Natural Medicines 57 (1), 7-11 (2003)

A New Method for Identifying Potential Remedies for Larva Migrans using Crude Drug Extracts (I)

Tadaaki Satou,[a] Nobuaki Akao,[b] Kazuo Koike,[a] Ie Watanabe,[a] Koichiro Fujita,[b] Tamotsu Nikaido*,[a]

[a]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

[b]Section of Environmental Parasitology, Division of Public Health, Graduate School of Tokyo Medical and Dental University, 1-5-45 Yushima Bunkyo-ku, Tokyo 113-8519, Japan

(Received September 4, 2002)

Parasitic diseases, which affect many people all over the world, have been studied extensively. However, identification of drug treatments for larva migrans of the dog roundworm, *Toxocara canis*, has been unsuccessful. In the present study, we conducted *in vitro* screening of potential treatments for larva migrans infections using a new testing standard that evaluates nematocidal, immunologic enhancement, and cytotoxic activity of the drugs *in vitro* considering in the targeted host. We examined 36 crude drugs extracted by methanol and water using this evaluation concept. As a result, eight methanol extracts and one water extract at a concentration of 0.1 mg/ml were selected as drug candidates.

Key words: *Toxocara canis*, crude drug, larva migrans

Toxocara canis infects 80% of dogs in tropical countries. Human infection occurs through accidental ingestion of the encapsulated egg of *T. canis* in soil, water, or food contaminated by the feces of infected juvenile dogs. Although the larva are initially locate in the small intestine, they may shift to other organs, including the putamen, without ever reaching the imago stage. The infection symptoms fall under two categories. Depending on the infected organ, visceral larva migrans (VLM) can cause fever, anorexia, splenohepatomegalia, skin exanthema, pneumonia, and asthma. The syndrome cures naturally in 6 - 18 months if egg intake stops. This type of infection rarely causes death, unless the brain or heart are infected. Ocular larva migrans (OLM), also called eye toxocariasis, rarely causes systemic symptoms. The OLM lesion is a granuloma reaction in the retina, which may cause visual deficit.[1)]

In the present study we initiated a search for drugs that treat *T. canis* infection because no drugs in current use are effective against this disease. Though the therapeutic effect is reported with albendazole, etc., it is not a cure.[2,3)] In albendazole, the following are reported: Examination by the rise in the solibility[4)] and by liposome with glucan.[5)]

In the meantime, in the search for parasitic disease drugs, drug candidates have traditionally been selected based on the strength of their nematocidal effects. However, this process may overlook candidates that have weaker nematocidal effect but have other beneficial effects. There exists an urgent need at present to accelerate drug discovery and therefore develop new search methodology. The method described in the present paper approaches drug discovery from a different angle, which evaluates nematocidal, immunologic enhancement, and cytotoxic activity of the drugs *in vitro*.

Materials and Methods

The parasite Second stage larvae of *T. canis* were collected using a previously described method[6)] and were maintained in Eagle's MEM1 medium (Nissui-Pharmaceutical) at 37°C. The medium was changed weekly.

Cell culture We used the mouse macrophage cell J774.1[7)] for analysis of immunologic enhancement, measuring nitrogen oxide and cytotoxicity in the activated

macrophages. These cells were maintained in RPMI 1640 medium (GIBCO BRL) containing 10% fetal bovine serum (Sanko-Junyaku) supplemented with L-glutamine, 100 units/ml penicillin (Meiji-Seika), and 100 μg/ml streptomycin (Meiji-Seika).

Anthelmintics The parasitic disease drugs albendazole (AZ), diethylcarbamazine (DC), ivermectin (IM), pyrantel pamoate (PP), and thiabendazole (TZ) were used in the present study for comparison. AZ, DC, and IM were obtained from Sigma. PP, an ascaridiasis remedy, was also obtained from Sigma. TZ, a toxocariasis remedy, was bought from Tokyo-Kasei. These compounds were dissolved in a small volume of ethanol (1%v/v).

Test crude drugs The following crude drugs (sample number, crude drug, species) were tested.

The drugs were prepared as follows. A 10 g sample of each drug was extracted twice using 100 ml hot methanol ("sample number" M). Alternatively, a 10 g sample was extracted twice using 100 ml hot water and then freeze-dried ("sample number" H). Table1 presents the plant source for each crude drug. These extracts were dissolved in a small volume of ethanol (1%v/v).

Table 1. The sample numbers of crude drug extracted in the methanol or water.

No.	Crude Drug	Species
1	檳榔子	*Areca catechu*
2	茵陳蒿	*Artemisia capillaris*
3	艾葉	*Artemisia* sp.
4	黄連	*Coptis japonica*
5	鶴虱	*Daucus carotra*
6	海人草	*Digenea simplex*
7	呉茱萸	*Evodia* sp.
8	茴香	*Foeniculum vulgare*
9	甘草	*Glycyrrhiza* sp.
10	薄荷	*Mentha arvensis* var. *piperascens*
11	黄柏	*Phellodendron amurense*
12	苦木	*Picrasma quassioides*
13	雷丸	*Polyporus mylittae*
14	使君子	*Quisqualis indica*
15	大黄	*Rheum* sp.
16	苦参	*Sophora flavescens*
17	山椒	*Zanthoxylum piperitum*
18	生姜	*Zingiber officinale*
19	紫苑	*Aster tataricus*
20	升麻	*Cimicifuga simplex*
21	桂皮	*Cinnamomum cassia*
22	山茱萸	*Cornus officinalis*
23	紫根	*Lithospermum erythrorhizon*
24	芍薬	*Paeonia lactiflora*
25	瓜呂実	*Trichosanthes kirilowii*
26	桑寄生	*Viscum* sp.
27	独活	*Aralia cordata*
28	射干	*Belamcanda chinensis*
29	陳皮	*Citrus unshiu*
30	紅花	*Carthamus tinctorius*
31	枳実	*Citrus* sp.
32	延胡索	*Corydalis turtschaninovii*
33	辛夷	*Magnolia* sp.
34	人参	*Panax ginseng*
35	瓜呂根	*Trichosanthes* sp.
36	釣藤鉤	*Uncaria* sp.

Nematocidal activity We used a modification of the method developed by Kiuchi et al.[6] For one assay, 20 second-stage *T. canis* larvae were incubated with a test solution in a 96-well flat-bottom plate at 37°C and the behavior of the larvae was observed under a microscope at 72 h after the start of incubation. Nematocidal activity was evaluated in terms of relative motility (RM): lower RM indicated stronger nematocidal activity. These values were calculated by averaging four motion scores.

Immuno-enhancement activity NO_2^- generation by activated J774.1 cells was measured using a method described previously.[8] J774.1 cells were washed and resuspended in the culture medium to 200 μl at 1 x 10^6 cells/ml, and this cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated in 5% CO_2/air for 2 h at 37°C. After the incubation, the medium was replaced by a medium containing 10 ng/ml lipopolysaccharide (LPS: WAKO). Recombinant murine interferon-γ (40 U/ml, IFN-γ: Funakoshi) and the extract samples (0.01 mg/ml, 0.1 mg/ml) were added to the cells. Absorbance at 550 nm was measured after 48 hours using isovolumic Griess reagent (1% sulfanilamide (Sigma), 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (Sigma) in 2.5% phosphoric acid), and compared to that of the control. The average absorbance from 10 wells was used to determine the NO_2^- generation rate.

Cytotoxicity J774.1 survival rates were used as indicators of cytotoxicity. After the NO_2^- generation rate was measured, the remaining cells were used to determine survival rate. These cells were washed and resuspended in the culture medium and evaluated using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetazolium bromide (MTT; Sigma) reduction assay.[9] Briefly, after termination of the cell culture, 10 μl of 5 mg/ml MTT in PBS was added to each well and the plate was incubated in 5% CO_2 / air for 4 h at 37°C. The plate

was read on a microplate reader at 550 nm. Cytotoxcity was determined by averaging the results from 10 assays.

Results and Discussion

In the present study, we examined the efficacy of extracts from 36 crude drugs that can be purchased in Japan. We selected two extraction methods (methanol and water). *In vitro* experiments were carried out using two concentrations (0.01 mg/ml and 0.1 mg/ml). Currently used drugs for treatment of *T. canis* larva migrans include AZ, TZ, IM, and DC. However, their therapeutic effects are limited. We used these drugs and PP (a human ascariasis drug) for comparison in our search for new drug candidates.

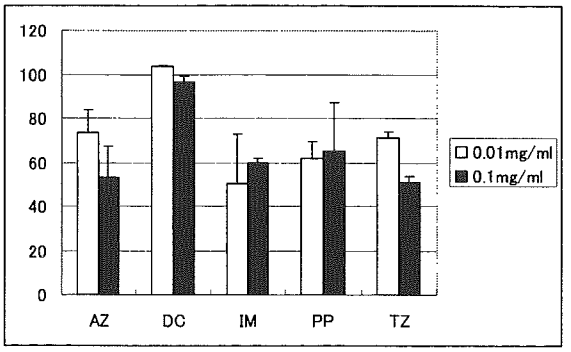


Fig.1 Nematocidal effects of existing anthelmintics at 0.1 mg/ml and 0.01 mg/ml using *T. canis* second-stage larvae. The vertical axis is the RM value (mean±SD %).

In our examination of nematocidal activity, we found that DC had no effect on the larvae. AZ, IM, PP, and TZ were also only weakly effective at both concentrations (Figure 1). AZ, which was the most prospective drug against this disease, exhibited an RM of 74±10% at 0.01 mg/ml, and 53±15% at 0.1 mg/ml. Drug candidates for treatment of *T. canis* larva migrans should not have adverse effects on the host, but instead they should enhance the host's immune system. In our search, we attempted to find a balance between nematocidal effects, cytotoxicity, and enhancement of the host's immune response. In order to widely choose the candidate, RM values below 80% were considered effective.

