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Research Note

Anthelmintic Effects of Bithionol-Antibody Complex on *Paragonimus westermani* Juveniles

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Bithionol, 2, 2'-thiobis (4, 6-dichlorophenol), has been used for the treatment of paragonimiasis (Yokogawa *et al.*, 1961a; Yokogawa *et al.*, 1961b). However, the anthelmintic drug gave rise to some side effects (Yokogawa *et al.*, 1961b). Therefore, for chemotherapy with available anti-worm drug, the present study was attempted to demonstrate whether anti-neutral thiol protease immunoglobulin-bithionol (Anti-NTP IgG-Bithionol) does specifically possess anthelmintic effect on *Paragonimus westermani* juvenile worms *in vitro* and *in vivo* as shown in oncostatic-antibody complexes (Dullens and Weger, 1980).

Metacercariae of *P. westermani* (triploid type) used in these experiments were isolated from the crabs, *Eriocheir japonicus* collected on the Tsushima Is., Japan. The excysted young flukes were removed from the cysts soaked in the Ringer's solution containing 0.1% sodium bicarbonate at 37°C. The neutral thiol protease of the larvae was purified by affinity chromatography on arginine-Sepharose CL-4B, gel filtration on Ultrogel AcA-54 and DEAE-toyopearl S column chromatography according to the procedure of Yamakami and Hamajima (1987).

Anti-neutral thiol protease immunoglobulin (anti-NTP IgG) as bithionol-carrier used in these experiments was prepared by the method of Hamajima *et al.* (1985) with some modification. Nineteen nmole portions of bithionol were coupled, respectively, to one milligram of anti-NTP IgG and of normal IgG, after each IgG were activated with carbonylating reagent, according to the procedure of Bethell *et al.* (1979). The inhibition effect of these drugs on *P. westermani* newly excysted juvenile worms were examined by observing the motility of 10 juvenile worms in 0.1 ml portion of culture medium (RPMI 1640, 90 μl and saline 10 μl) with penicillin G potassium (100 i.u./ml) and streptomycin (100 μg/ml) at U-bottom wells of assay plate for micro test, and the evaluation of the effect of the drug complexes on the motility of the worms *in vitro* was based on perfect stillness of the worms, and was expressed by time (hr) required for the effect as shown by Hamajima *et al.* (1979). The effect of the drugs on the juvenile worms *in vivo* was examined by the number of the worms recovered from ddy female mice weighing 30 g inoculated orally with 10 metacercariae of the worms per mouse at 30 days after the injection with the drugs.

The survived juvenile worms in the mice were obtained from the abdominal and pleural cavities, and from the abdominal wall, the intrapleural wall, the diaphragm of the minced muscles and the other organs soaked in Ringer's solution with penicillin G potassium (200 i.u./ml) and streptomycin (100 μg/ml) warmed at 37°C for 12 hours. The juveniles were observed under a binocular dissecting microscope. Deviation from the mean was expressed as a standard deviation, and Student's test was used to estimate the data of statistical significance. Differences between the number of the juvenile

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Table 1 Motility of *Paragonimus westermani* juvenile worms treated with bithionol-antibody complexes *in vitro*

Treatment	Time required for effect (hours) Mean±SD	probability
Anti-NTP IgG-Bithionol	12± 0.2	——
Anti-NTP IgG	162±18.4	<0.001
Normal IgG-Bithionol	18± 3.0	<0.01
Normal IgG	348±55.4	<0.001
Bithionol	16± 3.0	<0.05
Saline	624±36.1	<0.001

Each value represents the mean of six determinations in two experiments. The abbreviation is the standard deviation (SD).

worms recovered from mice injected with the drugs and that from control ones were considered to be significant when $P < 0.05$.

