Experimental Studies on Filaricidal Effect upon *Setaria digitata*

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Introduction

There are several kinds of man and animal filariasis in Japan. In veterinary community many investigators have hitherto reported achievements on the treatment of animal filariasis. Supatonin (1-diethylcarbamyl-4-methylpiperazine citrate), Stinal (natrium tartar emetic) have been used for the treatment of cerebro-spinal filariasis of sheep, goats, horses and cattle (lumbar paralysis) due to *Setaria digitata* and for that of dermal microfilariasis of cattle and horses (Kose and Kasen disease) due to onchocerca microfilariae. Filarsen (dichlorphenarsine hydrochloride), Stinal, Supatonin have been used for dirofilariasis of dog. On the treatment of human filariasis due to *Wuchereia bancrofti*, Mapharzol (oxophenarsenic hydrochloride) and Supatonin (especially the latter) are now widely used for chemotherapy in Japan.

In studies on the treatment of canine filariasis, Kume[1] has reported that arsenticals are effective for the extermination of mature worms but are not effective on microfilariae and that derivative of piperazine and antimonial compounds are effective for the extermination of microfilariae but are not effective on mature worms.

As described above, in the treatments of filariasis, the efficacy of drugs does not cover all sorts of worms and differs in accordance with the stage of the growth of worms.

This seems to mean that the exterminative effect of the drugs remains to be delicate and unknown.

The author, using these filaricides and intending to add somethings to its fundamental studies have made both *in vitro* and *in vivo* experiments on the killing effect upon mature worms and microfilariae of *S. digitata*, with some interesting results mentioned as follows.

Materials and Methods

Drugs used :

Drugs used were, as follows namely : Stinal (made in Gendai Seiyaku Co.) as one of the antimonial compounds, Supatonin (made in Tanabe Seiyaku Co.) as one of the piperazine compounds, Mapharzol (made in Daiichi Seiyaku Co.) and Filarsen (made in Fujita Seiyaku Co.) as arsenticals.

Worms used :

Setaria digitata generally occurs as a natural parasite of the cattle in Japan. Its adult worms were collected aseptically from the peritoneal cavities of a cattle killed at the slaughter house. By crushing the uteri of mature female, microfilariae were taken out of it.

In vitro experiments:

These drugs were dissolved in various degrees of dilution in 0.8 per cent saline fluid in petri dishes and watch glasses. Then, prepared mature worms and microfilariae were put into these petri dishes and glasses, which were incubated at 37°C. The worms were observed through naked eye or microscope to ascertain how long they would survive. When the worms completely stopped moving, it was regarded as the sign of their death.

In vivo experiments:

The author used 18 rabbits as experimental animals. 5-7 setaria adult worms were aseptically transplanted into the peritoneal cavities of 18 rabbits of 2.8-5 kg of body weight. Filaricides were administered immediately after transplantation. The group given Supatonin consisted of 5 rabbits, the method of dosage was daily intramuscular injection for 5-7 days. The group given Stinal consisted of 4 rabbits, the group given Filarsen consisted of 5 rabbits, the rabbit given Mapharzol was 1 in number and the methods of dosage were all intravenous injection for 5-7 days. All rabbits were sacrificed after treatment, and the efficacy of the drugs was examined. Three of the eighteen rabbits were left non-treated, as control. All the worms obtained from the peritoneal cavity at autopsy were put into 0.8 per cent saline fluid at 37°C to examine whether they were still alive or dead and embryos in the uteri of mature female were also examined through microscope. The number of circulating microfilariae in rabbits were estimated by means of thick blood smears prepared after being taken from the lungs at autopsy. After the life or death of the adult worms was examined, they were fixed with 10 per cent formalin solution and their section preparates were stained with hematoxylin and eosin to examine the changes due to drugs.

Experimental results

I Killing effect of filaricide upon *Setaria digitata* by *in vitro* studies.

When mature worms or microfilariae of *Setria digitata* were put into the petri dishes or watch glasses filled with 0.8 per cent saline fluid containing drugs of various degrees of dilution, they were active at first, but gradually they became inactive and finally stopped moving. The death of worms was decided when they completely stopped moving. As was seen in case of adult worms microfilariae were observed in watch glasses microscopically. By this method the author examined how long the worms would survive in each different degree of solution. The results were as follows. (Table 1. 2. 3. 4)

Table 1. Killing effect of Supatonin upon adults or microfilariae of *Setaria digitata in vitro* studies.