Based on RM values, none of the methanol and water extracts at 0.01 mg/ml satisfied the RM standard. (Figure 2, 3). However, the RM values for the 0.1 mg/ml of 1M, 2M, 3M, 4M, 5M, 7M, 8M, 9M, 10M, 13M, 15M, 17M, 18M, 21M, 28M, 29M, 32M, 33M, 1H were all lower than 80%. Especially, the RM values of extracts 2M, 7M, 18M, and 21M (0.1 mg/ml) were very low and all larvae died. The inhibition of the motion from these extracts greatly exceeded that from the available anthelmintics. The water extracts were less effective than the methanol extracts against the larvae. Only extract 1H (0.1 mg/ml) was selected as a candidate.

Therapeutic effects of nematocidal drugs are reportedly enhanced by combining albendazole and beta-glucan with immunomodulators.[10] Eosinophils, neutrophils, and macrophages initiate the anthelmintic response of the host organism. Eosinophils secrete the main basicity protein (MBP), which damages the parasite's exodermis. Neutrophils and macrophages then generate superoxide (O_2^-) and nitrogen monoxide (NO), to thwart the parasite. Therefore, effects on the immune system should be considered during drug evaluation, in addition to nematocidal activity. In the present study, we evaluated a drug's contribution to the immune system by analyzing the NO generation rate in J774.1 cells. The NO generation rate was estimated by measuring NO_2^- generation, which almost increases in parallel. First time, the above-mentioned results were compared to cytotoxicity (the cell survival rate) measurements. The extracts, that caused high cytotoxicity at concentrations, were eliminated from further consideration.

Cell survival, as determined by the MTT method, was used for the evaluation of cytotoxicity. Except for 0.1 mg/ml IM, the anthelmintics were all low cytotoxic (the cell survival rate was in the upper 50's%). However, high cytotoxicity was observed in response to 4M, 7M, 18M, 21M, and 32M (0.1 mg/ml). Therefore, these methanol extracts were eliminated from candidacy. Based on these results, 1M, 2M, 3M, 5M, 8M, 9M, 10M, 13M, 15M, 17M, 28M, 29M, 33M, and 1H were selected for further evaluation. An increase in the cell population was observed in response to 0.1 mg/ml 10M, 15M, and 28M.

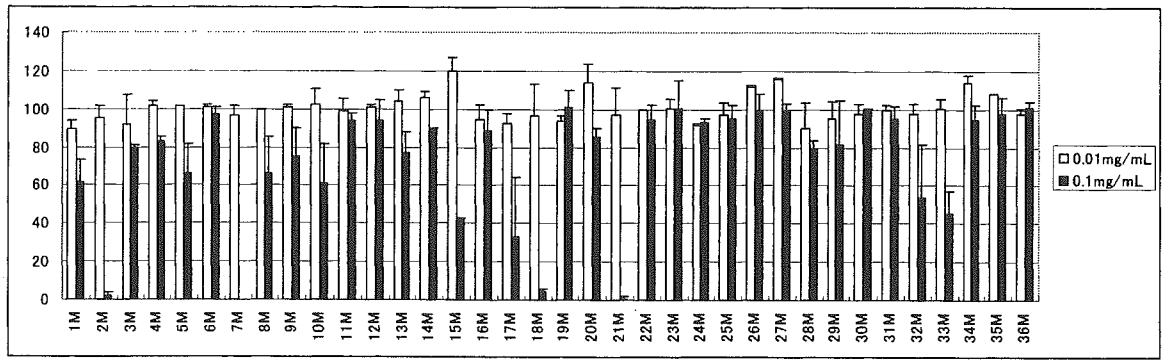


Fig.2 Nematocidal effects of crude drug methanol extracts (0.1 mg/ml and 0.01 mg/ml). The vertical axis is the RM value (mean±SD %).

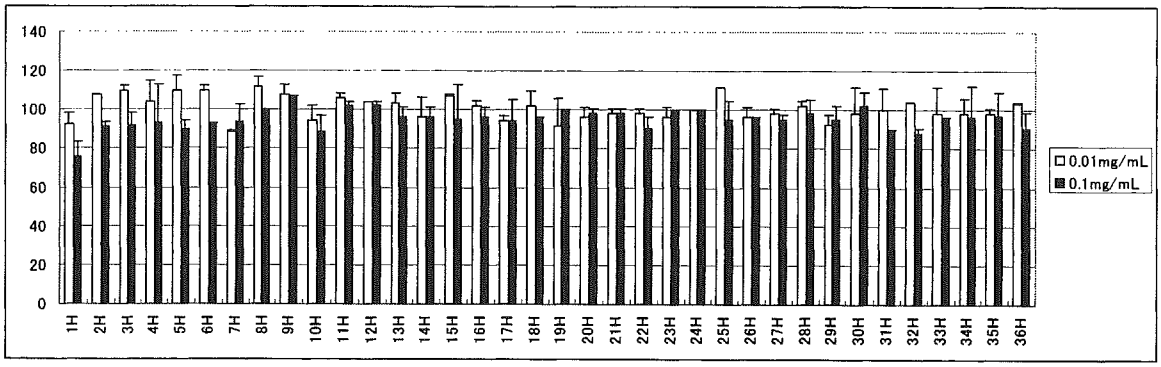


Fig.3 Nematocidal effects of crude drug water extracts (0.1 mg/ml and 0.01 mg/ml). The vertical axis is the RM value (mean±SD %).

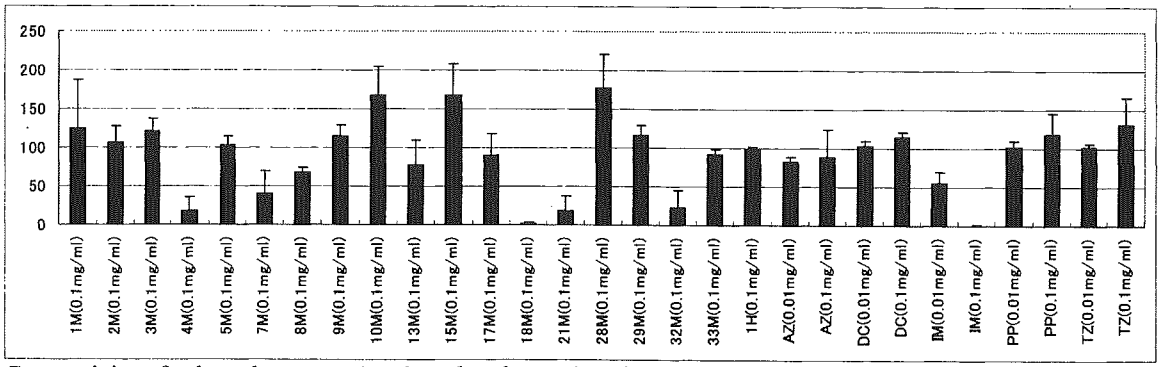


Fig.4 Cytotoxicity of selected extracts (methanol and water) and anthelmintics in the activated J774.1 cells. The vertical axis indicates the absorbance (mean±SD %) determined by MTT method.

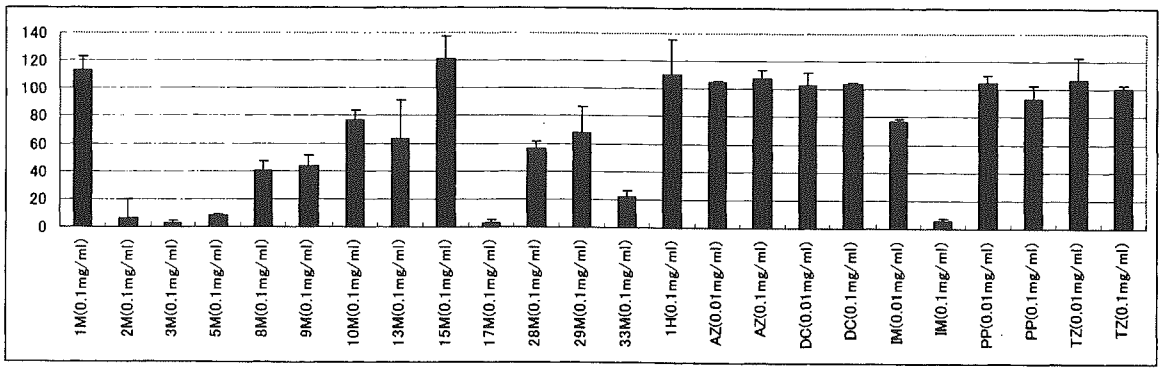


Fig.5 NO_2^- generation rate in activated J774.1 cells in response to selected methanol and water extracts. The vertical axis indicates the absorbance (mean±SD %) using by Griess reagent.

NO_2^- generation did not decrease in response to AZ (0.01 mg/ml and 0.1 mg/ml), DC (0.01 mg/ml and 0.1 mg/ml), IM (0.01 mg/ml), PP (0.01 mg/ml and 0.1 mg/ml) and TZ (0.01 mg/ml and 0.1 mg/ml). Because *in vivo* requirements for NO are unknown, we arbitrarily selected an NO_2^- generation rate of about 50%. As a result, extracts 1M, 8M, 9M, 10M, 13M, 15M, 28M, 29M, and 1H were selected as drug candidates.

The present approach identified several new drug candidates including these that could not be found using the conventional screening approach. However, the present study used only one cell type (J774.1), which limits extrapolation to *in vivo* responses. Therefore, in the following paper, we further refined candidate selection by evaluating the responses in additional cultured cells.

