Combination ivermectin plus diethylcarbamazine, a new effective tool for control of lymphatic filariasis

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Abstract

In 1993, a three arm double-blind controlled trial was implemented in French Polynesia, to compare the tolerance and efficacy of single doses of the combination ivermectin (IVR) 400 µg.kg^{-1} plus diethylcarbamazine (DEC) 6 mg.kg^{-1} vs. IVR 400 µg.kg^{-1} or DEC 6 mg.kg^{-1} alone, for treatment of *Wuchereria bancrofti* carriers. Of the 57 treated male patients in whom microfilaremia (mf) densities ranged from 22 to 4,709 mf/ml, three groups of 19 were randomly selected, and allocated to one of the three treatments. Twelve months after treatment 37 %, 16 % and 16 % of patients were mf negative in groups DEC, IVR and IVR plus DEC respectively. Mf percent return to pretreatment level was significantly lower in the group IVR + DEC (1.9 %) than for DEC 6 (14.7 %) or IVR 400 (11.6 %). Antigenemia percent return to pretreatment level was lower in the groups IVR + DEC or DEC 6 than for IVR 400. The combination IVR + DEC proved to be the most effective on macrofilariae and microfilariae (antigenemia and mf negative patients). The combination will be a very powerful tool for control of lymphatic filariasis. An annual filariasis day could be the most cost-effective strategy for administration of the drugs.

Introduction

Several therapeutic trials have indicated that single dose treatments with diethylcarbamazine (DEC) or ivermectin (IVR) were safe and effective against lymphatic filariasis due to *Wuchereria bancrofti* (Diallo et al., 1987; Kumaraswami et al., 1988; Cartel et al., 1992 b). Single doses of IVR at a dosage of 400 µg.kg^{-1} were reported to be safe (Cartel et al., 1992 a) and more effective than a dosage of 100 µg.kg^{-1} (Moulia-Pelat et al., 1993 b), and a recent study showed an additive effect of both drugs on the early clearance of microfilariae (mf) (Moulia-Pelat et al., 1993 c). We report here the results at twelve months of a three arm double-blind controlled clinical trial of single doses of the combination IVR 400 µg-kg^{-1} plus DEC 6 mg.kg^{-1} vs. IVR 400 µg.kg^{-1} vs. DEC 6 mg.kg^{-1}, conducted in French Polynesia in 1993–1994. Microfilaremia (mf) and antigenemia levels show clearly the activity of the drugs on microfilaria and macrofilaria.

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Patients and methods

Patients: Fifty seven apparently healthy male Poly-nesian *W. bancrofti* carriers living in Moorea island, in whom mf densities ranged from 22 to 4,709 ml/mlwere recruited and given a supervised single dose treatment. The subjects were retreated at M12 and will be reevaluated at the end of the second year.

Treatments: Patients were randomly allocated to one of 3 treatments: DEC 6 mg.kg^{-1} (group DEC), IVR 400 µg.kg^{-1} (group IVR) and IVR 400 µg.kg^{-1} plus DEC 6 mg.kg^{-1} (group IVR + DEC). All treatments were identically looking capsules. Neither the carriers nor the clinical investigator were aware of the nature of the treatment received.

Methods: Blood mf densities were determined using nucleopore filtration method from venous blood samples collected the day of the treatment and every three months. One millimeter of heparinized blood was collected and filtered through nucleopore membrane which was stained by Giemsa method for counting microfilariae.

Levels of soluble parasite antigenemia prior to drug administration and 1, 6, 9 and 12 months later were evaluated with two different protocols. One was a monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) that detects a 200-kDa *W. bancrofti* adult worm antigen in human serum (Weil et al., 1987). The other was the Og4C3 antigen capture ELISA (Trop-ag *W. bancrofti*, JCU Tropical Biotechnology Pty Ltd., Townsville, Australia) which detects adult worm antigens from *W. bancrofti* (More and Copeman, 1990). Sera were analyzed as described in Chanteau et al. (1994). Only 45 patients (15 in each group) has enough blood collected for this later dosage. Sera were tested without information treatment group assignment.

