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Schistosomicidal and antifecundity effects of oral treatment of synthetic endoperoxide N-89

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ABSTRACT

1,2,6,7-Tetraoxaspiro[7.11]nonadecane (N-89) is a chemically synthesized compound with good efficacy against malaria parasites. We observed strong anti-schistosomal activities of N-89 both *in vitro* and *in vivo*. In a murine model with experimental infection of *Schistosoma mansoni*, orally administered N-89 at the dose of 300 mg/kg resulted in a significant reduction in worm burden (63%) when mice were treated at 2-weeks postinfection. Strong larvicidal effects of N-89 were confirmed *in vitro*; schistosomula of *S. mansoni* were killed by N-89 at an EC50 of 16 nM. In contrast, no significant reduction in worm burden was observed when N-89 was administered at 5 weeks postinfection *in vivo*. However, egg production was markedly suppressed by N-89 treatment at that time point. On microscopic observation, the intestine of N-89-treated female worms seemed to be empty compared with the control group, and the mean body length was significantly shorter than that of controls. Nutritional impairment in the parasite due to N-89 treatment was possible, and therefore quantification of hemozoin was compared between parasites with or without N-89 treatment. We found that the hemozoin content was significantly reduced in N-89 treated parasites compared with controls ($P<0.001$). The surface of adult worms was observed by scanning and transmission electron microscopy, but there were no apparent changes. Taken together, these observations suggested that N-89 has strong antischistosomal effects, probably through a unique mode of drug efficacy. As N-89 is less toxic to mammalian host animals, it is a possible drug candidate against schistosomiasis.

1. Introduction

Schistosomiasis is a parasitic disease caused by trematode flatworms of the genus Schistosoma that is common in many tropical countries and affects more than 200 million people living in conditions of poor sanitation and/or with less developed social infrastructure [1–3]. The World Health Organization (WHO) is leading the global strategy of schistosomiasis control, with a focus on morbidity control through chemotherapy. Praziquantel (PZQ) is a safe and effective drug for schistosomiasis and has been the drug of choice since the late 1970s. This has raised concerns about the development of drug resistance, and suggestive cases of PZQ-resistant parasites have been reported in *Schistosoma mansoni* from African countries [4–6]. Therefore, the development of new antischistosomal drugs is a matter of priority, and new candidate compounds have been reported [7–9].

Artemisinin-derivatives (ADs) are compounds extracted from the plant *Artemisia annua* used in traditional Chinese herbal medicine, which have strong malaricidal effects [10–13]. Recent studies clearly showed that these compounds also had strong effects against schistosome parasites [14,15]. The most notable difference between PZQ and ADs is the developmental stages of the parasite at which the drugs show efficacy [16,17]. Adult worms are highly sensitive to PZQ, while the larval stages are less sensitive to the drug [18,19]. On the other hand, ADs are effective mainly against the larval stage parasites, while adult worms are less sensitive to treatment with these drugs. In this sense, PZQ is a therapeutic drug, while ADs are drugs for prophylaxis [20]. Therefore, it is recommended to use a combination of the two drugs [21,22].

Although the mechanism of the efficacy has not yet fully been elucidated, peroxide bridge is necessary for antimalarial activities of ADs [10]. Previously, we reported that synthetic endoperoxide (1,2,6,7-tetraoxaspiro[7.11]nonadecane: N-89) [23] has high antimalarial activity against *Plasmodium falciparum in vitro* and *Plasmodium berghei in vivo*, and it shows low levels of cytotoxicity in mice and rats (LD50: >2000 mg/kg) [23–25]. ADs are structurally complicated and their chemical synthesis is

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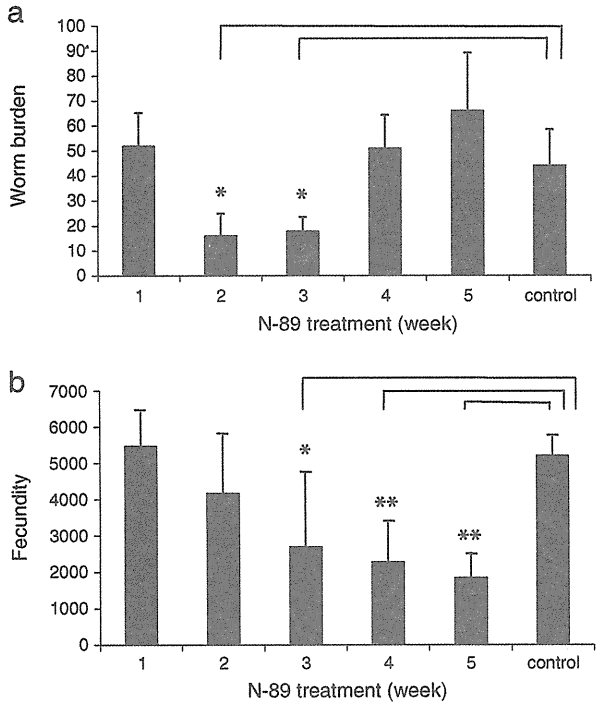

Fig. 1. *In vivo* effects of N-89 to *S.mansoni*. *S. mansoni*-infected mice were orally treated with N-89 from week 1 through week 5 postinfection. (a) *Y*-axis shows the number of worms that were collected by perfusion 9 weeks postinfection (**P*<0.001). (b) *Y*-axis shows the number of eggs produced per female worm. (**P*<0.05, ***P*<0.001).

not easy. On the other hand, N-89 is a compound with a relatively simple structure and is inexpensive to mass produce [23–25]. If N-89 also has strong effects against schistosome parasites, this will allow a new strategy of schistosomiasis control using a lower cost agent.

In this study, we found strong effects of N-89 against *S. mansoni* both *in vitro* and *in vivo*. The efficacies of N-89 were almost comparable to those of ADs. However, N-89 had additional effects that were not reported in the case of ADs, suggesting that N-89 may be a novel compound with unique antischistosomal activities.

2. Materials and methods

2.1. Parasites and animals

Puerto Rican strain *S. mansoni*, which was kept in our laboratory, was used for the present study. Female 5-week-old BALB/c mice were purchased from CLEA (Tokyo, Japan).

2.1.1. *In vivo* treatment of *S. mansoni*-infected mice with N-89

For *in vivo* study, mice were infected with 180 cercariae by the standard method in which mice were percutaneously exposed *via* the tail to cercariae for 1 h at room temperature [14]. BALB/c mice infected with *S. mansoni* were orally treated with N-89 suspended in olive oil at a dose of 300 mg/kg twice a day for two consecutive days. Mice were divided into 6 groups and treated with N-89 at various time points, *i.e.*, from week 1 through week 5 postinfection. To analyze parasite egg burden, eggs were recovered from the liver and intestine by the method reported previously [26]. Briefly, chopped liver and intestine were digested in 4% KOH at 37 °C for 1 h. After incubation, the digested samples were centrifuged at 1500 rpm for 5 min at room temperature, and pellets were resuspended in distilled water. Eggs were counted under a light microscope. Effects on pathological lesions after N-89 treatment were determined by observation of egg granulomas formed in the liver. Liver sections of Azan staining were prepared, and granuloma size was measured by using Image J image processing software (NIH). The mean size of 100 granulomas formed around a single egg in N-89 treated mice was compared to that in control (olive oil-treated mice). In addition, we calculated the body length of the worms using Image J. All *in vivo* experiments were approved by the Committee of Animal Rights and Ethics, Tokyo Medical and Dental University.

