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In Vitro Antiproliferative Effects of Neuroleptics, Antimycotics and Antibiotics on the Human Pathogens *Acanthamoeba polyphaga* and *Naegleria fowleri*

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Background. Using reproducible conditions *in vitro*, the aim of this study was to obtain a comparative evaluation of the efficacies of several tricyclic neuroleptics, antimycotics and antibiotics with antiproliferative activities against *Acanthamoeba polyphaga* and *Naegleria fowleri* trophozoites.

Methods. We used reproducible conditions *in vitro* to obtain results.

Results. The most effective drugs against *N. fowleri* expressed as (IC_{50}) were as follows: the antimycotics ketoconazole and amphotericin B, followed by trifluoperazine, mepacrine, chlorpromazine, miconazole, and metronidazole. The least effectives were rifampicin and pentamidine. The most potent growth inhibitors (MIC_{100}) against *N. fowleri* were the antimycotics amphotericin B and ketoconazole and the neuroleptic trifluoperazine. It was clear that there are major differences between the two amebas in their susceptibility to some of the drugs.

Conclusions. The drugs with the minimal inhibitory concentration (MIC) values could be considered alone or in combination as potential anti-amebic agents for the treatment of the diseases produced by these amebas.

Key Words: Acanthamoeba polyphaga, *Naegleria fowleri*, Neuroleptics, Antimycotics, Antibiotics, Antiparasitic agents.

Introduction

There are several species of free-living amebas in air, soil, and water environments that can infect humans, in addition to some species that live close to humans, such as those that inhabit air-conditioning units. Some of the species implicated in human pathogenicity are *Acanthamoeba cultbersoni*, *A. polyphaga*, and *A. castellanii*, among others. *Acanthamoeba* spp. has been recognized as an opportunistic pathogen and is frequently present in immunodeficient and chronically ill patients. The parasite invades the human body through a wound or through the nostrils and, once inside, can travel through the bloodstream to the lungs or organs and structures such as the central nervous system.

Currently, the medical treatment for some of these pathogens, such as A. keratitis, is difficult due to the parasites' resistance to treatment (1). However, satisfactory results have been seen in some patients using a combination of neomycin and propamidine isethionate against *A. keratitis* (2) and using biguanides such as chlorhexidine and polyhexamethylene (3).

Naegleria fowleri is also a free-living ameba that can be pathogenic to humans and is normally found in lakes, ponds, swimming pools and sewage water. Infection is transmitted via the nasal mucosa and olfactory nerve to the brain, producing a primary acute meningoencephalitis (PAM) that causes death of the host within a few days of the first symptoms being detected (4). Although amphotericin B treatment has proven effective, survival is problematic due to the fulminant nature of the disease.

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From a metabolic point of view, *N. fowleri* and *A. polyphaga* are aerobic organisms with mitochondria that have the capacity to synthesize glutathione in addition to glutathione reductase activity. In comparison, *Entamoeba histolytica* is microaerophilic, with no mitochondria, and has neither the capacity to synthesize glutathione nor to actuate glutathione reductase (5) and, therefore, lives exclusively as parasites in the human host.

The human parasite *E. histolytica* HK9 and HM1 trophozoites and the effect of various phenothiazine and tricyclic neuroleptic compounds have been investigated earlier (6,7). The inhibitory activity on cell proliferation and lytic effects on this human parasite by these compounds indicated that they could be considered potential anti-amebic agents.

In view of the previous results, the aim of the present study was to obtain a comparative evaluation of the inhibitory efficacies of neuroleptics, antimycotics and antibiotic agents against *A. polyphaga* and *N. fowleri* trophozoites.

Materials and Methods

Reagents

The following reagents were used: biotriptase peptone (Difco Laboratories, Detroit, MI), dextrose (Gibco BRL, Life Technologies, Gaithersburg, MD), sodium phosphate monobasic, potassium phosphate and trypan blue (Sigma Chemical Co., St. Louis, MO), fetal bovine serum (Equitech).

