Antifilarial activity of oral CGP 6140 against subperiodic *Brugia malayi* in the leaf-monkey (*Presbytis cristata*)

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Abstract

CGP 6140, the methyl-piperazine derivative of amoscanate was tested for its filaricidal activity against experimentally infected subperiodic *Brugia malayi* in the leaf-monkey, *Presbytis cristata*. The leaf-monkeys were each given the compound orally at 50 mg/kg body weight daily x 5 days or 25 mg/kg body weight daily x 5 days. Geometric mean microfilarial counts (GMMFC) at 5 to 8 weeks post-treatment were substantially higher than pre-treatment counts in animals in the treated and control groups. Final GMMFCs compared to that of pre-treatment were 189.1% in animals given 250 mg/kg total dose, 8035.2% in those given 125 mg/kg total dose, and 15459.1% in the control group. At autopsy, 1% of the infective dose was recovered as live adult worms from the group of animals given the higher drug dose compared to 6.5% from the control group and 9.9% in the group given the lower drug dosage. No substantial biochemical changes were observed in treated and control animals. The study showed that CGP 6140 has no microfilaricidal action, but has some adulticidal activity against subperiodic *B. malayi* in the leaf-monkeys, at a total oral dose of 250 mg/kg.

Key words: *Brugia malayi*; CGP 6140; filaricide; *Presbytis cristata*

Introduction

Lymphatic filariasis due to *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* infections is still a major global problem with an estimated prevalence of 119.1 million (WHO, 1998). Control and treatment of the infection has until recently been based mainly on diethylcarbamazine citrate (DEC). In the last couple of years ivermectin and its combination with albendazole and DEC have been field tested for the control of bancroftian and brugian filariasis. A combination of albendazole and ivermectin was found to be more effective than albendazole alone, albendazole with DEC, or DEC and ivermectin in clearing microfilaraemia and maintaining amicrofilaraemia in bancroftian filariasis (Ismail *et al.*, 1998). As albendazole has previously been shown to kill the adult worms of subperiodic *B. malayi* in leaf-monkeys (Mak *et al.*, 1984b), it is believed that the combination of albendazole and ivermectin killed both adult worms and microfilariae in bancroftian filariasis patients. In India, DEC combined with albendazole or ivermectin and given as a single dose was also shown to be highly effective in clearing *B. malayi* microfilariae, and maintaining extremely low levels of microfilaraemia even at a year post treatment (Shenoy *et al.*, 1999).

Although DEC has filaricidal activities against *W. bancrofti* and *B. malayi* (Chen, 1964; Edeson & Liang, 1959; Mak *et al.*, 1990), its macrofilaricidal activity is slow and multiple doses of the drug are required. The latter requirement is a problem faced in control programmes for lymphatic filariasis where mass drug therapy with DEC is the main approach. There is therefore a need for more effective adulticidal drugs, which can be combined with ivermectin, or DEC for the treatment of lymphatic filariasis.

CGP 6140 or amocarzine is a 4-methyl-1-[4-(-nitrophenylamino) phenylthiocarbamido] piperazine derivative of amoscanate. This compound has been evaluated and found to have antifilarial activity against *Litomosoides carinii* in *in vitro* study (Davis *et al.*, 1989), and in *Mastomys coucha* experimentally infected with various filarial parasites (Zahner & Schares, 1993). The compound exerts its antifilarial effect through its action on the mitochondrial function and acetylcholinesterase activity of the parasite (Kohler *et al.*, 1992).

We report here the activity of CGP 6140 against subperiodic *B. malayi* infection in experimentally infected leaf-monkey (*P. cristata*). The study was approved by the Animal Use Committee, Institute for Medical Research.

Materials and Methods

Presbytis cristata weighing 3-4 kg each were obtained from non-endemic areas of brugian filariasis and quarantined for three months in mosquito-proofed rooms as previously described (Mak *et al.*, 1984a). Each animal was inoculated subcutaneously with approximately 200 infective larvae (L3) of subperiodic *Brugia malayi* on the mid-ventral aspect of the right thigh. Animals were examined weekly, six weeks after infection, for microfilaraemia. Microfilaraemic leaf-monkeys were then randomly assigned to Groups I, II and III of three, two and four animals respectively, for the experiment.