The treatment effect on microfilaremia was assessed by measuring (i) the mf percent return (calculated from geometric means compared to the pretreatment geometric mean for mf counts in each group) and (ii) the mf clearance rate (proportion of carriers who remained mf negative after treatment).

The treatment effect on macrofilaremia was assessed by measuring (i) the decline in parasite antigen levels relative to pretreatment level for each treatment group and (ii) the proportion of carriers who remained antigen negative after treatment.

Side effects: During 2 days following the treatment at M12 adverse reactions were quoted as described in Glaziou et al. (1994) for intensity as: grade 1: mild (easily tolerated), grade 2: moderate (discomfort not enough to interfere

Treatment	Pre-Rx	Post treatment months (M)			
		M1	M6	M9	M12
DEC 6	100 % (266)*	11.3 %	6.3 %	7.9 %	14.7 %
IVR 400	100 % (363)*	0.6 %	3.1 %	8.6 %	11.6 %
IVR 400 + DEC 6	100 % (242)*	0.8 %	1.0 %	1.7 %	1.9 %
Statistical analysis between					
DEC and combination	$p > 0.05$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
IVR andcombination	$p > 0.05$	$p > 0.05$	$p < 0.01$	$p < 0.01$	$p < 0.01$

Table 1 Mf percent return of pretreatment level in 19 carriers of each three groups treated according to the time. (Moorea island, French Polynesia, 1994).

*Geometric means (mf/ml) densities at M0 (pretreatment).

with daily activity) and grade 3: severe (subjects unable to perform usual activity). Statistical analysis: Comparisons were made using standard bivariate statistical analysis.

Results

Groups homogeneity

Of the 57 carriers, 19 received a single dose of DEC 6 $mg.kg^{-1}$, 19 a single dose of IVR 400 $\mu g.kg^{-1}$ and the last 19 a single dose of the combination IVR 400 $\mu g.kg^{-1}$ + DEC 6 $mg.kg^{-1}$. Before treatment, in the 57 carriers, microfilaremia ranged from 22 to 4,709 ml/ml, and the geometric mean for mf counts (GMM) in the whole study population was 283 mf/ml. GMM per group were 266, 363 and 242 mf/ml (Table **1**), the difference was not statistically significant ($p = 0.7$). Age of patientsranged from 19 to 69 (mean 40), and did not significantly differ between groups ($p = 0.8$).

Side effects

At M12, side effects were experienced by 10 treated patients (18 %), of whom 7 suffered a grade 1, and 3 a grade 2 reaction. None of the reactions was considered serious. With regard to treatment, no significant difference of the mean grade was observed. The grade of adverse reaction was correlated with the pretreatment mf density level but not significantly ($p = 0.06$).

Microfilaria recurrence and negativation rate

The table 1 shows the percent return to pretreatment mf levels according to the treatment group and time. After 12 months, the combination of IVR + DEC proved to be the most effective with mf residual of 1.9 % compared with mf residual greater than 10 % for DEC 6 or IVR 400 alone. One year after the first treatment, 3 (16 %), 3 (16 %) and 7 (37 %) patients were found mf negative, from group DEC, IVR and IVR + DEC respectively ($p > 0.05$). All these mf negative patients remained mf negative during 12 months after the treatment. Pretreatment mf levels were significantly lower in patients that were mf negative at 12 months (106 mf/ml) than in the other patients (376 mf/ml; $p = 0.005$).

