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NOTES

Antifungal Activity in Human Cerebrospinal Fluid and Plasma after Intravenous Administration of *Allium sativum*

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Commercial *Allium sativum* (garlic) extract was given intravenously to two patients with cryptococcal meningitis and three patients with other types of meningitis. Plasma titers of anti-*Cryptococcus neoformans* activity rose twofold over preinfusion titers. Anti-*C. neoformans* activity was detected in four of five cerebrospinal fluid samples but not in pooled normal cerebrospinal fluid.

High dilutions of extracts of *Allium sativum*, or garlic, have been shown to possess fungistatic and fungicidal activity in vitro (1, 4, 7, 14, 15) and in vivo (3, 13). In the People's Republic of China, commercial *A. sativum* extracts are widely used to treat patients with systemic fungal infections (6, 10). In support of the use of *A. sativum* to treat cryptococcal meningitis, we found anti-*Cryptococcus neoformans* activity in plasma and cerebrospinal fluid (CSF) following intravenous (i.v.) administration of a commercial *A. sativum* extract.

Commercial garlic extract (Allidridium) was prepared by the Shanghai Second Pharmaceutical Factory, Shanghai, People's Republic of China. Although the extraction process was a commercial secret, factory scientists reported that the extraction contained active components of natural garlic, such as diallyl thiosulfinate (allicin), diallyl disulfide, and diallyl trisulfite. We had previously confirmed by gas chromatographic analysis that the major ingredient of the extract contained two sulfur atoms and was allicin or a derivative of allicin (12). Thirty milligrams of this oily extract was diluted into 2 ml of sterile distilled water that contained Tween 80, and the mixture was placed into ampoules.

The *C. neoformans* used in this study was isolated from the CSF of a patient with cryptococcal meningitis. High titers of *C. neoformans* capsular antigen were detected in the CSF of the patient and in a beef extract broth containing the isolate by the Cryptococcal Antigen Latex Agglutination System (Meridian Diagnostic, Inc., Cincinnati, Ohio). A final concentration of 5×10^5 yeast cells per ml, prepared from fresh cultures and determined microscopically, was used in all tests. The MICs of commercial garlic extract were determined by the broth dilution method of Al-Doory (2). Each twofold dilution was performed in duplicate. The minimum fungicidal concentration (MFC) was determined from subculture of the MIC broth tubes onto Sabouraud dextrose agar by the method of Al-Doory (2). Antifungal activity in plasma and CSF was determined by a modification of the method of Schoenknecht et al. (9). Citrated plasma was heated to 56°C for 30 min to inactivate the complement. Plasma or CSF was serially diluted in twofold dilutions into two tubes that each contained 1 ml of beef extract broth, 100 U of penicillin, and 200 μg of streptomycin. Control broth tubes lacked plasma and CSF. All tubes (except three sterility control tubes that contained plasma and broth, CSF and broth, or only broth) received 5×10^5 *C. neoformans* organisms. The highest plasma and CSF dilutions that contained MIC and MFC activities were then determined (2). The minimal lethal concentration was determined by the method of Pearson et al. (8). The rejection value for determination of 99.9% killing was 25 colonies when the inoculum was 5×10^5 CFU/ml; tests were performed in duplicate.

Five patients hospitalized at the Ren Ji Hospital, Shanghai, volunteered for this study. The patients were receiving commercial garlic extract (1 mg/kg of body weight per day diluted into 500 ml of saline and administered i.v. over 4 h). Patients did not receive other antimicrobial drugs. Diagnoses included cryptococcal meningitis (two patients), viral meningitis (two patients), and unknown chronic meningitis which was not cryptococcal meningitis (one patient). Blood was taken from three patients prior to *A. sativum* infusion. At the conclusion of the 4-h *A. sativum* i.v. infusion, 10 ml of blood was removed from the arm opposite the i.v. infusion site and placed into a citrate anticoagulant tube. A lumbar puncture was then performed. No CSF samples had any blood contamination. Two normal individuals not receiving *A. sativum* donated blood as controls. Leftover fresh CSF from several patients not receiving *A. sativum* was combined into two pools as CSF controls. Plasma and CSF samples were frozen at −30°C until assayed.

From January to July 1988, patients receiving i.v. *A. sativum* at the Ren Ji Hospital were evaluated for adverse effects. In addition, five Chinese neurologists were asked to recall any adverse effects they had encountered from the use of *A. sativum*.