Concentration mg/*l*	Time showing killing effect on adult (hours)	Time showing killing effect on microfilariae (hours)
800	1	3
400	2	7
200	3	over 10
100	20	over 20
50	over 20	over 20
20	over 20	over 20

Table 2. Killing effect of Stinal upon adults or microfilariae of *Setaria digitata in vitro* Studies.

Concentration mg/*l*	Time showing killing effect against adult (hours)	Time showing killing effect against microfilariae (hours)
40	3	3
20	3	4
8	5	6
4	7	8
2	over 18	over 20

Table 3. Killing effect of Filarsen upon adults or microfilariae of *Setaria digitata in vitro* studies.

Concentration mg/*l*	Time showing killing effect against adults (hours)	Time showing killing effect against microfilariae (hours)
60	1.5	3
12	2	7
6	2	8
3	4	over 8
1.5	4	over 8

Table 4. Killing effect of Mapharzol upon adults or microfilariae of *Setaria digitata in vitro* studies.

Concentration mg/*l*	Time showing Killing effect against adults (hours)	Time showing Killing effect against microfilariae (hours)
30		5
10	1.5	
5	3	6
1.25	4	over 8

The killing effect of various filaricides upon *Setaria digitata* by *in vitro* studies, was ascertained in case of both adults and microfilariae with the result that the drugs in high dilution killed adult worms and microfilariae more rapidly than those in low dilution. The difference of the killing effect upon *Setaria digitata* due to the drugs was not recognized and each of the drugs killed adult worms earlier than it killed microfilariae. Worms in control lived longer than those in the dissolued drugs.

But the result of this experiment did not show that a certain kind of drug is effective only to certain kinds of filaria and some drug is effective to either mature worms or microfilariae.

II Killing effect upon *S. digitata* transplanted into the peritoneal cavities of the rabbits.

The author made some experiments, *in vivo* studies, on the effect of Stinal, Supatonin, Filarsen, Mapharzol upon *Setaria digitata* transplanted in the rabbits.

The author paid special attentions to the following four items.

1. The effect of filaricides on the adult worms.

2. The effect of filaricides on the circulating microfilariae in blood.
3. The effect of filaricides on the embryos in the uteri.
4. Histopathological changes of the worms due to drugs.

1. The effect of filaricides on the adult worms. The results is shown in Table 5.

Table 5. The number of worms gathered in peritoneal cavities of the treated rabbits at autopsy.

No. of rabbits	Name of drug	No. of transplanted setaria worms	Total dose mg/k	times	Days from transplantation to autopsy	No. of worms gathered		
						surrived	dead	lost
1	Supatonin	♀ 7 ♂ 2	180	5	7	0	3	6
2	〃	♀ 8	300	5	5	1	2	5
3	〃	♀ 6	240	4	5	2	3	1
4	〃	♀ 6	500	5	6	1	2	3
5	〃	♀ 7	400	5	6	2	1	4
6	Stinal	♀ 5	10	5	6	4	1	0
7	〃	♀ 7	10	5	7	3	4	0
8	〃	♀ 6	12	4	5	0	5	1
9	〃	♀ 7	15	5	6	1	6	0
10	Filarsen	♀ 5	15	5	7	5	0	0
11	〃	♀ 7	15	5	6	0	6	1
12	〃	♀ 6	16	4	5	1	3	2
13	〃	♀ 6	15	5	6	2	4	0
14	〃	♀ 6	15	5	6	0	6	0
15	Mapharzol	♀ 6	15	5	6	3	1	2
16	Control	♀ 8 ♂ 2			7	1	7	2
17	〃	♀ 8			5	4	1	3
18	〃	♀ 6			6	0	6	0

'lost' means the transplanted worms which could not be fouund at autopsy.

