

REVIEW

DRUG TRIALS FOR TREATMENT OF HUMAN ANGIOSTRONGYLIASIS

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SUMMARY

Abdominal and cerebral angiostrongyliasis are two important infections produced by metastrongylid worms, the former occurring in Central and South America and the later in Asia and Pacific Islands. Drug treatment is a challenge since the worms and its evolving larvae live or migrate inside vessels and efficient killing of the parasites may produce more severe lesions. Larvicidal effect of certain drugs appears to be more easily accomplished but this outcome is not useful in abdominal angiostrongyliasis since clinical manifestations appear to result from sexual maturation of the worms. We review the drug trials in murine experimental models and conclude that most of them could not be considered good candidates for treatment of human infection, except for PF1022A, pyrantel and flubendazole.

KEYWORDS: Eosinophilia; Angiostrongyliasis; Tissue-dwelling parasites; Anthelmintic drugs; Zoonosis.

INTRODUCTION

Human angiostrongyliasis has been widely reported from various parts of the world. Among the food-borne zoonotic diseases, *Angiostrongylus cantonensis*⁴, the causative agent of human eosinophilic meningoencephalitis¹, and *Angiostrongylus costaricensis*³², the causative agent of human abdominal angiostrongyliasis have such a medical importance that studies have been carried out in order to search for suitable drugs. The trials of treatment have been done both on adults and larvae stages.

It is known that drug efficacy is influenced by various factors including route, dosage and regimen of the treatment. In addition, in the treatment of diseases produced by tissue-dwelling parasites, allergenic components of killed worms may cause adverse effects on the host^{7,8,17}. Therefore, effective treatment will be established after these factors are taken into account through basic studies using experimental models.

CHEMOTHERAPY AFTER WORM MATURATION

Many studies have examined the effects of various neuropharmacological agonists and antagonists on the motility of nematohelminths, suggesting the existence of excitatory cholinergic and inhibitory gabergic mechanisms.

Avermectin B1a, one of the parasitic macrocyclic lactones produced by actinomycetes of *Streptomyces avermitilis*, may act on the nervous system of various nematodes as well as other animals including crustacea

and mammals, possibly through an effect on γ -amino-butyric acid (GABA). It was also suggested that it could paralyze *Angiostrongylus cantonensis*, through a neuropharmacological mechanism including GABA and acetylcholine^{24,35}.

SANO *et al.*³⁶ developed an *in vitro* method to study the effects of drugs on the motility of small helminths and observed the paralyzing effects of avermectin B1a on *Angiostrongylus cantonensis*, using $3 \cdot 10^{-18}$ mg. The drug caused a sustained inhibition in the motility, with a relaxation of the parasite. ISHII *et al.*¹³ extended the *in vitro* studies to *in vivo* experiments in rats. Avermectin B1a was given intraperitoneally (ip) six weeks after infection, and a sustained paralysis of worm by this drug was suggested from the following aspects: the change of the first larval stage count in feces; histological observations of lung tissues of the host and the reproductive system of female worms, and the motility of recovered worms *in vitro*. When the drug was given per os (po), three days after infection, a significant reduction in the recovery rate of worms was observed.

ISHII *et al.*¹⁴ administered ivermectin ip to rats infected with *Angiostrongylus cantonensis*. They observed a significant reduction in the recovery of adult worms when the drug was given at 2 mg/kg 0.5 to 72 h and four weeks after infection, compared to the non-treated, infected control group.

TERADA *et al.*^{49,50} searched the effects of some alkaloids on the motility of some parasitic helminths, isolated frog rectus and mouse ileum. They verified that tuberosamine (TS) at 6.7×10^{-6} approximately

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2×10^{-5}), an alkaloid from *Stemona japonica*, abolished the motility of *A. cantonensis*. With N-methylcystisine (N-MC) and matrine (Mat), alkaloids from *Sophora flavescens*, the motility of *A. cantonensis* musculature was affected spastically by N-Mc (1.2×10^{-6} , approximately 1.2×10^{-4} M), and paralytically by Mat (10^{-5}), approximately 10^{-4} M. From the results on interactions between these alkaloids and known neuropharmacological agents, it was suggested that the effects of both alkaloids are elicited through a neuropharmacological mechanism in parasitic helminths and host tissues.