The MIC of commercial *A. sativum* extract against *C. neoformans* was 2.5 μg/ml. The MFC and minimum lethal concentration were 5.0 μg/ml. Table 1 shows the maximal dilution of plasma or CSF from patients and controls that possessed fungistatic or fungicidal activity against *C. neoformans*. We found that heat-inactivated plasma taken from patients before *A. sativum* infusion or from control subjects

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TABLE 1. Antifungal activity in human CSF and plasma

Source of plasma and CSF	Maximum dilution[a] of:						Pleocytosis and elevated CSF protein
	Plasma				CSF[b]		
	MIC		MFC				
	Before[c]	After[c]	Before	After	MIC	MFC	
Patient with:							
Viral meningitis	1:2	1:4	1:1	1:1	1:1	0	Yes
Unknown meningitis	ND[d]	1:16	ND	1:4	1:1	0	Yes
Convalescent-phase viral meningitis	ND	1:16	ND	1:4	0	0	No
Cryptococcal meningitis	1:2	1:4	1:1	1:2	1:1	0	Yes
Cryptococcal meningitis	1:4	1:8	1:1	1:2	1:1	0	Yes
Control							
Normal subject	1:4	ND	1:1	ND	ND	ND	
Normal subject	1:4	ND	1:1	ND	ND	ND	
Pooled CSF					0	0	Unknown
Pooled CSF					0	0	Unknown

[a] Numbers represent the maximum dilution of plasma or CSF that contained antifungal activity.
[b] Values obtained after i.v. administration of *A. sativum*.
[c] Before and after i.v. administration of *A. sativum*.
[d] ND, None donated.

contained low titers of antifungal activity (median dilution, 1:4) against *C. neoformans*. However, plasma from all patients who received *A. sativum* had titers of fungistatic and fungicidal activity twofold higher than pre-*A. sativum* infusion titers. Plasma titers as high as 1:16 were found. In four of five patients, the CSF contained fungistatic activity against *C. neoformans*. The one patient who lacked CSF fungistatic activity had CSF that was acellular and had normal CSF protein. The two pools of control CSF lacked anti-*C. neoformans* activity.

Adverse effects of i.v. commercial *A. sativum* administration occurred in less than 25% of patients and included abdominal discomfort, nausea, and thrombophlebitis at the i.v. site. No cases of anaphylaxis were recognized. Abdominal discomfort and nausea appeared to depend on the rate of drug delivery. In one patient, these symptoms developed when the infusion rate was 30 mg/h but not when it was 15 mg/h. Patients could tolerate i.v. *A. sativum* daily for at least 1 month without apparent major damage to liver, kidneys, or bone marrow. Since hemograms, liver function studies, and renal function studies were seldom repeated on patients during their hospitalization, it was impossible to determine whether the commercial *A. sativum* extract had mild effects on liver, kidneys, or bone marrow.

Extracts of garlic have been shown to contain several molecules that exhibit antifungal activity, e.g., allicin and ajoene (4, 14, 15). Therefore, we decided to measure antifungal activity by using a standard in vitro microbiological assay rather than attempt to directly measure the number of molecules of *A. sativum*. Using a biological assay, we found that plasma from normal individuals and from patients prior to *A. sativum* infusion possessed low titers of anti-*C. neoformans* activity. However, following infusion with *A. sativum*, the anti-*C. neoformans* titers always rose twofold above preinfusion levels. This study therefore confirms a previous study that demonstrated antifungal activity in serum following oral administration of raw garlic (5).

Four of five CSF samples from patients receiving *A. sativum* contained fungistatic activity against *C. neoformans*. Pooled CSF from patients not receiving *A. sativum* lacked anti-*C. neoformans* activity. Allicin, the major antifungal molecule in *A. sativum*, is highly lipid soluble (11), which suggests that it might cross an intact blood-brain barrier. However, the one patient whose CSF lacked detectable anti-*C. neoformans* activity had normal CSF values, which raises the possibility that disruption of the blood-brain barrier is necessary for detectable activity to appear in CSF.

The limitations of this study include the absence of control patients treated with an inert biological extract of *A. sativum*, the lack of chemical confirmation of any active *A. sativum* molecules in plasma or CSF, and the inability to be certain that equivalent doses of active drug were given to each patient. Although the postinfusion anti-*C. neoformans* titers were usually twofold higher in both plasma and CSF than the preinfusion titers and although the tests were always performed in duplicate, it should be noted that this difference may reflect only an error of the test.

Administration of commercial *A. sativum* appeared to cause few side effects, and those that appeared were quite similar to those encountered from eating fresh garlic (e.g., vomiting, diarrhea, nausea, anorexia, flatulence, weight loss, or garlicky body odor) (5).

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