Antitumoral activity of allicin from garlic

Eduardo PADILLA CAMBEROS1 & Claudia PADILLA CAMBEROS2

1Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco. Avenida Normalistas 800 C.P. 44270 Guadalajara, Jalisco, México.
2Centro Universitario de la Ciénega, Universidad de Guadalajara. México

Contactos | Contacts: Eduardo PADILLA CAMBEROS E-mail address: epadilla@ciatej.net.mx

Abstract
Epidemiological studies link increased garlic consumption with a reduced incidence of cancer in various human populations. Experimental carcinogenesis studies in animal models and in cell culture systems indicate that several allium-derived compounds exhibit inhibitory effects. To provide a better understanding of the effects of allium derivatives on the prevention of cancer, we examined allicin, the major component of garlic, for their effects on antitumoral activity in vitro and in L5178Y lymphoma bearing mice. We found that allicin decreased the growth of tumor cells whereas in vivo, the compound shown an antitumor effect in murine L5178Y lymphoma. Allicin enhanced the secretion of IL-2, IFN-gamma and TNF-alpha cytokines from mouse plasma. These cytokines are associated with the beneficial Th1 antitumor response, which is characteristic of effective cancer immunotherapies.

Keywords: allicin, cytotoxic, antitumor, lymphoma L5178Y.

Resumen
Los estudios epidemiológicos relacionan el aumento del consumo de ajo con una disminución en la incidencia de cáncer en diferentes poblaciones humanas. Los estudios experimentales de carcinogénesis en modelos animales y en los cultivos de células tumorales indican que varios compuestos derivados del ajo tienen efectos inhibitorios. A fin de proporcionar una mejor comprensión de los efectos de derivados del ajo en la prevención del cáncer, se evaluó la alicina el principal componente del ajo, en la actividad antitumoral in vitro y en ratones con linfoma. Se encontró que la alicina disminuyó el crecimiento de células tumorales, mientras que in vivo, el compuesto muestra un efecto antitumoral en el linfoma murino L5178Y. La alicina incrementó la secreción de las citocinas IL-2, interferón-gama y TNF-alfa obtenidas de plasma de ratón con linfoma. Estas citocinas están asociadas con la respuesta antitumoral benéfica Th1, que es característica de inmunoterapias efectivas para el cáncer.

Palabras Clave: alicina, citotóxico, antitumoral, linfoma L5178Y.

Recibido | Received: July 29, 2010.
Aceptado en versión corregida | Accepted in revised form: August 16, 2011.
Publicado en línea | Published online: September 30, 2011.
Declaración de intereses | Declaration of interests: This work was supported in part by CONACyT-SAGARPA Grant 185236.
Este artículo puede ser citado como / This article must be cited as: Eduardo PADILLA CAMBEROS1, Claudia PADILLA CAMBEROS. 2011. Antitumoral activity of allicin from garlic. Bol Latinoam Caribe Plant Med Aromat 10(5): 423 – 428.
INTRODUCTION
Garlic (*Allium sativum*) has been used among centuries for treating various diseases, its consumption has been related to reduce cancer risk. Some garlic constituents have been shown to alter activation of carcinogens and to cause growth inhibition of tumor cells (Reddy *et al*., 1993). Garlic extracts and components effectively block experimentally induced tumors (Oommen *et al*., 2004).

Allicin, the major component present in freshly crushed garlic, is formed from Garlic bulbs contain alliin. When crushed, the bulbs release the enzyme alliinase, which converts alliin to allicin (diallyl thiosulfinate) and other thiosulfimates. Allicin is one of the most biologically active compounds of garlic (Hirsch *et al*., 2000), it was reported to affect different biological activities such as antibacterial, antiparasite and antifungal activities. It was also shown that garlic compounds may reduce serum lipid levels, as well as inhibit platelet aggregation. Interestingly, allicin has radical scavenging properties in activated granulocytes and may also inhibit inducible nitric oxide synthase expression in activated macrophages (Lang *et al*., 2004).

Epidemiological studies and animal experiments have suggested that several garlic-derived compounds have potential anticarcinogens (Sun and Wang, 2003), inhibit the proliferation of cancer cells, and some of them cause apoptosis in tumor cells of different tissue origin (Kwon and Moon 2005; Arditti and Rabinkov 2005; Jakubikova and Sedlak 2006).

Therefore, the present study was carried out to determine the antitumor activity of allicin in murine test system L5178Y lymphoma and the role of cytokines involved in the immune Th1 response.

MATERIALS AND METHODS

**Materials**
Allicin (1 mg/ml) were purchased from from Chromadex Company (USA).

**Mice**
Male BALB/c mice (20-25 g), were obtained from the Zooterio Universidad de Guadalajara and maintained under conventional laboratory conditions, according to the guidelines for the use and care of laboratory animals and World Medical Association Declaration of Helsinki. Animal protocols were approved by the Biomedicine Sciences Committee.

**Cell viability assay**
Cell viability assays were performed as follows: cells obtained from lymphoma L5178Y were cultured in RPMI medium, at 37° C in a 5% CO2 incubator. Cells (1 x 10^5 cell/ml) were plated in 96-well plates. Twenty four hours later they were treated with allicin at a final concentration of 20, 40, 60, 80, 100 or 120 µg/ml. Allicin treated cells viability was determined by assaying for the reduction of MTT to formazan, and the absorbance was measured spectrophotometrically at 570 nm, and the cell survival was expressed as percentage over the untreated control.