2.1.2. *In vitro* treatment of *S. mansoni* with N-89

As N-89 seemed to be effective against larval stage parasites, we prepared schistosomula from the lungs of mice and incubated them in

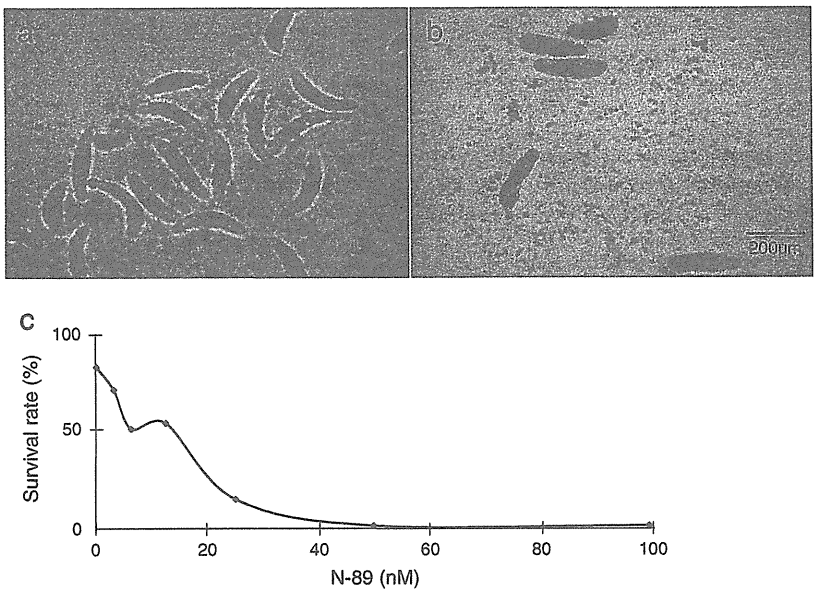

Fig. 2. Schistosomicidal effects of N-89 *in vitro*. (a) 14-day schistosomula were round-shaped and in a state of continuous contraction and extension when they are alive in the medium containing DMSO (2.5%) alone. (b) Schistosomula treated with 50 nM of N-89 were stiff and easily stained with trypan-blue. (c) Y-axis indicates the survival rate of 14-day schistosomula after treatment with serial dilutions of N-89.

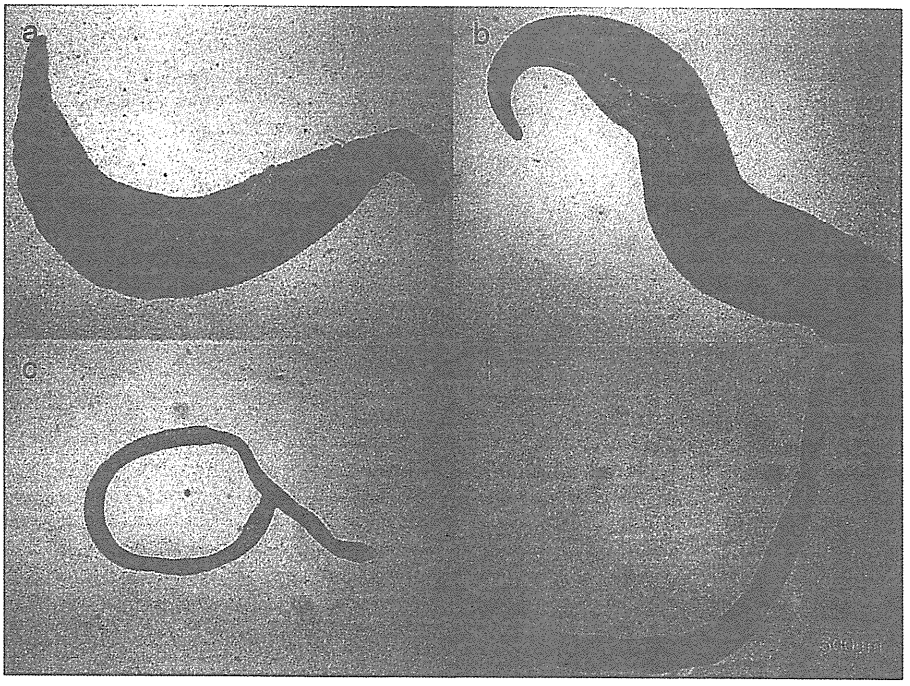

Fig. 3. Light microscopic observation of adult parasites after *in vivo* treatment with N-89. *S. mansoni*-infected mice were treated with or without N-89 5-weeks postinfection. Worms were collected 2 weeks after the treatment. 7-week *S. mansoni* worms were stained with hematoxylin–carmine solution. A male worm from mice treated with N-89 (a), a male worm from control mice (b), a female worm from mice treated with N-89 (c), a female worm from control mice (d).

RPMI-1640 (Wako, Osaka, Japan) supplemented with 10% FBS (JRH Biosciences, Kansas, MO), 150 U/ml of penicillin, and 150 μg/ml of streptomycin (Gibco, Gaithersburg, MD) in 24-well plates (Greiner, Ulm, Germany). N-89 was dissolved in dimethylsulfoxide (DMSO) and added 25 μl to the plates which contains 1 ml of RPMI at various concentrations from 3.12 to 100 nM. Plates were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air for 7 days. Survival of the treated schistosomula was determined by trypan blue dye-exclusion test. Based on the observations, we calculated the EC_{50} of N-89 against schistosomula of *S. mansoni in vitro*.

2.2. Morphological observation of adult parasites after treatment with N-89 in vivo

To observe the morphological changes after N-89 treatment, infected BALB/c mice were administered orally with N-89 at 5 weeks post-infection at a dose of 300 mg/kg, and 2 weeks later adult worms were recovered by portal perfusion. Recovered parasites were washed thoroughly with 0.85% NaCl and 0.45% Na-citrate in distilled water, and paired worms were fixed in 70% ethanol and stained with hematoxylin–carmine solution for light microscopic observation. Parasites were observed by scanning electron microscopy and transmission electron microscopy (Hitachi, Tokyo, Japan) according to the method reported previously [27,28].

2.3. Quantification of hemozoin contents of S. mansoni

Hemozoin was extracted from *S. mansoni* and quantified by the method reported previously [29–31]. Protein contents of worm homogenates were measured using a protein assay kit (Bio-Rad, Hercules, CA). Infected mice were administered orally with N-89 (300 mg/kg) at 5 weeks postinfection, and 2 weeks later adult parasites were tested for hemozoin contents. The worms used for the tests were paired to compare worms in the same/similar developmental stages. For each experiment, 15 to 30 worms were used from each mouse. Worms were homogenized in 1 ml of PBS (pH 7.2), and centrifuged for 10 min at 10,000×*g*. Insoluble pellets were washed with 0.1 M sodium hydrogen carbonate, and then dissolved in 0.1 N NaOH. Hemozoin was converted to heme in this treatment, and we then measured the converted heme as hemozoin in accordance with the reagent manufacturer's protocol (Hemin, Sigma-Aldrich, St. Louis, MO). Heme was quantified spectrophotometrically by measuring absorbance at 405 nm. Hemozoin content in the parasite was expressed as ng heme/mg protein.

2.4. Statistical analysis

Statistical analyses were performed by Student's *t* test. In all analyses, $P<0.05$ was taken to indicate statistical significance.

3. Results

3.1. Schistosomicidal effects of N-89 in vivo

Reduction of worm burden was observed when mice were treated 2 or 3 weeks postinfection, and the maximum effect of N-89 driven reduction in worm burden was observed at 2 weeks postinfection compared with the olive oil control group (Fig. 1a). Schistosomicidal effects became less apparent at 3 weeks postinfection, and there was no detectable reduction in worm burden when mice were treated at 5 weeks postinfection. However, egg production per paired female worm was significantly reduced when mice were treated with N-89 at 5 weeks postinfection. Reduction in egg production per female worm in the N-89-treated group was statistically significant in comparison to the olive oil control group (Fig. 1b). These observations indicated that the larval stage is the target for the killing effect of N-89, while this agent showed inhibitory effects on fecundity of adult worms without killing the parasite.