The drug CGP 6140 (Ciba-Giegy Ltd., Basel Switzerland) was suspended in 1% Tween 80 in small volume of distilled water, sonicated, and fed orally by stomach tube to Group I and II at 50 mg/kg daily x 5 days, and 25 mg/kg body weight daily x 5 days respectively. The control (Group III) animals were each given an equivalent volume of distilled water with 1% Tween 80.

One ml blood was collected from each animal of the three groups at pre-treatment and at weekly post-treatment until autopsy, for the screening of microfilaraemia using Nuclepore (R) membrane filtration. Haemoglobin, total leukocyte and differential counts, serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltranspeptidase (gamma GT), urea and bilirubin levels were measured at the same time points in all three groups of animals. All the animals were anaesthetised with ketamine hydrochloride during handling as described previously (Mak *et al.*, 1990).

Autopsy were performed at week 6 post-treatment on Group I animals and at week 8 on the other two groups, to recover worms using the method of Buckley & Edeson (1956). Five ml 1% Evans Blue was inoculated into the dorsum of feet of each animal to outline the lymphatics. The animals were killed with an overdose of pentobarbital sodium 30 minutes later.

Results

The geometric mean microfilarial count (GMMFC) at weekly post-treatment increased steadily in the two groups of *Presbytis cristata* treated with oral CGP 6140 as well as in the control group. GMMFCs at 5 to 8 weeks post-treatment were substantially higher than pre-treatment counts in all three groups of animals (Table 1). Final GMMFCs expressed as a percentage of pre-treatment were 189.1% in Group I animals, 8035.2% in Group II and 15459.1% in the control group.

At autopsy, live, active worms were recovered from all the infected animals. The mean $\pm$ S.D. number of worms recovered from Group I was fewer being 2 $\pm$ 1.0 compared to 18 $\pm$ 24.2 in Group II and 13 $\pm$ 7.6 in Group III representing 1.0%, 9.9% and 6.5% of the infective doses, respectively (Table 2).

The ALT was transiently raised in one of the two animals in Group II given a total CGP 6140 dose of 125 mg/kg. However, levels reverted to normal by week 4. There were no other significant changes in other biochemical parameters monitored.

Discussion

CGP 6140 has been evaluated for its antifilarial activities *in vivo* and *in vitro* in a number of filarial models (Court *et al.*, 1986; Townson *et al.*, 1988; Strote, 1987; Zanner & Schares, 1993). The present study is the first evaluation of the compound in a primate model of *B. malayi*. Davis *et al.* (1989) found that CGP 6140 caused rapid immobilisation *in vitro* of the adult filarial worm, *Litomosoides carinii*, by blocking of the respiratory chain. *Onchocerca volvulus* microfilariae showed a reduction of motility of up to 100% after 3 hours of *in vitro* exposure at levels of 5 and 10 µg/ml CGP 6140 (Strote, 1987). *O. volvulus* microfilariae and infective larvae exposed *in vitro* to CGP 6140 showed deleterious effects on the muscle cells and cytoplasm (Strote, 1989). The compound was also shown to have filaricidal activity *in vivo* against *B. malayi*, *B. pahangi*, *Acanthocheilonema viteae*, and *L. carinii* (Zanner & Schares, 1993).

In the present study the GMMFC in animals of the treated and control groups increased from the pre-treatment levels. Although the increase in GMMFCs in animals given a total dose of 250 mg/kg were much lower than those in the group given 125 mg/kg and the control animals, the pattern of microfilarial counts show that there is poor, if any microfilaricidal action of CGP 6140. The slight increase in GMMFCs in treated animals in Group I is due to death of adult worms and not to microfilaricidal activity. The reduction in microfilarial count due to macrofilaricidal activity of the drug has also been reported in the treatment of onchocerciasis patients in Latin America (Poltera *et al.*, 1991a).