References and Notes

1) Obwaller A., Jensen JE., Auer H., Huber A., Kraft D., Aspock H., *Parasite Immunol.*, **20**, 311-317 (1998).
2) Abdel-Hameed AA., *Am. J. Vet. Res.*, **45**, 1430-1433 (1983).
3) Delgado O., Botto G., Mattei R., Escalante A., *Ann. Trop. Med. Parasite.*, **83**, 621-624 (1989).
4) Torrado S., Lopez ML., Torrado G., Bolas F., Torrado S., Cadorniga R., *Int. J. Pharm.*, **156**, 181-187 (1997).
5) Velebny S., Tomasovicova O., Hrckova G., Dubinsky P., Helminthologia, **33**, 181-186 (1996).
6) Kiuchi F., Miyashita N., Tsuda Y., Kondo K., Yoshimura H., *Chem. Pharm. Bull.*, **35**, 2880-2886 (1987).
7) These cells were obtained from RIKEN Cell Bank, Japan.
8) Ding AH., Nathan CF., Stuehr DJ., *J. Immunol.*, **141**, 2407-2412 (1988). Cunha FQ., Assreuy J., Xu D., Charles I., Moncada S., *Eur. J. Immunol.*, **23**, 1385-1388 (1993).
9) Sargent JM., Taylor CG., *Bri. J. Cancer*, **60**, 206-210 (1989).
10) Hrckova G., Velebny S., *J. Helminth.*, **75**, 141-146 (2001).

Natural Medicines 57 (1), 23–26 (2003)

A New Method for Identifying Potential Remedies for Larva Migrans using Crude Drug Extracts (II)

Tadaaki Satou,[a] Nobuaki Akao,[b] Kazuo Koike,[a] Ie Watanabe,[a] Koichiro Fujita,[b] Tamotsu Nikaido*,[a]

[a]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

[b]Section of Environmental Parasitology, Division of Public Health, Graduate School of Tokyo Medical and Dental University, 1-5-45 Yushima Bunkyo-ku, Tokyo 113-8519, Japan

(Received September 4, 2002)

In the present study, we conducted *in vitro* screening of potential treatments for larva migrans infections using a new testing standard that evaluates nematocidal, immunologic enhancement, and cytotoxic activity of the drugs *in vitro* considering in the targeted host. We measured cytotoxicity by the MTT method and examined immunologic enhancement of activated neutrophiles (differentiated HL60) to identify crude drug candidates. We examined 36 crude drugs extracted by methanol and water. As a result, three methanol extracts (*Areca catechu*, *Rheum spp.*, *Citrus unshiu*) and one water extract (*A. catechu*) were selected by this method. As a result, arecoline (ARE), a component of *A. catechu* was identified as a potent remedy. However, a comparison of the efficacy of the extract and ARE suggested the existence of an additional active component. And the effectiveness as a drug of these extracts was also shown.

Key words: *Toxocara canis*, crude drug, larva migrans, arecoline

In the previous paper,[1] we examined the efficacy of methanol and water extracts from 36 crude drugs. The previous study tested two concentrations of each extract (0.01 mg/ml and 0.1 mg/ml) *in vitro*. The assays evaluated anti-nematode activity using *T. canis* second-stage larvae, and cytotoxicity and immunologic enhancement using the mouse macrophage cell J774.1. The following extracts were selected as drug candidates: 1M, 8M, 9M, 10M, 13M, 15M, 28M, 29M, and 1H (0.1 mg/ml). However, the previous study used only one cell type (J774.1), which limits extrapolation to other *in vivo* responses. In the present study, we examined cytotoxic and immunologic enhancement activity using other cultured cell HL60.

Materials and Methods

The parasite Second stage larvae of *T. canis* were collected using a previously described method[2] and were maintained in Eagle's MEM1 medium (Nissui Pharmaceutical) at 37°C. The medium was changed weekly.

Cell culture cell We used human acute myeloid leukemia cells, HL60,[3] for analysis of cytotoxicity and immunologic enhancement (superoxide production). These cells were maintained in RPMI 1640 medium (GIBCO BRL) containing 10% fetal bovine serum (Sanko Junyaku) supplemented with L-glutamine, 100 units/ml penicillin (Meiji Seika), and 100 μg/ml streptomycin (Meiji Seika).

Test compounds The parasitic disease drugs albendazole (AZ), diethylcarbamazine (DC), ivermectin (IM), pyrantel pamoate (PP), and thiabendazole (TZ) were used in the present study for comparison. AZ, DC, and IM were obtained from Sigma. PP, an ascaridiasis remedy, was also obtained from Sigma. TZ, a toxocariasis remedy, was bought from Tokyo Kasei. ARE hydrobromide was obtained from ICN. These compounds were dissolved in a small volume of ethanol (1%v/v).

Test crude drugs The following crude drugs (sample number, crude drug, species) were tested.

The drugs were prepared as follows. A 10 g sample of each drug was extracted twice using 100 ml hot methanol ("sample number" M). Alternatively, a 10 g sample was extracted twice using 100 ml hot water and then freeze-dried ("sample number" H). Table1 presents the plant source for each crude. These extracts were dissolved in a small volume of ethanol (1%v/v).

Table 1. The sample numbers of crude drug extracted in the methanol or water.

No.	Crude Drug	Species
1	檳榔子	*Areca catechu*
2	茵陳蒿	*Artemisia capillaris*
3	艾葉	*Artemisia* sp.
4	黄連	*Coptis japonica*
5	鶴虱	*Daucus carotra*
6	海人草	*Digenea simplex*
7	呉茱萸	*Evodia* sp.
8	茴香	*Foeniculum vulgare*
9	甘草	*Glycyrrhiza* sp.
10	薄荷	*Mentha arvensis* var. *piperascens*
11	黄柏	*Phellodendron amurense*
12	苦木	*Picrasma quassioides*
13	雷丸	*Polyporus mylittae*
14	使君子	*Quisqualis indica*
15	大黄	*Rheum* sp.
16	苦参	*Sophora flavescens*
17	山椒	*Zanthoxylum piperitum*
18	生姜	*Zingiber officinale*
19	紫苑	*Aster tataricus*
20	升麻	*Cimicifuga simplex*
21	桂皮	*Cinnamomum cassia*
22	山茱萸	*Cornus officinalis*
23	紫根	*Lithospermum erythrorhizon*
24	芍薬	*Paeonia lactiflora*
25	瓜呂実	*Trichosanthes kirilowii*
26	桑寄生	*Viscum* sp.
27	独活	*Aralia cordata*
28	射干	*Belamcanda chinensis*
29	陳皮	*Citrus unshiu*
30	紅花	*Carthamus tinctorius*
31	枳実	*Citrus* sp.
32	延胡索	*Corydalis turtschaninovii*
33	辛夷	*Magnolia* sp.
34	人参	*Panax ginseng*
35	瓜呂根	*Trichosanthes* sp.
36	釣藤鉤	*Uncaria* sp.

Nematocidal activity We used a modification of the method developed by Kiuchi et al.[2] For one assay, 20 second-stage *T. canis* larvae were incubated with a test solution in a 96-well flat-bottom plate at 37°C and the behavior of the larvae was observed under a microscope 72 h after the start of incubation. Nematocidal activity was evaluated in terms of relative motility (RM): lower RM indicated stronger nematocidal activity. These values were calculated by averaging four motion scores.

Cytotoxicity HL60 cells were washed and resuspended in the culture medium to 200 μl at 3 x 10^4 cells/ml, and this cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated in 5% CO_2 / air for 24 h at 37°C. After incubation, these diluted extracts were added to the test wells and EtOH:H_2O (1:9) was added into the control wells. The cells were incubated for 72 h in the presence of each agent, and cell growth was evaluated using a modified 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetazolium bromide (MTT; SIGMA) reduction assay.[4] Briefly, after termination of the cell culture, 10 μl of 5 mg/ml MTT in PBS was added to each well and the plate was incubated in 5% CO_2 / air for 4 h at 37°C. The plate was read on a microplate reader at 550 nm. Average absorbency was determined from 10 assays.

Immuno-enhancement activity Neutrophil-like cells were used for measuring the superoxide (O_2^-) generation rate and were prepared by differentiating HL60 cells in medium containing 1% dimethyl sulfoxide (DMSO: WAKO). The O_2^- generation rate was calculated by a method described previously.[5,6] The neutrophil-like cells were washed and resuspended in the culture medium to 200 μl at 1 x 10^6 cells/ml, and this cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated in 5% CO_2 / air for 2 h at 37°C. After the incubation, the extract samples were administered to the cells and then 20 μmol/ml cytochrome c from horse heart (SIGMA) was added. The absorbency at 550 nm was measured using 0.1 μg/ml phorbol 12-myristate 13-acetate (PMA: WAKO) to activate the neutrophils. For each sample, 10 replicates were measured after 0 and 60 minutes, and the average was calculated (O_2^- generation rate).

Results and Discussion

In the methanol and water extracts (0.01 mg/ml and 0.1 mg/ml), the nematocidal activities for the 0.1 mg/ml extracts 1M, 2M, 3M, 4M, 5M, 7M, 8M, 9M, 10M, 13M, 15M, 17M, 18M, 21M, 28M, 29M, 32M, 33M, 1H were all strong.[1] And, these crude drug extracts were tested as follows. The materials that were highly cytotoxic at the most effective nematocidal concentration could not be considered for further use because of host mortality. We examined compound toxicity using HL60 cells instead of the J774.1 cells.

The anthelmintics AZ (0.01 mg/ml) and IM (0.01 mg/ml) were highly cytotoxic. Several extracts that showed no toxicity in the J774.1 cell[1] were strongly cytotoxic to the HL60 cells. Because HL60 cells exhibit high sensitivity to cytotoxins, extracts that showed low absorbance were excluded from candidacy. Based on these results, the extracts 1M, 15M, 29M, and 1H (0.1 mg/ml) were selected as drug candidates (Figure 1).

