RESEARCH NOTE

IN VITRO DRUG SUSCEPTIBILITY OF *ACANTHAMOEBA CASTELLANI* TO CHLOROQUINE, IVERMECTIN AND FUNGIZON

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Abstract. *In vitro* sensitivity of *Acanthamoeba castellani* was tested to three drugs : Chloroquine, ivermectin and fungizone (amphotericin B). Sensitivity was demonstrated to the latter two compounds but not to chloroquine. Thus ivermectin and amphotericin ß show promise as therapeutic agents against this parasite.

Acanthamoeba castellani can cause granulomatous amebic encephalitis (Carter *et al,* 1981) and eye infection (Nagington and Richard, 1976). There is no satisfactory treatment for *Acanthamoeba* infections for both amebic encephalitis and optic keratitis as the parasites are highly resistant to chemotherapeutic agents, especially in the encysted stage (Ferrante *et al,* 1984). Different investigators have performed susceptibility tests with strains they have isolated, with conflicting findings. Culbertsoni *et al* (1965), demonstrated the protection of sulphadiazine against *Acanthamoeba* encephalitis in mice, and *in vitro* efficacy of sulphadiazine against the ameba has also been demonstrated, but these drugs have not been proven useful clinically (Ferrante *et al,* 1984).

Several antimalarial drugs, such as quinine, chloroquine and mefloquine have also been tested for their efficacy against *A. culbertsoni* infection and although both quinine and chloroquine were ineffective, the ameba was sensitive to mefloquine. In the present study we tested the *in vitro* activity of chloroquine, ivermectin and fungizone (amphotericin B/sodium desoxycholate/ml) against *A. castellani.*

Acanthamoeba castellani was obtained from Dr Mulkit Singh, Department of Microbiology, University of Singapore. The ameba was maintained in mycological peptone medium (4%) in 25mm^2 culture flasks at room temperature, and subcultured every 3 days. The subculture were carried out by decanting the tube in ice for 10 minutes and then flushing the wall of the culture flask using a pasteur pipette to detach the adherent ameba from the wall of the flask and transfering the contents into a centrifuge tube. After centrifugation at 7,000*g* for 10 minutes at 4°C, the ameba pellet was resuspended in 1 ml of culture medium and parasite counts determined using a Neubauer counting chamber. Approximately 2×10^4 amebae were inoculated into culture flask containing 10 ml culture medium together with the drug tested.

The antimicrobial agents used in the study were: ivermectin (Ivomec Injection: Merck, Rahway NJ, USA), chloroquine phosphate, and fungizone (205 µg amphotericin B and 205 µg sodium desoxycholate/ml as a solubilizer in distilled water, Flow Lab, USA).

Drugs were diluted in mycological peptone medium. Chloroquine was diluted from 4,000 µg/ml to 0.97 µg/ml, ivermectin from 1,000 µg/ml to 0.97 µg/ml and fungizone from 125 µg/ml to 0.97 µg/ml. The serial dilution of drugs was done in 25mm^2 culture flasks (Costar, USA) in 10 ml volume.

In vitro sensitivity of amebae to the antimicrobial agents was carried out as follows; A total of 2×10^4 trophozoite stage of the *A. castellani* were added into each drug dilution aseptically. The control consisted of trophozoites cultured in mycological peptone. The test suspensions were kept at room temperature (30°C) for 7 days. Cell counts were performed daily for 7 days. For cell counting, the culture flask was placed on a tray of ice for 10 minutes and the suspension mixed by Pasteur pipette and the walls of the culture flask were flushed so as to detach the amebae. The cells were then allowed to settle and mixed just before the parasite count was carried out in a Neubauer counting chamber. The cysts and trophozoites were

Table 1

In vitro sensitivity of *Acanthamoeba castelloni* to the antimicrobial agents.

