

Chemotherapy of *Clonorchis sinensis*

II. Clinical observations on the treatment of clonorchiasis patients with 1, 4-bis-trichloromethylbenzol

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Introduction

Many studies have been published on the treatment of clonorchiasis, but no reliable therapy is yet available.

The authors showed in Report I that 1, 4-bis-trichloromethylbenzol has exhibited an excellent therapeutic effect in the animals experimentally infected with *Clonorchis sinensis* and that the agent showed practically no toxicity.

In the present paper the results of the first application of the above mentioned agent in human clonorchiasis are described.

Materials and Methods

Drug used :

The agent used for the treatment of patients was 1, 4-bis-trichloromethylbenzol which Hoechst Co. of Germany placed at our disposal. The structural formula of this drug is shown in Fig. 1. It is a colorless, odorless powder

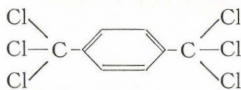


Fig. 1. Chemical structure of 1, 4-bis-trichloromethylbenzol

insoluble in water. The drug is already on the market as "Hetol" of Hoechst Co. for

the treatment of cattle and sheep liver flukes, *Fasciola hepatica* and *Dicrocoelium dendriticum*.

Preliminary test :

Though widely used already as an anthelmintic for animals, this drug has not yet been used in human patients. As a preliminary test, Hetol was administered to four volunteers consisting of the authors and their colleagues at doses of about 100 mg per kilogram of body weight per day (divided into 3 parts, after meals) every other day for 5 doses, and these recipients of the drug were subjected to various examinations as shown in Table 1.

Selection of patients for treatment with Hetol :

Eight clonorchiasis patients were selected for treatment with Hetol on an inpatient basis. For their age, occupation, and clinical findings, refer to Table 2. All eight patients live in Tokyo. Besides clonorchiasis, they had the following diseases: cholelithiasis (4 cases), diabetes (2 cases), duodenal ulcer (one case), and gastric cancer (one case). One of the authors, Dr. Matsumoto, is in charge of the treatment of gastrointestinal diseases in the Toranomon Hospital, Tokyo. From among his inpatients, those who were positive for *Clonorchis sinensis* eggs were selected for treatment with Hetol.

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Table 1. Liver function tests of the healthy individuals given daily dose of 100 mg/kg of Hetol every other day for 5 doses

Case No.	Age	Sex	Body weight (Kg.)	Doses of Hetol (100 mg/kg × 5)	Liver Function Test									
					Total protein	A/G	TTT	ZST	Al.-Phos.	Cholin-E.	T-Cholest.	GOT	GPT	
1	42	M.	52	5.0 g×5	{B	6.9	1.8	2.9	8.3	4.8	0.8	168	20	6
					{A	7.1	1.7	3.0	9.5	4.7	0.7	161	18	6
2	33	M.	75	7.0 g×5	{B	8.0	1.6	2.8	6.0	6.0	1.0	195	23	9
					{A	8.0	1.8	2.7	6.1	6.1	0.9	203	22	9
3	27	M.	67	6.5 g×5	{B	9.0	1.7	2.6	6.1	4.3	0.7	141	20	6
					{A	7.4	1.5	2.7	8.4	6.8	0.9	195	22	6
4	23	M.	52	5.0 g×5	{B	7.8	1.7	2.0	6.7	5.2	0.9	183	19	5
					{A	7.6	1.7	3.2	6.4	5.4	0.9	185	26	5

B: Before treatment

A: After treatment

Table 2. Cases treated with Hetol

Case No.	Age	Sex	Occupation	Residence	Clinical diagnosis
1	57	M.	Journalist	Tokyo	Clonorchiasis sinensis Cholelithiasis
2	63	F.	Publisher	"	Clonorchiasis sinensis Diabetes mellitus
3	73	M.	Hotel-keeper	"	Clonorchiasis sinensis Diabetes mellitus
4	27	F.	Public Official	"	Clonorchiasis sinensis Cholelithiasis
5	51	M.	"	"	Clonorchiasis sinensis Cholelithiasis
6	56	F.	Housewife	"	Clonorchiasis sinensis Cholelithiasis
7	36	M.	Public Official	"	Clonorchiasis sinensis Duodenal ulcer
8	50	M.	"	"	Clonorchiasis sinensis Gastric cancer