**Tumor models**
Animals, 10 per group, were inoculated i.p. with 0.1 ml of suspension ascitic fluid, containing L5178Y lymphoma 2 x 10^4 cell/mouse, on day 0. This tumour line was derived from murine thymic lymphoma (H-2d) haplotype (Puebla *et al*., 1998). The treatment with allicin (i.p. administration), started 24 h after inoculation, and it was extended over 7 days. Antitumor activity was evaluated by survival time represented as Kaplan-Meier curves.

**Cytokine secretion**
Mouse blood plasma of animals treated was obtained by cardiac punction. After centrifugation the supernatant was collected and the concentrations of IL-2, IFN-gamma and TNF-alpha determined by sandwich enzyme-linked immunosorbant assays using published protocols, performed according to the manufacturer's instructions (Katiai *et al*., 1998).

**Statistical analysis**
Results are expressed as means ± SD. Differences between means were determined using Student’s t-test.

RESULTS

**Inhibition of cell viability of allicin-treated tumor cells**
The L5178Y tumor cells were choosen to determine the antiproliferative activity of allicin. A typical dose-dependent inhibition of cell growth is shown in Figure 1 documenting the survival cell percentage plotted versus allicin concentration. After a 24 h incubation, increasing concentrations of allicin (0 -
120 μg/ml) led to a gradual decrease of the viable cells. ED50 value was 72 μg/ml.

**Figure 1**
Inhibitory effects of Allicin on the growth of L5178Y tumor cells, treated with various concentrations of Allicin. The viability was determined by MTT assay. Data are presented as the mean ± SD of the results for three independent experiments.

**Tumor growth inhibition in vivo**
The effect of allicin on tumor growth has been studied in L5178Y murine lymphoma model. Intraperitoneal injection of allicin was performed every day, following tumor cell inoculation. It resulted in an inhibition of tumor growth that has been quantified by survival time at the end of the experiment (Figure 2). Maximal antitumor effect was attained by administration of allicin at multiple doses of 20.0 mg/kg. At higher doses it was less or not effective.

**Allicin enhances the secretion of IL-2, IFN-gamma and TNF-alpha**
IL-2, IFN-gamma and TNF-alpha are important cytokines in antitumor response. Therefore, the effect of animals treated with allicin on cytokine secretion was studied. As shown in Figure 3, allicin enhanced the secretion of studied cytokines from mouse plasma.

**DISCUSSION AND CONCLUSION**
The many beneficial effects of garlic have been reported. They include anticancer activity, immunomodulatory effects, antibacterial and antiparasitic effects, and lowering serum cholesterol, triglycerides and blood pressure (Tattelman, 2005).

It is plausible that the different compounds comprising garlic ingredients are involved in eliciting their multiple effects. The most characterized non-protein fraction of garlic includes the organosulfur compounds. Allicin is the main biologically active component of this group and its action was attributed to either antioxidant activity or interaction with thiol containing proteins.

In this paper we reported the antitumoral and immunomodulatory properties of allicin. In vitro, allicin inhibited growth of L5178Y cells, whereas in vivo, the compound shown an antitumor effect in murine L5178Y lymphoma.
Kaplan Meier survival function in animals bearing L5178Y lymphoma, treated with allicin at different doses by ip injection for 7 days. The 20 mg/Kg group lived 10 days longer (P <0.001 compared with vehicle group).

The antiproliferative and cytotoxic effects of garlic thioallyl derivatives on tumor cell lines in culture has been reported (Pinto and Rivlin, 2001; Arditti and Rabinkov 2005). We postulated that the in vivo antitumor effect reported here is mediated by immune stimulation. This is supported by our findings demonstrating the immune stimulatory properties of allicin, with an enhanced IL-2, IFN-gamma and TNF-alpha secretion in plasma. These cytokines are associated with the beneficial Th1 antitumor response, which is characteristic of effective cancer immuno-therapies. The antitumor effect of allicin presented only at low doses was reported in melanoma and fibrosarcoma bearing mice supporting indirect antitumor activity of allicin (Patya et al., 2004).

This findings suggests that allicin represents a major water-soluble antiproliferative compound in garlic. Siegers and coworkers (Siegers et al., 1999) also suggested a major role for breakdown products of alliin, such as allicin or polysulfides, in the growth inhibitory effect of crude garlic preparations, although these authors did not test an isolated allicin preparation.

Recently, it has demonstrated that allicin was found to be a potent growth inhibitor in promyelocytic leukemia-derived, gastric, and colon cancer cells (Miron et al., 2008; Zhang et al., 2010; Bat-Chen et al., 2010).

In conclusion, the present study shows that allicin, the main biologically active substance of freshly crushed garlic, modulates cytokine patterns towards a Th1-type response. Additional studies are needed to clarify the mechanisms and intracellular mediators of the growth inhibitory activity of allicin.
Figure 3
Cytokine production by plasma of mice bearing L5178Y lymphoma and treated with the antitumor concentration of allicin. Data represent results of three experiments. Values are given as mean ± SD of each group. *Significant differences with control group, P < 0.01.
AKNOWLEDGEMENTS
This work was supported in part by CONACyT-SAGARPA. Grant 185236.

REFERENCES