3.2. In vitro effects of N-89 for schistosomula of S. mansoni

To confirm the direct effects of N-89 against the larval stage of *S. mansoni*, schistosomula were treated with serial dilutions of N-89 and

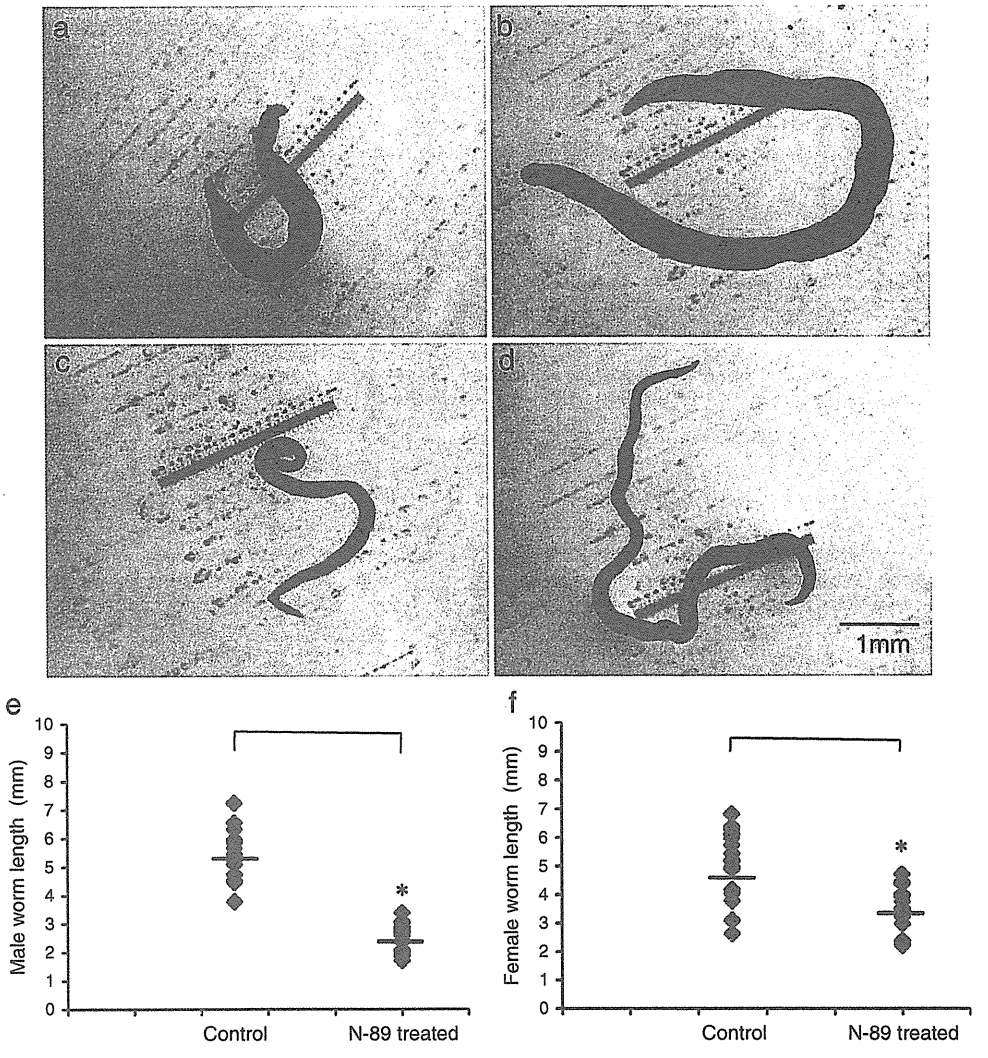

Fig. 4. The mean body length of the worms. All worms used were obtained in the same manner as described in Fig. 3. *Y*-axis indicates the length of male worms (a) (*$P<0.01$) and female worms (b) (*$P<0.01$).

cultured for 7 days *in vitro*. The schistosomicidal effects of N-89 were dose-dependent, and the EC_{50} against *S. mansoni* larvae was calculated as 16 nM (Fig. 2a–c). During the observation period, all schistosomula were alive and active under culture conditions containing DMSO alone (data not shown).

3.3. Pathological changes in the liver in infected mice treated with N-89

The sizes of granulomas formed around single schistosome eggs in N-89 treated mice was compared to that in control animals. The liver pathology of the mice treated at 5 weeks postinfection showed significantly smaller granulomas compared with controls ($P<0.001$) (data not shown).

3.4. Morphological changes of N-89 treated adult worms

To observe morphological changes of the parasite after N-89 treatment *in vivo*, we compared morphological profiles of the adult worms with or without N-89 treatment. The most obvious difference was noted in the intestine of female worms on light microscopic observation. Briefly, the dense substances, probably hemozoin, disappeared in N-89-treated worms (Fig. 3a–d). Furthermore, the mean body length of the treated worms was smaller than that of untreated controls (Fig. 4a–f). On TEM observation, the tegument morphology was compared between parasites with and without N-89 treatment. In both males and females, there were no marked differences between N-89-treated worms and control worms (Fig. 5a–d). In the SEM profiles, we found small surface changes, such as the disappearance of tubercles on the surfaces of males and shortened spines on females, but these changes were not as severe as the findings of previous studies for PZQ and ADs [28,32] (Fig. 5e–h).

3.5. Heme contents of adult parasites with and without N-89 treatment

As hemoglobin is the main source of nutrition for adult female worms, we measured hemozoin contents of parasites with and without N-89 treatment to examine whether nutritional impairment occurred in N-89-treated parasites. In the N-89-treated group, the mean heme content was 15 nmol heme/mg protein, while it was 89 nmol heme/mg protein in the untreated controls; this difference in heme content was statistically significant ($P<0.001$) (Fig. 6).

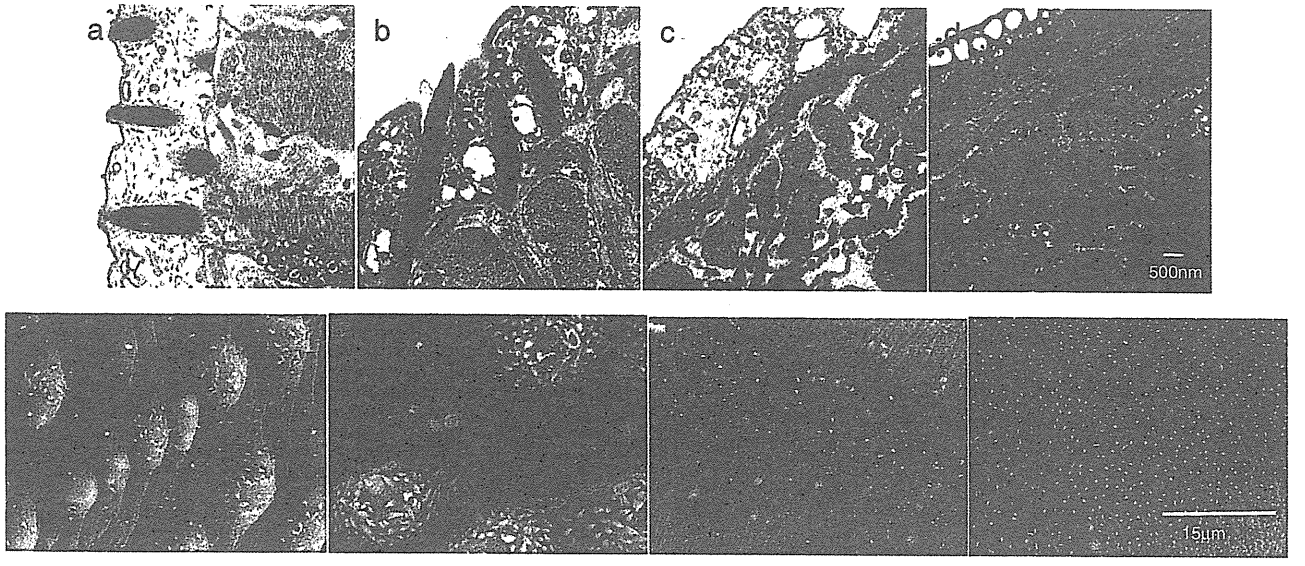

Fig. 5. EM observation of *S. mansoni* adult worms. All worms used were obtained in the same manner as described in Fig. 3. TEM observation of a male worm from mice treated with N-89 (a), a male worm from control mice (b), a female worm from mice treated with N-89 (c), and a female worm from control mice (d). SEM observation of a male worm from mice treated with N-89 (e), a male worm from control mice (f), a female worm from mice treated with N-89 (g), and a female worm from control mice (h).