Eosinophils, neutrophils, and macrophages initiate the anthelmintic response of the host organism. Eosinophils secrete the main basicity protein (MBP), which damages the parasite's exodermis. Neutrophils and macrophages then generate O_2^- and nitrogen monoxide (NO), to thwart the parasite. We examined the O_2^- generation rate of neutrophils and found that none of these extracts suppressed O_2^- generation (Figure 2). Extracts 1M, 15M, 29M, 1H will become the lead material of the drug. This result (1M, 15M, 29M, and 1H) was included for the result of previous paper (1M, 8M, 9M, 10M, 13M, 15M, 28M, 29M, and 1H).[1]

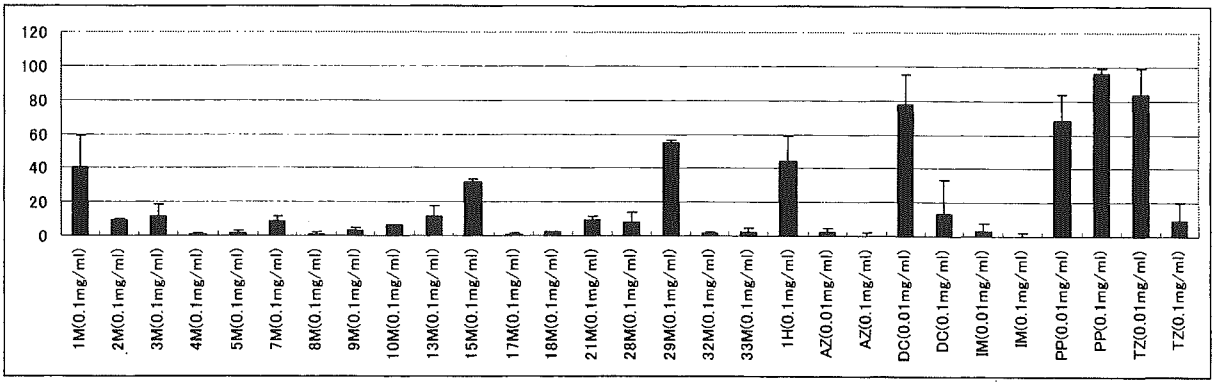


Fig.1 Cytotoxicity of selected extracts (methanol and water) and anthelmintics in the HL60 cells. The vertical axis indicates the absorbance (mean±SD %) determined by MTT method.

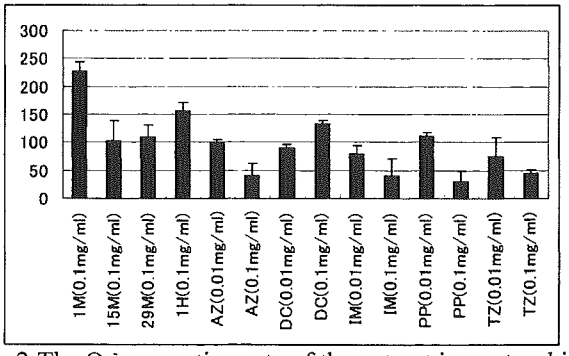


Fig.2 The O_2^- generation rate of the extract in neutrophils. The vertical axis indicates the absorbance rate (mean±SD %) using by cytochrome c.

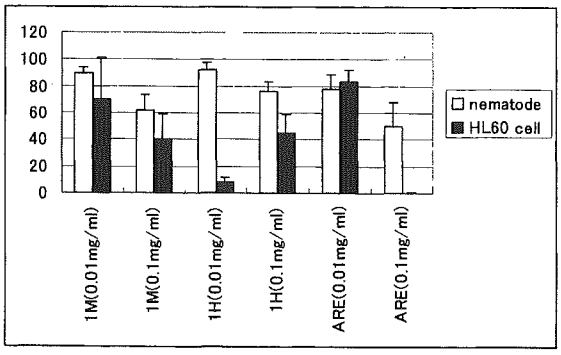


Fig.3 Nematocidal and cytotoxic activities of 1M, 1H, ARE (0.01 mg/ml and 0.1 mg/ml). The vertical axis of "nematode" indicates the RM value (mean±SD %), and that of "HL60 cell" indicates cytotoxicity in HL60 cell.

In contrast, the O_2^- generation rate increased remarkably in response to 1M and 1H (0.1 mg/ml). Therefore, extract 1 is regarded as the best drug candidate, because the O_2^- generation rate increased in response to both the water and methanol extracts.

ARE, which was a main component of 1M and 1H, and these extracts (0.01 mg/ml and 0.1 mg/ml) were examined for nematocidal and cytotoxic activity (Figure 3). As a result, ARE showed nematocidal activity which was equivalent to 1M and 1H at 0.01 mg/ml and 0.1 mg/ml. This chemical compound seems to be a cause of the activity in 1M and 1H. On the other hand, the compound as an active element except for ARE will exist, because nematocidal activity of 1M and ARE were almost the same in 0.01 mg/ml and 0.1 mg/ml. Further 0.1 mg/ml ARE showed strong cytotoxic activity, while 0.1 mg/ml 1M had a cell proliferation rate of about 40%. Also ARE is known to be one of the parasympatho-mimetic constituent's.[7] These facts have shown that the extract is a more nearly ideal drug than the pure chemical compound in this case.

These 0.01 mg/ml 1M, 0.1 mg/ml 1M, 0.1 mg/ml 1H and 0.01 mg/ml ARE with no cytotoxic activity did not suppress the immune activity in neutrophiles. From the above fact, it is considered that 1M, 15M, 29M, and 1H become the lead candidates for a *T. canis* larva migrans remedy.

It is important to do the correlation of the result of *in vivo* and *in vitro*. The *in vivo* experiment is the progressing in our laboratory.

References and Notes

1) The paper reference before one.
2) Kiuchi F., Miyashita N., Tsuda Y., Kondo K., Yoshimura H., *Chem. Pharm. Bull.*, **35**, 2880-2886 (1987).
3) These cells were obtained from RIKEN Cell Bank, Japan.
4) Sargent JM., Taylor CG., *Bri. J. Cancer*, **60**, 206-210 (1989).
5) Collins SJ., Ruscetti FW., Gallagher RE., Gallo RC., *Proc. Natl. Acad. Sci. U. S. A.*, **75**, 2458-2462 (1978).
6) Daval J-L., Ghersi-Egea J-F., Oillet J., Koziel V., *J. Cereb. Blood Flow Metab.*, **15**, 71-77 (1995).
7) Chu NS., *J. Biomed. Sci.*, **8**, 229-236 (2001).

＜症例報告＞

肝内に多発小結節像を呈した犬回虫症の1例

広岡　昌史[1)]　堀池　典生[1)]　金子　恵理[1)]　阿部　雅則[1)]
道堯浩二郎[2)]　坪井　敬文[3)]　赤尾　信明[4)]　恩地　森一[1)]

要　旨：症例は53歳男性．狩猟を趣味としており猪肉を生食する機会があった．平成12年11月の検診で腹部超音波検査にて肝内に多発するSOLを指摘され精査目的で当科に紹介され入院した．入院時検査所見では白血球，好酸球，IgEが著明に増加していた．腹部超音波検査，CT検査，MRI検査で肝内に多発する径1cmまでの結節をみた．SOLに対し超音波誘導下肝生検を施行．好酸球浸潤を伴う肉芽腫の所見であった．ELISA法にて寄生虫抗体を検査し，犬回虫に対し陽性であった．寒天ゲル内二重拡散法では患者血清は犬回虫抗原に対し沈降線をみた．以上より犬回虫幼虫による肝内への幼虫内臓移行症と診断し，メベンダゾール200 mg/日を3週間投与した．10カ月後の超音波，CT検査では肝内のSOLは消失したが白血球，好酸球は若干の改善にとどまっている．肝内に多発する小結節像をみた場合，本疾患を念頭に置き診療する必要があると考えた．

索引用語：　犬回虫症　　内臓幼虫移行症

緒　言

これまで犬回虫症は，幼小児が砂場で遊びその土壌中に存在する虫卵を経口摂取することによると考えられてきた[1~4)]．しかし，最近嗜好の多様化やペットブームの影響で，これまで少なかった成人の回虫症の報告例が増加してきている[5~8)]．今回著者らは犬回虫幼虫により肝内に多発する結節像を呈した1例を経験したので報告する．

症　例

患者：53歳，男性．

職業：ガス器具販売員．

主訴：肝内結節精査目的．

既往歴：17歳，虫垂炎．28歳，腎炎．38歳，A型肝炎．狩猟を趣味とし年に2回程猪肉の生食をしていた．最後に猪肉の生食を行ったのは平成12年7月であった．

家族歴：特記すべきことなし．

飲酒歴：日本酒1~2合/日/30年．

現病歴：平成12年11月の近医での検診で，腹部超音波検査にて肝内に多発する結節像があり転移性肝癌を疑われた．上下部消化管内視鏡検査，胸腹部CT検査，頭部MRI検査を施行したが原発巣となる病変はなく，精査加療目的で当科に紹介され平成13年3月30日当科に入院した．

入院時現症：特記すべきことなし．

入院時検査成績：白血球12300/μlで，好酸球分画は55.0%と増加していた．またIgEは8542 IU/mlに上昇していた．

A case of Toxocara cani infection with multiple hepatic nodules

Masashi HIROOKA[1)], Norio HORIIKE[1)], Eri KANEKO[1)], Masanori ABE[1)], Kojiro MICHITAKA[2)], Takahumi TSUBOI[3)], Nobuaki AKAO[4)], Morikazu ONJI[1)]

[1)]Third Department of Internal Medicine, [2)]Endoscopy center, [3)]Department of Molecular Parasitology, Ehime University School of Medicine

[4)]Section of Environmental Parasitology, Division of Public Health, Graduate School of Tokyo Medical and Dental University

