In Vitro Susceptibility of Angiostrongylus cantonensis to Chemotherapeutic Compounds

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ABSTRACT

In vitro susceptibility of fifth-stage larvae of Angiostrongylus cantonensis to four different anthelmintic drugs was investigated. Larvae of Taiwan strain of A. cantonensis were collected from experimentally infected rats. They were washed, grouped, and cultivated in the incubation medium in the wells of a 24-well tissue culture plate. Four synthetic compounds commonly used against nematode parasites were compared to determine the susceptibility of these larvae to different concentrations of drugs. These are quinacrine dehydrochloride atabrine (atabrine), mebendazole, praziquantel, and diethylcarbamazine (DEC). Atabrine and DEC caused 50% inhibition (ID$_{50}$) in half an hour at a concentration of $10^{-8}$ mg/ml or more. Atabrine paralyzed the parasite completely four hour after incubation. Mebendazole and praziquantel had ID$_{50}$ in 4 hr at a concentration of 1 mg/ml. Compare to mebendazole, praziquantel showed even less larvicidal effect on A. cantonensis. The results obtained from this in vitro study may help in the preliminary screening for an adequate treatment of clinical cases.
INTRODUCTION

_Angiostrongylus cantonensis_ is the primary cause of eosinophilic meningitis and eosinophilic meningoencephalitis in man in Taiwan and in other Asian-Pacific regions (5,6,8,10). Adult worms of _A. cantonensis_ live in the pulmonary arteries of rodents. Human infections occurred when the third-stage larvae from the intermediate molluscan hosts are ingested by humans. These larvae migrate to the meninges, spinal cord, and brain, undergo two further stages of development during a 2 week period, and cause meningeal symptoms associated with eosinophilia of the cerebrospinal fluid and peripheral blood (3). In severe cases, death occurs between 2-4 weeks. In spite of the importance of the disease and its wide distribution, no satisfactory medical treatment for angiostrongyliasis has been developed so far. Corticosteroids have been given in combination with analgesics and the results were statistically insignificant from the controls (4). Much remains to be learned to bring a possible treatment of angiostrongyliasis under control. This investigation is designated to compared the effects of four chemotherapeutic compounds on the fifth-stage larvae of _A. cantonensis_ by the _in vitro_ microculture drug assay.

MATERIALS AND METHODS

Parasite

A long-term laboratory-reared strain of _Angiostrongylus cantonensis_, originally isolated from _Achatina fulica_ collected from Nei-hu, Taipei, was used in this investigation. It was maintained in this laboratory by cycling through the planorbid snails, _Biomphalaria glabrata_, and adult Sprague-Dowley rats. In this study, rats were experimentally infected by stomach intubation of 200 third-stage larvae. They were sacrificed 16 days after infection and fifth-stage larvae were collected carefully from brains. The parasites were washed and incubated in Earle solution (NaCl 8.8, KCl 0.4, MgSO₄·7H₂O 0.2, NaH₂PO₄·H₂O 0.4, NaHCO₃ 1.05 and glucose 1.0 g/l, pH 7.4-7.5) at 35°C for experimental use. The Earle
solution used was added with 100 unit/ml of dicillin and 100 ug/ml of streptomycin sulfate.

**Nematocide**

Four different nematocides were employed in the *in vitro* pharmacological test. These include quinacrine dehydrochloride atabrine (atabrine), mebendazole, praziquantel, and diethylcarbamazine (DEC). They were prepared in five different concentrations, 2, 2x10⁻², 2x10⁻⁴, 2x10⁻⁶, and 2x10⁻⁸ mg/ml, in normal saline.

**In vitro sensitivity test**

Parasite susceptibility to anthelmintic drugs was measured by the visualization method of Tomosky et al. (9) and Sono et al. (7) with minor modifications. Polystyrene microplate containing 24 flat-bottomed wells were used. Ten larvae in 1 ml of Earle solution was added into each well and incubated at 35°C for 30 min prior to the addition of 1 ml tested solution. The parasite were observed under a dissecting microscope 1/2, 1, 2, 4, 8, and 32 hr after incubation at 35°C. The criteria for estimating the effect of tested compound include the S type movement in the head, the scissor-like movement in body, and the rolling movement in tail. Only these larvae showed essentially normal in appearance and gross morphology and exhibited all three types of movement were scored as lively. Abnormal larvae exhibited only scissor-like movement occasionally in a period of 5 min were counted as dead.

**RESULTS**

The *in vitro* paralyzing effects of atabrine, mebendazole, praziquantel, and DEC on *Angiostrongylus cantonensis* larvae were plotted in Figures 1-4. The activity of fifth-stage larvae was depressed when the parasite was incubated in the presence of atabrine (Figure 1) or DEC (Figure 2). The time required to cause 50% inhibitory effect (*ID₅₀*) at 10⁻⁸ mg/ml or more was half hour. Mebendazole and praziquantel showed little effect on the motility over the same period of time (Figures 3 and 4). Subsequent observations indicated that the condition of
the organisms gradually deteriorated with time. Atabrine caused a complete paralysis 4 hr after incubation. Most larvae died 32 hr after incubation in the control group though they are quite normal when observed at the 8th hour. Mebendazole and praziquantel had ID$_{50}$ in 4 hr at a concentration of 1 mg/ml. Concentration of mebendazole which leads to the slow death of the helminths by inhibition of the glycogen uptake was not as critical as other drugs we tested. Compare to mebendazole, praziquantel was even less effective against the fifth-stage larvae of *A. cantonensis*.