Antimicrobiol agents	Drug conts µg/ml	No. of amebae × 10^4 days of culture						
		1	2	3	4	5	6	7
Chloroquine	4,000	0.2	0.2	0.6	2.0	4.2	9.8	19.0
	2,000	0.2	0.2	1.0	1.8	5.4	27.2	41.8
Chloroquine	1,000	0.6	0.2	0.8	2.0	9.8	6.0	3.8
Ivermectin	1,000	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloroquine	500	0.2	0.4	2.2	7.6	22.6	45.6	38.6
Ivermectin	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloroquine	250	0.4	0.6	1.6	8.6	28.0	43.4	46.0
Ivermectin	250	0.2	0.0	0.2	0.2	0.0	0.0	0.2
Chloroquine	125	1.2	0.8	4.6	22.4	58.2	93.8	91.4
Ivermectin	125	0.2	0.0	0.2	0.6	0.8	0.4	2.0
Fungizone	125	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloroquine	62.5	0.6	1.2	2.2	22.4	22.6	78.2	76.4
Ivermectin	62.5	0.6	0.2	0.4	0.6	3.0	4.2	11.8
Fungizone	62.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloroquine	31.25	1.2	0.8	5.0	19.2	34.8	84.2	88.6
Ivermectin	31.25	0.6	0.2	2.0	6.6	13.8	56.4	50.6
Fungizone	31.25	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Chloroquine	15.62	0.4	12.0	12.0	50.0	66.2	130.0	136.0
Ivermectin	15.62	0.6	0.4	2.4	5.6	17.0	54.6	49.6
Fungizone	15.62	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloroquine	7.81	0.0	1.0	1.0	26.0	36.0	50.6	80.0
Ivermectin	7.81	0.6	0.5	4.8	15.2	19.8	53.0	53.4
Fungizone	7.81	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Chloroquine	3.90	0.2	1.2	12.0	40.0	40.8	56.0	80.4
Ivermectin	3.90	0.6	0.8	1.8	10.2	15.0	65.0	52.0
Fungizone	3.90	0.0	0.0	0.2	0.2	0.2	0.2	0.2
Chloroquine	1.95	0.2	0.8	3.2	15.4	36.8	56.4	82.8
Ivermectin	1.95	0.2	1.4	1.4	16.8	25.6	28.0	50.0
Fungizone	1.95	0.8	0.1	1.2	0.3	5.0	6.0	6.3
Chloroquine	0.97	0.4	1.0	8.0	18.8	31.0	46.2	80.6
Ivermectin	0.97	0.2	0.6	3.4	15.0	20.2	26.8	50.8
Fungizone	0.97	0.2	0.2	0.6	4.0	4.5	cont	cont
Control		0.2	0.7	7.1	17.9	40.9	46.0	65.1

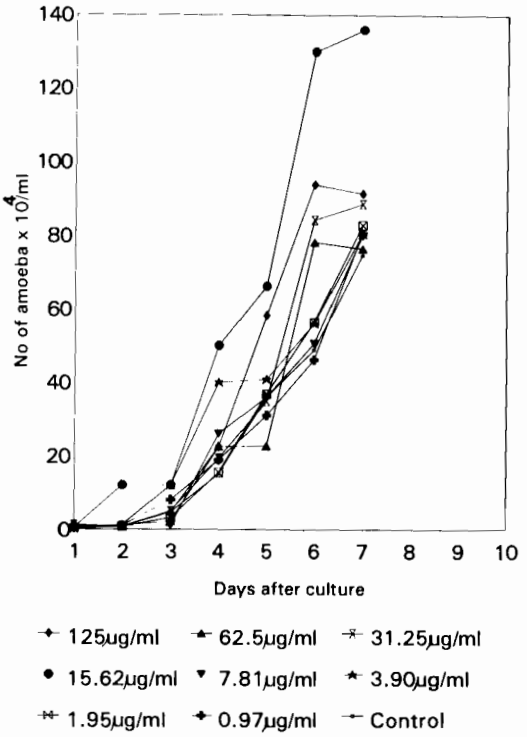

Fig 1– Effect of chloroquine on the growth of *Acanthamoeba castellani.*

counted separately. Parasites were counted daily from each drug dilution and the percentage inhibition was calculated as follows:

$$\% \text{ inhibition} = (A\text{-}B) \div A \times 100$$

where A = No. of amebae/ml in normal culture medium,
B = No. of amebae/ml in medium with drug.

