and Milner 1997, Fan et al. 1985, Weisberger and Pensky 1958). The mechanism of direct tumor cell inhibition has not yet been determined. Perhaps the most important action of garlic in the inhibition of cancer and the topic of this review is potentiation of the immune system.

**Aged garlic extract as an immunotherapy for bladder cancer**

Lau et al. (1986) compared intranasal and intraperitoneal garlic extract therapy with effective immunotherapies for bladder cancer, BCG, Corynebacterium parvum (CP) and KLH in the transplantable murine bladder tumor model MBT2. These experiments demonstrated that intranasal immunotherapy with each of these agents significantly inhibited tumor growth (P < 0.05). Maximal inhibition of tumor growth was seen with CP (250 μg) and garlic extract (25 mg). Significant reduction in tumor growth was observed with intraperitoneal CP treatment. Intraperitoneal garlic appeared to reduce tumor growth, although not to the level of statistical significance, and intraperitoneal BCG had no effect.

Examination of hematoxylin and eosin–stained sections of the transplanted tumors demonstrated necrosis and infiltration with macrophages and lymphocytes. Intraperitoneal BCG and CP induced granuloma formation as well, but no granuloma were seen after treatment with garlic extract. Intraperitoneal garlic treatment produced tumor necrosis and infiltration with macrophages and small lymphocytes, suggesting an immune response.

This group (Marsh et al. 1987) evaluated these same treatments intravesically after intravesical tumor transplantation. The efficacy of CP, garlic and BCG, but not KLH, was confirmed. Maximal inhibition of tumor growth was again observed with CP and garlic. Comparing treatment schedules of 1, 6, or both 1 and 6 d after tumor transplantation, only garlic treatment achieved statistical significance when given as a single treatment 6 d after transplantation.

Microscopic examination of the bladders revealed infiltration of macrophages and small lymphocytes in animals treated with CP, BCG and, to a lesser extent, KLH. Topical garlic treatment resulted in extensive macrophage and neutrophil infiltration, with few lymphocytes. Splenic weights were significantly increased in all treatment groups relative to untreated controls. Splenic phagocytic function and natural killer (NK) cell cytotoxicity were reported to be significantly increased with both CP and garlic immunotherapy.

These experiments suggested that garlic treatment effectively inhibited growth of transitional cell carcinoma in vivo. In view of the recognized toxicity of BCG therapy and the absence of observed toxicity with garlic treatment in these studies, garlic therapy could be an effective treatment alternative for patients with bladder cancer. Data suggested that immune mechanisms might be responsible for the observed beneficial response to garlic.

To further establish garlic as a safe and effective treatment for bladder cancer, we evaluated intranasal and oral AGE treatment of transitional cell carcinoma in the murine model (Riggs et al. 1997). We confirmed that intranasal garlic extract was highly effective in the treatment of subcutaneously transplanted MBT2 bladder cancer. Inhibition of tumor growth was highly significant (P < 0.001) and similar to that of BCG (Table 1). Unfortunately, in contrast to the previous investigators, we observed that repeated intranasal garlic injection was toxic, resulting in death of up to 42% of treated mice. Reduction in the dose and frequency of intranasal garlic injection reduced the toxicity, but our enthusiasm for a clinical trial of intravesical garlic was diminished in view of the newly discovered risk. Because oral garlic has been used for thousands of years, we sought to evaluate oral garlic in the treatment of transplanted bladder cancer. Remarkably, oral AGE when added to drinking water in doses of 5, 50 and 500 mg/100 mL inhibited the growth of transitional cell carcinoma in a dose-dependent manner (Table 2). Significant inhibition of tumor growth was seen at the 50 and 500 mg/mL dose (P<0.023 and P<0.001, respectively), and significant improvement in survival (10 of 20, 50% vs. 4 of 20, 20% control, P=0.048) was seen with the 500 mg/mL dose. No adverse effects of oral garlic administration were seen. Animal weights did not differ among groups and water intake was highest in the group with the highest concentration of AGE. Because no toxicity was observed and antitumor effect was highest in the group with the highest oral garlic intake, it is possible that higher doses may be even more effective. Studies to identify the optimal oral dose of garlic in the treatment of bladder cancer are in progress.