[1)]愛媛大学第3内科，[2)]同　光学医療診療部，[3)]同　寄生虫学教室

[4)]東京医科歯科大学医歯学総合研究科国際健康開発講座国際環境寄生虫病学分野

＜受付日2002年11月5日＞

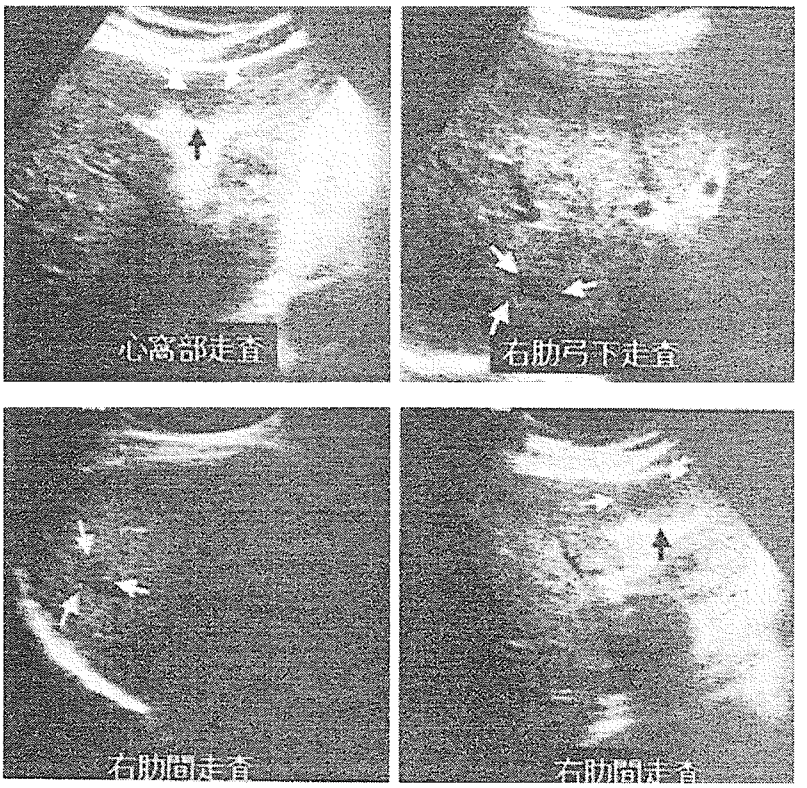


Fig. 1　入院時腹部超音波検査：腹部超音波検査にて肝両葉に径1cm程で内部均一なSOLが多発していた．

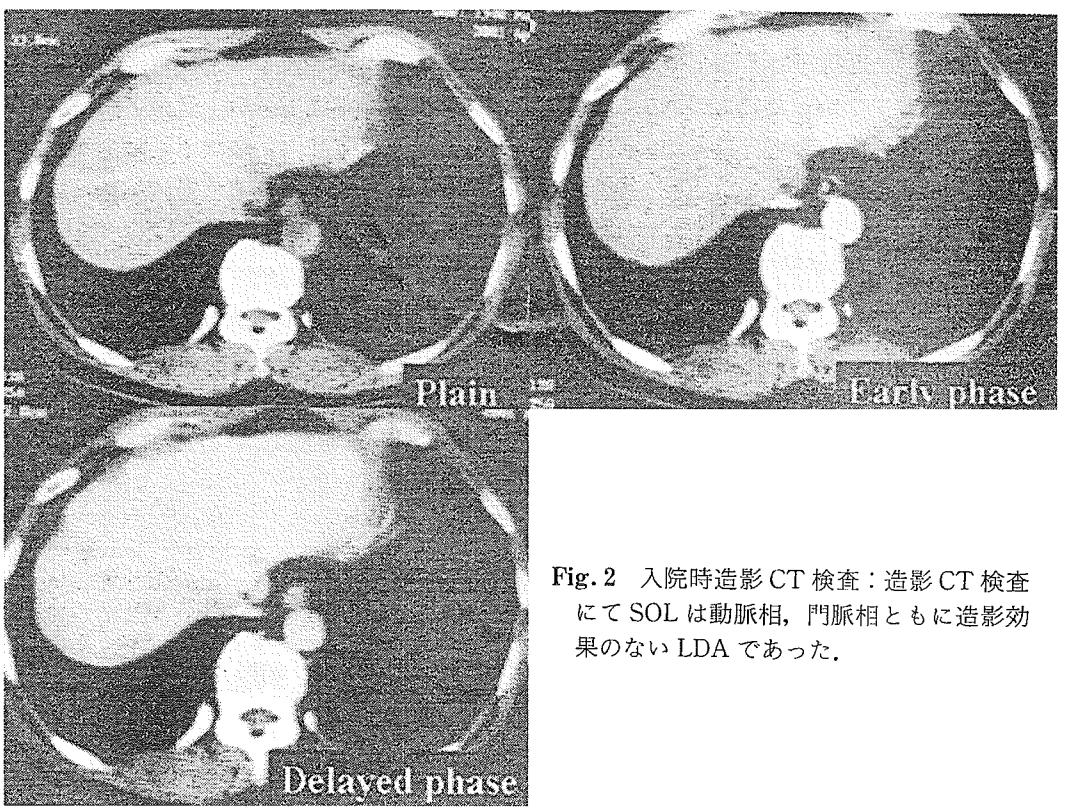


Fig. 2　入院時造影CT検査：造影CT検査にてSOLは動脈相，門脈相ともに造影効果のないLDAであった．

腹部超音波検査：肝両葉に径1cm程の低エコー結節を多数みた(Fig. 1)．形は円形で辺縁は整，境界は明瞭で内部はほぼ均一であった．

腹部CT検査：造影CTでは平衡相にて同部位に1cm程のlow density areaがあり，動脈相，門脈相にて造影効果はなかった(Fig. 2)．

腹部MRI検査：T1強調像にて1cm程のhigh intensity，T2強調像にてiso intensityの結節が多数あった(Fig. 3)．

肝組織像：エコーガイド下に低エコー結節像を狙撃生検した．肝実質に著明な好酸球の浸潤を伴う肉芽腫がみられた(Fig. 4)．

血清学的検査：ELISA法にて犬回虫幼虫の排泄物に対して犬回虫抗体は陽性であった．また寒天ゲル内二重拡散法にて患者血清と犬回虫幼虫排泄物との間に濃い沈降線があった(Fig. 5)．