4. Discussion

Rational drug design should be applied to develop new agents for use against schistosomiasis. As PZQ is the only drug available for controlling disease activity, the appearance of drug-resistant strains is a non-negligible concern. New drug candidates must be developed to address this concern, and ADs are promising candidates for this purpose. However, it should be noted that ADs are used for malaria therapy because of the recent WHO recommendation for use of artemisinin-based combination therapy (ACT). ADs are drugs prepared from plant materials. Due to their structural complexity, these compounds are not easy to chemically synthesize, and the distribution of the product depends on the supply of herbal plant materials. On the other hand, mass production of N-89 is not difficult, and it can be prepared at a much lower cost than ADs. No serious toxicity has been noted for N-89 in animal [23–25]. As N-89 is effective for reducing egg fecundity but not worm burden when it is administered 5 weeks post infection, it can supplement the effect of praziquantel that is effective for reducing worm burden.

The results of the present study suggest that N-89 is a novel antischistosomal compound with a unique mechanism of action compared to other drugs used to combat schistosomiasis, such as PZQ and ADs. Due to the structural similarity, we postulated that N-89 would have both antimalarial and antischistosomal effects in the same manner as observed for ADs. However, reference to previous publications regarding ADs indicated that there were marked differences in its anti-schistosomal effects. That is, N-89 showed two modes of antischisto-somal effect — larvicidal effects and antifecundity effects. Previous reports have indicated no such dual modes of drug efficacy for ADs [17]. Thus, it is possible that N-89 has functions distinct from those of ADs.

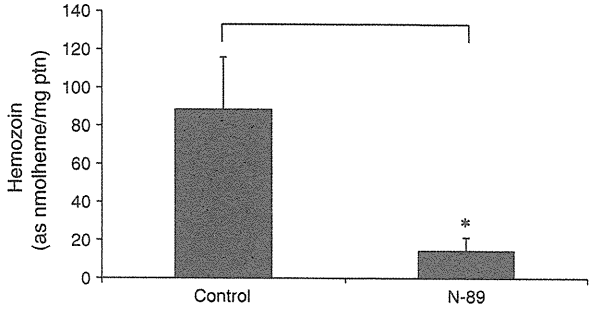

Fig. 6. Heme contents of adult parasites obtained after *in vivo* treatment with N-89.7-week worms collected in the same manner described in Fig. 3 were examined for quantification of hemozoin contents. Y-axis indicates the hemozoin contents (as nmol heme/mg protein) (*$P<0.001$).

It is still necessary to elucidate the detailed mechanisms of action for the two different effects of N-89. Considering the presence of endoperoxide structures in N-89, it is possible that oxygen stress generated by N-89 may be a factor involved in the schistosomicidal effects. Recent studies demonstrated the importance of the redox system for parasite survival [33,34]. However, no direct evidence in support of this possibility is available, nor killing effect of the worms was observed when *Sm*-infected mice were treated with N-89 at 5 week postinfection. In spite of this situation, we observed the reduction of egg fecundity. Morphological observations in the present study suggested that N-89 treatment induce nutritional deficits in the worms, as heme contents in N-89-treated female worms were significantly reduced compared to controls. This may be related to the antifecundity effect of the drug against female worms. It is well discussed that host hemoglobin derived from the host blood is essential for growth, development and reproduction of schistosomes [35,36]. It is possible that N-89 inhibits a process for hemoglobin usage in female worms, and more direct evidence may be obtained by testing the effects of N-89 on the biological pathways involved in hemoglobin uptake. It has been suggested that proteolysis of hemoglobin was important for worm development in male and female, and production of yolk protein in developing egg was also important for female worm [37]. The two modes of drug efficacy in N-89 raise questions regarding why the larval stages were destroyed, while the adult stage was resistant to this drug. In other cases, such as vaccine efficacy, lung stage parasites are the targets for the killing effects [7], although these are immune-mediated mechanisms. Analysis of the direct target molecules for N-89 could provide valuable information for the development of therapeutic strategies. Studies to elucidate these points using other approaches, such as proteomic analysis, are currently underway in our laboratory.

In conclusion, N-89 is a promising compound for use as an antischistosomal drug, which may supplement the effects of PZQ

through mutually different modes of efficacy. Strategies using N-89 as supplemental effect for praziquantel or ADs would be helpful to avoid the development of drug-resistance. Therefore, N-89 is a good candidate partner for its efficacy, safety, and its low cost of mass production.