Antigenemia recurrence and negativation rates

Table **2** shows the percent return to pretreatment antigen levels according to the treatment group and time. After 12 months, whatever the antigen assay used, the treatment with DEC 6 and the combination IVR + DEC were significantly more effective than the treatment with IVR 400 ($p < 0.05$). There was no significant difference in antigenemia recurrence between the two treatments DEC 6 and IVR + DEC. However, the decrease of antigenemia was higher in all the treatments protocols and at all times when using the 200-kDa *W. bancrofti* antigen ELISA than the Og4C3 antigen assay.

At M12, one year after the first treatment, 6 (43 %), 4 (27 %) and 5 (38 %) people were found antigen negative, with the 200-kDa *W. bancrofti* antigen ELISA, in groups treated with DEC, IVR and IVR + DEC respectively ($p > 0.05$). All these antigen negative patients remained antigen negative between the sixth and the twelfth month. Pretreatment antigen levels were significantly lower in patients found antigen negative at M12 (10 ng/ml) than in the other patients (39 ng/ml; $p < 10^{-4}$). No patient were found antigen negative at M12 using the Og4C3 antigen assay.

Correlation between microfilaremia and antigenemia according to the time

At M0, M6, M9 and M12, whatever the treatment, there was a positive correlation ($p < 10^{-4}$) between microfilaremia and antigenemia, using the 200-kDa *W. bancrofti* antigen assay. When microfilaremia increased, antigenemia increased. At M0, 44 of 45 mf positive subjects were antigen positive (sensibility of 97.8 %, 95 % confidence interval: 93.5–100 %). At M1, whatever the treatment, there was no correlation between mcirofilaremia and antigenemia ($p = 0.43$). Indeed, many subjects weremf negative at M1 (17/45) and 13 of these 17 mf negative were antigen positive, 1 with DEC, 5 with IVR and 7 with IVR + DEC (geometric mean: 8 ng/ml, range 5–75).

There was also a good positive correlation ($p < 10^{-6}$) at M0 between the microfilaremia and antigenemia, using the Og4C3 antigen assay.

Antigen test	Treatment	Pre-Rx	Post treatment months (M)			
			M1	M6	M9	M12
A	DEC 6	100 % (26)*	42 %	22 %	15 %	18 %
	IVR 400	100 % (20)*	85 %	46 %	41 %	43 %
	IVR 400 + DEC 6	100 % (27)*	44 %	16 %	14 %	17 %
B	DEC 6	100 % (5812)**	78 %	62 %	54 %	50 %
	IVR 400	100 % (5459)**	96 %	80 %	72 %	94 %
	IVR 400 + DEC 6	100 % (5319)**	82 %	60 %	52 %	52 %

Table 2 Antigenemia percent return of pretreatment level in 15 carriers of each three groups treated according to the time. (Moorea island, French Polynesia, 1994).

A: 200 kD W. bancrofti antigen assay.* Geometric means antigenemia (ng/ml) at M0 (pretreatment). B: Og4C3 antigen assay.** Geometric means antigenemia (Antigen Units/ml) at M0 (pretreatment).

Discussion

Few side effects were reported after the first treatment at M0 (Glaziou et al., 1994) and the second treatment at M12; they consisted of a mild influenza-like syndrome. No difference was found between treatment groups and greater reactions were observed in patients with higher mf levels as at M0. This observation is consistent with other studies (Cartel et al., 1990; Sabry et al., 1991). The decrease in the proportion of the side effects after several treatments was important for the people's compliance during a trial or a mass treatment.

