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Experimental chemotherapeutic effects of niclofolan (Bayer 9015, Bilevon) on the animals infected with *Paragonimus westermani* or *P. iloktsuenensis*

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INTRODUCTION

Paragonimiasis has been known as a medically important endemic disease linked with eating habit of fresh water crustaceans, particularly in Far East Asian countries for a long time.

Bithionol is the drug of choice in paragonimiasis and has been used extensively in the past 15 years. However, its multiple doses and its side reactions are hampered in mass treatment with bithionol in the field. Therefore, it is desirable to find out new nontoxic drug which is used against paragonimiasis with a single dose.

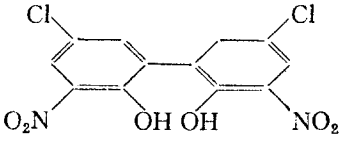
Recently, Rim (1975) proved that niclofolan was highly effective for human paragonimiasis with a single dose of 2.0 mg/kg of body weight, and it was also effective experimentally infected dogs and cats with *P. westermani*. And he also proved that niclofolan was highly effective for *Clonorchis* infection in both experimentally infected animals and clinical cases (Rim, 1975; Rim et al., 1975).

The first study on the anthelmintic effects of niclofolan was done by Kuttler et al.(1963) who reported the effectiveness with a single dose of niclofolan in sheep. After that, niclofolan has also been reported to be highly effective against *F. hepatica* infection in sheep with a dose of 3 to 8 mg/kg of body weight (Lee et al., 1966; Boray et al., 1967; Hildebrandt, 1968; Güralp, 1969) and similarly, in cattle when a single dose of 2 to 6 mg/kg was given orally or 3 to 4 mg/kg injected intraluminally (Gründer and Redlich, 1967).

The present studies were undertaken to evaluate the therapeutic results of niclofolan in rats experimentally infected with *P. iloktsuenensis* and to compare the therapeutic effects of *P. westermani* with that of *P. iloktsuenensis* in the experimentally infected animals.

MATERIALS AND METHODS

Drug used: The tested compound is known by the investigational name BAY 9015 and the generic names niclofolan and menichlopholan. The chemical formula is 5,5′- dichloro-2,2′- dihydroxy-3,3′- dinitrobiphenyl. It is an almost tasteless and odorless, yellowish crystalline substance, practically insoluble in water but soluble in organic solvents. The structural formula of the compound is the following:



Drug used was prepared with a suspension of niclofolan in Tween 80. The drug was instilled directly into the stomach by tube.

Infection of animals: Dogs, cats and rats were served as experimental hosts. Four dogs weighing from 5 to 8 kg were infected with 63 to 69 metacercariae of *P. westermani*. Five cats to about 1 kg of body weight were infected each with 30 metacercariae of *P. westermani*. On the other hand, five rats weighing about 150 gm were given each with 10 metacercariae of *P. iloktsuenensis*.

In dogs experimentally infected with *P. westermani*, niclofolan was administered on the 74 th day after infection in 2 dogs with the doses 3×1.0 mg/kg and 2×2.0 mg/kg body weight respectively, and the other 2 dogs were not medicated as control. Among five experimentally infected cats, 4 cats received niclofolan on the 59th day after infection at the dose of 2.0 mg/kg body weight one or two times, and one being as control was not medicated. In the rats experimentally infected with *P. iloktsuenensis*, 4 rats received the niclofolan at the dose rates of 1.0 mg/kg or 2.0 mg/kg body weight on the 50th day after infection, and one was served as control. All the animals were sacrificed after treatment and examined pathologically and parasitologically.

The efficacy was evaluated by the number of worms detected and their vital condition at necropsy 7 to 61 days after the last medication.

RESULTS

Effect on the infected dogs with *P. westermani*: The dog infected with 64 metacercariae of *P. westermani* was given 1.0 mg/kg of niclofolan on each of 3 successive days from the 74th day after infection. The dog was sacrificed on the 7th day after the last medication. Only one worm was found alive (Table 1). And 5 worm-cysts which contained the necrotic masses of the worm were found in the lungs. Daily dose of 2.0 mg/kg of niclofolan was given for 2 successive days to the dog from 74 days after infection with 63 metacercariae of *P. westermani*. The dog died of the pneumonitis on the 61st days after the last medication of niclofolan. At necropsy, neither living or dead worm was found in the lungs, however several localized foci found in the lungs seemed to be the fibrous granulations and with many *Paragonimus* eggs. Two of the control dogs which one with 63, the other with 69 metacercariae were infected respectively were also sacrificed on the 59th and the 128th day after infection. 47 and 57 living worms were found in their lungs respectively.