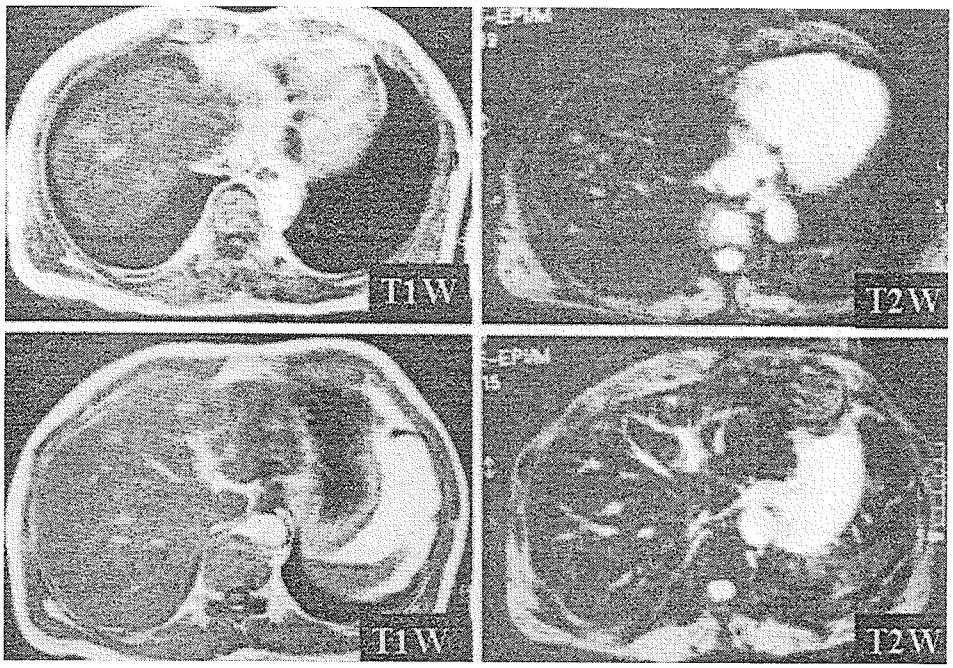


Fig. 3　入院時MRI検査：MRIではCTで描出された部位に一致し，T1Wでhigh intensity，T2Wでlow intensityのSOLをみた．

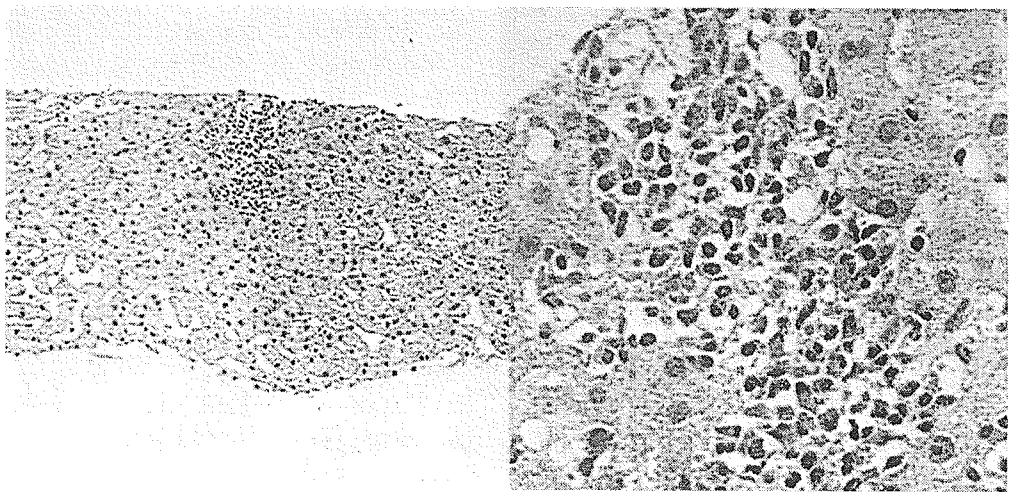


Fig. 4　超音波誘導下肝生検：腫瘍部の狙撃生検ではHE染色にて好酸球浸潤を伴う肉芽腫の所見であった．

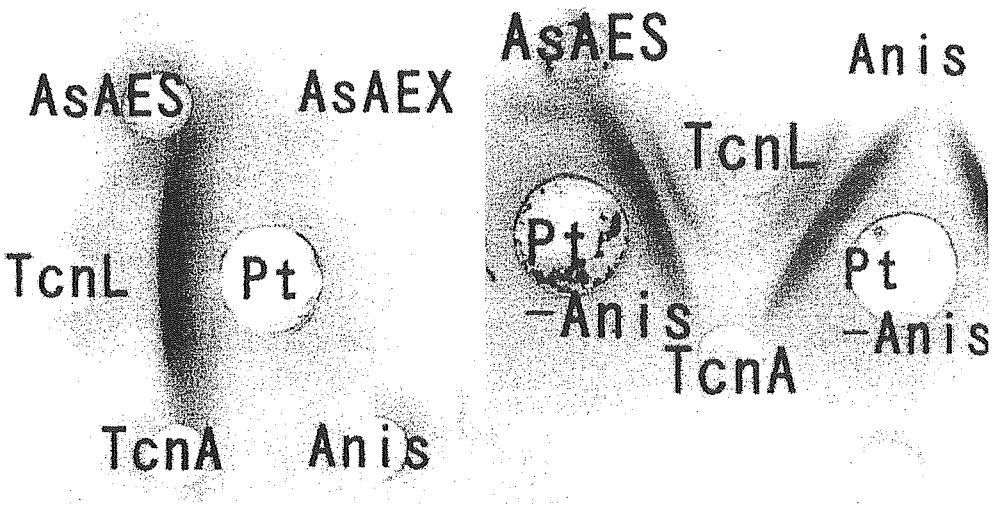


Fig. 5　寒天ゲル内二重拡散法：患者血清のみ(左)，患者血清にアニサキス幼虫抗原吸収後(右)．いずれもTcnL(犬回虫幼虫排泄物抗原)に濃い沈降線を形成した．AsAES；ブタ回虫成虫排泄物抗原，AsAEX；ブタ回虫成虫抽出抗原，TcnL；イヌ回虫幼虫排泄物抗原，TcnA；イヌ回虫成虫抽出抗原，Anis；アニサキス幼虫抽出抗原．

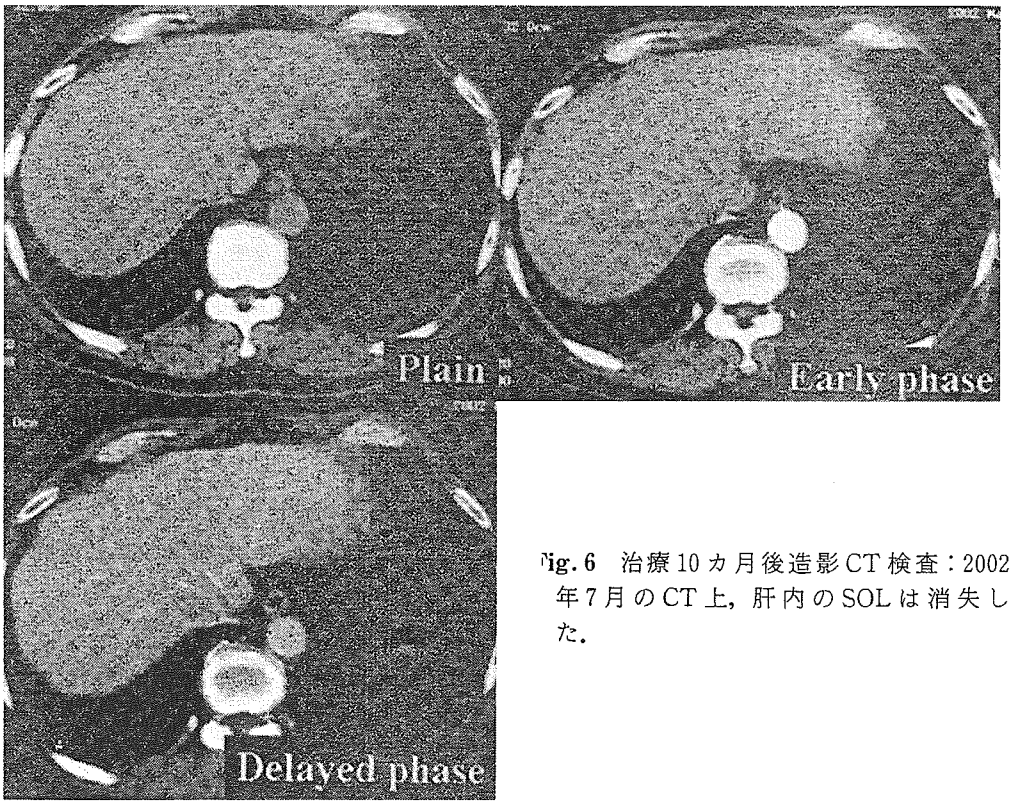


Fig. 6　治療10カ月後造影CT検査：2002年7月のCT上，肝内のSOLは消失した．

経過：猪肉の生食，白血球と好酸球の増加，血清IgEの上昇，画像所見から肝に多発した結節は犬回虫幼虫による内臓幼虫移行症と診断した．眼底，頭部，肺について検査したが病変はなかった．治療としてmebendazoleを1日200 mg，3週間経口投与した．6月10日の時点で肝内結節は変化していなかったが白血球10100/μl，好酸球 4000/μl と改善したため外来にて経過観察とした．2002年7月外来受診時の腹部CT検査では肝内の結節像は消失したが白血球8052/μl，好酸球1932/μl と依然高値であるため厳重に経

過観察中である(Fig. 6).

考　察

内臓幼虫移行症は1952年にBeaver[1]らが初めて報告して以来，主に幼小児が土壌中もしくは犬猫に接触することにより虫卵を経口摂取し，腸管で孵化した幼虫が腸管壁に侵入し，主として血流によって諸臓器に運ばれることで起こるとされてきた[2~4]．しかし近年本邦で報告された症例の多くは成人に発症しており，鶏や牛のような待機宿主と思われる動物の肉や肝の生食を行った症例が多い[5~8]．本症例は感染経路は不明であるが狩猟を趣味とし猪肉の生食を行ってきたことから，待機宿主に存在する虫卵あるいは幼虫を摂取した可能性が考えられる．

犬回虫症の確定診断には組織学的に結節中に虫体を証明することが必要であるが困難なことが多い．そのため診断にあたり免疫血清学的診断法が用いられる．また組織内に本症例同様好酸球浸潤を伴う肉芽腫を来すことが多い．

本疾患の画像診断上の特徴としてはいくつかの報告がある[9~13]．径1 cm程度で腹部超音波検査では円形の均一な低エコー，CTではlow density area，MRIでは様々な像を呈することが多いとされる．本症例でも同様の所見であった．鑑別診断としては画像的には白血病，ATL，リンパ腫などの腫瘍細胞の浸潤，肝膿瘍，転移性肝癌などが挙げられ，組織学的には結核，サルコイドーシスなどがあり注意を要する．

治療としてはthiabendazoleやdiethylcarbamazineなどが一般に試みられ，最近ではalbendazole[14]やmebendazole[15]の効果が報告されている．本症例でもmebendazoleを投与し効果がみられた．

本疾患の症状として軽度の腹痛，全身倦怠感などがあるが，無症状であることが多い．しかし諸臓器に幼虫が迷入した結果蕁麻疹，胃腸炎，肺炎，脳髄膜炎，眼症などを来すことがある．このため本疾患に対しての的確な診断と治療が必要となる．また待機宿主となりうる動物の肝・筋肉などの生食を避けることが本疾患を防ぐ上で重要であると考えられた．本症例は2002年7月の時点で15カ月の経過を追跡しており，このような長期経過観察例の報告はない．画像上肝内結節の消失をみたが白血球，好酸球，IgEは治療前より改善しているものの依然高値であり厳重な経過観察の予定である．

まとめ

肝内に多発小結節像を呈した犬回虫症の1例を経験した．本画像所見をみた場合，本疾患を念頭に置き鑑別診断を行う必要があると考えられた．

本論文の要旨は第77回消化器病学会四国地方会(平成14年6月，松山市)にて発表した．

文　献

1) Beaver PC, Synder CH, Carrera GM, et al：Chronic eosinophilia due to visceral larva migrans. Pediatrics 9：7－19，1952
2) 吉村裕之：小児寄生虫病－蟯虫症，犬回虫移行症及びクリプトスポリジウム症を中心に－．小児保健　31：303－310，1990
3) Mok CH： visceral larva migrans. A discussion based on review of the literature. Clin Pediatrics 7： 565－573，1968
4) Shimokawa H, Nakashima T, Akagi K, et al：visceral larva migrans by toxocara cati. Fukuoka Acta Med 73：64－69，1982
5) 伊藤孝一郎，酒井健二，岡嶋泰一郎，他：鶏肝や牛肝の生食により発症したと考えられる内臓幼虫移行症の3例．日内誌　75：759－766，1986
6) 三ツ木健二，梅井利彦，井上孝利，他：肝に肉芽腫形成による結節像を多発性に認めた猫回虫による内臓幼虫移行症．日内誌 77：1742－1743，1988
7) 中辻祐司，重本昌三，神代尚芳，他：血清学的に内臓幼虫移行症が疑われた兄弟例．日内誌　78：35－40，1989
8) 八幡勝也，奥野府夫，平野芳昭，他：肝内に多発性微小結節を認めた猫回虫内臓幼虫移行症の1例．肝臓　30：1537－1542，1989
9) Clarke HM, Hinde FR, Manns RA, et al：Case report： Hepatic ultrasound findings in a case of toxocarasis. Clin Radiology 46：135－136，1992
10) 中島　収，渡辺次郎，田口　順，他：肝の凝固壊死を呈する肉芽性結節に関する臨床病理学的研究．肝臓　35：527－535，1994
11) 平田経雄：猫回虫移行による肝肉芽腫の超音波所見．日医放誌　47：1606－1608，1987
12) 西方宏昭，平田泰彦，島村隆二，他：猫回虫内臓幼虫移行症の肝内好酸球肉芽腫を各種画像診断にて解析し得た1例．日消誌　88：2697－2702，1991