These patients cannot be considered to be completely suitable as subjects for the first treatment of human clonorchiasis. But, in Tokyo it is almost impossible quickly to find new cases of infection with *Clonorchis sinensis* for treatment on an impatient basis. The patients selected up for treatment with Hetol had other diseases in addition to clonorchiasis sinensis as mentioned already, and this fact may be regarded as having the advantage of showing what effect Hetol has on these other diseases in addition to its effect on clonorchiasis sinensis itself.

Dosage and Administration :

The dosage for the patients was the same as that employed in the preliminary test: 100 mg/kg of Hetol (divided into 3 parts taken after meals) was administered every other day for 5 doses. However, the dosage was not always precisely 100 mg/kg, being occasionally a little less than that.

Evaluation of the efficacy of drug :

The drug effectiveness was judged on the basis of the results of fecal examinations. For fecal examinations AMS III centrifugation tech-

nique was used; the sediment was examined microscopically, the eggs of *Clonorchis sinensis* were counted, and doubling the number the E. P. G. (Eggs Per Gram of Feces) was worked out. Fecal examinations were carried out 2 or 3 times before treatment, every day during treatment, twice a week until the 6th week after treatment, and twice each at 2, 4, 6, and 9 months after treatment.

Results

1) Preliminary test with Hetol:

The four volunteers already mentioned (all are physicians) who received 100 mg/kg of Hetol (divided into 3 parts taken after meals) every other day for 5 doses were examined for subjective symptoms. Blood was withdrawn before administration and on the completion of administration (which was the 9th day after the first administration). The serum so obtained was used in liver function tests. These tests con-

sisted of total protein, A/G ratio, thymol turbidity test (T.T.T.), Kunkel's zinc sulfate test (Z. S. T.), alkali-phosphatase (AL-P), cholinesterase (choline-E), total cholesterol (T-chol.), glutamic oxalacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT). The results are shown in Table 1. In all four volunteers, there was practically no difference in the results of these tests before and after the administration of Hetol. All four, during the administration of Hetol, were engaged in their daily routine work in their laboratory with no deleterious side effects noted at all.

2) Results of treatment of patients with clonorchiasis sinensis:

The age, sex, occupation, abode and clinical diagnosis of the patients are presented in Table 2. All eight patients live in Tokyo. Their admission diagnosis, besides clonorchiasis sinensis, was cholelithiasis in 4 cases, diabetes in 2 cases, duodenal ulcer in one case and gastric

Table 3. Results of treatment with Hetol.

Case No.	Age	Sex	Body weight (Kg.)	Total doses of Hetol every other day for 5 doses	Side effect	Stool examination*									
						Before treatment	After treatment								
							1w.	2w.	3w.	4w.	5w.	6w.	2m.	3m.	6m.
1	57	M.	64	30.0 g (6.0 g×5)	Malaise	++	++	++	+	-	-	-	-	-	-
2	63	M.	64	30.0 g (6.0 g×5)	Slight dizziness Epigastric discomfort	+++	+++	+++	-	-	-	-	-	-	-
3	73	M.	53	26.5 g (5.3 g×5)	None	+	+	+	-	-	-	-	-	-	-
4	27	F.	48	20.0 g (4.0 g×5)	Sore tongue	++	++	++	-	-	-	-	-	-	-
5	51	M.	61	30.0 g (6.0 g×5)	None	+	++	+	+	-	-	-	-	-	-
6	56	F.	53	25.0 g (5.0 g×5)	Sore tongue	+++	++	++	+++	-	-	++	++	++	++
7	36	M.	60	30.0 g (6.0 g×5)	None	++	++	+	-	-	-	-	-	-	-
8	50	M.	50	25.0 g (5.0 g×5)	None	++	++	++	+	+	-	-	-	-	-

*: AMS III centrifugation technique: 2 stools were examined each time. 0.5 gram of stool was used.

w.: week + : 1-9 Eggs per gram feces.
m.: month ++ : 10-99 ,,
 +++ : 100- ,,

Table 4 Clinical examinations of patients treated with Hetol before and after treatment.