The worm-cysts were filled with the necrotic masses with many eggs of *P. westermani* and localized by the fibrous granulation tissues. Around the worm-cyst, granuloma were found with the giant cells of Langhans and foreign body types. Some of these engulfed the ova. The

Table 1. Results of treatment with niclofolan on 4 dogs experimentally infected with *Paragonimus westermani*

Doses	No. of metacer. infested	Days to treatment after infection	Days from treatment to autopsy	No. of worms recovered at autopsy
Control	63	59	—	47
Control	69	128	—	57
1.0 mg/kg×3 daily	64	74	7	1
2.0 mg/kg×2 daily	63	74	61	0

Table 2. Results of treatment with niclofolan on 5 cats experimentally infected with *Paragonimus westermani*

Doses	No. of metacer. infested	Days to treatment after infection	Days from treatment to autopsy	No. of worms recovered at autopsy
Control	30	66	—	14
2.0 mg/kg×2 every other day	30	59	7	0
2.0 mg/kg×1	30	59	7	4
2.0 mg/kg×2 every other day	30	59	7	2
2.0 mg/kg×1	30	59	7	0

Paragonimus ova were also seen scattered in the lung tissue accompanying plasma cells, lymphocytes and occasionally brown hemosiderine pigments. The surrounding lung tissue showed hemorrhagic pneumonitis.

Effect on the infected cats with *P. westermani*: Out of 5 cats infected with 30 metacercariae of *P. westermani*, 2 were given niclofolan at the dosage of 2.0 mg/kg of body weight for 2 times on alternate days and the other 2 were given niclofolan with a single dose of 2.0 mg/kg of body weight. All cats including control were sacrificed on the 7th day after the last medication. The findings at necropsy are shown Table 2. As the result, 14 living adult worms were found in the control cat which was not drug administered. Four living adult worms were found in the cat received a single dose of niclofolan 2.0 mg/kg and 2 degenerated dead worms were found from the cat received 2 doses of niclofolan on alternative days. On the other hand, neither living nor dead worm was found at necropsy in the cats received a single dose or 2 doses on alternative days. But there were several worm-cysts filled with the necrotic masses of the degenerated worms.

The cut surfaces of the worm-cysts were usually oval or rectangular in shape. Within these cystic spaces there are dark brownish necrotic masses with many eggs of *P. westermani*. The lung tissue surrounding the cyst was generally dark and hemorrhagic. The dead worms showed severe cloudiness and degenerated atrophy of the bodies. The uteruses of these dead worms were all undistinguished and no eggs were found them. Noticeable atrophy of the integuments and lysis or necrosis in the parenchymal cells of ovaries and testes were observed.

The pathological change in the foci of the lungs were very similar as those of the treated dogs with niclofolan.

Effect on the infected rats with *P. iloktsuenensis*: Five experimental rats were infected with 10 metacercariae of *P. iloktsuenensis* equally. Each 2 rats were given 1.0 mg/kg body weight with 2 or 3 doses and 2.0 mg/kg body weight with a single or 2 doses of niclofolan respectively and they were sacrificed 2 weeks after medication. The control rats without treatment were also scarificed at almost the same time. The number of the survival worms discovered from the treated rats and that of the control rat was compared as shown in Table 3.

In the dose of 1.0 mg/kg body weight, 2 dead worms were found from the rat which received niclofolan daily for 3 days, but no worm was found in the rat which received 2 doses of alternative days. On the other hand, 3 living worms were found in the rat which was given a single dose of niclofolan 2.0 mg/kg body weight. However, 2 worm-cysts in which necrotic masses of the degenerated worms were found in the rat received 2 doses of 2.0 mg/kg body weight by alternative days. In the control rat, 6 living adult worms were found in the lungs.

Table 3. Results of treatment with niclofolan on rats experimentally infected with *Paragonimus iloktsuenensis*

Doses	No. of metacer. infested	Days to treatment after infection	Days from treatment to autopsy	No. of worms recovered at autopsy
Control	10	67	—	6
1.0 mg/kg×2 every other day	10	50	15	0
1.0 mg/kg×3 daily	10	50	14	2
2.0 mg/kg×1	10	50	17	3
2.0 mg/kg×2 every other day	10	50	15	0

In examination of the rat materials, grossly the lungs showed slightly elevated and dark reddish brown, moderately firm masses on the surface of the lungs. Many *P. iloktsuenensis* ova with or without yolk content were visible in the cystic lumen, along with ceratinized materials, dark coarsely granular debries and brown hemosiderine pigments. The ova were also seen scattered in the lung tissues accompanying plasma cells, lymphocytes and eosinophils. The inner surface of the cystic space was lined by stratified squamous epithelium instead of ciliated columnar epithelium. The granuloma were found in the vicinity of the cysts, along with giant cells of Langhans and foreign body types. However, it is evident that the pathological changes were seen in much the same way as those in dogs and cats.

The special attentions were paid to the side effects during the course of treatments in all animals. All the animals treated with niclofolan in the present study were not affected in their appetite and body weight during the course of treatment.

DISCUSSION

Emetine hydrochloride had been reported as an effective drug by Ikeda(1915) and had been believed to be slightly effective and the drug of choice until 1960. However treatment was followed by a higher recurrence rate and had considerable toxicity. Ro and Yokogawa (1941) reported the superior efficacy of emetine when it was used in combination with prontosil. Chloroquine was reported by Chung et al. (1954), who stated that the chloroquine attained a high concentration in the lungs and had an effect on emetine resistant cases. However according to a following study by Sadun and Buck(1960), only 12.5 per cent of 116 treated patients became egg negative 6 months after the treatment.