13) 島村隆二，田中　実，大坪亮一：超音波が診断に有用であった内臓幼虫移行症の1例．超音波医学 18：795－800，1991
14) Bhatia V, Sarin SK：Hepatic visceral larva migrans：evolution of the lesion, diagnosis, and role of high dose albendazole therapy. Am J Gastroenterol 89：624－627，1994
15) Krcmery V Jr, Gould I, Sobota K, et al：Two cases of disseminated toxocarasis in compromised hosts successfully treated with mebendazole. Chemotherapy 38：367－368，1992

症　例

著明な好酸球増多を認めたタイ肝吸虫症の1例

[1]東京都立荏原病院感染症科，[2]東京医科歯科大学国際健康開発学講座国際環境寄生虫学分野，
[3]マヒドン大学熱帯医学校
西浦　博[1][3]　角田　隆文[1]　赤尾　信明[2]

（平成15年3月17日受付）
（平成15年6月20日受理）

Key words：　eosinophilia, *Opisthorchis viverrini*

1. 序　文

タイ肝吸虫（*Opisthorchis viverrini*）は，主にタイ・ラオス・カンボジアを中心として分布しており，タイでは感染者は700万人と推定されている[1]．特にタイ北東部やラオスではKoi-plaと呼ばれるコイ科の淡水魚料理の生食習慣が主たる感染源と考えられている．タイ肝吸虫症はわが国で一般的に言われる肝吸虫（*Clonorchis sinensis*）の感染と類似の生活史・感染様式・臨床症状を呈するが，前者については病初期より重篤な症状のために特に医療機関で治療を必要とすることが多いことで知られている[2]．今回，発熱と好酸球増多の精査中にタイ肝吸虫症と診断した症例を経験したので報告する．

2. 症　例

患者：26歳女性，公務員（2002年6月よりラオス赴任）．

主訴：発熱．

既往歴，家族歴：特記すべきものなし．

生活習慣：2002年6月から8月にかけてKoi-pla複数回摂取，8月から9月にかけて右足背裂傷のある状態でメコン川上流にて遊泳．

海外渡航歴：多数．最近1年間のうちにラオス，タイ，カンボジア，ミャンマー滞在．

現病歴：2002年10月中旬から39℃台の発熱あり，赴任中のラオスで診療所受診した．この際，扁桃炎疑いと診断されAMPC等処方されるも改善を認めなかった．発熱以外に自覚症状はなかったが，その後も37℃以上の発熱が持続した．改善が認められないため，メリオイドーシスやリケッチア症等が疑われ，LVFX，MINOなど抗菌薬変更し投与されるも変化は認められなかった．10月30日の血算検査においてWBC：31,000/μl（Neutro：15%，Eosino：76%）と著明な好酸球増多を認めたが，その際に施行された糞便虫卵検査（直接法のみ）では虫卵検出されず，胸部X線においても異常所見を認めなかった．12月12日，外務省医務官より帰国命令あり，熱源および好酸球増多の精査加療目的で当院紹介受診した．

入院時現症：身長：159cm，体重：56kg，体温：37.2℃，血圧：98/64mmHg，脈拍：66/min整，結膜に貧血・黄疸なし，胸腹部に異常所見を認めなかった．

入院時血液検査所見（Table 1）：血液一般では白血球数の上昇を認め（15,700/μl），分画では好酸球が42%と著明な上昇を認めた．生化学ではアルカリホスファターゼが748 IU/*l*であり，γ-グルタミン酸トランスペプチダーゼ（γ-GTP）が190 IU/*l*，レクチンアミノペプチダーゼが150 IU/*l*と，胆道系酵素の上昇を認めた．血清学的にはCRPが2.8mg/dlであった．

別刷請求先：（〒145-0065）東京都大田区東雪谷4丁目5番地10
東京都立荏原病院感染症科　西浦　博

平成15年9月20日

Table 1 Laboratory Examination on admission

〈Blood cell count〉		〈Biochemistry〉		〈Serology〉	
WBC	15,700 /μl	TP	7.6 g/dl	HBsAg	(−)
Neutro	43 %	Alb	4.1 g/dl	HCVAb	(−)
Eosino	42 %	T. Bil	1.2 mg/dl	ANA	(−)
Mono	4 %	D. Bil	0.7 mg/dl	AntiMt. Ab	(−)
Lymph	10 %	BUN	9.7 mg/dl	CRP	2.8 mg/dl
RBC	449 × 10^4 /μl	Cre	0.6 mg/dl	sIL-2RcAb	1,570 U/ml
Hb	13.7 mg/dl	AST	90 IU/l	〈Endocrinology〉	
Ht	40.6 %	ALT	128 IU/l	ACTH	47.5 pg/ml
Plt	26.7 × 10^4 /μl	γGTP	190 IU/l	Cortisol	19.6 pg/ml
〈Coagulation〉		Al-p	748 IU/l		
PT	100 %	LAP	150 IU/l		
		ChE	266 IU/l		
		T. chol	207 mg/dl		

Fig. 1 CT scan of the abdomen
Multiple cystic lesion in the right lobe of the liver (S8) can be observed.

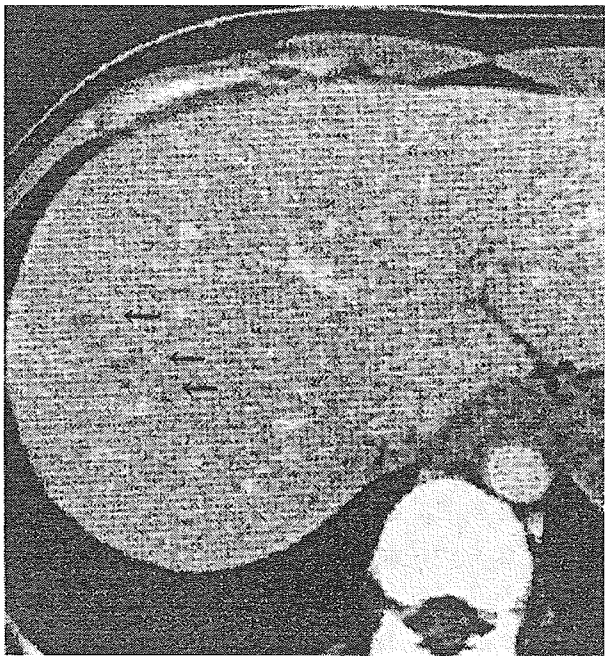
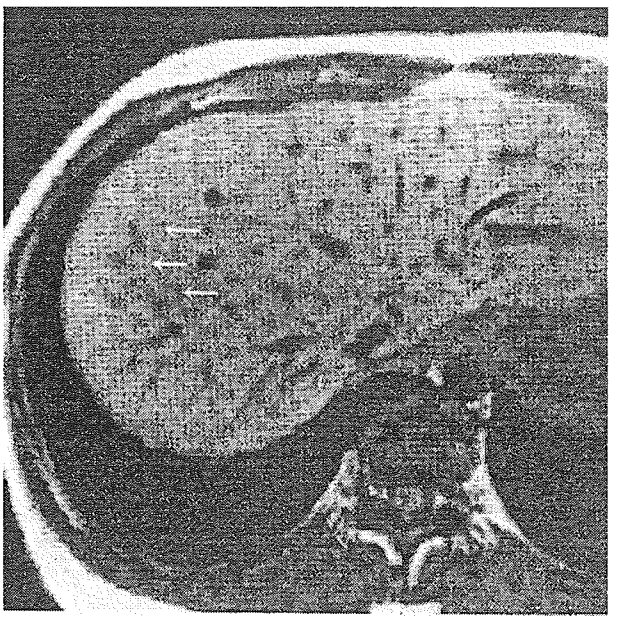


Fig. 2 MRI (T1W1) of the abdomen
Multiple cystic lesion in the right lobe of the liver (S8) can be observed. Edge of the cystic lesions was enhanced by dynamic contrast method in T2W1.



胸部単純X線写真：異常所見を認めず.

腹部超音波所見：肝辺縁の鈍化を認めたが，肝内部エコーは均一であった．肝実質に明らかな占拠性病変を認めなかった.

腹部CT所見（Fig. 1）：肝・脾の軽度腫大を認めた．造影後CTにおいて，肝右葉，特にS8優位に径3mmから5mm大の不整形の低吸収域を散在性に認めた．胆道系の明らかな拡張を認めず，膵に腫瘤性病変を認めなかった.

腹部MRI所見（Fig. 2）：肝S8優位にT1強調像にて斑状の不整形低信号域を認め，T2強調像では同部位は不明瞭であった．肝内血管走行の偏位は認めなかった．ダイナミック造影にて肝実質の不均一な増強効果を認め，T1強調像にて低信号部分は徐々に増強された.

便虫卵集卵法（Fig. 3）：平均計測値（n＝21）26.3 μm×14.4μmと小さく，淡黄色で小蓋を有する虫卵を認めた.

Fig. 3　*Opisthorchis viverrini* eggs in stool examination
Mean size in diameter was 26.3μm by 14.4μm (n = 21).