Naturally rabbits are not proper host for *Setaria digitata*, and the worms in the peritoneal cavities die, naturally, within about one week after transplantation. Consequently all the treated rabbits were sacrificed within one week after transplantation. Although daily dose of Supatonin (36 mg/K-80 mg/K), Stinal (2 mg/K-3 mg/K), Filarsen (3 mg/K-4 mg/K), Mapharzol (3 mg/K) was more than the maximum dose used for filariasis, there were no toxic symptoms. Live worms got at autopsy moved actively, when put in 0.8 per cent saline fluid, and continued moving for several hours at room temperature, while dead worms showed no such movement at all.

Between the treated rabbits and the non-treated ones no difference in the number of the survived worms was recognized at autopsy. As for dead worms, the author could not decide whether their death was due to the effect of filaricide or natural asthenia. But it was remarkable that there were many lost worms in the group of Supatonin.

2. The effect of filaricides upon the circulating microfilariae in blood.

Microfilariae appear in the peripheral blood of rabbits within two days after transplantation of adult worms. The author prepared three blood smears out of the blood of the lungs at autopsy and these preparates were stained with Giemsa's stain after hemoglobinolysis. The results obtained are as shown in Table 6.

3. The effect of filaricides upon embryos in the uteri.

As living embryos could be seen within the uteri of mature females under higher

magnifications, it was readily decided whether the embryos were dead or alive. The results obtained are as shown in Table 6.

Table 6. Microfilaricidal effect in rabbits, and embryocidal effect within uteri of mature females.

No. of rabbits	Name of drug	No. of microfilariae in blood smears *	Death or life of microfilariae **	
			In uteri of living mature females	In uteri of dead mature females
1	Supatonin	−	−	−
2	〃	−	±	−
3	〃	−	±	−
4	〃	+	−	
5	〃	+	+	−
6	Stinal	+	+	−
7	〃	+	+	
8	〃	+		−
9	〃	−	−	
10	Filarsen	+	+	
11	〃	++	−	−
12	〃	++		+
13	〃	++	+	
14	〃	++		−
15	Mapharzol	+	+	
16	Control	+++	+	−
17	〃	++	+	−
18	〃	+++	+	−

* Remarks:
+=1-2
++=3-5
+++=over 5

** Remarks:
+=Alive
−=Dead
±=Half dead

Generally, the rabbits given the drugs showed a reduction of the number of circulating microfilariae as compared with the control rabbits, especially the rabbits given Supatonin or Stinal showed a remarkable reduction of the number of circulating microfilariae.

In the group given Supatonin and Stinal, embryos in the uteri of mature females were observed to be dead or half dead occasionally.

In view of these facts, it may be said that Supatonin and Stinal are effective not only for circulating microfilariae but also effective for embryos in the uteri of mature females.

4. Histopathological changes of worms due to drugs.

After deciding death or life of the adult worms in 0.8 per cent saline fluid, the living worms and the dead worms were examined histopathologically. Histological changes obtained from the survived worms and the dead worms are as shown in Table 7, 8.

Live worms:

Although setaria worms were alive macroscopically, some histopathological changes were detected in the internal organs. All the worms in the treated rabbits given filaricides showed slight regressive changes in body-structure. In group given Supatonin and Stinal, somatic layer, uteri and oesophagus swelled; and nuclei showed karyopyknosis. Embryos in the uteri were dead or half dead and showed a reduction in the number. Especially it was observed that there occurred necrosis of the parenchyma cell of ovaries and eggs. On the contrary, the group of Filarsen revealed slighter regressive changes than the group of Supatonin and Stinal, and especially the uteri of female worms were observed to be

Table 7. Histopathological changes of survived worms

No. of worms	Name of drug	Somatic layer	Uteri	Oeso-phagus	Intestine	Embryos in utei	Decrease of embryos in uteri	Embryos in eggs	Mature eggs	Immature eggs	Ovar-ies
1	Supatonin	+	+	+	+	++	±	++	++	++	++
2	〃	±	+	±	+	++	±	++	++	++	++
3	〃	±	±	±	±	±	±	±	±	+	±
4	〃	+	+	—	—	±	+	+	±	±	±
5	〃	+	+	—	—	+	++	+	±	±	±
Mean	〃	+	+	±	±	±	++	±	±	±	±
6	Stinal	+	+	+	+	±	+	+	±	±	
7	〃	—	+	+	+	++	±	++	+	±	±
8	〃	+	+	+	+	+	+	±	±	±	±
9	〃	+	+	±	+	+	±	±	±	±	±
10	〃	+	+	+	+	±	++	±	±	±	±
11	〃	—	—	—	—	+	+	+	+	±	±
Mean	〃	+	+	+	+	±	++	±	±	±	±
12	Filarsen	±	±	+	+	±	—	±	+	+	±
13	〃	+	+	+	+	+	—	+	+	±	
14	〃	±	±	±	±	+	+	+	+	+	±
15	〃	—	—	±	—	—	—	—	—	+	
16	〃	—	+	+	±	—	—	—	—	+	±
Mean	〃	±	±	±	±	±	—	±	±	+	±
17	Control (Rabbit)	—	—	—	—	—	+	—	—	+	±
18	Control (Cattle)	—	—	—	—	—	—	—	—	—	—