**TABLE 1**

**Effects of subcutaneous therapy with bacillus Calmette-Guérin (BCG) or Allium sativum (AS) using the MBT2 model**

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor incidence (d 21)</th>
<th>Treatment-related death</th>
<th>Tumor volume (d 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>11/12 (91.6%)</td>
<td>0/12 (0.0%)</td>
<td>6743.8 ± 617.8</td>
</tr>
<tr>
<td>BCG 107 cfu</td>
<td>3/12 (25.0%, P = 0.01)</td>
<td>0/12 (0.0%)</td>
<td>859.7 ± 892.9 (P &lt; 0.001)</td>
</tr>
<tr>
<td>AS 25 mg</td>
<td>2/7 (28.6%, P = 0.01)</td>
<td>5/12 (41.7%)</td>
<td>338.0 ± 412.7 (P &lt; 0.001)</td>
</tr>
<tr>
<td>AS 12 mg</td>
<td>4/9 (44.4%, P = 0.03)</td>
<td>3/12 (25.0%)</td>
<td>658.0 ± 654.2 (P &lt; 0.001)</td>
</tr>
<tr>
<td>AS 6.3 mg</td>
<td>2/7 (28.6%, P = 0.01)</td>
<td>5/12 (41.7%)</td>
<td>639.2 ± 667.4 (P &lt; 0.001)</td>
</tr>
<tr>
<td>AS 3.1 mg</td>
<td>4/7 (57.1%, NS)</td>
<td>5/12 (41.7%)</td>
<td>941.1 ± 715.3 (P &lt; 0.001)</td>
</tr>
</tbody>
</table>

1 Treatment days were 1, 3, 5 and 7.
2 cfu, colony-forming unit; NS, not significant.
**Evidence for immunologic antitumor action of garlic**

We observed that garlic has direct dose-dependent toxicity to cultured transitional cell carcinoma cells, but only at doses ≥15 mg/mL, concentrations that are not practical with systemic administration (Riggs et al. 1997). The remarkable efficacy of oral, intravesical and intravesical AGE is therefore clearly not related to direct cytotoxicity alone. The alternative antitumor mechanisms of detoxification of carcinogens, antioxidant activity and inhibition of procarcinogens similarly cannot explain the inhibition of growth of transplanted cancer. Of the currently recognized effects of garlic, only immune stimulation can logically explain the observed inhibition of growth of transplanted cancer.

What evidence supports immune stimulation as an important antitumor effect of garlic? One approach to answer this question is to compare the reported effects of garlic with a recognized, Food and Drug Administration–approved, clinically useful cancer immunotherapy such as BCG.

The effects of garlic on murine transitional cell carcinoma are remarkably similar to those of BCG. Both inhibit tumor growth, and microscopic examination of the site of tumor transplantation reveals infiltration with macrophages and lymphocytes. BCG, but not garlic, induces granuloma formation. In animal studies, both BCG and garlic induce hypertrophy of the reticuloendothelial system as measured by splenic hypertrophy. Garlic, like BCG, increases NK cell activity.

Intravesical BCG administration results in the release of cytokines in the urine, and elevation of urinary cytokines, particularly IL-2, tumor necrosis factor-α (TNF-α), and INF-γ, is associated with antitumor activity. In animal studies, AGE is reported to induce the release of IL-2, TNF-α, and INF-γ (Kyo et al. 1998). Enhanced phagocytosis, one of the first immunostimulatory actions reported with BCG, is seen with garlic administration (Kyo et al. 1998). Additional activities seen with both BCG and garlic include enhanced killer cell activity and immunoproliferation of lymphocytes in response to mitogen stimulation (Kyo et al. 1998). These effects, particularly the pattern of cytokine release, suggest that garlic, like BCG, stimulates a Th1 cellular immune response that is characteristic of effective antitumor immunotherapies.

The component in garlic that is responsible for the effective immune stimulation is not known conclusively, and it is likely that multiple ingredients are immunologically active. A protein fraction from garlic is known to augment in vitro macrophage cytotoxicity and phagocytosis as well as stimulate lymphocyte proliferation (Hirao et al. 1987). The protein fraction 4 (F4) from garlic has been demonstrated to enhance the cytotoxicity of human peripheral blood lymphocytes against NK-sensitive (K562) and NK-resistant (M14) cell lines (Moriioka et al. 1993). These effects were markedly augmented by the addition of low doses of IL-2. The combination was also more effective in inducing lymphokine-activated killer cell activity. F4 enhanced IL-2 or concanavalin A–induced proliferation of lymphocytes and IL-2 receptor expression. The enhanced cytotoxicity induced by F4 and F4 plus IL-2 was abolished by anti-IL-2 antibody, suggesting that the activity of F4 is mediated by IL-2 (Moriioka et al. 1993). These data suggest that the F4 fraction of garlic is an efficient immunopotentiator that may be effective in cancer immunotherapy.

