

A Simple Method for Screening of Macrofilaricidal Compounds-The Inhibitory Effect of Substances on Phosphatase Activity of Adult *Dirofilaria immitis*

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Abstract: A simple and reproducible screening method of macrofilaricides is described. The method evaluated in the present paper is based on inhibitory activity of the drugs on acid-phosphatase of *Dirofilaria immitis* adult worms. The potent inhibitor of acid-phosphates, NaF, inhibited completely the enzymic activity at 1 mM. trimelarsan, suramin and flubendazole inhibited partially the enzymic activity. Their inhibitory activity is comparable to that of Na₂HAsO₄, a specific inhibitor of phosphatase. Diethylcarbamazine and levamisole did not inhibit the phosphatase of *D. immitis*.

Key words: *Dirofilaria immitis*, phosphatase, inhibitors, macrofilaricide

A series of physiological and biochemical studies on *Dirofilaria immitis* demonstrated the possible transcuticular absorption of nutrients of nematodes (Yanagisawa and Koyama, 1970; Chen and Howells, 1981). And the presence of high concentration of phosphatase(s) in the hypodermis (Maki and Yanagisawa, 1980a) supports the absorption of some molecules through the body surface. Therefore inhibitors of phosphatase are expected to have adulticidal action. In fact some arsenicals, the potent inhibitors of the phosphatase, are potential macrofilaricides (Hayasaki, 1993), although they are not for clinical use due to the sever side reaction.

Despite extensive studies, diethylcarbamazine is the only drug which shows adulticidal activity against lymphatic filarial worms. And no macrofilaricidal drugs are available for the treatment of onchocerciasis. Therefore new, powerful and safe macrofilaricides are highly expected to be developed (WHO, 1984).

In the present study, we describe a simple method for the screening of macrofilaricidal drugs based on the inhibitory activity of the drugs against phosphatase of adult *D. immitis*.

Female and male adult *D. immitis* were collected from the pulmonary artery and heart

of stray dogs in Kanagawa Prefecture, Japan. The homogenate containing phosphatase(s) was prepared according to the method reported elsewhere (Maki and Yanagisawa, 1980b). The chemicals tested were NaF (Kanto Chemical Co., Inc.), Na₂HAsO₄ (Wako Chemical Co., Inc.), flubendazole (Janssen Pharmaceutica), diethylcarbamazine (Tanabe Pharmaceutical Co. Ltd), levamisole (Kyowa Hakko Kogyo Co., Ltd), suramin (Bayer Co. Ltd.) and trimelarsan (Rhone-Poulenc Pharmaceutica). These drugs were dissolved in 1% Tween 80. Inhibitory effect of these drugs on phosphatase were evaluated as follows. An assay tube contains 0.1 ml of *D. immitis* homogenate, 0.1 ml of a given test substance, 0.7 ml of 0.4M Tris-maleate buffer (pH7.3) and 0.1 ml of 10 mM *p*-nitrophenyl phosphate. The control tube contains homogenate inactivated by being kept in boiling water for 1 min. This mixture was incubated at 37C for 30 min in a water bath. The enzymic reaction was stopped with the addition of 4 ml of 10% TCA. The tube was kept in iced water for about 10 min. Then it was centrifuged at 2,000 rpm for 5 min. The supernatant was transferred to a new tube. Addition of 2 ml of saturated Na₂CO₃ to the tube developed yellow color. The yellow color was photometrically measured at 405 nm. The protein concentration of *D. immitis* homogenate was determined by the Lowry method using bovine serum albumin as a standard. The enzymic activity was expressed provisionally with delta 405/30 min/mg protein. The calculation of % inhibition of the phosphatase activity was [OD405/30 min/mg of control tube - OD405/30 min/mg of test tube / OD405/30 min/mg of control tube] x 100.

Although the preliminary study showed that the enzymic activity of *D. immitis* acid phosphatase is highest at pH 5, enzymic activity in the present study was measured at pH7.3. The reason is that the drugs probably act against filarial worms at physiological pH.

The specific inhibitors of acid-phosphatase, NaF and Na₂HAsO₄ inhibit the acid-phosphatase of *D. immitis* adult worms (Table 1). NaF showed 100% inhibition at 1 mM. Na₂HAsO₄ showed 100% inhibition at 10 mM. Among anthelmintics examined, trimelarsan, suramin and flubendazole inhibited partially enzymic activity at 1 mM. Their ability to inhibit

Table 1. Effect of substances on 1 mM *p*-nitrophenyl phosphate hydrolysis by *Dirofilaria immitis* at pH 7.3

Substances tested	Concentration mM	% Inhibition	
		male	female
NaF	1	100	100
Na ₂ HAsO ₄	10	74	87
	1	23	26
	0.1	11	0
Trimelarsan	1	21	33
Suramin	1	17	25
Flubendazole	1	17	22
Diethylcarbamazine	10	0	0
Levamisole	10	0	0

enzymic action is comparable to those of Na_2HAsO_4 . Since trimelarsan contains arsenical, its inhibitory action is expected. However, we can not give any explanation for inhibition of suramin and flubendazole. While diethylcarbamazine and levamisole did not inhibit the enzymic activity. The new macrofilaricidal drugs are urgently needed to be developed. However, the methods of screening of macrofilaricidal drugs have some weakness both *in vivo* and *in vitro*. For *in vivo* study the huge number of infected animals is required and the recovery of adult worms from animals is troublesome. For *in vitro* study again the numerous adult worms must be recovered from many infected animals.

The present study dealt with the new method of screening of macrofilaricides based on the inhibitory action of drug against phosphatase of adult worms. This method is so simple and reproducible that it might allow us to screen numerous candidate compounds including the traditional medicinal plants with ease and accurately. However this method can not necessarily screen all macrofilaricides.

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