TERADA *et al.*⁴⁰ examined the effects of various cholinergic agents on the motility of *A. cantonensis* to define their neuropharmacological properties. It was suggested that the excitatory cholinergic mechanism in this parasite was mediated through nicotinic receptors, and it was basically similar to that reported in *Ascaris suum*. The same group demonstrated spastic paralysis caused by pyrantel tartrate, another gabergic anthelmintic, through direct stimulation of the nicotinic cholinergic receptors in *A. cantonensis*⁴¹.

TERADA *et al.*⁴² compared the paralysis of *A. cantonensis* produced by avermectin B1a and ivermectin to that produced by phenylephrine (an alpha-adrenergic agonist) and strychnine (a cholinergic inhibitor) and concluded that a gabergic mechanism is involved in the paralyzing action of both macrocyclic lactones.

The reproductive functions of *A. cantonensis* are apparently affected by milbemycin D, another macrocyclic lactone derivative, through an indirect mode of action including paralysis and inhibition in food intake and energy and/or synthetic metabolism³⁸. The *in vitro* motility of females recovered from rats was inhibited, and almost all females and males were semitransparent colourless. Results obtained from sectioned worms showed little content in the digestive tract and uteri. In addition, there were few eggs and first-stage larvae in the lung tissues of treated rats. Inhibitory and stimulatory actions of milbemycin oxime on the motility of *A. cantonensis* and *Dirofilaria immitis* are probably mediated through gabergic and cholinergic mechanisms²⁶. TERADA & SANO⁴⁶ studied the effects of santonin on the motility of *A. cantonensis* and some other parasitic nematodes. Santonin paralyzed the worms at lower concentrations (10^{-9} – 10^{-7} M), whereas a slight stimulatory effect on the motility was also seen at its higher concentrations (10^{-6} – 10^{-4} M). The results suggested that santonin has inhibitory and stimulatory actions through stimulating gabergic and cholinergic mechanism in the parasite.

CUCKLER *et al.*⁵ conducted studies to determine whether thiabendazole, a potent and broad-spectrum anthelmintic, would affect *Angiostrongylus cantonensis*. They showed that this drug was highly effective in controlling the early invasive stages of the rat lungworm. Also depending upon the dosage, time, and duration of medication, the worms were killed, their migration to the lungs was delayed and the oviposition was prevented.

MORERA & BONTEMPO³³ tested diethylcarbamazine (DEC), thiabendazole and albendazole as treatment of cotton rats (*Sigmodon hispidus*) infected with *A. costaricensis*, 40 days after infection. Several trials were carried out, varying the dose and the timing of the treatment. They demonstrated that the parasites were actively moving instead of being killed and also that lesions were more severe after treatment.

TERADA & SANO⁴⁷ examined the effects of diethylcarbamazine (DEC) on the motility of adult *A. cantonensis* and *Dirofilaria immitis*. They suggested that the DEC inhibitory and stimulatory action was produced through the gabergic and cholinergic mechanisms in both adult parasites. Other neuropharmacological agents, including eserine, phenylephrine and dibenamine, did not have any effect on the motility of irradiated and non-irradiated *Angiostrongylus cantonensis* adult females¹⁵.

TERADA *et al.*⁴⁵ compared *in vitro* effects of 19 anthelmintics on the motility of *A. costaricensis* and *A. cantonensis*. Phenolic compounds (hexylresorcinol, bithionol and niclosamide) and levamisole were all effective on the motility of *A. costaricensis*, but other derivatives having piperazine (diethylcarbamazine and piperazine), lactone (santonin, avermectin B1a and ivermectin) and benzimidazole (mebendazole and thiabendazole) had no efficacy at all.

