

MACROFILARICIDAL EFFECTS OF CHLOROQUINE ON ADULT *ONCHOCERCA VOLVULUS* BY LOCAL INFILTRATION OF PALPABLE ONCHOCERCAL NODULES

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The macrofilaricidal effects of local infiltration of high concentrations of chloroquine into the capsule of onchocercal nodules on adult worms of Onchocerca volvulus was determined. Six weeks post infiltration, histological examination of single nodules showed all adult worms to be dead. With nodule conglomerates, there was localized action of chloroquine only on the adult worms in the infiltrated nodule, with no diffusion of the drug to adjacent nodules. Chloroquine infiltration of young, recently formed nodules to reduce the adult worm load of infected individuals may be an alternative method to costly nodulectomy.

Key-words: Onchocerciasis. Nodules. Infiltration. Chloroquine.

Prior to the introduction of ivermectin in the control of onchocerciasis, nodulectomy was used as the principal method of disease control in Mexico, Guatemala, and Ecuador. The widespread use of nodulectomy, particularly for the removal of head nodules, was associated with decreasing rates of blindness in Guatemala^{2 3}; and in Ecuador was shown to reduce dermal and ocular microfilarial loads^{4 7}.

In the hyperendemic areas of Ecuador, new nodules were seen to develop rapidly, even within 6 to 8 months following nodulectomy (unpublished observations). Therefore effective control could be only achieved by costly annual nodulectomy campaigns. For this reason, alternative methods to reduce the adult worm burdens were sought that were equally effective as nodulectomy, but less expensive.

In vitro studies have shown that chloroquine is toxic for both *Onchocerca volvulus* microfilariae and adult worms⁸, that higher drug concentrations are required to kill the adult worms than the microfilariae, and that this macrofilaricidal effect is pH dependent¹⁵. Clinical studies show that ingestion of oral chloroquine, though it has a suppressive effect on dermal microfilarial numbers, and

though there is a rapid accumulation of chloroquine in the tissues of adult *O. volvulus*¹⁰, does not appear to have any demonstrable effect on adult worms⁵. However, nothing is known concerning the status of the adult worm when bathed in high concentrations of chloroquine *in situ* by infiltration of nodules with the drug.

Reported here are the findings on the macrofilaricidal effects of local infiltration of high concentrations of chloroquine into the capsule of palpable onchocercal nodules in patients living in a hyperendemic area for onchocerciasis in Ecuador.

MATERIALS AND METHODS

Study population. The study was performed in the hyperendemic region for onchocerciasis located in the Santiago River Basin in the province of Esmeraldas⁶. Chachi Indian males, aged 20 to 40 years, with previously documented positive skin snips for *O. volvulus* (range 11-390mf/mg), were recruited from 4 communities. Two different clinical types of palpable nodules were selected for chloroquine infiltration: single nodules with less than 1 year of evolution (28 patients) and conglomerates of nodules [eg., large, irregular nodules indicating nodule aggregates (24 patients)]. An additional further 40 patients from the same communities with single and conglomerate nodules were recruited as controls.

Nodule infiltration. Nodule infiltration was performed as follows: the skin above the nodule

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was disinfected with 70% alcohol followed by povidone-iodine solution, after which chloroquine sulphate solution, concentration 150mg/ml (Winthrop, USA), was infiltrated using an insulin syringe with 26 gauge needle. The volume of chloroquine infiltrated was dependent upon the size of the nodule, with an average of 15 to 50mg of chloroquine injected per nodule. In the control patients, the nodules were infiltrated with equivalent volumes of physiological saline.

Histopathology. Six months following infiltration, all nodules were surgically removed as previously described⁷. An additional 36 single nodules and 40 conglomerate nodules, in which no infiltration was done, were also removed. These served as internal non-treated controls. Nodules were placed in 4% buffered formaldehyde and examined histologically at the Hospital Vozandes in Quito. The nodule specimens were coded and analysed in a *blinded fashion*. Tissues were processed by standard paraffin embedding and sectioning processes and histological evaluation of each nodule was done as previously recommended¹². Only when the histological results were reported was the code broken and a comparative analysis done.

Ethical clearance was obtained from the Ethical Committee of the Hospital Vozandes and the study protocol was approved by the Chachi Indian Federation. Individual informed verbal consent was obtained from each study participant.