The combination IVR 400 plus DEC 6 showed promising results for an annual strategy with a mf percent return less than 2 % after only one treatment and 37 % of carriers still mf negative twelve months after treatment. The effects of the drugs were stronger on low carriers. The pattern of antigenemia percent return was similar with the two antigen assays. However, the 200-kDa adult worm antigen assays appears to be a more sensitive indicator of drug effect than the Og4C3 antigen assays. This could be due to the fact that this last assay is based on an adult worm antigen from an other filarial species. The 200-kDa *W. bancrofti* antigen-test also showed more complete clearance in low carriers and the residual antigen level between M6 and M12 with DEC (22–18 %) or IVR + DEC (16–18 %) was very attractive. Therapeutic success in bancroftian filariasis has generally been measured in terms of mf percent return of mf pretreatment level and negativation rates, because there has been no way to assess the direct effect of therapy on adult worms. The development of parasite antigen detection methods has provided a new approach to the assessment of treatment efficacy in filariasis (Weil et al., 1988). Our study confirmed (i) the sensibility of this antigen-test 97 %, and (ii) the positive correlation between microfilaremia and antigenemia as reported by Ramzy et al., 1991. One month after treatment, differences in antifilarial activities of the two drugs were already evident: there were 12 (IVR or IVR + DEC) mf negative and antigen positive patients at M1. This, reflects the dramatic activity of IVR on microfilariae, but also its weak activity on macrofilariae. The discrepancy between mf and antigen data is consistent with previous studies that suggest that DEC has a greater macrofilaricidal effect than IVR (Weil et al., 1991). Prior studies showed slight reductions in antigen levels after IVR. Antigen data from the Moorea trial suggests that IVR had a more significant partial macrofilaricidal activity in this trial than in the trial conducted in Haiti (Weil et al., 1991). If real, this difference could be due to a difference in dosage (IVR 400 as a single dose in Tahiti vs. one or two IVR 200 after a pretreatment dose of IVR 20 in Haiti) or to different levels of infection before treatment. Kinetics of both drugs on filariae are different; IVR induced an immediate and nearly complete elimination of mf, whereas DEC led to a sustained decrease in both mf and antigenemia (Kazura et al., 1993). The summation of the IVR microfilaricidal activity (reduction of transmission) and the DEC macrofilaricidal activity (parasitological cure) is an important argument to use the combination IVR + DEC as a single dsoe. Moreover, IVR alone seemed to have a macrofilarial effect, probably a sterilization which did not last longer than 6 months (increase of microfilaremia after six months). With combination therapy, the mf reduction was sustained between 6 and 12 months as a result of all these activities: negativation of microfilaremia, sterilization of adult worms and macrofilaricidal effect. As the drugs were more effective on low carriers whether in comparison with antigenemia or microfilaremia, elimination of lymphatic filariasis as a public health problem might be achieved using repeated single doses of combination IVR + DEC. Combination of IVR + DEC greatly reduces the human reservoir of mf for at least 12 months after treatment, a result not achieved by treatments with IVR or DEC alone. Therefore, this treatment will maintain such a low level of mf density in the population that it should result in a dramatic decrease in transmission by the vector. This strategy is very attractive for countries, such as French Polynesia, where the vector control is very difficult to implement.

A single dose of the combination could be administered to the whole population during an annual 'filariasis day'. But a first problem will be the compliance with treatments. The second problem will be the strategy: which one of the two following methods is the most cost-effective for administration of the drugs? House-to-house survey with supervised treatment; or mass treatment on a filariasis day with unsupervised treatment. The best method may depend upon local mf prevalence. With a high level of mf carriers (10 % or more), a house-to-house survey will be necessary to reach all the population and all reluctant carriers (with explanations on adverse reactions and its decrease after several treatments) during 2 or 3 years. After a filariasis day with health education messages will also be efficacious. The last question will be:when can you stop your programme? At which level of mf prevalence will the disease disappear? Maybe when the geometric mean of the antigenemia level falls to near zero.

In conclusion, important sustained reductions in microfilaremia and antigenemia were observed in the three treatment groups. The combination IVR 400 + DEC 6 was the most effective at one year, combining all effects of the drugs on both microfilariae and macrofilariae. These results suggest that combination therapy will be a very powerful tool for the control of the lymphatic filariasis. An annual filariasis day could be the most cost-effective strategy for administration of the drugs. With an effective annual mass chemoprophylaxis, the national objective of French Polynesia will be the total control of filariasis by the year 2000.

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