Cell Culture Conditions

A. polyphaga (kindly provided by Patricia Bonilla from ENEP, Universidad Nacional Autonoma de Mexico, Iztacala, Tlalnepantla Estado de Mexico, Mexico) was grown axenically at 28–30°C in axenic liquid medium which contained biotriptase peptone, sodium phosphate monobasic, potassium phosphate and 10% inactivated fetal bovine serum (FBS). The total amount of trophozoites present in 100 mL of normal culture harvested at the 72 h stage was approximately 31. 5×10^6.

Highly pathogenic *N. fowleri* ATCC 30808 (kindly provided by Dr. Mineko Shibayama, Centro de Investigaciòn y de Estudios Avanzados, Instituto Politécnico Nacional, Mexico, D.F., Mexico) was grown axenically at 37°C in culture medium containing Bacto casitone, 2% pancreatic digest of casein and 10% inactivated FBS. The total number of trophozoites present in 40 mL of normal culture medium, harvested at the 60 h stage, was approximately 25. 4×10^6.

Pharmaceutical Agents

Neuroleptics. Trifluoperazine (MW 480.4) and chlorpromazine (MW 355.3), both of which are typical antipsychotic drugs of the phenothiazine group, were used.

Antimycotics. Amphotericin B (MW 924.1), ketoconazole (MW 531.4) and miconazole (MW 479.1) were used, all of which affect the integrity of the plasmatic membrane producing loss of cell components and consequently inhibiting the growth of the fungus.

Amphotericin B, as with other polyene antifungals, associates with ergosterol, a membrane chemical of fungi, forming a pore that leads to K^+ leakage and fungal cell death. However, the actual mechanism of action may be more complex and multifaceted.

Ketoconazole is an imidazole antimycotic. Its fungistatic effect is based primarily on enzyme inhibition due to a bond with the cytochrome P450. It thus inhibits, among other activities, the biosynthesis of ergosterol and thereby alters the permeability of the fungal cell membranes. The discovery of its hepatotoxic potential has been detrimental to its initially promising use as an antimycotic agent. Miconazole is also an antimycotic agent with the same characteristics as ketoconazole.

Antibiotics. Rifampicin, INN or rifampin, USAN (MW 823.0) is used against tuberculosis and inhibits RNA synthesis by binding directly to the RNA polymerase of the pathogen; pentamidine isethionate, 1,5-bis(4-amidino phenoxypentane) (MW 592.7) binds to the parasite's DNA, inhibiting its replication; quinacrine or mepacrine dihydrochloride (MW 472.9) is supposed to increase the pH of the intracellular vacuoles and favors protein degradation by acid hydrolases within the lysosomes; and metronidazole is an antiprotozoal and antibacterial agent (MW 171.2) that inhibits DNA synthesis. All the above drugs used were provided by Sigma Chemical Co.

Determination of the Inhibitory Concentration IC_{50} and MIC

Experiments were begun using all agents mentioned above and their inhibitory concentration (IC_{50}), and MIC was established. IC_{50} corresponds to a decrease in 50% of the population of trophozoites of *A. polyphaga* and *N. fowleri* in the culture. MIC is defined as the lowest concentration of drug that inhibits $>99\%$ of the cell population.

Care should be exercised in interpreting the IC, because it is a culture definition. In cells and tissues the situation may be different since they may be resistant to the passage of drugs.

To establish the IC_{50} for each drug, the recommendations published by the National Committee for Clinical Laboratory Standards, NCCLS were followed (8).

For the preparation of the inoculate, test tubes were used with 3 mL sterile culture medium inoculated with 25×10^3 and 35×10^3 trophozoites of *A. polyphaga* and *N. fowleri*, respectively. A series of dilutions were made starting from a stock of 5120 μg/mL for each drug, resulting in a range of concentrations per tube of 0.125 μg/mL, 0.25 μg/mL, 0.5 μg/mL, 1.0 μg/mL, 2.0 μg/mL, 4.0 μg/mL, 8.0 μg/mL, 16.0 μg/mL and 32.0 μg/mL.