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References

[1] Chitsulo L, Engels D, Montresor A, Savioli L. The global status of schistosomiasis and its control. Acta Trop 2000;77:41–51.
[2] Esrey SA, Potash JB, Roberts L, Shiff C. Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. Bull World Health Organ 1991;69:609–21.
[3] McCullough FS, Gayral P, Duncan J, Christie JD. Molluscicides in schistosomiasis control. Bull World Health Organ 1980;58:681–9.
[4] Fallon PG, Tao LF, Ismail MM, Bennett JL. Schistosome resistance to praziquantel: fact or artifact? Parasitol Today 1996;12:316–20.
[5] Liang YS, Coles GC, Doenhoff MJ, Southgate VR. In vitro responses of praziquantel-resistant and -susceptible *Schistosoma mansoni* to praziquantel. Int J Parasitol 2001;31:1227–35.
[6] Ismail M, Botros S, Metwally A, William S, Farghally A, Tao LF, et al. Resistance to praziquantel: direct evidence from *Schistosoma mansoni* isolated from Egyptian villagers. Am J Trop Med Hyg 1999;60:932–5.
[7] Xiao S, Tanner M, N'Goran EK, Utzinger J, Chollet J, Bergquist R, et al. Recent investigations of artemether, a novel agent for the prevention of schistosomiasis japonica, mansoni and haematobia. Acta Trop 2002;82:175–81.
[8] Hamed MA, Hetta MH. Efficacy of citrus reticulata and mirazid in treatment of *Schistosoma mansoni*. Mem Inst Oswaldo Cruz 2005;100:771–8.
[9] Angelucci F, Sayed AA, Williams DL, Boumis G, Brunori M, Dimastrogiovanni D, et al. Inhibition of *Schistosoma mansoni* thioredoxin–glutathione reductase by auranofin: structural and kinetic aspects. J Biochem 2009;284:28977–85.
[10] Dhingra V, Rao KV, Narasu ML. Current status of artemisinin and its derivatives as antimalarial drugs. Life Sci 2000;66:279–300.
[11] Ridder S, Kooy F, Verpoorte R. *Artemisia annua* as a self-reliant treatment for malaria in developing counties. J Ethnopharmacol 2008;120:302–14.
[12] Krishna S, Bustamante L, Haynes RK, Staines HM. Artemisinins: their growing importance in medicine. Trends Pharmacol Sci 2008;29:520–7.
[13] Jonathan LV, Sarah AB, Reto B, Susan AC, Francis CKC, Jacques C, et al. Identification of an antimalarial synthetic trioxolane drug development candidate. Nature 2004;430: 900–4.
[14] SH L, Kumagai T, Ai QH, Yan XL, Ohmae H, Yabu Y, et al. Evaluation of the anthelmintic effects of artesunate against experimental *Schistosoma mansoni* infection in mice using different treatment protocols. Parasitol Int 2006;55:63–8.
[15] Utzinger J, Xiao S, Keiser J, Chen M, Zheng J, Tanner M. Current progress in the development and use of artemether for chemoprophylaxis of major human schistosome parasites. Curr Med Chem 2001;8:1841–60.
[16] Xiao SH, Mei JY, Jiao PY. *Schistosoma japonicum*-infected hamsters (*Mesocricetus auratus*) used as a model in experimental chemotherapy with praziquantel, artemether, and OZ compounds. Parasitol Res 2011;108:431–7.
[17] Xiao SH. Development of antischistosomal drugs in China, with particular consideration to praziquantel and the artemisinins. Acta Trop 2005;96:153–67.
[18] Stelma FF, Talla I, Sow S, Kongs A, Niang M, Polman K, et al. Efficacy and side effects of praziquantel in an endemic focus of *Schistosoma mansoni*. Am J Trop Med Hyg 1995;53:167–70.
[19] Goennert R, Andrews P. Praziquantel, a new broad-spectrum antischistosomal agent. Z Parasitenk 1977;52:129–50.
[20] Xiao SH, Jennifer K, Jacques C, Juorg U, Yuxiang D, Yvette E, et al. *In vitro* and *in vivo* activities of synthetic trioxolanes against major human schistosome species. Antimicrob Agents Chemother 2007;51:1440–5.
[21] Xiao SH, Utzinger J, Chollet J, Tanner M. Effect of artemether administered alone or in combination with praziquantel to mice infected with *Plasmodium berghei* or *Schistosoma mansoni* or both. Int J Parasitol 2006;36:957–64.
[22] Inyang-Etoh PC, Ejezie GC, Useh MF, Inyang-Etoh EC. Efficacy of a combination of praziquantel and artesunate in the treatment of urinary schistosomiasis in Nigeria. Trans R Soc Trop Med Hyg 2009;103:38–44.
[23] Aly NS, Hiramoto A, Sanai H, Hiraoka O, Hiramoto K, Kataoka H, et al. Proteome analysis of new antimalarial endoperoxide against *Plasmodium falciparum*. Parasitol Res 2007;100:1119–24.
[24] Kim HS, Nagai Y, Ono K, Begum K, Wataya Y, Hamada Y, et al. Synthesis and antimalarial activity of novel medium-sized 1,2,4,5-tetraoxacycloalkanes. J Med Chem 2001;44:2357–61.
[25] Kim HS, Begum K, Ogura N, Wataya Y, Nonami Y, Ito T, et al. Antimalarial activity of novel 1,2,5,6-tetraoxacycloalkanes and 1,2,5-trioxacycloalkanes. J Med Chem 2003;46:1957–61.
[26] Sabah AA, Fletcher C, Webbe G, Doenhoff MJ. *Schistosoma mansoni*: chemotherapy of infections of different ages. Exp Parasitol 1986;61:294–303.
[27] Wang W, Itoh S, Matsuda A, Ichinose S, Shinomiya K, Hata Y, et al. Influences of mechanical properties and permeability on chitosan nano/microfiber mesh tubes as a scaffold for nerve regeneration. J Biomed Mater Res A 2008;84:557–66.
[28] Shaw MK, Erasmus DA. *Schistosoma mansoni*: structural damage and tegumental repair after in vivo treatment with praziquantel. Parasitology 1987;94:243–54.
[29] Oliveir MF, d' Avila JCP, Torres CR, Oliveira PL, Tempone AJ, Rumjanek FD, et al. Haemozoin in *Schistosoma mansoni*. Mol Biochem Parasitol 2000;111:217–21.
[30] Oliveira MF, d' Avila JC, Tempone AJ, Soares JB, Rumjanek FD, Fereira-P A, et al. Inhibition of heme aggregation by chloroquine reduces *Schistosoma mansoni* infection. J Infect Dis 2004;190:843–52.
[31] Correa Soares JB, Menezes D, Vannier-Santos MA, Ferreira-Perreira A, Almeida GT, Venancio TM, et al. Interference with hemozoin formation represents an important mechanism of schistosomicidal action of antimalarial quinoline methanols. PLoS Negl Trop Dis 2009;3:e477.
[32] Xiao S, Shen B, Chollet J, Utzinger J, Tanner M. Tegumental changes in adult *Schistosoma mansoni* harbored in mice treated with artemether. J Parasitol 2000;86: 1125–32.
[33] Kumagai T, Osada Y, Kanazawa T. 2-Cys peroxiredoxins from *Schistosoma japonicum*: the expression profile and localization in the life cycle. Mol Biochem Parasitol 2006;149:135–43.
[34] Kumagai T, Osada Y, Ohta N, Kanazawa T. Peroxiredoxin-1 from *Schistosoma japonicum* functions as a scavenger against hydrogen peroxide but not nitric oxide. Mol Biochem Parasitol 2009;164:26–31.
[35] Zussman RA, Bauman PM, Petruska JC. The role of ingested hemoglobin in the nutrition of *Schistosoma mansoni*. J Parasitol 1970;56:75–9.
[36] Lawrence JD. The ingestion of red blood cells by *Schistosoma mansoni*. J Parasitol 1973;59:60–3.
[37] Sauer MCV, Senft AW. Properties of a proteolytic enzyme from *Schistosoma mansoni*. Comp Biochem Physiol 1972;42B:205–20.

住血吸虫症に対するプラジカンテル投与法に関する考察 －1回投与か分割複数回投与か－

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Key Words: 日本住血吸虫症，メコン住血吸虫症，プラジカンテル，分割投与，薬剤耐性

はじめに

住血吸虫症の治療薬として開発されたプラジカンテルは，吸虫のみならず消化管に寄生する条虫にも効果がある．また，副作用が問題となることも殆どないので，住血吸虫症対策をはじめ，世界中の寄生蠕虫症対策で，流行地の住民を対象にした集団治療に広く使われてきた[1)]．色々な投与量や分服法が試されてきたが，住血吸虫症の集団治療の場合は，いずれの住血吸虫種に対しても，コンプライアンスの良さから40mg/kgの1回内服法が選択されることが多い．アフリカでのマンソン住血吸虫症治療例では，以前から，標準的なプラジカンテル治療を行っても，完全に治療できない例もあることが指摘されていたが，最近，耐性を疑わせる例が報告される国や地域が増える傾向が指摘されている[2)3)4)]．また，カンボジアでのメコン住血吸虫症対策でも，プラジカンテルによる集団治療後が毎年行われている4村落で，2005～2006年は，虫卵陽性者が認められなかったのに，2007年以降は，3村落で虫卵陽性率が3～5%に戻るなど，プラジカンテルによる治療効果の低下が疑われている（図1）．

そこで，2009年6月，カンボジアのメコン住血吸虫症対策の現場で，プラジカンテル集団治療後にも虫卵検査が陽性であった例を中心に，再度プ

How to use praziquantel to treat *Schistosoma japonicum* or *S. mekongi* infections ?