Figure 1. Effects of atabrine on the fifth-stage larvae of *Angiostrongylus cantonensis*. 
Figure 2. Effects of diethylcarbamazine on the fifth-stage larvae of Angiostrongylus cantonensis.

Figure 3. Effects of mebendazole on the fifth-stage larvae of Angiostrongylus cantonensis.
DISCUSSION

The methodology we developed in this study is well suited for examining and screening anthelmintic activity of compounds in vitro. The presence of humoral and cellular immune factors, undetected drugs and variable quantities of nutrients in in vivo test system may have a pronounced effect on the test result. The use of in vitro system may avoid these influences. In this study, the ability of four anthelmintic compounds to kill *A. cantonensis* was assayed in in vitro system.

Atabrine is generally used against malaria and intestinal tapeworm infections. It inhibits enzymes which have flavin as prosthetic group or as coenzymes. In this study it showed apparently the highest paralyzing effect on the fifth-larvae of *A. cantonensis*. It should receive most attention in the in vivo pharmacological experiments.

Diethylcarbamazine (DEC) is the only compound for the practical treatment of human filariasis at present. It has a direct vermicidal effect against both adult and larval worms. It
is sometimes referred as microfilaricide. However, therapeutic concentration of DEC have no significant action on any kind of microfilariae. In this in vitro study, the fifth-stage larvae of *A. cantonensis* were paralyzed by DEC. The fourth moult into fifth-stage larvae in the life cycle of *A. cantonensis* is found in the brain of mammalian hosts 8-12 days after infection by third stage larvae. These larvae are recognized as young adults which undergo no further moult but migration in the host rats. Compare to microfilariae the fifth-stage larvae are more like adults biochemically in a nematode life cycle. The opinion is also supported by the results of this study.

Mebendazole is a synthetic compound usually used in the treatment of intestinal parasitic infections, especially ascariasis, enterobiasis, trichuriasis, and hookworm infections. The mode of action is by inhibition of the glucose uptake by the helminths, which result in a depletion of glycogen and adenosin triphosphate contents, necessary for parasite survival, which leads to the slow death of the worms. Hayashi et al. (1) reported in detail the effects of mebendazole against *A. cantonensis* in rats. According to their description, the drug was most effective in the third and fourth larval stages in the rat brain, while no wormicidal effect against the tissue-dwelling adults was observed. No explanation can be given for this difference, but it may be associated with differences in the structure and the function between stages. Since the nematocidal effect of mebendazole is due to its ability to inhibit the glucose uptake which causes the gradual depletion of energy, as we expected, the time required for the onset of the drug action was longer than other drugs we tested.

Praziquantel is acylated heterocyclic compound commonly used for the treatment of trematodiasis and cestodiasis. Though the intimate way of action is still under investigation, studies have shown that the compound acts on the carbohydrate metabolism of the parasites.
Results of this study suggested that it might have very little value for the treatment of angiostrongyliasis in vivo. It showed no wormicidal effect when compared with the control.

Among non-synthetic drugs, avermectin Bl a produced by actinomycetes has been studied to measure its in vivo (2) and in vitro (7) effects on the adult and larval stages of A. cantonensis. Although it showed both in vivo and in vitro paralyzing effects against the parasite, it was not the drug of choice in the treatment of angiostrongyliasis. Through an effect on amine-butyric acid, it inhibits the nervous system of the mammalian host as well as the nematode parasite.

This study provided a rapid visual observation method which made the examination of many subjects at the same time for a long period possible. The results obtained from this in vitro study may help in the preliminary screening for an adequate treatment of clinical cases. However, the toxicity and therapeutical efficacy of these drugs need further in vivo investigations.

ACKNOWLEDGEMENTS

This investigation was supported in part by the research grant NSC77-0412-B110-01 from the National Science Council, Republic of China.

REFERENCES


4. Punyagupta, S. 1979. Angios-


廣東住血線蟲對化學藥物之體外感受性

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摘 要

本研究由實驗感染的大白鼠收集台灣品系廣東住血線蟲之第五期幼蟲，並測定其在體外對四種常用驅蟲藥物的感受性。幼蟲清洗、分組後在24 種組織培養皿中浸於 Earle 培養液內。將 atabrine、mebendazole、praziquantel 及 DEC 等四種治療線蟲感染的合成藥物配置成不同濃度測定幼蟲對藥物的感受性，結果顯示在半小時內 1 mg/ml 的 atabrine 或 DEC 均可抑制蟲體運動。mebendazole 或 praziquantel 無法達到此效果，四小時後 atabrine 處理組所有幼蟲完全麻痹，而以 mebendazole 或 praziquantel 處理者則可達到半數以上（ID₅₀），其中 praziquantel 的效果比 mebendazole 又略差。本研究所得結果，在治療藥物的篩選工作上極有幫助。