In Latin America, Poltera *et al.* (1991b) observed the effectiveness of amocarzine on onchocerciasis patients given 3 mg/kg x twice daily after food for 3 days. At 4 months post-treatment, 73% of 1477 female worms recovered at nodulectomy were found to be dead. Mean microfilarial skin count was reduced be-

Table 1. Effect of oral CGP 6140 on microfilarial count of subperiodic *Brugia malayi* in *Presbytis cristata**

Animal No./Sex	Microfilarial count per ml blood at weeks post-treatment♠								
	0	1	2	3	4	5	6	7	8
Group I (50 mg/kg/daily x 5 days)									
556F	22	143	41	63	546	110	205		
561F	80	78	99	243	193	131	128		
567M	57	76	31	184	66	212	26		
GMMFC#	46.4	94.7	50.1	141.2	192.1	145.6	87.7		
Group II (25 mg/kg daily x 5 days)									
763F	130	673	1160	904	2302	2000	2298	1812	1728
780F	1	3	12	6	110	148	62	170	484
GMMFC	11.4	45.2	117.4	74.1	501.2	543.6	375.8	555.9	912
Group III (Control)									
523F	8	81	128	51	128	237	225		
547F	19	78	56	42	113	264	73		
579M	1	1	118	12	50♣				
764F	8	102	283	114	714	479	804	1259	909
GMMFC	5.9	28.3	111.5	41.5	150.4	737.1	602.5	1259	909

*Group I animals and some control animals followed up only to 6 weeks post-treatment; ♠Nucleopore membrane filtration of blood; # Geometric mean microfilarial count, except for week 7 & 8 in Group III; ♣Died from viral infection

Table 2. Adulticidal effect of oral CGP 6140 against subperiodic *Brugia malayi* in *Presbytis cristata*

Group & Sex	Reg No. Infective dose	Number of L3 recovered	Live worms as % of L3	Number of liveworms
I	556F	196	2 (1M, 1F)	1.0
I	561F	198	1 F	0.5
1	567M	200	3 (2M, 1F)	1.5
II	763F	200	37(15M,22F)	18.5
II	780F	194	2 (1M, 1F)	1.0
III	523F	191	24 (10M, 14F)	12.6
III	547F	200	7 (2M, 5F)	3.5
III	579M	199	9 (3M, 6F)	4.5
III	764F	198	12(2M, 10F)	6.1

M = male; F = female; RN = registration number

tween 6-11% of pre-treatment within a week.

Skin punch biopsies performed on onchocerciasis patients given 3 mg/kg of CGP 6140 twice daily x 3 days, revealed microfilariae in the upper and deeper dermis degenerated after treatment, thus showing marked microfilaricidal effect on *O. volvulus* (Zak *et al.*, 1991). However, Awadzi *et al.* (1997) found the drug effect to be different in African patients. They compared the effect of the combination of ivermectin (150 µg/kg body weight single dose) with amorcazine (3 mg/kg twice daily x 3 days), with ivermectin alone or amorcazine alone. While ivermectin was found to have

potent microfilaricidal and poor macrofilaricidal activities, amorcazine had poor microfilaricidal and adulticidal activities against onchocerciasis in Ghana.

In this study, the antifilarial activity of CGP 6140 was evaluated for the first time in a non-human primate model of *B. malayi*. The drug was shown to have adulticidal activity when given at 50 mg/kg body weight daily for 5 days. The mean number of live adult worms recovered at autopsy was only 1% of the infective dose compared to 6.5% in the control group. Perhaps a twice-daily regimen would be more effective as this has been shown to give a better drug profile (Lecaillon *et al.*, 1990). These authors showed that in onchocerciasis patients there was rapid absorption of CGP 6140 when given orally at single 100-1600 mg doses. The plasma concentration was proportional to the dose and the terminal elimination half-life was about 3 hrs. They suggested that a twice-daily administration would maintain the minimum concentration at about 10% of the Cmax. In our study the animals were treated with 25 - 50 mg/kg body weight daily for 5 days.

Drug combinations of a macrofilaricide and a mainly microfilaricide have been shown to be useful in the treatment of lymphatic filariasis (Ismail *et al.*, 1998; Shenoy *et al.*, 1999). It remains to be seen whether a combination of CGP 6140 with ivermectin or DEC would be as useful.

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