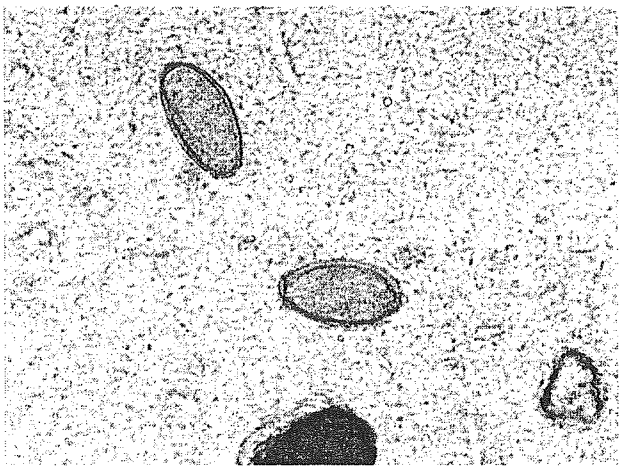


Fig. 4　Multiple-dot ELISA
Serum was negative for all species examined.

Normal human serum	+ + +	IgG	
Dirofilaria immitis	−	−	*Paragonimus westermanii*
Toxocara canis	−	−	*Paragonimus miyazakii*
Ascaris suum	−	−	*Fasciola* spp.
Anisakis simplex	−	−	*Clonorchis sinensis*
Gnathostoma doloresi	−	−	*Spirometra erinacei-europaei*
Strongyloides ratti	−	−	*Cysticerus cellulosae*

血清免疫学的検査（Fig. 4）：Multiple-dot ELISA 法では日本住血吸虫成虫抗原を含め，陽性反応を認めなかった．

以上より，海外渡航歴と摂食歴，肝内の多発散在性不整形病変および便虫卵検査から，本例はタイ肝吸虫症と診断した．肝右葉にタイ肝吸虫によると思われる病変を認めることから，プラジカンテル（ビルトリシド®）40mg/kg（1,800mg 分 3）2 日間の内服治療を行った．治療後は速やかに解熱を認め，患者の希望にて退院し再度ラオスに赴いた．1 カ月後の 2003 年 1 月 31 日に評価目的で施行した血液検査では WBC：6,700/μl（Neutro：58%，Lymph：32%，Eosino：6%，Mono：4%），CRP：0.2mg/dl と改善を認め，同日採取した糞便は Hospital for Tropical Diseases（Mahidol University, Thailand）の協力を得て施行した糞便検査で虫卵の陰転化を確認した．

3. 考　察

タイ肝吸虫の感染急性期によると考えられる著明な好酸球増多を認めた 1 例を経験した．本邦での *O. viverrini* 感染症は，われわれの調べた範囲では，今日までにタイ人を含めてタイ旅行者症例が数例報告[3)4)]されているが，①ラオスからの輸入感染例，②重度好酸球増多については本報が初例である．ラオスにおける疫学研究[5)]によると，糞便中の虫卵陽性率は都市部・僻地で大きな差はなく 52.9% から 60.7% とされており，35 歳から 54 歳の年齢群においては 80% 以上の有病率が観察されており，タイ肝吸虫症は淡水魚の生食習慣に深く関係した深刻な公衆衛生課題となっている．

本症例の好酸球増多について，発熱以外に特異的所見・異常を認めなかったため，原因疾患として組織侵襲性蠕虫症以外にもアレルギー疾患・膠原病・内分泌疾患・血液疾患を含めた好酸球増多の鑑別診断[6)]を頻度順に実施した．その鑑別診断の過程で，淡水魚（とりわけ Koi-pla）の生食歴からタイ肝吸虫症が疑われ，足背裂傷のある状態でメコン川遊泳していたことからメコン住血吸虫症が疑われた[7)]．その後，糞便中に認めた虫卵がわが国で見られる肝吸虫（*C. sinensis*）に類似した形態を呈するが比較するとより小さく，疫学的には *O. viverrini* の浸淫地に矛盾しないことから，本症例はタイ肝吸虫症であると診断した．10 月中旬時点でのラオスにおける 39℃ 台発熱がタイ肝吸虫によるものかどうかは判断が難しいが，プラジカンテル投与後に速やかに解熱し好酸球正常化・糞便虫卵陰転化が確認されたことから，来院後の微熱の持続はタイ肝吸虫症によるものであったと考えられた[8)]．画像所見上，肝右葉に不整形の低吸収域を散在性に認め，胆道系酵素の上昇を認めたことから，本症例でもタイ肝吸虫はグリソン鞘内の中・小胆管に寄生することによって小胆管中の胆汁うっ滞が生じていたものと推測された．しかし，他に報告されている[9)10)]ような肝内外胆道系や胆

平成15年 9 月20日

嚢内の結石，総胆管壁や総肝管壁の石灰化などの慢性炎症性反応を示す所見を認めないことから，本例は初感染であり比較的急性期に今回のような好酸球増多を来たしたものと考えられた．

現在，輸入寄生虫症は年々増加の一途を辿っており，感染地・病原体ともに多様化する傾向にある[11]．好酸球増多を来たした場合はもちろんであるが，日常から海外渡航歴・摂食歴・行動歴を含む詳細の病歴聴取の上，疫学的調査に基づいた鑑別診断を行うことは非常に重要である．日常の検査においても，従来本邦に見られない寄生虫症の輸入の可能性が高いことを常に念頭において対応していく必要があると考えられる．

謝辞：最後に，診療記録を提供していただき詳細に渡り御指導下さいました元在ラオス日本大使館医務官の宇高真智子先生，ご助言いただきました東京女子医科大学国際環境・熱帯医学教室の山浦常講師，諸検査に協力いただきましたマヒドン大学 Hospital for Tropical Diseases スタッフの皆様に深謝致します．

文 献

1) World Health Organization：Control of Foodborne Trematode Infections. WHO Technical Report Series. 849. WHO, Geneva, 1995.
2) Pungpak S, Viravan C, Radomyos B, Chalermrut K, Yemput C, Plooksawasdi W, *et al.*：*Opisthorchis viverrini* infection in Thailand：Studies on the morbidity of the infection and resolution following praziquantel treatment. Am J Trop Med Hyg 1997；56（3）：311—4.
3) Tanaka M, Ohmae E, Nara T, Utsunomiya H, Taguchi H, Irie Y, *et al.*：Treatment of opisthorchiasis with praziquantel. Jpn J Trop Med Hyg 1989；17（1）：81.
4) 田中真奈実，大前比呂思，奈良武司，宇都宮弘美，田口浩之，入江勇治，他：プラジカンテルで完治し得たタイ肝吸虫症の1例．日熱帯医会誌 1988；16（Suppl）：71.
5) Kobayashi J, Vannachone B, Sato Y, Manivong K, Nambanya S, Inthakone S：An epidemiological study on *Opisthorchis viverrini* infection in Lao villages. Southeast Asian J Trop Med Public Health 2000；31（1）：128—32.
6) Rothenberg ME：Eosinophilia. New Eng J Med 1998；338（22）：1592—600.
7) Ittiprasert W, Butraporn P, Kitikoon V, Klongkamnuankarn K, Pholsena K, Vanisaveth V, *et al.*：Differential diagnosis of *Schistosoma mekongi* and trichinellosis in human. Parasitology International 2000；49（3）：209—18.
8) Pungpak S, Radomyos P, Radomyos BE, Schelp FP, Jongsuksuntigul P, Bunnag D：Treatment of *Opisthorchis viverrini* and intestinal fluke infections with Praziquantel. Southeast Asian J Trop Med Public Health 1998；29（2）：246—9.
9) Pungpak S, Bunnag D, Harinasuta T, Sornmani S, Kanda T：Ultrasonographic evidence of biliary tract stones in opisthorchiasis patients after treatment with praziquantel. Jpn J Trop Med Hyg 1989；17（Suppl）：81.
10) Mairiang E, Haswell-Elkins MR, Mairiang P, Sithithaworn P, Elkins DB：Reversal of biliary tract abnormalities associated with *Opisthorchis viverrini* infection following praziquantel treatment. Trans R Soc Trop Med Hyg 1993；87（2）：194—7.
11) 小島荘明：輸入寄生虫症のガイドライン．輸入寄生虫症の動向と問題点．Modern Physician 1999；19（4）：386—8.

A Case of Severe Eosinophilia in the Acute Phase of *Opisthorchis viverrini* Infection

Hiroshi NISHIURA[1)3)], Takafumi TSUNODA[1)] & Nobuaki AKAO[2)]
[1)]Department of Infectious Diseases, Tokyo Metropolitan Ebara General Hospital
[2)]Section of Environmental Parasitology, Department of International Health Development, Division of Public Health, Graduate School, Tokyo Medical & Dental University,
[3)]Bangkok School of Tropical Medicine, Faculty of Tropical Medicine, Mahidol University

A 26-year-old woman Japanese public official servant in Lao People's Democratic Republic was introduced to our hospital on December 12, 2002, because of two months duration of low grade fever and severe eosinophilia. There was no significant finding in physical examination. Laboratory tests showed leukocytosis (15,700/μ*l*) with severe eosinophilia (42%), and no abnormal lymphocyte was observed. Furthermore, elevation in alkaline phosphatase (748 IU/*l*), gamma-glutamyl transpeptidase (190 IU/*l*), leucine aminopeptidase (150 IU/*l*), and slight elevation in CRP (2.8mg/dl) were pointed out. A computed tomographic scan of the abdomen obtained multiple cystic lesions in the right lobe of the liver. On parasitological study, a stool specimen examined by the formalin-ether concentration method revealed positive for *Opisthorchis viverrini* eggs. She was orally administered with 40mg/kg of praziquantel a day for two consecutive days. Following check-up by medical-affairs official at Embassy of Japan in Lao PDR confirmed the normal eosinophil count and her fever disappeared. It was considered eosinophilia in this case was induced by acute phase of severe ophisthorchiasis.

〔J.J.A. Inf. D. 77：677～681, 2003〕

平成15年9月20日