Chloroquine was used at a concentration from 4,000 µg/ml to 0.97 µg/ml. For each concentration tested, the parasite growth was observed to increase from day 3 onwards (Table 1, Fig 1). The growth of parasite was clearly observed in the presence of chloroquine at 125 µg/ml and below. Progressive inhibition was observed between 250 to 4,000 µg/ml.

Ivermectin concentration tested was from 1,000 µg/ml to 0.97 µg/ml. From 1,000 µg/ml to 250 µg/ml drug concentration, there was appreciable inhibition of growth of parasite (Table 1). As the drug concentration decreased from 125 µg/ml to 0.97 µg/ml, an increase in parasite growth was seen (Table 1, Fig 1).

Fungizone concentration was tested from 125 µg/ml to 0.97 µg/ml. There was growth of parasite from 1.95 µg/ml drug concentration to 0.97 µg/ml concentration (Table 1, Fig 1). There was a 100% percentage inhibition of parasite using fungizone in all the drug concentration from 125 µg/ml to 15.62 µg/ml tested (Table 2). Fungizone showed a high inhibitory action from concentration 7.81 µg/ml to 0.97 µg/ml.

The results presented here indicate *in vitro* response of *A. castellani* to the three compounds, chloroquine, ivermectin and fungizone. Chloroquine at high concentration (4,000 µg/ml to 250 µg/ml), showed some degree of inhibition. Both invermectin and fungizone showed a promising inhibitory effect on the growth of parasite. Ivermectin at a concentration 1,000 µg/ml to 500 µg/ml was effective in inhibiting the growth of the parasite while fungizone (amphotericin B/sodium desoxycholate/ml), showed 100% inhibition from concentrations of 125 µg/ml to 15.62 µg/ml. Amphotericin B, a drug of considerable toxicity, is the antinaeglerial agent for which there is evidence of clinical effectiveness (Anderson and Jamieson, 1972a). *In vitro* drug sensitivity of *N. fowleri*

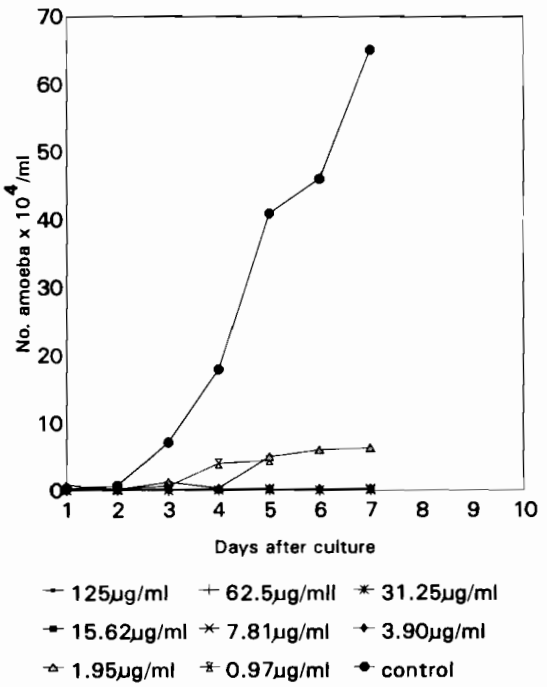

Fig 2– Effect of fungizone on the growth of *Acanthamoeba castellani.*

Table 2

In vitro sensitivity of *Acanthamoeba castelloni* to the antimicrobial agents.