As shown in Table 1, the times required for the inhibition effect of anti-NTP IgG-bithionol (0.1 mg protein), normal IgG-bithionol (0.1 mg) and bithionol (1.9 nmoles) on the motility of *P. westermani* newly excysted juvenile worms in the culture medium (0.1 ml) were shorter than did anti-NTP IgG (0.1 mg protein), normal IgG (0.1 mg) and saline solution (0.01 ml) as control, respectively ($P < 0.001$). In particular, anti-NTP IgG-bithionol exhibited a more rapid inhibition effect on the worms than did normal IgG-bithionol and bithionol ($P < 0.05-0.01$). Thus, anti-NTP IgG-bithionol was found in this experiment to be effective in inhibiting the motility of the worms *in vitro*. As shown in Table 2, all mice were infected with the worms. However, the total number of the worms recovered in six mice was lower than that of saline solution (0.1 ml) as control when anti-NTP IgG-bithionol (3 mg), normal IgG-bithionol (3 mg) and bithionol (57 nmoles) were injected in the mice ($P < 0.05-0.01$). While, the reduction rate of the worms was higher than those of normal IgG-bithionol and bithionol when anti-NTP IgG-bithionol was injected in the mice ($P < 0.05$). Thus, anti-NTP IgG-bithionol was found in this experiment to exert its anthelmintic effect in mice.

From these results, it seems likely that the inhibition effect on the worm motility *in vitro* and the reduction of the worm recovery *in*

Table 2 Number of *Paragonimus westermani* juvenile worms recovered from mice inoculated with 10 metacercariae and injected with bithionol-antibody complexes at 2 days* and 4 day† after the infection

Injection	No. of mice examined	Total no. of the worms recovered and the recovery rate (%)	% worm reduction compared to control	probability
Anti-NTP IgG-Bithionol	6	33 (55)	37	——
Anti-NTP IgG	6	43 (72)	17	<0.05
Normal IgG-Bithionol	6	41 (68)	21	<0.05
Normal IgG	6	42 (70)	19	<0.05
Bithionol	6	41 (68)	21	<0.05
Saline	6	52 (87)	0	<0.01

Each datum was based on six mice examined.
* intraperitoneal injection, † caudal intravenous injection

vivo were occurred by contact with bithionol molecule of the conjugates and the drug to the worms, respectively. Moreover, it seems probable that the bithionol molecule of anti-NTP IgG-bithionol did effectively react against the worms, in the fact that the conjugate was able to bind to the worms carrying the corresponding antigen by antibody-antigen interaction both *in vitro* and *in vivo*. Similar result was obtained by Garnett *et al.* (1983) for the cytotoxicity of a drug-carrier-antibody conjugate on the human osteogenic sarcoma cell line *in vitro*. In addition, Kanellos *et al.* (1985) reported identical result on the inhibition of methotrexate-monoclonal antibody conjugates on the growth of human colon carcinoma *in vitro* and *in vivo*.

On the other hand, the injections of anti-NTP IgG (3 mg) and normal IgG (3 mg) were found in this experiment to be more effective than that of saline solution (0.1 ml) for the reduction of the number of the worms in the mice ($P < 0.05$). This result is similar to that reported in the reduction of the number of *Schistosoma japonicum* worms recovered from mice injected with normal and immune sera of rabbits in comparison with the saline solution (Sadun and. Lin, 1959). From these results, it seems likely that polymorphonuclear leucocyte, macrophage and lymphocyte migrations for the phagocytosis *in vivo* could be mediated by a certain immunoglobulin-derived chemotactic factor produced by these immunoglobulins and inflammatory neutral SH-dependent protease from polymorphonuclear leucocytes migrated by *P. westermani* metacercarial protease (Hayashi *et al.*, 1974; Hamajima and Yamakami, 1987). On the basis of these results, it is suggested that the cytotoxicity of the conjugate against the juvenile worms may probably be brought by the attachment of the bithionol molecule to the worms, and by the increase of phagocytic cells to destroy the worms owing to immunoglobulin-derived chemotactic factor.

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