FLUBENDAZOLE, A POTENTIAL USEFUL CHEMOTHERAPEUTIC AGENT FOR TREATMENT AND CONTROL OF FILARIAL INFECTIONS

Mackenzie CD, Specht S, Geary T, O'Neill M, Marsh K, Agnew D, Ceballos L, Lanusse C, Hoerauf A, Geary J, Wanji, S, McCall J, Behan A, Ames K, Scandale I, Silber S. (MSU, Bonn MH, McGill U, AbbVie, UBA-Tandil, REFOTDE, TRS, DNDi, & J&J/JRD)

Abstract

The search for a macrofilaricide that can enhance the goal of elimination of filarial infections, and the diseases they cause, is a current and relevant goal. The most attractive approach to macrofilaricide discovery is to re-purpose agents already in use in humans. High amongst these potential candidates is flubendazole, a drug that was shown in a screening survey in the early 1980s to be highly effective against *Onchocerca volvulus* in humans but was difficult to administer due to the properties of the formulation used. The need to reformulate this agent to overcome the issue of insolubility and make it suitable for use as an oral administration was a goal regarded as important for field program use of this anthelmintic in filariasis. We discuss here results obtained with a new formulation of flubendazole that achieves, after single oral doses of 2-6 mg/kg, a plasma concentration of between 1-3 µg/ml with a peak plasma level at 3-5 hr which decreases to control levels by 12-20 hr in rodents. Using the *Litomosoides sigmodontis* model in gerbils, we achieved >90% killing of adult worms when the animals were assessed 9 weeks after treatment with 6 mg/kg of the oral formulation of flubendazole given daily for 5 days. In vitro incubation of *Brugia* spp. with pharmacologically-relevant concentrations of flubendazole caused alterations in the internal organs of the adult worms. Histological observations in both filarial species exposed to flubendazole in vitro and in vivo showed that several structures in the worms were affected by the drug; the early developing forms in the uterus were the most affected component, and hypodermal cells were also significantly damaged when worms were incubated at concentrations of flubendazole equivalent to the plasma levels obtained after oral dosing. The beneficial effect of treatment with oral flubendazole on host tissue pathology induced by the presence of the parasite was marked when comparing untreated animals to flubendazole-treated animals 9 weeks after dosing. The degree of tissue and organ change was scored subjectively, and the histopathology occurring examined and scored. The very significant reduction in pathology in animals treated with 6 mg/kg flubendazole suggests that the death of adult worms induced by this drug is not likely to invoke severe adverse events in the host. Our study shows that oral flubendazole can safely kill the majority of adult filariae in this experimental model.

Background

Chemotherapy remains the main approach to treatment, control, and elimination of filarial infections aided where suitable by adjunct activities such as vector control and enhanced program management. The current needs in this area of tropical medicine are specifically aimed at three types of filariae:

1. Onchocerciasis - An infection targeted for elimination. We have anti-microfilarial agents (ivermectin, possibly moxidectin) but no macrofilaricidal agent that is optimally suitable for field distribution to large numbers of people.
2. Lymphatic filariasis - An infection also targeted for elimination. We have albendazole, ivermectin and diethylcarbamazine, which are effective, but need chemotherapeutic options that could significantly reduce program duration.
3. Loiasis - An infection co-endemic in some areas with the other infections. Although not targeted for elimination, it can cause severe adverse reactions, including death, in people being treated with anti-microfilarial drugs.

THE OPTIMAL DRUG TARGET

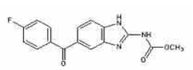
The central pathogenic entity in onchocerciasis is the microfilarial stage; adults are most important as sources of this L1 stage. The ultimate aim of chemotherapy of onchocerciasis is to permanently reduce the number and prevent the accumulation or production of microfilariae.

The central pathogenic entity is microfilariae, their destruction and the pathological responses associated with this parasite stage.
The importance of microfilariae can be summarized as follows:
1. It is the stage that is transmitted to the vector (and thus central to the breaking of transmission and elimination)
2. It is the stage that causes ocular and dermal damage in onchocerciasis
3. It is the stage that causes the pathology in Post-treatment *Loa* encephalopathy syndrome.

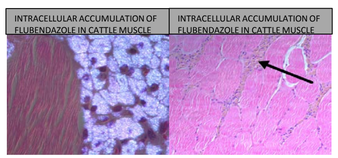
Strong preliminary efficacy data obtained during a screening study of benzimidazoles in the late 1970s led to the identification of flubendazole as been a prime candidate for repurposing as a macrofilaricide that could greatly enhance efforts to achieve disease elimination for both onchocerciasis and lymphatic filariasis . However, efficacy was only achievable by parenteral dosing, as commercial formulations provided very low oral bioavailability (Mackenzie & Geary, 2011).

Flubendazole

Flubendazole is a benzimidazole developed in 1971 as an analogue of mebendazole; it has a molecular weight of 313.28, and is composed of C 61.34%, H 3.86%, F 6.06%, N 13.41%, O 15.32%.

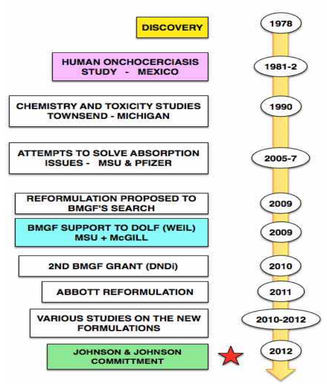


The compound is comparatively insoluble and this has led to difficulties in the past in developing oral formulations. Used as a depot (intramuscular or subcutaneous injection) in experimental models, it is the "gold standard" positive control for studies in animal models of filariases.



