Flubendazole: a candidate macrofilaricide for lymphatic filariasis and onchocerciasis field programs

Expert Rev. Anti Infect. Ther. 9(5), 497–501 (2011)

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"Although flubendazole faces … important challenges with regard to safety and formulation, the potential benefits that could result relatively quickly from a safe, usable formulation of flubendazole make this a top priority for the filarial world today."

A safe, field-usable chemotherapeutic agent that will rapidly kill adult filarial worms is urgently needed in tropical medicine. Ivermectin, distributed as Mectizan® by Merck & Co. Inc., has had an enormous impact on two major human filarial infections of developing countries, onchocerciasis and lymphatic filariasis [1]. However, this agent works primarily against the microfilarial stage and lacks the ability to rapidly kill the adult parasites. Since the adult worms can survive for many years producing offspring, it has been necessary for control programs to continue drug distribution for more than a decade, for instance, until the adult worms eventually die; a labor-intensive and expensive proposition. Other agents used in filarial control programs, such as diethylcarbamazine and albendazole, may be more effective macrofilaricides than ivermectin, but for various reasons are not suitable, or are unable, to fill the role of a being rapidly acting macrofilaricide. Thus, a drug administered once, or at least in multiple doses over a very short period, that safely kills adult filarial worms would be a major contributor to the current efforts to rid the world of filarial infections and the diseases they cause. A useful field agent has typically been required to be administered in an oral dosage form, but a truly safe agent administered by another route, including parenteral approaches, could be acceptable and may even be advantageous.

Given the challenges of discovery and development of agents for human use, a drug as described previously is arguably most likely, at least at present, to come from the benzimidazole group of anthelmintics. Although several benzimidazoles are currently employed in human chemotherapy, there are other potential candidate macrofilaricides in other drug classes. However, time is of the essence in finding a new drug for use in ongoing filarial control programs, and the first priority is to consider the benzimidazoles as the most likely source of a macrofilaricide. This group has provided many important effective agents for both veterinary and human medicine over the past 50 years, beginning with thiabendazole and now most prominently including albendazole and mebendazole for human parasites and a whole range of agents in veterinary medicine [2]. Benzimidazoles work by interfering with the equilibrium among tubulin subunits, tubulin and microtubules. Not surprisingly, benzimidazoles can affect host tubulin as well as that of the parasites, are typically positive in mammalian cell cytotoxicity assays and cause chromosomal nondisjunction during mitosis [3]. However, the benzimidazole anthelmintics show a differential preference for binding to nematode tubulin compared with mammalian tubulin [4], an important factor for the development of a drug against nematodes in mammals. Benzimidazoles

Keywords: filariasis • global control programs • macrofilaricides • onchocerciasis

Expert Reviews

 10.1586/ERI.11.30 ISSN 1478-7210

are also antifungal agents as well as anthelmintics, a fact that may be important in filarial conditions such as elephantiasis that involves secondary infections often involving fungi; albendazole is one of the two drugs used in the global lymphatic filariasis elimination program.

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We believe that the most appealing benzimidazole with regard to filarial parasites is flubendazole, as it is highly active against filariae in a number of hosts. It has the typical benzimidazole structure but with an added fluorine as the major structural difference from other benzimidazoles. It is a very efficacious macrofilaricide in a variety of experimental animals, with perhaps its most dramatic and relevant action being its ability to completely eliminate adult *Dirofilaria immitis* from dogs after a single injection [McCall JW, Pers. Comm.]. Flubendazole was developed by Janssen in the mid-1970s and is currently licensed in Europe for use as an anthelmintic in humans for intestinal nematodes (5 mg/kg for 3 days). Flubendazole is a potent and efficacious anthelmintic for gastrointestinal nematode infections in swine, poultry and domestic animals, as well as against lungworms in swine. It is usually administered over 3 days at approximately 5 mg/kg, but is probably also efficacious even as a single dose at this same rate [5]. In a number of experimental filarial rodent models, flubendazole was found to have essentially 100% efficacy as a macrofilaricide at reasonable doses and schedules. A trial in human onchocerciasis was also carried out in Mexico in the early 1980s [6] with promising results. However, wider testing in humans was restricted at that time by problems associated with the route of administration and the relatively unsophisticated carrier agent used at that time, some 39 years ago. In addition, the introduction of ivermectin at this time lessened the urgency to replace diethylcarbamazine for onchocerciasis control with a new macrofilaricide.