Case No.	Blood examinations				Liver function tests										Urinalysis				
	Hb	R.	W.	Eos. (%)	Total prot.	A/G	I.I.	TTT	ZST	Al-Phos.	Chol.-E.	Cholest.	GOT	GPT	LDH	Protein	Sugar	Urobilinogen	Sedi-ment
A	16.6	487	5200	0	7.6	1.6	6	1.0	5.5	8.7	0.9	240	27	28	183	-	-	+	-
1 B																			
C	16.1	499	7200	0	7.4	1.8	3	1.7	6.9	11.0	0.6	225	17	12	-	-	+	-	
A	16.4	499	3400	1	8.0	1.0	5		5.0	1.0	254	9	6	-	-	+	+	-	
2 B																			
C					8.0	1.7		3.5	6.0	6.9	0.8	10	5	-	-	+	+	-	
A	13.1	394	5300	1	6.3	1.2		2.9	8.6	5.8	0.7	253	10	10	122	-	##	+	
3 B																			
C																			
A	12.6	426	8600	1	7.7	1.3	4	2.7	8.5	4.5	1.1	121	11	5	152	-	-	+	
4 B	13.0	438	4800	8	8.5	1.4	5	5.0	10.5	4.1	0.8	212	15	8	206	-	-	+	
C																			
A	16.8	492	5300	2	8.6	1.5	6	4.7	9.3	11.0		191	24	12	191	-	-	+	
5 B	15.2	441	5900	3	7.8	1.6	4	5.0	7.6	10.0		200	21	20	199	-	-	+	
C	15.7	475	5400	2	8.4	1.6	3	4.0	7.5	9.3	0.8	214	20	14	252	-	-	+	
A	13.9	399	8400	1	8.0	1.4	4	3.5	10.0	11.0	1.2	198	10	5	93	-	-	+	
6 B	14.8	414	10600	0	1.8	1.5		4.1	10.5	11.0	1.1	196	9	8	174	-	-	+	
C	14.4	383	8300	4	7.4	1.2	2	2.9	7.4	7.2			8	4	181	-	-	+	
A	17.2	498	4500	1	7.5	1.7	4	2.3	9.5	8.5		174	12	10	186	-	-	+	
7 B	17.9	496	4100	2	7.6	1.8	3	4.0	7.6	7.0		172	10	7	177	-	-	+	
C	15.4	497	5500	0	7.6	2.0	3	2.8	7.6	5.6	0.8	195	12	10	237	-	-	+	
A	10.2	446	7100	4	7.6	1.2	4	5.0	13.1	6.0	0.6	195	12	52	144	-	-	+	
8 B	11.7	453	5200	4	7.7	1.2	5	5.7	13.3	9.7	0.5	166	35	40	127	-	-	+	
C																			

A: before treatment
 B: immediately after treatment
 C: one month after treatment

cancer in one. Case No. 1 had undergone cholecystectomy. Case No. 6 with cholelithiasis and Case No. 8 with gastric cancer were both operated upon after the completion of the Hetol administration.