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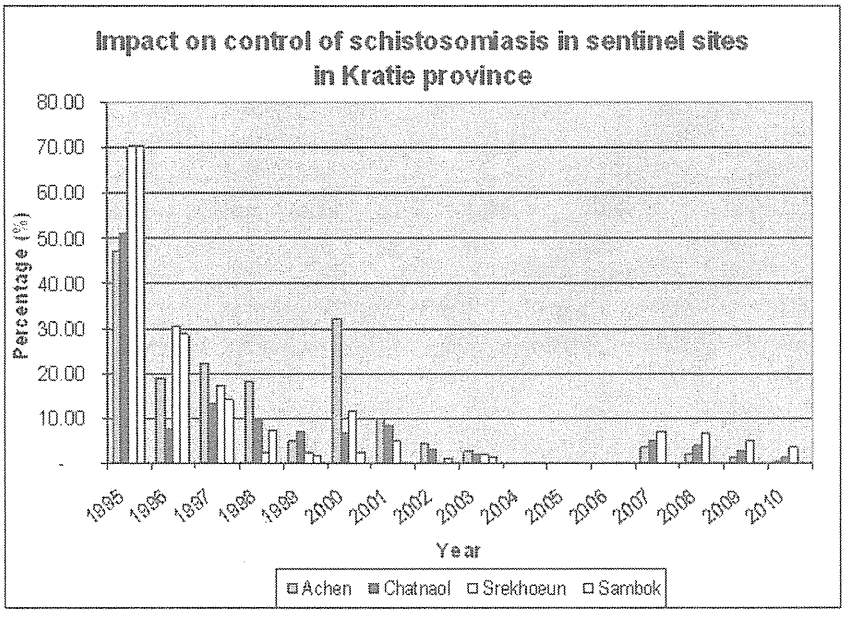

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図 1　カンボジア、クラチエ省におけるメコン住血吸虫卵陽性率の推移
ー年 1 回のプラジカンテル集団治療の成果と最近の停滞ー

ラジカンテルの効果を確認した．また，日本住血吸虫症について，中国とフィリピンにおけるプラジカンテル治療例を中心に，文献での報告例や日本国内での推奨使用例も含めて考察を行った．

方法

2009 年 3〜5 月にかけて，カンボジア王国，クラチエ省のメコン住血吸虫症の浸淫地では，全住民を対象とした寄生蠕虫症対策として，プラジカンテル（用量 40mg/kg の 1 回投与）とメベンダゾール（用量 500mg の 1 回投与）による集団治療が行われた．その効果判定のために，集団治療が行われた地域にある 6 つの小学校で，治療後 4〜6 週間で，計 939 人の児童を対象とした Kato-katz 法（3 日間連続）による糞便検査を行った．その検査で陽性を示した例に対しては，集団治療時の他の薬剤の服用状況について確認するとともに，総量 60mg/kg/日で 2 分割投与とする方法でプラジカンテル再投与を行った．そして，やはり 4〜6 週間後に，治療効果の判定を Kato-katz 法（3 日間連続）にて行った．

結果

939 人のうち，プラジカンテル投与（用量 40mg/kg の 1 回投与）後も，各々3 人の児童からメコン住血吸虫とタイ肝吸虫の虫卵が検出された．また，メベンダゾール 500mg を 1 回投与したにも関わらず，2 人から鞭虫卵が，85 人から鉤虫卵が検出された（表 1）．また，リファンピシンやクロロキン，或いはシメチジンといったプラジカンテルと相互作用を示す薬剤については，いずれの例も内服歴はなかった．

メコン住血吸虫とタイ肝吸虫については，同一村落で複数の感染者が見つかったが，別の児童が感染しており，特に感染者が同一人物に集積する傾向はみられなかった．最も感染者が多かった鉤

表１　カンボジア クラチエ省のメコン住血吸虫症有病地における集団治療
（プラジカンテル+メベンダゾール）１ヶ月後の糞便検査　－2008 年５月－

陽性者数（%）

村　落 学校名	検査検体数 被検者数	メコン 住血吸虫	タイ 肝吸虫	回 虫	鞭 虫	鉤 虫
Rokakandal	134	0	0	0	0	11 (8.2)
Sambox	137	0	0	0	0	8 (5.8)
Sre Khoeun	112	2 (1.8)	2 (1.8)	0	0	10 (8.9)
Chartnol	149	0	0	0	0	11 (7.4)
Sambo	165	0	1 (0.6)	0	1 (0.6)	17 (10.3)
K. Krabei	121	0	0	0	1 (0.8)	16 (13.2)
Achen	121	1 (0.8)	0	0	0	12 (9.9)
合計	939	3 (0.3)	3 (0.3)	0	2 (0.2)	85 (9.1)

虫についても，メコン住血吸虫，タイ肝吸虫など他の寄生吸虫卵検査で陽性を示した例は少なく，混合感染例はタイ肝吸虫との混合感染１例にとどまった（表 2）．一方，鞭虫卵の陽性者は，鉤虫卵の陽性者と陰性者で１人ずつみられた．

また，プラジカンテル再投与（用量 60mg/kg/日で２分割投与）後の検査では，メコン住血吸虫卵，タイ肝吸虫卵とも陰性であった．

表２　鉤虫卵陽性者における他の寄生蠕虫卵の検出状況

	鉤虫陽性例 (85 例)	鉤虫陰性例 (854 例)	合計 (939 例)
メコン住血吸虫	0	3	3
タイ肝吸虫	1	2	3
鞭　虫	1	1	2
回　虫	0	0	1
寄生蠕虫卵陰性例	83	848	930

考察

プラジカンテルは，日本国内ではビルトリシドとして市販されており，添付文書では，肝吸虫症と肺吸虫症で，総量 80mg/kg（1 回 20mg/kg を 1 日 2 回 2 日間），横川吸虫症で，総量 20～40 mg/kg（1 回 20mg/kg を 1 日 1～2 回）によるする内服法が，適当な例としてあげられている[5]．本来，住血吸虫症治療薬として開発されたプラジカンテルが，国内での感染例がないことより，住血吸虫症が保険適応疾患として記載されていないのは，奇異な印象を受けるが，国内で入手しやすい治療指針では，１日に複数回の投与で，２日以上の投与期間を推奨するものが多い[6]．一方，欧米の標準的な内科学書や熱帯医学のテキストでは，単回投与か１日で投与をやめる方法が記載されるのが一般的である[6]．

プラジカンテルによる治療効果の減弱は，1990 年代からアフリカのマンソン住血吸虫症でよく知られており，一つはセネガル北部にある伝播の高い高度浸淫地で，もう一つはエジプトである[2)3)]．ところが，最近は，ケニアといった東アフリカの国でも，プラジカンテルの感受性低下が疑われる例が報告されるようになった[4]．これらの地域では，プラジカンテルによる治療を 2～3 回繰り返した後でも，1～2%の患者は治癒しなかった[2]．

今回のカンボジアのメコン住血吸虫症有病地での調査では，プラジカンテルを 40mg/kg の用量でする集団治療後，メコン住血吸虫，タイ肝吸虫とも 0.3%の例で，糞便検査で虫卵が陽性となったが，プラジカンテルを総量 60mg/kg/日で２分割という内服法で再投与したところ，全例で治癒が

確認された．一方，最近の中国での日本住血吸虫症治療例では，プラジカンテルを総量 80 mg/kg/日で 2 分割投与したにもかかわらず，185 例のうち 1 例で，治癒が確認されなかった[7]．もっとも，中国の別の報告では，用量 40mg/kg の 1 回投与で治療を受けた 584 人のうち，6 週間後の糞便検査で治癒が確認されなかった 19 例について，再度同じ用量でのプラジカンテル投与により，6 週後全例で治癒が確認されている[8]．住血吸虫の高度流行地にあっては，薬剤の感受性低下と再感染を厳密に区別することは難しい．特に，乾季に感染リスクが急速に増し新感染もその時期に集中するメコン住血吸虫については，プラジカンテルによる治療困難例と再感染例を区別することは，再治療の効果判定も含め，乾季の終わり頃でなければ困難である．少なくとも現時点では，アジア地域における住血吸虫に対するプラジカンテルの耐性出現は，はっきり確認されたとは言えず，臨床的にもまだ大きな問題とはなっていない．