TERADA *et al.*⁵¹ did some preliminary trials on treatment after worm maturation in abdominal angiostrongyliasis in mice, using mebendazole, one of the effective antilarval drugs against nematodes, including *A. costaricensis*. No definitive conclusion about killing efficacy was made and several topics were suggested to be addressed in forthcoming studies, like the influence of immune status of the host on the efficacy of the drug.

Mebendazole at 10 mg/kg nd with four intermittent doses of 5 mg/kg given weekly led to a lowest worm recovery of *A. costaricensis*, in groups treated with four daily successive doses, suggesting involvement of different killing mechanisms. Otherwise, since the drug is not highly effective to kill the worms, the inhibition of egg formation and/or oviposition would be more desirable for treating abdominal angiostrongyliasis after worm maturation⁵⁴.

KACHI *et al.*¹⁸ examined the effects of PF1022A, a cyclodepsipeptide isolated from a mycelial cake of *Mycelia sterilia*, on adult *Angiostrongylus cantonensis* in the pulmonary arteries and larvae migrating into the central nervous systems of rats, although the compound does not pass the blood-brain barrier. The treatment killed especially female worms. This gender selective effect would be attributable to non-neuropharmacological mechanisms²⁰.

CHEMOTHERAPY BEFORE WORM MATURATION

Considering the migration paths of developing larvae of *Angiostrongylus cantonensis* in vertebrates and the probable association of worm maturation and pathogenic changes caused by *Angiostrongylus costaricensis*, it is important to test the efficacy of drugs on the early stages of infection, with both nematodes.

HAYASHI *et al.*⁹ examined the effects of mebendazole against *A. cantonensis* in rats. The drug was more effective in the larval stage (3rd and 4th stages) than in the adult stage. Reduction rates higher than 90% were observed in groups treated with ≥ 3 mg/kg, that was similar to its clinical dosis (3 -10 mg/kg) for human intestinal nematodes. When mebendazole suspended in propylene glycol was given orally or intraperitoneally, no difference in reduction rate was seen between two routes.

MAKI & YANAGISAWA²⁸ compared the effects of flubendazole and thiabendazole on the larvae of *Angiostrongylus cantonensis* and other helminths. Thiabendazole had no larvicidal effect on *A. cantonensis* at

the doses of 10 mg/kg/day for six consecutive days. On the other hand, no larvae were found in the brain of the mice treated with flubendazole at 5 mg/kg/day for six days. The same high efficacy of flubendazole (50 mg/kg po) was seen in mice with different worm burdens (50, 250 or 500 larvae of *A. cantonensis*) with almost complete larvicidal effect, irrespective of the worm burden. The results also suggested that worm density had an effect on the fecundity of this parasite²⁹. This effect may be due to fibrous changes in host lung tissues and not to changes in the physiology of the worms. The density-dependent effects on fecundity play a role in the population dynamics of both the parasite and host as regulatory mechanisms in the field.

MAKI & YANAGISAWA³⁰ gave flubendazole and mebendazole po at 10 mg/kg/day 5-7 days post-infection (total 30 mg/kg), resulting in 93-100% reduction of *A. cantonensis* larvae in mice and rats. No significant difference was observed between the effects of the two drugs. It was possible to treat *A. cantonensis* adults in rats by administering flubendazole or mebendazole at 10 mg/kg/day for 10 consecutive days. The drugs exhibit better anthelmintic efficacy in a divided than in a single dosis regimen.

ISHIH *et al.*¹⁶ obtained significant inhibition on egg development of *Angiostrongylus costaricensis* *in vitro* caused by pyrantel ($10^{(-9)} - 10^{(-80)}$ g/ml) and levamisole ($10^{(-9)} - 10^{(-8)}$ g/ml). None of the eggs developed to first-stage larvae in higher concentrations of these anthelmintics ($10^{(-7)}$ g/ml). Furthermore, incubation with these drugs at $10^{(-8)}$ g/ml for at least 3 h or at $10^{(-4)}$ g/ml for 1 h caused irreversible effects on egg development.

