Treatment of Hookworm Infection with 2,6-Diiodo-4-Nitrophenol

Chin-Thack Soh, M.D., D.M.Sc. Moo-Joon Cho, M.D.
Yong-Hee Rho, M.D. & Jae-Heung Lee

Department of Parasitology, Yonsei University Medical College, Seoul, Korea

Introduction

2,6-diiodo-4-nitrophenol(DNP) has been used for the elimination of dog hookworm. Koutz and Groves(1961) reported that it was more effective in *Ancylostoma caninum* than *Uncinaria stenocephala*.

Wood et al(1961) also found the single subcutaneous dose of 7.5 mg/kg was enough to eliminate *A. caninum* and *A. brasiliense*, and obtained similar results by oral or intramuscular administration. Later, Wood and Pankavich(1961) found the drug did not eliminate the immature stage of hookworm while full effectiveness was recorded after 21 days or more of infection. Darne and Webb(1964) reported that *Spirocerca lupi* was sensitive to the chemical, whereas *Dipylidium caninum* and *Dirofilaria immitis* were apparently ineffective.

Recently, Huang and Khaw(1964) tried the drug as a clonorchicidal agent, and found that 4 injections(7.5 mg/kg, each) of DNP given subcutaneously every other day led to cure of clonorchiasis in dogs, but they reported that nitrophenol was highly toxic and its clinical application required careful further investigations.

The present studies were designed to test and confirm the efficacy of the drug to dog and human hookworms and the toxicity to the host.

1) *Ancylostoma caninum*

Materials and methods:

Parenteral administration:

4 to 6 months old young dogs(male 3, female 5) were infected with 1,000 to 2,000 of canine hookworm filariform larvae. One month later, all the fecal samples from these dogs showed egg-positive. Various doses of 3.5% DNP solution (0.6~6.0 c.c.), Keun-Il Co., were injected percutaneously, and the stools were examined from the third day of the injection until sacrifice of the animals.(Table 1.)

No. 4 died with unclear pathological reason 12 hours after the injection, and the other animals were sacrificed 9 days after the injection. The small intestine was examined in order to find out the adult worm in it, and also the organs (small intestine, liver and kidney) were fixed in 10% formalin for routine pathological examinations.

Oral administration:

60 mg of single dose was given to two hookworm infected dogs (Nos. 7,8). The follow-up studies were all the same with the parenteral administration.

Results:

Nos. 1, 2, 3, 5 and 6 showed egg-positive in stool from the third day of the injection. In No. 4 dead adult worms were found in the large intestine but not in small intestine.

The side effects seemed not to be so serious except in No. 1 and No. 5 which showed the complication of vomiting several times on the 1st day and recovered within 48 hours.

Table 1. Parasitological and pathological examination of hookworm infected dogs which were injected with 3.5% 2,6-diiodo-4-nitrophenol solution.

No.	Sex	Wt. (kg)	Dose (c.c.)	Eggs in feces			Pathological changes, postmortem				
				before injection (EPG)	after injection(day)			No. of worm in small intestine	small intestine	liver	kidney
				3	6	9					
1	M	15.0	0.6	17,600	0	0	0	0	Petechiae	—	desquamation of tubular epithelium.
2	F	6.6	1.2	43,600	0	0	0	0	—	congestion	—
3	M	9.0	2.1	8,400	0	0	0	0	—	—	—
*4	M	9.0	3.0	4,800				0	Petechiae	congestion	desquamation of tubular epithelium
5	F	8.1	3.0	5,600	0	0	0	0	—	—	—
6	F	12.0	6.0	152,000	0	0	0	0	—	cirrhosis	congestion
**7	F	10.0	60mg	+	0			0	—	—	—
**8	F	10.0	60 //	+	0			0	—	—	—

Remarks: *died 12 hours after the injection

**Oral administration

No systemic pathological changes were observed except in No. 4. However, the cirrhotic changes in liver due to the chemical was not considered because of the short duration of only 9 days after the injection.

No hookworm was found in intestine of oral group on 6 days after the administration and also no pathological changes were observed in liver, kidney and small intestine (Table 1).

Summary:

Single dose(0.6~6.0 c.c.) of 2,6-diiodo-para-nitrophenol solution(35 mg/cc) was injected subcutaneously in dogs infected with *Ancylostoma caninum*.

Form the third day after the injection, no eggs were found by fecal examination and no significant pathological changes were observed on postmortem examination. The range of the drug amount was 20 to 200 mg/capita, dog.

Oral administration of 60 mg single dose was also effective in eliminating the dog hookworm without giving any side-effect.

II) Human hookworm

Materials and Methods:

Parenteral administration:

82 cases of hookworm infection were treated in the present study. 20% 2,6-diiodo-4-nitrophenol solution(200 mg/c.c Keun-il co.) in single dose was injected percutaneously on upper arm or gluteal region in 13 to 70 years old age groups.