The results of fecal examinations after the treatment with Hetol are shown in Table 3. As can be seen in the table, in all the eight cases treated, the feces became negative for *Clonorchis* eggs within 3 to 5 weeks after the beginning of treatment. The time when the feces became negative for eggs was as follows: in four cases, No. 2, No. 3, No. 4 and No. 7, at 3 weeks after treatment (to put it more exactly, when examined at 22 and 23 days after the beginning of treatment); in 3 cases, No. 1, No. 5 and No. 6, at 4 weeks after treatment (when examined at 29 and 30 days after the beginning of treatment), and only in case No. 8 at 5 weeks after treatment (when examined at 36 and 37 days after the beginning of treatment). Follow-up examination for more than 6 months after treatment were made in 7 cases out of 8 cases except one case of No. 4 at 3 months after treatment. With the exception of case No. 6, none of all the above cases was found positive for eggs in the above examinations. The follow-up studies are still continuing at this writing. Case No. 6 was negative for *Clonorchis* eggs in the fecal examinations at 4 and 5 weeks after treatment. But, after this case underwent cholecystectomy, the feces examined became positive again for *Clonorchis* eggs. In repeated fecal examinations thereafter, this case was always positive for eggs, though the EPG was much less than before treatment. Presumably, the disease has returned in this case. The seven other cases remained negative for *Clonorchis* eggs for more than three months after treatment may be regarded as having obtained a complete cure.

Side effects of Hetol are shown in Table 3. Four of the eight cases had no subjective complaint at all of side effects. The complaints of side effects in the remaining four cases were no worse than languor, mild giddiness, and rough tongue. No severe side effect at all was observed.

The results of hematologic examinations, liver function tests and urinalysis before, immediately after and one month after treatment are shown in Table 4. The liver function tests on three occasions, i. e. before, immediately after and one month after treatment, could not be carried out in all subjects. However, the values obtained in the tests showed nothing particularly abnormal as shown in Table 4. Only the L. D. H. (lactic dehydrogenase) value was somewhat elevated after treatment in case No. 5 and case No. 7. The reason for this elevation is not yet clear. The urinalysis in case No. 3 showed at one month after treatment the presence of a small amount of protein, but this was not observed thereafter. Case No. 2 and case No. 3 complicated by diabetes, suffered no deleterious effect from the treatment with Hetol.

Discussion

Eight patients of clonorchiasis sinensis were treated with Hetol 100 mg/kg every other day for 5 doses. In all cases *Clonorchis* eggs in feces became negative at from 3 to 5 weeks after the beginning of treatment. Fecal examinations thereafter found one of the cases positive again for *Clonorchis* eggs at 6 weeks, but the remaining 7 cases have stayed negative for eggs for 3 to 9 months already.

There is no generally accepted theory on the time when clonorchiasis sinensis can be defined as cured following treatment. The authors' experiences in this connection are that, in the instance of relapse because of insufficiency of treatment, the eggs usually appear in feces again within one to two months after their temporary disappearance. Therefore, when *Clonorchis* eggs have not been found in feces for three months or more as experienced in the present study, complete cure may be regarded as having been achieved. We will of course continue follow-up studies further. However, the fact that cure has been obtained in 7 (87%) of the eight cases will have to be considered to be a very good result as compared with the results obtained with other drugs.

Crane *et al.* (1955) reported that their treatment of 34 patients with chloroquine diphosphate resulted in complete cure in 12 patients (35%). Yamaguchi *et al.* (1962) who carried out a mass treatment of 344 clonorchiasis patients with dithiazanine iodide reported that about 70 percent of the patients became negative for eggs soon after treatment. But no follow-up study has been done by them. In our use of chloroquine or dithiazanine iodide, not even a single case could be cured completely.

The Hetol dosage and its manner of administration in the present study cannot necessarily be regarded to be best. Particularly, in mass treatment shorter periods of time for treatment are most desirable. The authors' recent trials have given fairly good results with the administration of 50 mg/kg daily for 5 days. That administration every day even at a low dosage is more effective than administration every other day, can of course be presumed also from the results described in the previous Report I. Another report on the studies of the dosage and the mode of administration of Hetol will be published soon. Liver function tests and other clinical laboratory examination before, immediately after and at one month after the treatment with Hetol showed nothing abnormal in any of the cases. In the present series of patients who all had other disease in addition to clonorchiasis, such as cholelithiasis, diabetes, duodenal ulcer and gastric cancer, the use of Hetol produced no signs suggesting the exacerbation of those other diseases.