Antimicrobiol agents	Drug conts µg/ml	% Inhibition days of culture 2	4	7
Chloroqiune	4,000	71	88.8	71
	2,000	71	89.9	35
Chloroquine	1,000	71	88	48
Ivermectin	1,000	100	100	100
Chloroquine	500	42.8	57.5	40.7
Ivermectin	500	100	100	100
Chloroquine	250	14	51	29
Ivermectin	250	100	98.8	99.6
Chloroquine	125	0	0	0
Ivermectin	125	100	96.6	96.9
Fungizone	125	100	100	100
Chloroquine	62.5	0	0	0
Ivermectin	62.5	71	96.6	81.9
Fungizone	62.5	100	100	100
Chloroquine	31.25	0	0	0
Ivermectin	31.25	71	63.1	22.2
Fungizone	31.25	100	100	100
Chloroquine	15.62	0	0	0
Ivermectin	15.62	42.8	68.7	23.8
Fungizone	15.62	100	100	100
Chloroquine	7.81	0	0	0
Ivermectin	7.81	28.5	15	17.9
Fungizone	7.81	100	100	99.6
Chloroquine	3.90	0	0	0
Ivermectin	3.90	0	40.9	20.9
Fungizone	3.90	100	98.8	99.6
Chloroquine	1.95	0	13.9	0
Ivermectin	1.95	0	6.1	23.0
Fungizone	1.95	85.7	98.3	90.3
Chloroquine	0.97	0	0	0
Ivermectin	0.97	14	16.2	21.4
Fungizone	0.97	71	77.6	cont

0 - no inhibition (parasite growth is equal or more than the control)
cont - contamination
The results are express as the mean of 2 experiments

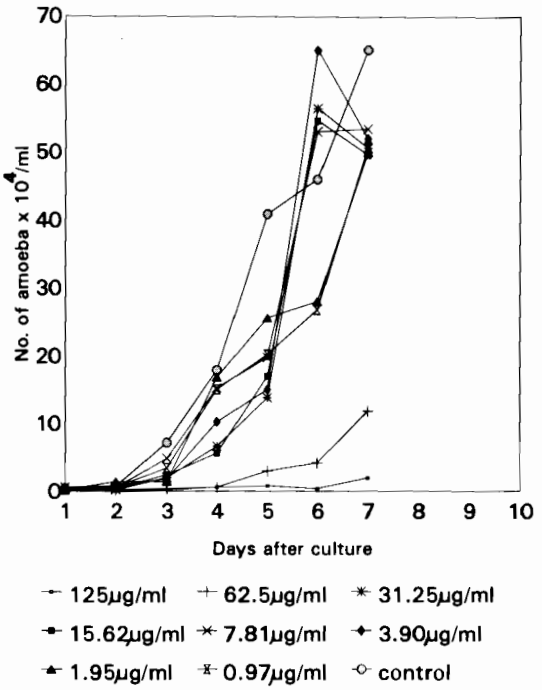

Fig 3– Effect of ivermectin on the growth of *Acanthamoeba castellani.*

demonstrated that the ameba was extremely susceptible of amphotericin B (minimal inhibitory concentration, (MIC), 0.15 µg/ml (Ferrante, 1982). Amphotericin B is a polyene compound that acts on the plasma membrane of microorganism and disrupting its selective permeability and causing leakage of cellular compounds (Kobayashi and Medoff, 1977). Since *in vitro* evaluation of amphotericin B to *Naegleria* proved to be successful (Ferrante, 1982), we decided to use the drug in this experiment to test the sensitivity of our isolate of *A. castellani.* The results showed that amphotericin B has a promising effect on *A. castellani,* with percentage inhibition of 100% or slightly less in all the concentration of drug tested.

Antimalaria drugs such as artemisinin have demonstrated amebicidal activity (Cooke *et al,* 1987) and in this study we examined the amebicidal activity of chloroquine to *A. castellani.* One of the mechanisms of action of chloroquine is that it acts as a weak base. It accumulates in parasite lysosomes, resulting in their alkalinization (Homewood *et al,* 1972) which is toxic to the lysosome membrane (Ginsburg and Krugliak, 1988). Another well-known mechanism of action of chloroquine is that it intercalates with the parasite DNA (Meshnik, 1990). Chloroquine works well for malaria parasites but it is not effective against *A. castellani.* Schmidt and coworkers (1978) stated that chloroquine displayed a very poor activity to *Acanthamoeba.*

The findings are important because they show that *A. castellani* has a marked sensitivity to amphotericin B but is resistant to chloroquine. The other drug that has potential against this parasite is the antihelminthic, ivermectin.

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