No.1-8 were the group who had ordinary lives at their homes, No.9-25 were the patients admitted to ward for other diseases(Table 2), and No.26-82 were out-patients who visited the clinic due to some other physical trouble.

The stool examinations were done by brine floatation method 2 to 3 weeks after the single injection in Nos. 1-24, and 7-180 days in 26-27. Pulse and blood pressure were checked just before and 5 minutes after the injection. Urine protein and urobilinogen were examined before and 5 days after the injection.

Oral administration:

60 mg of 2,6-diiodo-4-nitrophenol tablet was given to hookworm infected cases.

The duration of administration was from 1 to 4 days and each day 360 mg was given in three divided doses between meals.

The period of stool examination after the treatment varied because the subjects were out-patients. The other examinations were all the same as with parenteral use.

Results:

65 out of 82 hookworm positive cases (79%) in parenteral administration became egg-negative on stool examination within 2 to 3 weeks after the injection. No. 13 continued eggpositive until the 14 th day after the drug administration, although the egg counting method was not applied.

Table 2. Parasitological and clinical examination of hookworm infected cases who were injected with 1 c.c. of 20% 2,6-diiodo-4-nitrophenol solution

No.	age	sex	eggs in faces		pulse/min.		blood pressure/mmHg		urineprotein		urine urobili.		chief complains before injection
			before	after	before	after	before	after	before	after	before	after	
1	60	F	800(EPG)	—									health
2	34	F	600 //	—									//
3	19	F	400 //	—									//
4	44	M	200 //	—									//
5	70	M	1400 //	—									//
6	42	F	600 //	—									//
7	41	M	600 //	—									//
8	23	F	8400 //	—									//
9	58	M	+	—	70	80	140-90	150-90	—	—	—	—	dyspepsia
10	32	F	+	—	80	80	120-80	140-90	—	—	—	—	dyspepsia
11	30	F	+	—	84	92	130-80	110-70	—	—	+	+	liver cirrhosis
12	30	F	+	+	92	92	150-110	140-100	++	++	—	—	nephritis
13	19	M	+	—	84	88	130-90	130-90	—	—	—	—	gastritis
14	54	M	+	—	120	120	100-70	100-70	+	+	+	+	heart failure
15	30	F	+	—	80	88	120-80	100-80	—	—	—	—	dyspepsia
16	45	F	+	—			130-80	110-80	++	++	—	—	nephritis
17	49	F	+	—			130-70	150-80	—	—	—	—	gastritis
18	35	F	+	—	100	112	110-75	120-80	—	—	—	—	neuritis
19	62	M	+	—	80	80	130-90	150-100	—	—	—	—	gastritis
20	35	F	+	—	88	88	130-100	160-100	—	—	—	—	dyspepsia
21	33	F	+	—	84	84	110-80	110-80	—	—	—	—	neurasthenia
22	26	M	+	—	80	80	120-95	140-100	—	—	—	—	dyspepsia
23	37	F	+	—	100	120	120-80	120-80	—	—	—	—	neurasthenia
24	26	M	+	—									bronchial asthma
25	19	F	+	—									anemia
26	32	M	+	—									ancylostomiasis
27	28	M	+	—									anemia
28	13	M	+	—									gastritis
29	14	F	+	+									gastritis
30	20	M	+	—									enteritis
31	46	M	+	—									bronchitis
32-82			+(50)**	+(15)**									

Nos. 1-8 from the model village in Koyang

Nos. 9-31 from the Southern Seoul City Hospital

Nos. 32-82 from the Eastern Seoul City Hospital

* The examination was done 174 days after the infection

** The number in parenthesis is the hookworm positive cases.

No systemic damages nor aggravation were observed in any case by the application of the drug, even in the cases of hepatic, cardiac, respiratory, kidney and psychosomatic troubles.

In every case, slight to severe local pain was followed by injection. for 2 to 5 minutes. No. 13 complained of paralysis on the same side of right leg for 3 days. No. 28 complained the same side-effect for three months showing progressive

atrophy of 1,2 finger on the same side arm, and could not be confirmed whether it was recovered because he did not visit any more. No. 32 complained of mild paralysis on the same side of arm which continued for one month.

71% of the oral administration became negative, It is uncertain whether the reason was due to only one day administration or the short duration after the treatment. No manifestation of liver

and kidney damage was observed, and the side effects were almost null or transient.

Summary

Single dose(1.0 c.c) of 20% 2,6-diiodo-4-nitrophenol solution was injected to 82 hookworm infected cases and 79% of them became egg-negative within 2-3 weeks after the injection. Mild to severe local pain were experienced for several minutes in all cases. Two cases complained of paralysis of the arms of the same side. The tablet of the same formula was given orally to 21 cases for 1-4 days. The 2-4day course with 360mg/day were enough to eliminate the parasite, and

showed no systemic side effects.