RESULTS

Single nodules. A total of 36 single nodules were infiltrated with chloroquine. Within 4 months of infiltration, 11 (30.6%) of the single palpable nodules completely disappeared. Six months after infiltration, all the remaining 25 nodules (69.4%), were found to be reduced in size and harder upon palpation when compared to their initial size and texture. Upon extirpation they were found to have fibrotic adhesions to the fascia. No significant changes were noted in the size and texture of the 36 nodules infiltrated with saline.

Histopathological changes observed in single nodules infiltrated with chloroquine, with saline, as well as those not infiltrated are given in Table 1. Significant morphological changes were seen in nodules infiltrated with chloroquine in comparison to those infiltrated with saline and those not infiltrated. In the

Table 1 - Histopathological changes observed in single onchocercal nodules infiltrated with chloroquine, with saline, and those not infiltrated, province of Esmeraldas, Ecuador.

Histopathological Changes	Chloroquine (n = 25)		Saline (n = 36)		Non-infiltrated (n=36)	
	n°	%	n°	%	n°	%
Adult parasite						
Viable	0	0	27	75	28	78
Dead	25	100	9	25	8	22
Degenerated changes	25	100	9	25	8	22
Calcified	0	0	1	3	0	0
Worm remnants	0	0	0	0	0	0
Degenerative mf <i>in utero</i>	25	100	8	22	8	22
Degenerative embryo <i>in utero</i>	25	100	8	22	8	22
Male	0	0	0	0	0	0
Female	25	100	35	97	36	100
Nodule						
Necrotic center	25	100	8	22	8	22
Granulation tissue	19	76	5	13	6	17
Eosinophils	25	100	7	19	6	17
Polymorphs	25	100	6	17	7	19
Lipophages	23	92	4	11	5	13
Giant cells	22	88	5	13	4	11
Lymphoplasmocytes	14	56	3	8	3	8

chloroquine infiltrated nodules, all (100%) of the adult worms were classified as dead as compared to 25% in the saline infiltrated nodules. In non-filtrated nodules, 22% of the worms were classified as dead. There was complete

degradation of the internal structures, eg., uterus and intestine, of the worms in nodules infiltrated with chloroquine. The walls of the worms were vacuolated with massive cellular infiltration of lymphocytes and macrophages

around the degenerating worm (Figure 1). Lipid-filled histocytes were frequently found. In the control nodules, infiltrated with saline or non-infiltrated, 75% of the nodules showed no morphological changes indicative of damage to the adult worms.

Conglomerate nodules. A total of 40 conglomerate nodules were infiltrated with chloroquine. The infiltration was difficult in that only the larger superficial palpable nodule(s) could be infiltrated. The smaller deeper nodules located beneath the superficial nodules could not be palpated nor infiltrated. After 6 months post-infiltration, there was

basically no change in the size or form of the conglomerate. No significant changes were noted in the 40 control nodule conglomerates infiltrated with saline.

Histological studies of infiltrated nodules showed morphological changes indicating death of the adult worm similar to that seen in single nodules. However, no effect on the adult worms was seen in the adjacent nonpalpable noninfiltrated nodules, suggesting limited diffusion of the drug. In the control nodules, no morphological changes were noted in the adult worms in either those infiltrated with saline or those not infiltrated.