Similar depot effects occur in other animals. Here, following i.m. administration to a cow, flubendazole is present in macrophages lying between the muscle sheaths without causing major pathological changes in the associated muscle tissue (2 months after injection)

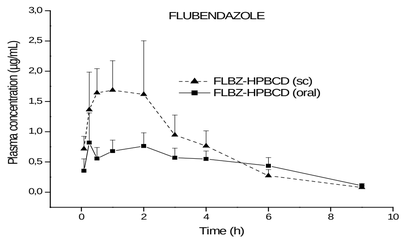
The History of Flubendazole in Human Filariasis



★ 2014 - JRF (J&J) produce several new formulations that are suitable for oral administration

The Pharmacodynamics of Different Flubendazole Preparations

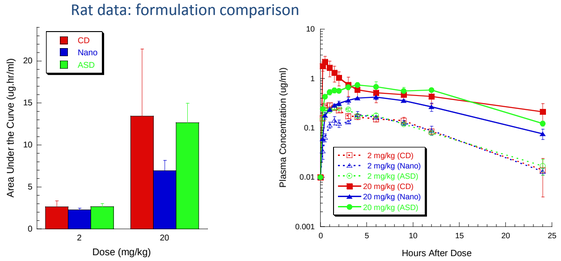
A



Jird plasma levels of flubendazole after oral and subcutaneous formulation

Rat data: formulation comparison

B



Formulation	2 mg/kg $t_{1/2}$*	C_{max}	T_{max}	AUC	20 mg/kg $t_{1/2}$	C_{max}	T_{max}	AUC
CD (Cyclodextrin Solution)	3.5	0.29 *(0.04)*	0.8	2.63 *(0.71)*	-,-	2.19 *(1.07)*	0.5	13.44 *(7.97)*
CD (Cyclodextrin Solution) (n=2)					-,-	1.58 *(0.30)*	0.5	8.87 *(1.18)*
Nano (Nanosuspension)	4.4	0.19 *(0.05)*	4.7	2.28 *(0.19)*	7.0	0.46 *(0.04)*	5.0	6.94 *(1.22)*
ASD (Amorphous Solid Dispersion)	4.5	0.29 *(0.02)*	1.7	2.66 *(0.36)*	6.5	0.78 *(0.23)*	4.7	12.65 *(2.31)*

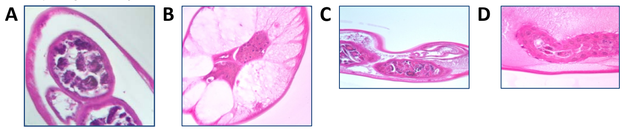
Data provided as Mean (*SD; n=3*); º harmonic mean ; t1/2 [hr]; Cmax [µg/mL]; Tmax [hr]; AUC [µg●hr/mL];

All formulations administered using a 10 ml/kg dose volume in non-fasted rats

In Vitro Studies

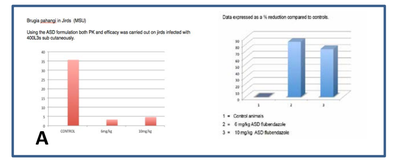
Flubendazole, a tubulin binding agent, was shown to damage various organs and cells within filariae by Franz et al. in 1990. Damage was seen in three locations. The primary damage seen at any concentrations was in the uterus and particularly in fertilized oocytes and early morulae stages, but also was seen at higher concentrations in the dysmorphic sausage forms of developing microfilariae.

Morphological Changes in adult female worms

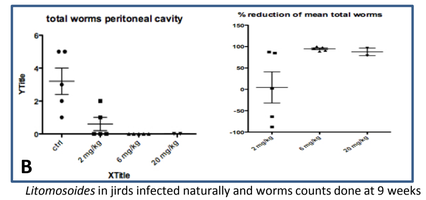


A. 10 ug/ml flubendazole in vitro showing embryo damage. **B.** Damage to adult female body wall. **C.** Degenerating developing microfilarial forms in utero **D.** Damaged uterine wall .

In Vivo Studies



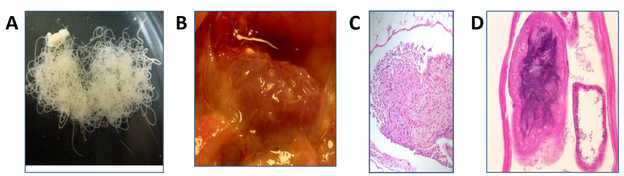
Brugia pahangi in jirds. Worm counts at 8 weeks post-treatment



Litomosoides in jirds infected naturally and worms counts done at 9 weeks post treatment.

Host Responses After Flubendazole Treatment

Tissue responses 9 weeks after treatment are considerably reduced compared to control animals.



A. The mass of worms removed from control animals at 9 weeks. **B.** The presence of worms and granulomas in the pleural cavity of control animals. **C**. A microfilaria-containing granuloma from the peritoneal cavity of *Brugia* -infected jirds 8 weeks after treatment with flubendazole. **D.** Uterine pathology in a female worm removed from the peritoneal cavity of a flubendazole-treated jird. **E.** Pathological score based on degree of tissue pathology visible macroscopically. Destruction of the adult worms does not cause adverse reactions, and markedly reduces the overall pathological status of the infection.

Major Conclusions

1. Flubendazole can be formulated to produce plasma levels of 1-2 ug/ml following oral dosing (2-6 mg/kg).
2. Five daily doses of oral flubendazole at 6 mg/kg reduce *Brugia* and *Litomosoides* adult parasites in jirds by >90% compared to control animals assessed 9 weeks after treatment
3. Safety studies with new formulations need to be completed. However, studies so far show similarities between flubendazole and other benzimidazoles used in humans, e.g., albendazole.
4. Approval for human use is being actively pursued with the goal of first-in-human studies in the next 1-2 years.

Associated References

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