MAKI & YANAGISAWA³¹ determined the effect of flubendazole po administered at 10 mg/kg/day for five consecutive days (the 11th; 20th or 40th post-infection) on the number of first-stage larvae of *Angiostrongylus cantonensis*, released in the faeces of rats. Faecal examination for five months showed that L1 release ceased one week after conclusion of treatment and resumed one to two months later in 86% of the rats.

In order to study the sensitivity of the developing larvae and adult of *A. cantonensis*, flubendazole and mebendazole were administered at 10 mg/kg to rats 3 and 10 days post-infection and to those harbouring the adult worms after 70 days post-infection. Almost all of the larvae were eliminated from the rats medicated 3 days post-infection. The larvicidal effects of the drugs administered 10 days post-infection were not so high. On the other hand, when the drugs were administered 70 days post-infection, no effects were seen on the number, body size and weight of recovered worm and the release of L1, concluding that the developing larvae were more sensitive to the drugs²⁷.

KANDA & MAKI²³ observed the *in vitro* egg release of *A. cantonensis* from rats treated with flubendazole and demonstrated that the drug, even at the single dose as low as 10 mg/kg, affected the physiology of adult worms in rats. No direct evidence was obtained on effects of flubendazole po administered to rats on eggs and the first-stage larvae. The authors hypothesized that flubendazole might affect the formation of fertilized eggs from early embrionated stages and/or the release of the eggs from the worms immediately after the conclusion of the treatment, leading to the reduction in the number of the L1. Testing these hypothesis is worthy of further investigations.

KAMATH *et al.*²² observed the larvicidal effect of perbendazole given po and subcutaneously to mice infected with *A. cantonensis* at different stages of infection. The subcutaneous route of administration was more effective than the oral one. They also showed the complete larvicidal effect of the drug at early stages of infection.

KAMATH *et al.*²¹ verified that the combined therapy of levamisole and flubendazole was 100% efficient against *A. cantonensis* infection in mice. The drugs were evaluated on the 15th day post infection. Both drugs given prior to experimental infection showed no prophylactic activity.

The larvicidal effect of albendazole was studied in the experimental infection with *Angiostrongylus cantonensis* in mice treated with dosages of 5, 10 and 25 mg/kg/day beginning 5, 10 or 15 days post-infection, respectively, for 7, 14 or 21 consecutive days. It was effective when given within 15 days post-infection¹⁰.

Levamisole was another anthelmintic tested against larval stages of *A. cantonensis* in rats and *A. costaricensis* in mice and the results suggested that the drug had conspicuous *in vivo* effects against larval stages of *A. costaricensis* as well as *A. cantonensis*¹². ISHII¹¹ also verified the effects of levamisole on the first-stage larva of *A. cantonensis* and suggested that levamisole affected the L1 output through a direct paralyzing action on the worm and an indirect pathway through inhibition of energy metabolism.

ISHII *et al.*¹⁴ administered ivermectin intraperitoneally to rats infected with *Angiostrongylus cantonensis*. When the drug was given at 2.0 mg/kg after 3 weeks post infection, a significant inhibition of the first larval stage output in rat faeces was observed.

TERADA *et al.*³⁹ examined the anti-larval effects of milbemycin D on *A. cantonensis* in rats and mice and on *A. costaricensis* in mice. Compared with non-treated control group, significant reductions in the first stage larval counts in feces (LPG/female), number of recovered worms, host lung-body weight ratio, body weight, and mortality were seen in the groups receiving 10 successive daily doses of 5 mg/kg, both in rats and mice. Some effect was also seen with five or 20 successive doses. From these results, it was suggested that milbemycin D had conspicuous *in vivo* effects against larval stages in both nematodes.