filled with active embryos.

Dead worms:

Swelling of protoplasma, karyopyknosis of nucleus in somatic layer, oesophagus, and intestine were observed, and these regressive changes were remarkable both in treated groups and non-treated ones. Especially in cases given Supatonin and Stinal microfilariae in the uteri of the worms reduced in number or died and the eggs and the ovaries were tended to suffer necrosis. On the contrary, the uteri of the non-treated group and of the group given Filarsen were filled with microfilariae, and embryos and eggs were stained comparatively well, showing slightly regressive changes. In short, it may be said that the functions of the organs of the adult female worms in producing microfilariae are to be destroied by the working effect of Supatonin and Stinal.

Discussion

Antimonial compounds and arsenticals have been used for the treatment of both animal and human filariasis. Hewitt[2][3] reported the marked filaricidal action of Hetrazan (1-diethylcarbamyl-4-methlpiperazine hydrochloride) working upon the naturally acquired filarial infections in cotton rats and dogs. Hewitt and his co-workers[4] have stated that Hetrazan is active against both adult worms and microfilariae of *Wuchereria bancrofti*. On the other hand, among the derivatives of piperazine Supatonin (1-diethylcarbamyl-4-methylpiperazine citrate, made in Japan) has been used for the treatment of animal and human filariasis in Japan, and it has been making not a small contributions to the prevention and treatment of human and animal filariasis.

It is generally recognized that antimonial compounds are microfilaricidal and arsenti-

Table 8. Histopathological changes of dead worms

No. of worms	Name of drug	Somatic layer	Uteri	Oeso-phague	Intestine	Embryos in uteri	Decrease of embryos in uteri	Embryos in eggs	Mature eggs	Immature eggs	Ovar-ies
1	Supatonin	±	++	++	±	+	+		+	+	+++
2	〃	+++	+++	++	++				++	++	+++
3	〃	+++	+++	++	+++		+++	+++	+++	+++	+++
4	〃	+++	+++	+++	+++	+++	+++	++	+++	+++	+++
5	〃	+	+++	+++	++	++	+++	+++	+++	+++	+++
Mean	〃	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
6	Stinal	+++	+++	+++	++	+++	+++	+++	+++	+++	+++
7	〃	+++	+++	+++	+++	+++	+++	+++	++	+++	+++
8	〃	+++	+++	+++	+++	++	++	++	++	+++	+++
9	〃	+++	+++	+++	+++		+++		++	+++	+++
10	〃	+	+	++	++	++	+		++	+++	+++
11	〃	+++	+++	+++	+++		+++		++	++	+++
12	〃	+++	++	+++	+++	++	+		++	+++	+++
13	〃	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
Mean	〃	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
14	Filarsen	+++	++	+++	+++	+	+	+++	+++	+++	+++
15	〃	+++	++	+++	+++	+	—	+	++	+++	+++
16	〃	++	++	++	++				+	+	++
17	〃	+++	++	+++	+++	++	—	+	+	++	+++
18	〃	++	++	++	++		++		++	++	+++
19	〃	++	++	++	++	+	—	+	+	++	
20	〃	++	++	+++	++	+	—	+	+	++	+++
21	〃	+++	++	+++	+++	++	—	++	+	++	+++
22	〃	+++	+++	+++	+++	++	—	++	++	++	+++
Mean	〃	+++	++	+++	++	++	—	+	+	++	+++
23	Control (rabbit)	+++	+++	+++	+++	+	—	+	+	++	++
24	〃	+++	+++	+++	+++	+	—	+	+	++	+++
25	〃	+++	+++	+++	+++	+	—	+	+	++	++
26	〃	++	++	++	++	+	—	+	+	+	++
Mean	〃	+++	+++	+++	+++	+	—	+	+	+	++