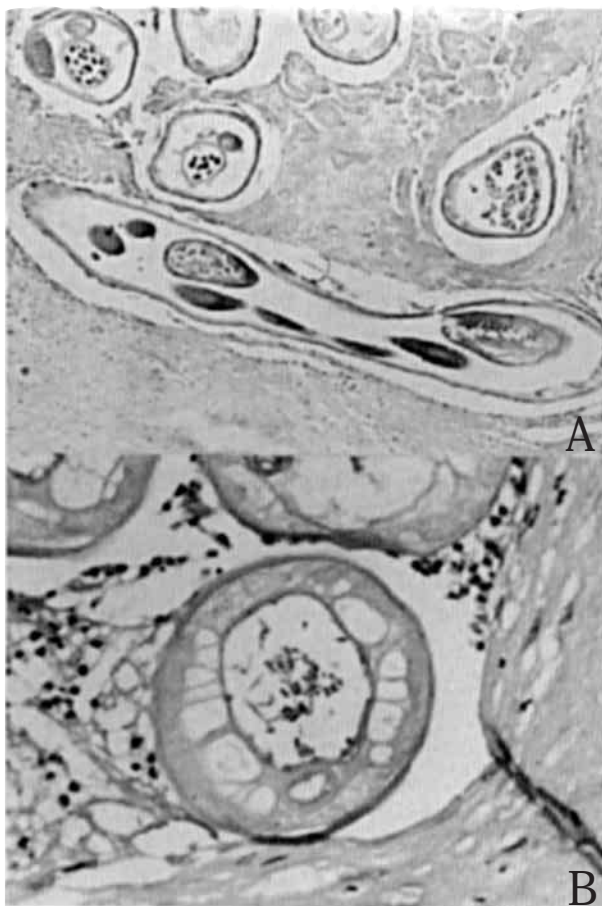


Figure 1 - A. Normal healthy female *Onchocerca volvulus* in nodule without treatment. B. Dead female of *O. volvulus* post chloroquine infiltration showing vacuolar degeneration of hypodermis, desintegrating mf in genital tract, inflammatory reactions surrounding the worm.

DISCUSSION

The infiltration of nodules with chloroquine had a definite macrofilaricidal effect on the adult worm. This is in contrast to the results observed when chloroquine was taken orally⁵, where no pathomorphological changes in extirpated nodules were noted. No pathological changes were seen in nodules infiltrated with saline, indicating that the signs of toxicity, degradation and intra-uterine microfilarial degeneration seen in this study were probably due to chloroquine infiltration. Great effort was taken upon infiltration to minimize any mechanical damage to the worm, to ensure that the changes documented were due basically to the substances injected. The use of a 26 gauge needle, penetration of only the nodule's capsule, and slow infiltration of the drug, greatly reduced the possibility of inflicting damage or death to the adult worm other than the action of chloroquine. This was evidenced by the fact that the adult worms in the nodules infiltrated with saline apparently remained healthy. In cases where a large gauge needle was used, with deep penetration of the nodule and manipulation, and with a rapid *slam* injection of a substance (saline), most of the worms were mechanically damaged or killed (unpublished observation).

In vitro studies have been done to determine the efficacies of quinoline-containing anti-malarial compounds, other than chloroquine, against filarial worms. Amodiaquine has been shown to have anti-filarial activity against laboratory models such as *Litomosoides carini*⁹, *Brugia pahangi*¹⁴ and *Wuchereria bancrofti*¹¹. However, clinical studies in Nigeria have shown amodiaquine to be ineffective against human onchocerciasis⁹. Mefloquine has anti-filarial activity *in vitro* against *Brugia pateri* and *Brugia malayi*¹⁷. However, other than chloroquine, few compounds have been studied both *in vitro* and *in vivo* to determine their antifilarial activities against *Onchocerca volvulus*^{5 8 10 14 15 16}. Chloroquine, which is readily available in injectable form in Ecuador, was chosen for this study as an extension of these former studies.

It was interesting that there was no evidence of secondary infection or formation of an abscess in any of the infiltrated nodules studied. There was no perforation of any nodule with spontaneous suppuration as seen at times with suramin treatment¹. In young nodules (those between 3 to 5 months of

evolution according to the history given by patients), the nodule disappeared with the death of the adult worm. The capsule surrounding the worm at this stage of development dissolved. However if the nodule had more than 5 months of evolution, the nodule did not dissolve with the death of the worm but became a small hard fibrotic mass. It is possible over a longer period of time, depending on the anatomic site, that this fibrotic mass could increase in size.

The effect of the chloroquine was very local. There was no evidence of diffusion to adjacent nodules. The effect seen in each nodule was a result of infiltration in that given nodule. The utility of chloroquine infiltration as an alternative method for nodulectomy is evident. It could be used successfully in an area where a surveillance program for the detection of newly formed nodules exists. If the nodules have a 3 to 5 months' evolution, there is a good chance that these nodules will completely disappear. However, in nodules with several years of evolution, and with which the formation of satellite nodules may have occurred (unpublished observations), chloroquine infiltration would be of limited benefit.

RESUMO

O efeito macrofilaricida da infiltração local, com uma alta concentração de cloroquina, dentro da cápsula do nódulo oncocercótico sobre o verme adulto de Onchocerca volvulus foi determinado. Seis semanas depois da infiltração, estudos histopatológicos de nódulos simples demonstraram todos os vermes adultos mortos. Em conglomerados de nódulos a ação da cloroquina foi só sobre os vermes adultos do nódulo infiltrado, não acontecendo a difusão da droga aos nódulos adjacentes. A infiltração de cloroquina a nódulos novos ou de recente formação reduz a carga de vermes adultos dos indivíduos parasitados e pode ser uma alternativa para os altos custos das nodulectomias.

Palavras-chaves: Oncocercose. Nódulos. Infiltração. Cloroquina.

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