As noted, flubendazole is highly efficacious in various experimental filariasis models, including the feline *Brugia pahangi* model, a host in which it occurs naturally. Efficacy varies with parasite species, location in the host and host species (Table 1). It should be noted that flubendazole is highly efficacious and potent as a macrofilaricide in these models only when given parenterally (in keeping with its very low oral bioavailability in standard formulations). Given parenterally, flubendazole is arguably the best macrofilaricide tested in animal models. Importantly, no adverse reactions were reported in any of these animal studies. An important observation, relevant to current problems faced by the global control and elimination programs for human lymphatic filariasis and onchocerciasis, is that in cats and jirds infected with *Brugia* spp. [7,8], flubendazole is active against adult worms but poorly active against the microfilarial stage. The significance of this observation lies in the fact that a major problem for filarial control programs using ivermectin is that individuals coinfected with high levels of circulating *Loa loa* microfilariae may suffer severe adverse events. Over 124 people have died in the past 10 years, usually with signs and symptoms of CNS pathology [9] related to microfilarial death. An agent that will kill adult filariae but not microfilariae may be a breakthrough for this important practical problem, which currently limits ivermectin distribution programs in many African countries.

Following the encouraging findings in rodent models, a study was carried out in Mexico in the early 1980s in which several potential macrofilaricides, including flubendazole, were tested in humans infected with *Onchocerca volvulus* [6]. This study was terminated early due to problems associated with reactions at the intramuscular injection site where the flubendazole, in its oil-based carrier, was administered. Nevertheless, efficacy data on adult *O. volvulus* worms in surgically removed nodules from these patients suggested that flubendazole is a potent macrofilaricide. At 3 weeks after initiation of treatment (750 mg once per week for 5 weeks), significant degeneration of the adult worms was detected [6]; at 5 weeks (Table 2), there was very effective destruction of the adult worms compared with the other antifilarial agents [Mackenzie CD, Martinez-Palomo A. Unpublished Data].

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Flubendazole is currently registered for human use in Europe for treatment of gut-residing nematodes, an action that does not require efficient uptake into the host's circulation. A challenge for ensuring its suitability for filariasis will be to develop a new formulation that will produce blood and tissue levels of flubendazole sufficient to destroy tissue-resident parasites, such as the filariae. Efficacy against filariae was not observed following oral dosing of flubendazole in any of the early animal studies, but it should be noted that none of these studies used any of the new formulation methods now common in the pharmaceutical industry. Encouraging results come from Lanusse's group [10], which showed that the tissue-residing stage of the cestode *Echinococcus granulosus* can be killed by orally administered flubendazole formulated with the now commonly used excipient, hydroxypropyl-β-cyclodextrin [11]. Newer formulations such as this could greatly enhance the likelihood of developing flubendazole as a suitable macrofilaricide for human filariasis for oral dosing. A hydroxypropyl-β-cyclodextrin formulation might indeed be suitable given the increased degree of bioavailability it provides; for example, it markedly enhances the bioavailability of albendazole [12], mebendazole [13] and flubendazole [10]. This material is approvable and is a gold-standard reagent for enhancing bioavailability of lipophilic drugs and can be used in both liquid and solid dosage forms.

As the target (infective adult filariae) is complex and biochemically resourceful (many nematodes have the ability to

switch biochemical pathways when stressed), it is likely that a relatively long duration of exposure to the drug will be needed. This may involve the need for dosing on multiple (e.g., 3–5) days to maintain lethal levels of the agent for the required period of time. For many nematodes, acute exposure to benzimidazoles has few noticeable effects, even at very high concentrations; this is true for flubendazole in various adult filariids [14,15]. As the drug acts by disrupting the tubulin–microtubule equilibrium in cells, leading to cessation of nutrient transport and eventual cell death, these effects take time to become evident. *In vitro* experiments have shown that flubendazole concentrations as low as 100 ng/ml (incubated for 32 h) disrupt tissue structure in parasitic nematodes in the same clade as filariae [16].

In addition to pharmacodynamic challenges, there are other hurdles to developing a safe and effective formulation of a drug for the treatment of complicated infections, such as lymphatic filariasis and onchocerciasis. A primary concern with the benzimidazoles is safety. As these drugs interfere with microtubules, they have the potential to interfer with host cells, especially during cell division. Thus, the use of drugs such as albendazole is generally contraindicated for pregnant women; this is likely to apply with a new flubendazole formulation that provides for systemic exposure. However, it should be noted that albendazole has been used very successfully in mass drug programs across the world since 1999 and that inadvertent treatment studies in pregnant women have not detected adverse effects on the unborn child [17]. Nevertheless, a major hurdle for a formulation that produces enhanced bioavailable flubendazole will need to be carefully evaluated for embryotoxicity. It may turn out that flubendazole is only useful for filarial infections in males and females outside childbearing age. However, such a product would still be a useful advance for control programs.