cals are adultfilaricidal. Kume[1] reported that Supatonin showed killing effect upon microfilariae of *Dirofilaria immitis* in dogs but not upon the adult worms. Sato[5][6] and Katamine[7] used Supatonin for the treatment of human filariasis and observed the appearance of microfilariae in blood for a long time, noticing the fact that microfilariae did not increase in number with the conclusion that Supatonin may be effective for mature worms as well as for microfilariae.

As *Setaria digitata* can be alive in the peritoneal cavity of rabbits for 1-2 weeks, the author conducted, both *in vitro* and *in vivo* studies on the effects of various filaricides on adult worms and on the microfilariae of this worm. The killing effect of antimonial compounds, arsenticals and piperazine compounds upon *Setaria digitata*, by *in vitro* studies, was ascertained to be effective both to adult worms and microfilariae; and the drugs in high dilution killed adult worms and microfilariae more rapidly than those in low dilution.

Cuberson and Rose[8] conducted in vitro siudies with *Litomosoides carnii* using antimonial compounds, Otto and Maren[9] conducted the same studies using phenyl orsenoxide with *Dirofilaria immitis*, Hewitt[2] also conducted the same studies with *Litomosoides carinii* using Hetrazan. These investigators reported that the drugs in high dilution killed worms more rapidly than those in low dilution. Heweitt[2] stated that in order to observe filaricidal effects autopsy should be performed in 1-2 weeks after cessation of treatment. As *Setaria digitata* can be alive in the peritoneal cavity of rabbits only for a short period, all

experimental rabbits were sacrificed within one week. Between the treated rabbits and non-treated ones no difference in the number of the survived worms was recognized at autopsy. Judging from this fact, it may be said that to adult worms these drugs have no rapid effect but only have slow one.

The rabbits given Supatonin or Stinal showed remarkable reduction of the number of circulating microfilariae and this fact agrees with the results of studies on other filariae. Hitherto, efficacy of filaricide on adult worms has been observed through naked eye. After deciding the death or life of adult worms at autopsy, the author made section preparates in order to examine the changes due to drugs. Although setaria worms were alive at autopsy, their internal organs showed histopathological changes. The worms in a group given Supatonin or Stinal, showed regressive changes, especially in female genitial organ. These regressive changes include not only the necrosis of the parenchyma cell of ovaries and eggs but also the reduction of the number of embryos in the uteri. On the contrary, the changes of the worms given Filarsen and Mapharzol are very slight.

The above-mentioned changes of dead worms in groups given Supatonin, Stinal are very serious, but those of dead worms given Filarsen are just similar to changes in natural death and are very slight.

Judging from these facts, it may be said that Supatonin and Stinal destroy the functions of the organs of the female worms in producing microfilariae and these drugs, if they were used for a long period of time, may finally kill adult worms.

Summary

The author have conducted, both *in vitro* and *in vivo* studies on the effect of filaricide upon adults and microfilariae of *Setaria digitata*. From the results of the examination of the direct effect of Supatonin, Stinal, Filarsen, Mapharzol on adults and microfilariae of *Setaria digitata in vitro* studies, it was found that these filaricide had a killing effect on both adult worms and microfilariae of *Setaria digitata* and each of the drugs killed adult worms earlier than it killed microfilariae.

The experimental chemotherapy with the use of filaricides for the rabbits transplanted with *Setaria digitata* were carried out.

Between the treated and non-treated rabbits the filaricidal effect on the adult worms of *Setaria digitata* was not obvious. This may be due to the fact that adult worms of *Setaria digitata* in an peritoneal cavity of rabbits cannot be alive for a long time. The effects of Supatonin and Stinal upon the circulating microfilariae in blood were more remarkable than arsenticals. Among the histopathological findings, regressive changes of female genitial organ were observed. These changes were seen in the group given Supatonin and Stinal, especially Supatonin.

It may be said that the functions of adult female worms in producing microfilariae are destroied by the working effects of Supatonin and Stinal.

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