Comment

The minimum curative dosage of the chemical shall be the next subject to be solved. During the present survey, M. J. Cho, one of authors, applied the DNP solution to hookworm infected children aging 5-11 years old with the half dose (0.5 cc, 20% DNP solution) and resulted 76% elimination.

He also observed another 22 cases(4-10 years old), of hookworm infection who took the 60-240 mg tablet orally in total, resulted 50% negative conversion.

Table 3. Oral administration of 2,6-diiodo-4-nitrophenol to hookworm infected cases

No. of cases	age yr.	days of therapy (360 mg/capita)	stool examination			chief complaints before injection	protein(U) urob.(U)				side effects
			before	after	days*		B.	A.	B.	A.	
1	46	1	+	+	7	helminthiasis secondary anemia	-	-	-	-	-
2	32	3	+	-	16	neuralgia	+	-	-	-	-
3	33	2	+	-	14	gastritis	-	-	-	-	-
4	34	4	+	-	20	gastritis, anemia	±	±	-	-	-
5	54	3	+	-	18	ulcerative colitis	±	-	+	-	-
6	19	2	+	-	37	helminthiasis	-	-	-	-	-
7	27	2	+	-	37	gastroenteritis	-	-	-	-	-
8	33	4	+	-	16	minimal tb.	±	±	+	-	-
9	37	3	+	-	15	duod. ulcer	±	±	-	-	nausea, diarrhea
10	23	4	+	-	30	helminthiasis et duod. Ulcer	+	-	-	-	-
11	34	4	+	-	25	nephritis	±	-	-	-	-
12	50	4	+	-	27	bronchitis	+	+	+	-	-
13	34	2	+	-	15	hyperacidity	+	+	-	-	-
14	39	2	+	-	21	duod. ulcer	±	-	-	-	indigestion
15	39	2	+	-	20	nephritis	±	+	+	+	headache
16	34	2	+	-	25	heart neurosis	±	-	-	-	-
17	42	2	+	-	20	pellagra	+	+	+	+	-
18	40	2	+	-	15	neuralgia	+	+	-	-	-
19	20	2	+	-	17	gastric ulcer	±	+	-	-	epigastric pain
20	45	2	+	-	14	duod. ulcer	-	+	-	-	-
21	34	2	+	-	18	bronchitis, gastritis	+	-	-	-	-
22-40			+	+							
41-72			+	-							

Remarks: Protein(U).....Protein in Urin

B. ....Before

Urob(U)....urobilinogen in urin

A. ....after

*Oral administration was carried on mainly in Suwon Christian Hospital by one of the author: Dr. Y.H.Roh

References

1. Koutz, F.R. & Groves, H.F.(1961): *Preliminary*

report on the anthelmintic properties of Disophenol on hookworms (Ancylostoma caninum and Uncinaria stenocephala) of dogs. p. 14(2), 35-37,

2. Wood, I.B., Pankavich, J.A., Wall ACE WS, Thorson, R.E., Burkhart, R.L., Waletzky, E.(1961): *Disophenol, and injectable anthelmintic for canine hookworm. J. Amer. Veter. Med. Associ.* 139(10); 1101-1105.
3. Wood, I.B. and Pankavich, J.A.(1961): *The differential susceptibility of the hookworm, Ancylostoma caninum, during its maturation in dogs to the anthelmintic action of disophenol. J. of Parasit.* 47 (4. sect. 2) 20
4. Darne, A. and Webb, J.L.(1964): *The treatment of Ancylostomiasis and of spirocercosis in dogs by the new compound, 2,6-diiodo-4-nitrophenol. Veterinary Record*, 76(6); 171-172
5. Huang, S.W. and Khaw, O.K.(1965): *Experimental treatment of Clonorchiasis in cats and dogs with Disophenol. Chinese Med. J. Republic of China.* 12:68-79,

> 國文抄錄 <

2, 6-Diiodo-4-Nitrophenol(DNP)의 鉤虫驅虫實驗成績

延世大學校 醫科大學 寄生蟲學教室

蘇鎮璋・趙武駿・盧廣熙・李在興

1. 犬鉤虫感染成犬 8마리中 6마리에 3.5% DNP 液을 單回 0.6~6.0 c.c. 皮下注射하였고 2마리에는 60 mg 를 單回量 經口投與하였는데 全例 完全驅虫되었고 小腸, 肝, 腎에서 特異한 病理的 所見을 볼 수 없었다.
  2. 82 名의 十二指腸虫感染者에 20% DNP 液 1.0 c.c.를 單回皮下注射하여 79%의 驅虫成績을 올렸고 21 名에는 DNP 粉末 360 mg 를 一日量으로 하여 1~4 日 繼續經口投與하였던바 1 日投與 1 例를 除外하고 全例에서 完全 陰轉成績을 얻었다.
- 全例 注射局部에 數分間의 疼痛을 느꼈고 그中 2 例는 注射側上肢의 麻痺症勢를 呼訴하였으나, 腎, 肝機能에는 異常이 없었다.