In Vitro and In Vivo Activity of a Synthetic Halogenated Quinoline Against *Cryptococcus neoformans*

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The minimum inhibitory concentrations of a halogenated quinoline, 3-amino-7-chloro-3,4-dihydro-1-hydroxycarbostyryl (CBS), against nine clinical strains of *Cryptococcus neoformans* were determined by in vitro testing. The CBS was fungistatic at a minimum concentration of 0.2 µg/ml at 48 h for several strains. In vivo toxicity studies were carried out in mice. Mice were also infected with *C. neoformans* strain Price and injected with various concentrations of CBS. Mean life expectancy of treated groups of animals was increased over infected untreated controls.

In 1975, Davis et al. (1) reported the synthesis of 3-amino-7-chloro-3,4-dihydro-1-hydroxycarbostyryl (CBS), a halogenated quinoline, which was effective in preventing in vitro growth of *Escherichia coli* ATCC 9723 at 1 µg/ml, *Lactobacillus plantarum* ATCC 8014 at 0.6 µg/ml, and *Leuconostoc dextranicum* ATCC 8086 at 1 µg/ml. More recently, activity against gram-positive and gram-negative bacteria, as well as the yeast *Candida albicans*, has been demonstrated (2). Kobayashi and Medoff (3) have reviewed current antifungal therapy and the need for new antifungal agents. This communication reports the results of in vitro and in vivo analysis of CBS against nine clinical isolates of the organism *Cryptococcus neoformans*.

The nine human isolates of *C. neoformans* were obtained from the Missouri State Chest Hospital, Mt. Vernon, Mo. Stock cultures were maintained on Sabouraud dextrose (SAB) agar slants at 25°C. For in vitro studies, inocula were obtained from 125-ml screw-cap Erlenmeyer flasks containing 10 ml of SAB broth and incubated for 3 days at 36°C and 120 rpm on a gyratory shaker. A 0.1-ml sample of inoculum, containing approximately 10⁷ yeast cells, was placed in screw-cap tubes (25 by 150 mm) of SAB broth with either 0, 0.2, 0.4, 0.6, 0.8, or 1.0 µg of CBS per ml (10 ml final volume). All tubes were incubated at 36°C and 120 rpm on a gyratory shaker for 48 h. The minimum inhibitory concentration of CBS for each strain tested was determined at 48 h by plate counts on SAB agar. The minimum inhibitory concentration was defined as the drug concentration that inhibited increase in colony-forming units through the course of the study.

Four strains of *C. neoformans* were inhibited at a CBS concentration of 0.2 µg/ml. Three additional strains were inhibited by 0.4 µg of CBS per ml, and the final two strains were inhibited by 0.6 and 0.8 µg of CBS per ml, respectively.

For in vivo studies four groups of BALB/c mice (20 mice per group) were infected via the tail vein with (per mouse) 2.24 × 10⁵ cells of a virulent encapsulated strain of *C. neoformans* (Price). Three groups of mice received single daily intraperitoneal injections of CBS, initiated at 72 h and terminated at 16 days postinfection. The dosage levels were 200 (high dose [H]), 100 (medium dose [M]), and 25 (low dose [L]) mg/kg per dose, and were delivered in 0.2, 0.1, and 0.1 ml, respectively. The fourth group of infected animals were infected untreated (IU). A group of 10 uninfected drug control (DC) mice underwent the H dose regimen, and a group of 10 uninfected solvent control (SC) mice received 0.2 ml of sterile water according to the above injection schedule.

Brains and spleens were aseptically removed from moribund animals during the course of the experiment, homogenized, and plated on SAB agar for cultural recovery of *C. neoformans*. At 45 days postinfection, all surviving animals were sacrificed, and organs were plated. There were no deaths in the DC or SC group prior to day 45. One mouse in the IU group, five mice in the H group, and three mice in the M and L groups survived until day 45. Ninety-five percent of the IU mice died between days 11 and 22 postinfection, as did the majority of mice in the H (55%), M (65%), and L (80%) groups.

The mean life expectancy of the IU group was

16.8 $\pm$ 1.6 days. The L group had a mean life expectancy of 20.4 $\pm$ 2.6 days, the M group had 22.0 $\pm$ 2.8 days, and the H group had 26.4 $\pm$ 2.9 days. Statistical analysis of these data using Student's unpaired *t* test indicate the mean life expectancy of the H group was significantly different from that of the IU group (0.005 < *P* < 0.01).

Table 1 shows the percentage of organs positive for *C. neoformans* for each group of mice. Only one spleen from the IU group was negative for *C. neoformans*, but the brain from that animal was positive, as were all of the other recovered brains from this group. Two spleens of animals from the H group were negative for *C. neoformans*, but all brains were positive. The negative spleens were from two animals sacrificed at day 45 postinfection. The M group had three mice with negative spleens, and two of these animals had negative brains as well. These three animals were sacrificed at the termination of the experiment. The spleen from one animal in this group was not recovered, but the brain from that animal was positive. Eighty-four percent of the recovered spleens and 90% of the brains were positive in this group. Ninety percent of the spleens and brains in the L group were positive for *C. neoformans*. The two negative spleens and brains were from the same animals, again from two of the animals sacrificed at day 45. *C. neoformans* was not recovered from any DC or SC animals.

DC mice showed no ill effects from drug treatment. Hematocrit values from additional groups of five DC and SC mice were obtained 15 days after initiation of drug therapy. DC mice had hematocrits of 51.7 $\pm$ 0.8%, and SC mice had hematocrits of 52.3 $\pm$ 1.3%. These values are not significantly different (*P* > 0.5).

The results of in vitro studies suggest that CBS is fungistatic and not fungicidal under the conditions of the experiment. No attempt was made to determine fungicidal concentrations.

Unpublished studies in our laboratories have shown that seven of the nine strains of *C. neoformans* used are inhibited by amphotericin B concentrations ranging from 0.2 to 0.6 μ g/ml. These values compare favorably with the minimum inhibitory concentrations determined for CBS.

The lack of noticeable toxicity of CBS to mice at fairly high concentrations is encouraging. This

TABLE 1. *Organs positive for C. neoformans (Price) upon culture on SAB agar with 20 U of penicillin and 40 U of streptomycin*

Group ^a	% Spleens positive for <i>C. neoformans</i>	% Brains positive for <i>C. neoformans</i>
IU	95 ^b	100 ^c
H	90	100
M	84 ^{c, d}	90 ^d
L	90 ^d	90 ^d
SC	0	0
DC	0	0

^a Twenty animals in IU, H, M, and L groups, respectively. SC and DC groups had 10 animals each.

^b Animal with negative spleen had *C. neoformans* recovered from the brain.

^c Organ from one animal not recovered. Percentage based on 19 animals.

^d Brain and spleen from same two animals were negative for *C. neoformans*.

is especially important since amphotericin B may be toxic in therapeutic concentrations (4-6). The information on CBS toxicity is incomplete and warrants further investigation. Although treatment with CBS did not mediate complete protection for all infected mice, a clear increase in mean life expectancy was noted. No information is available concerning mode of action, bio-transport and transformation, or serum levels of CBS.

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