

Während der Drucklegung dieser Arbeit wurde uns eine Veröffentlichung über Flavonoidglyka bei *Clerodendranthus spicatus* (Thung.) C. Y. Wu bekannt (8). Dieser Name ist ein Synonym zu *Orthosiphon spicatus*. Beschrieben werden die Flavonoide Eupatorin und Sinensetin sowie Scutellarein-6,7,4'-trimethylether und Scutellarin-6,7-dimethylether. Damit werden unsere Ergebnisse bezüglich der Scutellareinderivate bestätigt.

Literatur

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Antiviral Properties of Garlic: *In vitro* Effects on Influenza B, Herpes Simplex and Coxsackie Viruses

Yen Tsai¹, Linda L. Cole², Larry E. Davis³, Steven J. Lockwood^{3,4}, Victoria Simmons³ and Gaynor C. Wild

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The spice, garlic or *Allium sativum*, has been employed for centuries as an herbal or traditional medicine to treat infectious diseases (1,2). In the Peoples Republic of China, cloves of garlic are eaten and concentrated extracts of garlic are given intravenously to treat cryptococcal meningitis (3) and a variety of viral infections. Although the anecdotal evidence suggests that garlic extract has antiviral properties, little scientific data is available to support these clinical impressions. We found that garlic extract produced by the Shanghai Tenth Pharmaceutical Factory possessed *in vitro* antiviral activity against influenza B and herpes simplex viruses but not against coxsackie B1 virus.

¹ Department of Neurology, Shanghai Second Medical College, Shanghai, Peoples Republic of China, Departments of Microbiology^{2,3}, Neurology³ and Biochemistry⁴, University of New Mexico School of Medicine, Albuquerque, New Mexico, U.S.A. and the Neurology Service³, Albuquerque Veterans Administration Medical Center, Albuquerque, New Mexico

⁴ Reprint requests and further correspondence to: Larry E. Davis, M. D., Department of Neurology, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

Methods

Influenza B/Lee/40 virus was grown in the allantoic cavity of embryonated chicken eggs and plaque counts were determined using a modification of the method of Tobita (4,5). Herpes simplex virus (HSV) type 1 was grown in rabbit skin cells and plaque counts were determined using the method of Lancz (6). Stocks of coxsackie B1 virus were grown in HeLa cells and plaque counts were determined using the method of Holland and McLaren (7).

Garlic extract (30 mg in 2 ml sterile ampules) was obtained from the Shanghai Tenth Pharmaceutical Factory, Shanghai, Peoples Republic of China (Allitridium, Lot #781227 and 791219). Although the extraction procedure is a commercial secret, factory scientists report that it contains most of the biologically active components of natural garlic including diallyl thiosulfinate (allicin), diallyl disulfide, and diallyl trisulfite. We confirmed that the major ingredient contained 2 sulfur atoms and was allicin or a derivative of allicin by gas chromatographic analysis.

In vitro experiments were performed to determine the antiviral activity of the garlic extract against all three viruses. Experiments were done in duplicate unless noted, and each virus was studied on two to three separate occasions using different ampules of garlic extract. The garlic extract was diluted serially in phosphate buffered saline (PBS), pH, 7.5. As

a control, PBS was mixed with virus stock. All tubes were incubated at 37°C for 6 or 24 hours. After incubation, tenfold dilutions of each test mixture was made in PBS containing 0.25% bovine serum albumin. For each dilution of virus-garlic mixture or virus-PBS control mixture, 0.1 ml was inoculated onto appropriate cell monolayers for plaque assay.

To determine the toxicity of the garlic extract on the different tissue culture cell lines, serial dilutions of the garlic extract were made in PBS and 0.1 ml was inoculated onto the various cell monolayers. The cells were observed for signs of cytopathic effect.

Results

It was found that concentrations of garlic extract above 1.5 mg/ml produced microscopic cell toxicity with cells rounding, shrinking and dying. Therefore, concentrations at or below this value were always used in the antiviral studies. Incubation of garlic extract at concentrations of 0.15 mg/ml or greater with influenza B/Lee/40 significantly reduced the infectivity titer of the influenza B/Lee virus. ($P = < .0001$, two way analysis of variance) (Figure 1). Garlic extract at concentrations of 0.015 mg/ml or higher significantly reduced the infectivity titer of the herpes simplex virus ($P = < .001$ one way analysis of variance) (Figure 1). Significant antiviral activity was found with either a 6 or 24 hour incubation. Garlic extract at concentrations as high as 1.5 mg/ml did not reduce the infectivity titers of coxsackie B1 virus. (Figure 1). In two separate experiments no concentration of garlic significantly reduced the infectivity titer of coxsackie B1 virus. There were no differences found between the two lots of garlic extract.

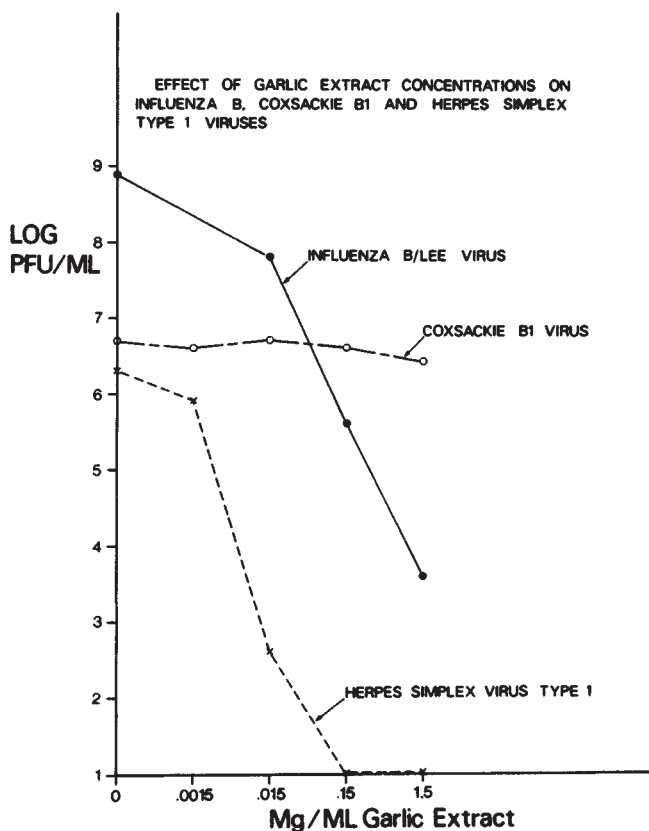


Fig. 1. Graph based on average of three separate experiments for herpes simplex and influenza B virus and two separate experiments for coxsackie B1 virus. All virus-garlic mixtures were incubated at 37° C for six hours.

Discussion

The garlic extract possessed *in vitro* antiviral activity against influenza B virus, *herpes simplex* virus type 1, but not

against coxsackie B1 virus. The antiviral activity was found to occur at concentrations below those that caused toxicity to tissue culture cells. Hence, these antiviral concentrations might be achieved in

man or animals without significant host toxicity. Since our studies were done only *in vitro*, further studies are needed to determine whether garlic or garlic extracts possess significant antiviral activity in experimental animals or man. Nevertheless, these studies lend credence to the historical usefulness of garlic as an antiviral agent.

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