Although the F4 fraction of garlic is clearly an immune stimulant, it is not the only immunologically active ingredient in garlic. Therefore, F4 may not be entirely responsible for the observed beneficial response in transplanted tumors. In studies of the effect of diallyl disulfide on the growth of transplanted human colon carcinoma cell lines in immunologically compromised nude mice, Sundaram and Milner (1996) found diallyl disulfide to be as effective as 5-fluorouracil (5-FU) in inhibiting tumor growth. Combining the diallyl disulfide and 5-FU did not increase the effect, but concurrent diallyl disulfide treatment did significantly reduce the depression of leukocyte counts and splenic weight associated with chemotherapy administration (Sundaram and Milner 1996). In another study, Geng et al. (1997) examined the effects of S-allyl cysteine, a water-soluble constituent of garlic that inhibits chemical carcinogen-induced colon and mammary tumors in animals and inhibits the growth of neuroblastoma and melanoma in vitro. In studies of human T cells, S-allyl cysteine was found to inhibit activation of the nuclear protein of the Rel oncogene family (nuclear factor-κB). This protein, which is induced by TNF-α or H2O2, regulates immune function, inflammation and cellular growth (Geng et al. 1997). These studies suggest that low-molecular-weight compounds as well as proteins found in garlic have antitumor and immune effects.

**Prevention of immune suppression**

Immune surveillance offers protection from cancer and from impairment of immune defenses, as occurs with conditions ranging from abnormailities such as acquired immunodeficiency syndrome (AIDS) to the normal aging process. In addition to enhancing NK function in AIDS patients, garlic is reported to improve age-related deterioration of learning behavior and impairment of immune response in a mouse model (Zhang et al. 1997). The most common carcinogen, ultraviolet irradiation, appears to be inhibited by garlic. UV irradiation damages DNA and induces a specific defect in T-cell immunity, impairing the recognition of UV-induced malignancy. Most interestingly, oral garlic administration is found to protect from photodamage (Reeve et al. 1997). Induction of an impaired immune response by the tumor itself is...
an effective means to escape destruction by host surveillance mechanisms. It is not known whether garlic can reduce the inhibition of immune response induced by tumor, but the observed responses are certainly compatible with this hypothesis. Protection from immune suppression is potentially an important mechanism in preventing the development of malignancy. For example, in our experience with maintenance BCG immunotherapy in patients with superficial bladder cancer, stimulation of the immune system for a period of 3 y not only protects from recurrence of bladder cancer, but significantly reduces the incidence of other malignancies as well (Lamm et al. 1999). Additional evidence that garlic potentiates immune responses is provided from other studies as well. In a study of the effect of garlic on the neuroendocrine and immunomodulation network, Zhang et al. (1997) reported that AGE improves age-related deterioration of learning behavior as well as impaired immune response in a mouse model. Garlic increased not only lymphocyte proliferation, as seen in other studies, but antibody production as well (Zhang et al. 1997).

Further study will be required to identify the active ingredients in garlic that are responsible for the observed antitumor activity and immune stimulation. Data now suggest that low-molecular-weight sulfur compounds and F4 have immunostimulating properties and also that garlic can detoxify chemical carcinogens to prevent carcinogenesis and directly inhibit the growth of cancer cells. Garlic appears to stimulate immunity including macrophage activity, NK and killer cells, and lymphokine activated killer cells, and it increases production of IL-2, TNF, and INF-γ. These cytokines are associated with the beneficial Th1 antitumor response, which is characteristic of effective cancer immunotherapies. Like BCG, garlic stimulates proliferation of macrophages and lymphocytes, and also protects against suppression of immunity by chemotherapy and UV radiation. Garlic is clearly not a panacea for cancer, but its broad range of beneficial effects warrants serious consideration in clinical trials for the prevention and treatment of cancer.

**LITERATURE CITED**