PF1022A killed developing larvae of *A. costaricensis* after five successive doses and it was well absorbed either po or by parenteral administration⁴³. This larvicidal activity was observed with any of the four forms of PF1022A, although forms alpha and III were more efficient against tissue-dwelling nematodes than the form I and form II when given po²⁰.

CHEMOTHERAPY AND IMMUNE STATUS OF THE HOST

Great interest has been recently directed towards advances in understanding the interactions between chemotherapeutic actions of anti-parasitic drugs and immune status of the host. Most of the studies using such approach have been carried out on anti-protozoan and anti-trematode chemotherapy.

Immunosuppression may reduce the efficacy of treatment in experimental hosts infected with several parasitosis, including

schistosomiasis, malaria, trypanosomiasis, filariasis⁶ and strongyloidiasis³⁷. The enhancement of drug efficacy through immunopotentiality was also demonstrated for some diseases caused also by tissue parasites like schistosomiasis²⁵ and visceral leishmaniasis². However, individual variability of the host's immune response prevents a clear control for demonstration of the drug effects.

The fungal metabolite cyclosporin A (CsA) has been used as a tool of investigating cellular immune mechanisms in various parasitic infections⁵². YOSHIMURA *et al.*⁵⁵ tried to determine the possible effects of cyclosporin A (CsA) on *Angiostrongylus cantonensis* infection and eosinophilia in mice. The data indicated a direct damaging activity of CsA against certain developmental stages of the parasite.

TERADA & SANO⁴⁸ did trials to assess protective resistance against reinfection with *Angiostrongylus costaricensis* in mice whose primary infection was treated with milbemycin D, a larvicidal anthelmintic. The protection was more remarkable in three situations: 1) when the larvicidal treatment was done later; 2) with high inocula at the primary infection; 3) when the infection and larvicidal treatment was repeated. The protective resistance lasted at least six months after primary infection.

TUNGTRONGCHITR *et al.*⁵³ investigated the relation between immunopotentiality and efficacy of mebendazole in sensitized mice infected with adult *A. costaricensis*. A significant decrease in the establishment of infection and in worm growth was probably the result of development of a protective humoral immunity in the sensitized mice.

CONCLUSION

Although the many experimental studies on drug treatment of *Angiostrongylus* sp. infections in mice and rats, many aspects are still open to investigation before studies on human infection are carried out. Several compounds have a significant larvicidal effect, what may be specially useful in treating cerebral angiostrongyliasis. Otherwise, in abdominal angiostrongyliasis, the most striking clinical manifestations occur after worm maturation and there is also the concern about the possibility of extended lesions secondary to parasite's death inside blood vessels. Longitudinal seroepidemiological studies are urgently required to further clarify the natural history of human infection in both cerebral and abdominal angiostrongyliasis. This knowledge is critical for future phase II and III studies with candidate drugs.

RESUMO

Ensaio com drogas para o tratamento da angiostrongilíase humana

Angiostrongilíase abdominal e cerebral são duas infecções importantes produzidas por metastrongilídeos, a primeira delas ocorrendo nas Américas Central e do Sul e a segunda, na Ásia e Ilhas do Pacífico. O tratamento é complicado pelo fato das larvas e vermes adultos viverem e migrarem dentro dos vasos sanguíneos, sendo que as lesões podem se agravar com a morte desses parasitos. Os efeitos larvicidas de determinadas drogas parecem ser mais eficazes, porém não são úteis no tratamento em angiostrongilíase abdominal, onde as manifestações clínicas podem resultar da maturidade sexual dos vermes. Este estudo faz uma revisão das drogas utilizadas para o tratamento de ambas as

parasitoses, em modelos experimentais murinos, realizados antes e depois da maturação dos vermes. A maioria das drogas testadas não pode ainda ser considerada candidata a ensaios de tratamento em infecções humanas, com exceção do PF1022A, pirantel e flubendazol.

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