It is said in connection with clonorchiasis sinensis that, in mild infection, there are no particular symptoms peculiar to this disease. In the present series of patients who were all mild infections as shown in their E. P. G. which were from a few hundred to a few dozen, no particular symptom was observed that could be considered due to clonorchiasis sinensis. No difference was observed either before and one month after treatment in respect of the blood picture or liver function test results. Hepatopathy such as cholelithiasis and diabetes was present in 6 of the 8 cases constituting the

present series. This high incidence of hepatopathy in the present series is seemed to be incidental because these patients were selected from among the patients visited our specialized clinic for gastrointestinal diseases, especially liver diseases and this high incidence has not been found to have any inherent relationship with *Clonorchis sinensis* infections. Nevertheless, we intend looking further into this matter.

It is of interest that the same drug referred to as hexachloroparaxyolol, at much higher dosage (500 mg/kg daily or on alternate days for 5 to 24 days), was tested on 71 patients in China; all were cured and in some cases large numbers of dead worms were passed in the feces (Chung *et al.*, 1965).

Let us end this report with a brief remark on the course of infection with *Clonorchis sinensis* in those 8 cases. As already stated, they all live in Tokyo, and it is almost sure that they must have been infected in Tokyo. Besides the 8 patients constituting our present series, in the Toranomon Hospital where the authors work, routine fecal examinations of the inpatients of the Internal Medical Department find one or two patients per month positive for eggs of *Clonorchis sinensis*. The fact that the incidence of clonorchiasis sinensis is almost regularly observed in Tokyo which is not an endemic area of clonorchiasis sinensis, is highly interesting. The actual incidence of the disease in Tokyo may be interpreted as associated with the fact that in some Tokyo restaurants such freshwater fish as carp or the crucian carp are served in the form of slices of raw fish as "Sashimi" or "Arai" dishes which in this country are considered to be rather high-class and find acceptance among the upper classes. This may be of some importance in the epidemiology of clonorchiasis sinensis in this country.

Summary

Eight inpatients with clonorchiasis sinensis in the Toranomon Hospital of Tokyo were treated as the first human cases to receive 1,4-bis-trichloromethylbenzol (Hetol). Hetol 100 mg/

kg (divided in 3 parts taken after meals) was administered every other day for 5 doses in all. For 3 to 9 months after the completion of treatment fecal examinations were carried out. On the lapse of 3 to 5 weeks after treatment, the feces became negative for eggs in all of the 8 cases, and 7 cases have since been continuing negative for eggs and even at this writing which is more than 3 to 9 months after treatment. In only one case in whom negativity for eggs was once achieved a small number of eggs was later detected again. Elaborated examinations for side effects due to Hetol revealed nothing abnormal in liver function test and in other respects.

The results thus far described have led to the conclusion that Hetol is safe to use and is more effective than the conventionally used drugs.

However, further studies may be suggested the feasibility of reducing the dosage and shortening the period of time required for its administration. Studies on these possibilities are under way at this moment.

The authors are grateful to Dr. Lämmler who has kindly provided us with literatures on Hetol, and also wish to express their gratitude Dr. Shigeo Okinaka, Director of the Toranomon Hospital, for the constant encouragement granted in our present studies.