ただ，プラジカンテルの投与量については，使用当初より，用量 40mg/kg の 1 回投与法よりも，用量 40～60mg/kg/日の用量で 分割する投与法で，高い治療効果が得られていた可能性もある．最近になるまで，用量 40mg/kg での 1 回投与をプラジカンテル治療の標準としてきた中国では，住血吸虫による中枢神経症状は難治とされてきたが，病院受診例を中心に，用量 60mg/kg/日の用量で 1～3 分服する投与法が選択されることが多かったフィリピンでは，中枢神経症状は，後遺症を残すことなく改善することが殆どであった[8)9)]．プラジカンテルには，Ca チャンネルを介して成虫の筋麻痺を起こし虫体の排泄を促す，よく知られた作用以外に，成熟卵に対して生体内で孵化させる働きもある[1)10)]．プラジカンテル投与後に肝線維化や門脈壁肥厚の改善傾向がみられるのは，プラジカンテルによって，肝線維化の主因である虫卵結節から，成熟虫卵が排泄されることで説明できるが，同様な作用は，中枢神経症状の原因となっている脳内の虫卵結節に対してもおきていると思われる．この際，脳－血液関門を通ってプラジカンテルの濃度が中枢神経内でも増加するには，分割投与したほうが望ましいのかもしれない．さらに，成虫が体内から排出された初回治療後にも体内に残存する虫卵排泄を目指し，虫卵の成熟を待って初回治療の 2～3 週後に，再度プラジカンテルの服用を推奨する報告もある[6]．

マラリア原虫をはじめ，単細胞の原虫では，従来から薬剤耐性株の出現と拡散が，問題となっていたが，多細胞で一般に寿命が長い寄生蠕虫では，薬剤耐性が臨床的に問題となることは従来殆どなかった．しかし，ヒトよりも駆虫薬の使用頻度がはるかに多かった家畜では，既にベンズイミダゾール系薬剤に対する耐性が，各地で報告され，ヒツジの線虫 *Haemonchus contortus* のベンズイミダゾール耐性に関する研究では，薬剤耐性と関連した遺伝子変異も明らかになっている[11]．現在，寄生蠕虫症対策の為にプラジカンテルやメベンダゾールといったベンズイミダゾール系薬剤の使用が急速に拡大していることから考えて，これらの抗寄生蠕虫薬に対する耐性は，アジアも含め地球規模で，経時的・組織的なモニタリングをしていかねばならない．

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文　献

1) 大前比呂思（2010）：プラジカンテル，メベンダゾール，アルベンダゾール，トリクラベンダゾール．Medical Practice. 27, 1549-1554.
2) Ismail, M. *et al.* (1999) : Resistance to

praziquantel: direct evidence from *Schistosoma mansoni* isolated from Egyptian villagers. Am J Trop Med Hyg, 60, 932-935.

3) Van Lieshout, L. *et al.* (1999) : The contribution of host-related factors to low cure rates of praziquantel for the treatment of *Schistosoma mansoni* in Senegal. Am J Trop Med Hyg, 61, 760-765.
4) Sandra, D.M. *et al.* (2009) : Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of *Schistosoma mansoni*. Plos Neg Dis, 3 e504, 1-10.
5) バイエル薬品　ビルトリシド添付文書（2009年9月改訂）1 - 3.
6) Chigusa, Y. (2010) : Praziquantel : Presribed dosage for schistosomiasis refereedtoin the book, TODAY's THERAPY from 1979 to 2010 in Japan, Annual Report of Institute of International Education and Research, 2, 67-74.
7) Edmund, Y.W. *et al.* (2011) : Human schistosomiasis resistance to praziquantel in China: Should we be worried ? Am J Trop Med Hyg, 85, 76-82.
8) Ting, Lei. *et al.* (2008) : Surgical treatment of epilepsy with chronic cerebral granuloma caused by *Schistosoma japonicum*. Epilepsia, 49, 73-79.
9) Watt, G. *et al.* (1986) : Praziquantel treatment of cerebral schistosomiasis. Lancet, 8506, 529-532.
10) Matsuda, H. et al. (1983) : Mechanism of action of praziquantel on the eggs of *Schistosoma japonicum*. Jpn J Exp Med 53, 271-274.
11) Sangster, N.C. and Gill, J. (1999) : Pharmacology of anthelmintic resistance. Parasitol Today, 1999, 15, 141 - 146.

$$\text{PT-INR} = \left(\frac{\text{被検血漿 PT（秒）}}{\text{標準正常血漿 PT（秒）}} \right)^{\text{ISI}}$$

図 1　PT-INR の算出方法

適治療域と考えていたワルファリンの投与量が，実際には効果が不十分な量であったというような事態も発生し，血栓症や塞栓症などの合併症を引き起こしてしまう可能性も考えられる．このような状態，すなわち ISI の値によって PT-INR 値が見かけ上高くなってしまう場合を，「人為的ワルファリンの不応性」という．人為的ワルファリンの不応性は，標準血清などによる感度の補正を行うことでもある程度改善できるが，その詳細については文献[1]を参照していただきたい．

文献

1）島津千里，鈴木節子，風間睦美，他：WHO 標準トロンボプラスチンによる INR 表示血漿の評価．日本検査血液学会雑誌，1（2）：145～147，2000.

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新たに 4 類感染症に追加されたチクングニア熱

はじめに

チクングニア熱は，トガウイルス科アルファウイルス属チクングニアウイルスによる急性の発熱性疾患である．ヤブカ（ネッタイシマカやヒトスジシマカ）によって媒介され，4～7 日の潜伏期を経て発症することが多い．

発熱，関節痛，発疹が 3 主徴であり，同じくヤブカで媒介されるデング熱（フラビウイルス科デングウイルスによる）と症状がよく似ている．多くは 7 日以内に症状は消失するが，多関節痛が長期間続くこともある．

チクングニア熱は基本的に熱帯感染症という性格をもつが，近年流行地は拡大しており，再興感染症として注目されている．わが国でも，2011 年 2 月から 4 類感染症および検疫感染症として，国内での発生・蔓延を防ぐ体制が強化された．

世界的再興と温帯での国内伝播

2004 年にケニアで始まったチクングニア熱の流行は，2005～2006 年には仏領レユニオン（推定患者数 27 万人）やインド（推定患者数 140 万人），2009 年までには東南アジアの広い地域に拡大した．これに伴い，海外旅行者による輸入症例も増加し，一部の温帯地域でも国内伝播が報告されている．2007 年には，イタリアで 295 例のチクングニア熱が確認され，インドからの旅行者が発端症例と推定されている．2010 年には，フランスや中国でも報告された．

このように広範な地域に流行が拡大した背景として，ヒトスジシマカでの増殖活性が増加したウイルス株の出現などがいわれている．

4類感染症への追加による影響

国立感染症研究所ウイルス第一部第2室のまとめでは，4類感染症指定以前に，18例の輸入症例が把握されている．ヒトスジシマカが東北以南に広く分布しているわが国においても国内伝播が発生する可能性がある．4類感染症に指定されたことで，医師による届出が義務づけられ，媒介蚊の駆除対策などが法的に可能となった．届出の要件となる検査診断（分離・同定法による病原体の検出，PCR法による血清中の病原体遺伝子の検出，ELISA法による血清中のIgMまたはIgG抗体の検出）を行えるところは限られるが，サーベイランスが強化されることになる．今のところ，温帯では一時的な定着にとどまり，患者発生も小規模であるが，今後の動向が注目される．

文献

1) Charrel, R. N., de Lamballerie, X., Raoult, D.：Chikungunya outbreaks—the globalization of vectorborne diseases. *N. Engl. J. Med.*, **356**（8）：769～771, 2007.
2) Rezza, G., Nicoletti, L., Angelini, R., et al.：Infection with chikungunya virus in Italy：an outbreak in a temperate region. *Lancet*, **370**（9602）：1840～1846, 2007.
3) Randolph, S. E., Rogers, D. J.：The arrival, establishment and spread of exotic diseases：patterns and predictions. *Nat. Rev. Microbiol.*, **8**（5）：361～371, 2010.
4) Mizuno, Y., Kato, Y., Takeshita, N., et al.：Clinical and radiological features of imported chikungunya fever in Japan：a study of six cases at the National Center for Global Health and Medicine. *J. Infect. Chemother.*, 2010（Epub ahead of print）.