In 1961, Yokogawa and his colleagues have shown the efficacy of treating human paragonimiasis with bithionol (2, 2′-thio-bis-4, 6-dichlorophenol), administered orally in the daily dose of 2.0 to 2.5 gm (for adults), 1.5-2.5 gm on alternate days (for children) for 5, 10 and 15 times. All cases treated were cleared of evidence of the infection and no relapses had occurred a year following chemotherapy(Yokogawa et al., 1961 a & b, 1962). The side effects of some gastrointestinal disturbances and skin eruptions resulting from administration of the drug were only temporary. Complement-fixation tests and chest X-ray films became negative, indicating eradication of the disease. Subsequently, extreme investigations of bithionol in human cases(Yokogawa et al., 1963 a,b; Katamine, 1961; Miyazaki, 1961; Kang, 1962 and Chyu et al., 1964) have strongly indicated that the drug is promising the therapeutic agent, showing a high efficacy and lower toxicity compared with the other drugs previously used. Therefore, bithionol is the drug of choice in the paragonimiasis and has been used extensively in the past 15

years. However, its multiple doses and its toxicities are hampered in mass treatment with bithionol in the field.

Recently, Rim (1975) proved that niclofolan was highly effective for human paragonimiasis at a single dose 2.0 mg/kg body weight, and also he proved that niclofolan was highly effective for *Clonorchis* infection in both experimentally infected animals and clinical cases.

Extensive experimental research on niclofolan has done with *F. hepatica*. Stašaitis and Kublickiene(1974) reported that at 48 hours after treatment of 5 week old immature stages of *F. hepatica*, only traces of RNA in the sub-integument was detected and the amount of DNA in the intestinal cell nuclei was reduced. According to Andrews(1975) biochemical enzymatic studies have revealed that niclofolan induced adenosine triphosphate activity in the mitochondria of the livers of rats to the same extent as disophenol(van Miert and Gremeveld, 1969). In histochemical investigations Panitz and Knapp(1970) found that niclofolan inhibits tricholinesterase activity and that there is also evidence of an inhibiting effect of succinic dehydrogenase.

Niclofolan has remarkable therapeutic effect on *Paragonimus* infection in animals by killing the flukes in the lungs. This destructive activity of niclofolan and pathological changes in the lungs are similar to that of bithionol(Yokogawa et al, 1961).

Therefore, it can be concluded from the present study that the administration of niclofolan in daily 3 doses of 1.0 mg/kg body weight or in two doses of 2.0 mg/kg body weight were the most effective against *P. westermani* in dogs and cats, and against *P. iloktsuenensis* in the rats.

SUMMARY

The new drug niclofolan (Bayer 9015, Bilevon), 5,5′-dichloro-2,2′-dihydroxy-3,3′-dinitrobiphenyl, has been used in the treatment of *Paragonimus westermani* in dogs and cats and *P. iloktsuenensis* in rats.

It was proved that administration of daily dose of 1.0 mg/kg body weight for 3 days or in 2 doses of 2.0 mg/kg body weight by alternate days were evidently effective for the infected dogs, cats and rats with the lung flukes, and toxic manifestations were not found.

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=國文抄錄=

肺吸蟲(*Paragonimus westermani* 및 *P. iloktsuenensis*) 感染 實驗動物에 있어서의 niclofolan (Bayer 9015, Bilevon)의 治療効果에 관한 硏究

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1961년 이래 肺吸蟲症 治療劑로서 bithionol이 현재까지 사용되어 왔으나, 그 副作用과 投藥期間이 길어 실제에 있어서 集團的으로 投藥하기 곤란하였다. 따라서 bithionol 이외의 副作用이 적고, 單時日에 治療가 可能한 藥劑의 開發이 요구되던 중 최근 niclofolan 2.0 mg/kg 1회 服用으로 肺吸蟲에 대한 治療效果가 특히 優秀하다는 것이 알려졌다.

著者는 肺吸蟲을 感染시킨 實驗動物에 있어서 niclofolan의 治療效果를 보기 위하여 實驗을 실시하였다. 4마리의 개에 각각 *P. westermani*의 被囊幼蟲 63~69個를 먹여 感染시켰고, 5마리의 고양이에 *P. westermani*의 被囊幼蟲 각각 30개씩 먹여 感染시켰다. 그리고 *P. iloktsuenensis* 被囊幼蟲 10個씩을 5마리의 쥐에 각각 感染시켰다. 개는 感染시킨지 74日, 고양이는 59日 및 쥐는 50日만에 각각 niclofolan 1.0 mg/kg 및 2.0mg/kg를 1~3일간 여러가지 方法으로 投與한 바, 모두 顯著한 殺蟲效果를 나타내었다.