What will it take to determine if flubendazole is an important answer to the needs of filarial control and elimination programs? Scientifically, it will initially require the determination of the blood and tissue levels needed for macrofilaricidal efficacy; closely related is the need to determine the levels that induce toxicity. Both issues are central to moving forward with the development of flubendazole. Based on recent experimental data from animal models, it is highly likely that current modern formulation techniques, including micronization, hydroxypropyl-β-cyclodextrin complexing or another new approach, will be able to provide the blood levels needed to kill adult worms. The testing of newly developed formulations for efficacy against filariae itself poses some challenges. Filarial infections are generally host specific and thus each filariae–host model is, to some degree, unique in form and properties. Flubendazole in a new formulation should be evaluated in a range of filarial models to encompass all the variations and characteristics of these infections and to make predictions about the pharmacokinetic parameters likely to be required for efficacy in human infections; this would allow formulations to be evaluated on the basis of pharmacokinetic data rather than efficacy *per se*, which requires extended periods of time post-treatment. A combination of many disciplines and institutions will be needed, including, as with the pioneering onchocerciasis ivermectin control program, 'public–private partnerships' between the pharmaceutical industry, nongovernmental organizations and academic scientists. Drug companies have the expertise needed to develop new formulations and are central to the final production phase needed; field-based expertise (Ministries of Health, nongovernmental development organizations and academics) are essential for developing a practical field-based mass drug administration intervention and will be important partners in any successful effort.

The benefit of developing a safe and practical agent that needs distribution only once, or perhaps twice, is substantial when compared with what is currently in place, for instance, annual distribution for 8–12 years in filarial control and elimination programs; a highly effective macrofilaricide would still be important even if a three-to-five daily course of treatment is needed. Significant saving in the financial and human costs of distributing drugs would be realized.

Table 1. Summary of lowest effective dose of flubendazole in filarial animal models.

Parasite	LED_{90} × 5 (mg/kg)	LED_{90} × 1 (mg/kg)	Ref.
Jird			
Brugia pahangi†	1.5	25	[7]
B. pahangi†	1.56	ND	[18]
B. pahangi†	2.5	ND	[19]
B. pahangi‡	12.5	ND	[19]
B. pahangi†	20¶	ND	[20]
B. pahangi§	10¶	ND	[21]
Dipetalonema viteae	100¶	ND	[22]
Acanthocheilonema viteae	1.56	ND	[18]
Rat			
Brugia pahangi‡	25	ND	[23]
B. malayi	12.5	50	[23]
A. viteae	3.1	1.6	[23]
Litomosoides carinii#	12.5	12.5	[23]
Mouse			
Onchocerca lienalis††	100	ND	[24]
Cat			
B. pahangi‡	ND	100	[7]

†Adult parasites in the peritoneal cavity.
‡Adult parasites in the lymphatics.
§L3 larvae.
¶Not titrated, only dose reported.
#Multimammate rat.
††Microfilariae transplanted into the skin.
Most of the efficacies reported at these doses in these studies were 100%. Efficacy determinations are dependent on the time of necropsy; efficacy is higher (i.e., number of worms observed) in jirds necropsied 8 weeks post-treatment compared with 6 weeks post-treatment [McCall Pers. Comm.].
LED_{50}: Lowest dose that was at least 90% effective.

Table 2. Effect of flubendazole and diethylcarbamazine on adult *Onchocerca volulus* isolated from human nodules.

Status of parasites	2 months post-Rx		3 months post-Rx	
	DEC†	*FLUB*†	*DEC*	*FLUB*
Degenerated adults	12	10	12	27
Intact adult worm	44	11	16	0
Females with empty uteri	6	1	5	0
Females with only oocytes	8	6	14	0
Reduction in dermal microfilariae‡	Yes	No	Yes	No

†DEC (100 mg) was administered twice daily for 14 days and 750 mg FLUB was injected intramuscularly once a week for five doses.
‡There was no significant ocular or skin pathology related to microfilarial death in those receiving FLUB. The only significant post-FLUB treatment reactions were associated with inflammation at the injection site. Dermal microfilarial loads stayed at pretreatment levels in the FLUB-treated individuals for approximately 6 months.
DEC: Diethylcarbamazine; FLUB: Flubendazole; Post-RX: After last treatment with flubendazole.
Data taken from [6] and [Mackenzie CD, Martinez-Palomo A. Unpublished Data].

Flubendazole has great potential as a macrofilaricide. Its reformulation using modern pharmaceutical platforms should be expedited to enable efficacy testing as soon as possible. Although flubendazole faces, as does any new anthelmintic, important challenges with regard to safety and formulation, the potential benefits that could result relatively quickly from a safe, usable formulation of flubendazole make this a top priority for the filarial world today.

Five-year view

In 2016, it is quite possible, should flubendazole be reformulated successfully into an easily administered and safe agent, that national lymphatic filariasis and onchocerciasis control and elimination programs will be dramatically shortened and medical staff freed for other important medical challenges.

Financial & competing interests disclosure

Charles D Mackenzie is supported in part by a grant from the Bill and Melinda Gates Foundation (Seattle, WA, USA). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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