References

- 1) Chu, S. (1957): Chloroquine in the treatment of clonorchiasis. A report of 90 cases. Chinese Med. Jour., 75. 473-484.
- 2) Chung, H. L., Hsu, C. P., T'sao, W. C., K'o, H. Y., Kuo, C. H., Hsu, H. C., Li, H. H., Cheng, S., Chang, H. Y., Yuan, C. T. and Chang, Y. C. (1965): Hexachloroparaxylol in treatment of clonorchiasis sinensis in animals and man. Chinese Med. Jour., 84. 232-247.
- 3) Crane, P. S., Bush, O. B. and Won, P. C. (1955): Treatment of clonorchiasis with chloroquine and methiscol. Royal Soc. of Trop. Med. and Hyg., 49. 68-70.
- 4) Lämmler, G. (1960): Chemotherapeutische Untersuchungen mit Hetol einem neuen hochwirksamen Leberegelmittel. Dtsch. Tierärztl. Wschr., 67. 408-413.
- 5) Lämmler, G. (1964): Studies in the experimental chemotherapy of opisthorchiasis. International Congress of parasitology. Rome.
- 6) Plotnikov, N. N. and Yoldigina, Z. S. (1962): Advances in experimental treatment of opisthorchiasis. (Preliminary report). Meditsinskaya Parazitologiya i Parazitarnie Bolezni. Moscow, 31. 680-682.
- 7) Yamaguchi, T., Uehara, K., Shinoto, M., Takagi, K., Horie, N., Minoda, H., Fukunaga, M. M., Kunishige, A. and Yanagawa, H. (1962): Chemotherapy of clonorchiasis sinensis with di-thiazanin iodide. Tokushima Jour. of Experimental Medicine, 8. 307-334.
- 8) Yokogawa, M., Koyama, H., Yoshimura, H. and Tsai, C. S. (1965): Chemotherapy of clonorchiasis sinensis. I. Therapeutic effect of 1, 4-bis-trichloromethylbenzol in animals experimentally infected with *Clonorchis sinensis*. Jap. Jour. Parasit., 14. 233-242.

肝吸虫症の治療に関する研究

II. 肝吸虫症の新しい治療剤 1,4-bis-trichloromethylbenzol
(Hetol) による臨床治療成績

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著者らは 1, 4-bis-trichloromethylbenzol (Hetol) を今回始めて人体肝吸虫症の治療に用いることを試みた。これを臨床治療に用いるに先立ち、教室の健康人 4 名がまづ 1 日量 100 mg/kg を隔日に 5 回服用し、諸種副作用を検査したが、本剤服用による影響と思われるものは認められなかった。そこで虎の門病院に入院中の肝吸虫症患者 8 名に Hetol を前記と同様の方法で投与した。治療前、治療直後、1 カ月後に血液検査、肝機能検査、尿検査などの臨床検査を行ったが、いずれもほぼ正常範囲内の値を示している。また 8 名のうち 4 名は胆石症、2 名は糖尿病、1 名は胃癌、残りの 1 名も十二指腸潰瘍を合併していたが、舌があるという訴えをした者が 2 名あるのみで、服薬を中止する程大きな障害を認めたものは

1 名もなかった。さらに治療前、治療中および治療後の糞便内の EPG を、治療中は毎日または隔日、治療 1 カ月後までは 3 ないし 7 日おきに、その後は 1 カ月に 1 ないし 2 度観察した。8 名とも EPG は服薬中は大きな変動をみせなかつたが、治療終了後 5 日ないし 6 日頃より減少し始め、治療終了後 10 日ないし 24 日目にはいずれも陰転した。しかし内 1 名は治療後 30 日を経て胆摘手術を行った所、再び虫卵が検出され始めた。他の 7 名は治療後すでに 3~9 カ月以上を経ているがいずれも虫卵は見出されず、完全治癒したものと思われる。今後さらに例数を増して投与量および投与方法に関して臨床的に詳細な検討を試みるつもりである。

誤文訂正 (本誌 14 巻 3 号)

頁	行	誤	正
225	Fig. 6 の説明	~the quantity of wheat~	~the quantity of <u>washed</u> wheat~
229	下から 4 行目	indispesable	indisp <u>en</u> sable
264	results 3) の全文を下記の如く訂正		

3) From the geographical distributions of patients (Fig. 3), it was supposed that filariasis used to be prevalent all over the village. Considering the life span of the adult filaria and the fact that the distribution of patients by age was limited to older ages, (47~74), the infection of microfilaria seemed to have been ceased long before, say 15~20 years, among the inhabitants in this village.