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感染症サーベイランスーその役割と展望

それぞれの機関が果たす役割

医療機関

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要　旨　医療機関は自施設の医療関連感染サーベイランスを実施するほか，感染症発生動向調査の重要な情報源にもなっている．特に国内排除を目指す麻疹や薬剤耐性菌のサーベイランスにおいて，医療機関の果たす役割は大きい．

はじめに

感染症サーベイランスは，ある特定の集団における感染症に関する情報を継続的・体系的に集めて分析し，それを必要とする者に還元することである[1]．医療機関は，感染症法などに基づいて行われる感染症サーベイランスの重要な情報源になるとともに，自施設の入院患者や医療従事者に発生する医療関連感染のサーベイランスを実施することも求められている．施設内伝播が生じやすいインフルエンザや麻疹などにおいては，両者が連動するようなサーベイランスも重要である．本項では感染症サーベイランスにおける医療機関の役割について概説する．

■医療関連感染サーベイランス

1975～85年に米国で行われた研究（the Study on the Efficacy of Nosocomial Infection Control：SENIC）によれば，医療関連感染の約30%は予防可能であり，医療関連感染のサーベイランスが有効な感染防止策の主要な構成要素としてあげられた[2]．これ以降，医療機関でサーベイランスを含めた感染防止策の導入が図られていくが，免疫不全患者の増加や侵襲的な医療行為の増加などに伴い，医療関連感染を減少させることは容易ではない．薬剤耐性菌に関するメディアの関心は高く，医療の質向上の観点からも医療関連感染への国民的な関心が高まっている．国際的にもこのような傾向にあり，欧米では自施設の医療関連感染の発生率について当局への届出が義務づけられるようになってきている[3]．

我が国では，厚生労働大臣が定める院内感染防止対策の基準に『当該保険医療機関内において（病院である保険医療機関においては，当該病院にある検査部において），各病棟の微生物学的検査に係る状況等を記した「感染情報レポート」が週1回程度作成されており，（中略）当該レポートは，入院中の患者からの各種細菌の検出状況や薬剤感受性成績のパターン等が病院または有床診療所の疫学情報として把握，活用されることを目的として作成される』とあり，病院で薬剤耐性菌のサーベイランスを行うことが求められている[4]．

また，2006年に改正された医療法施行規則では，医療機関等の管理者に院内感染対策のための体制確保が義務づけられた．院内感染対策の指針策定，院内感染委員会の開催，従業者の研修，感染症の発生状況の報告などが示されている．この

改正通知に添付されているガイドライン案では，中小病院においても対象別サーベイランスを可能な限り実施することが望ましいとされている[5]．このような法令や指針に従うばかりでなく，自施設の感染防止策につながるような内容のサーベイランスを行うことが大切である．

1. 医療関連感染で重要な薬剤耐性菌のサーベイランス

感染症法ではバンコマイシン耐性黄色ブドウ球菌（VRSA），バンコマイシン耐性腸球菌（VRE）感染症が全数報告，メチシリン耐性黄色ブドウ球菌，多剤耐性緑膿菌（MDRP），多剤耐性アシネトバクター属菌による感染症が定点報告対象になっている．最近，新しいカルバペネマーゼ（NDM-1やKPC型）を産生する腸内細菌科の細菌も注目され，全国的な実態調査も行われた[6]．

分離菌の種類や推移が検体別，病棟別に把握され，情報が還元される必要がある．比較的少ない労力で実施することができ，ある程度薬剤耐性菌による医療関連感染の発生数のベースラインを把握することができる．何らかの感染症が疑われて採取された検体からの分離菌を検査室で監視する受動的サーベイランスであるため，感染対策担当者の病棟回診などを併用することで，より詳しい情報が得られる．

薬剤耐性菌は保菌者も感染源になるため，患者，医療従事者（場合により医療器具などの環境を含める）の保菌状況を把握し，感染対策に役立てようとするのが積極的監視培養（active surveillance culture）である．保菌者を特定した後に接触感染予防策をとるなど感染防止策が実行可能な場合において，有効な手段となりうる[7]．

2011年2月に公表された院内感染対策中央会議による提言によれば，アウトブレイクと仮定する目安としては1例目の発見から4週間以内に，同一病棟において新規に同一菌種による感染症の発病症例（菌種によっては保菌者を含む：VRSA，MDRP，VRE，多剤耐性アシネトバクター・バウマニ等）が3例以上特定された場合などとされている[8]．ここにあげられている薬剤耐性菌は国内での検出は少なく，感染防止策として積極的監視培養の対象となる代表と考えられる．

2. 対象別サーベイランス

医療関連感染のうち，血管内留置カテーテル感染，尿道留置カテーテル感染，手術部位感染，人工呼吸器関連肺炎が最も重要である[9]．これらは厚生労働省院内感染対策サーベイランス事業（JANIS）の対象疾患でもあり，症例定義が示されている．全病棟を対象にこれら四大医療関連感染の包括的サーベイランスを行うのは労力が大きいため，集中治療室などの感染リスクが高い部門に限って実施することも行われている．いずれも感染対策担当者がサーベイランスシートなどを用いて調査を行い，発生率などの指標が算定され，情報を医療現場に還元する．

3. 症候群サーベイランス

本来，バイオテロを早期に検出することを目的とした症候群サーベイランスは医療関連感染のサーベイランスとしても有用である．

1)急性呼吸器症状サーベイランス

2002～03年に流行した重症急性呼吸器症候群（SARS）は医療機関内で伝播し，8,000名を越える報告患者のうち21%が医療従事者であった．世界保健機関は発熱，急性呼吸器症状がある医療従事者や入院患者の多発を早期に検出することをSARSアラートとして提唱した．インフルエンザ迅速診断キットの陽性率を同時にみることでインフルエンザの施設内伝播の早期検出に役立つと考えられる[10]．

2)下痢症サーベイランス

嘔吐，下痢症状を呈する入院患者，医療従事者での発生数を監視し，クロストリジウム関連腸炎，ノロウイルス胃腸炎などを早期に把握することを目的とする．こちらも抗原検査を併用することで情報の精度を高めることができる．

4. 職業感染に関連したサーベイランス

患者血液および体液の曝露は血液媒介病原体（HIV，HBV，HCV など）の職業感染の原因となる．このような事例の発生数，発生状況，使用された鋭利器材の種類などの情報を収集し，防止策に活用する．バージニア大学で開発された分析用データベース（エピネット）の日本版が普及しており，職業感染制御研究会によってエイズ拠点病院を中心に全国的なサーベイランスが行われている．

5. ケアプロセスに関連したサーベイランス

感染防止に有効であることが示されているプロセスの遵守率などをサーベイランスすることも医療関連感染を減らすための対策を立てる上で重要と考えられる．例えば，擦式手指衛生消毒薬の使用量，中心静脈カテーテル挿入時のマキシマルバリアプレコーション遵守率，医療従事者のインフルエンザワクチン接種率などである[11]．抗菌薬の種類別使用量も院内分離菌の抗菌薬感受性試験成績と併用されることで，抗菌薬適正使用に向けた重要な情報となる．

■感染症発生動向調査事業における医療機関の役割

感染症法に基づく全国的な感染症サーベイランスである感染症発生動向調査事業においても医療機関の役割はきわめて大きい．まず，報告すべき感染症が医療機関で正しく診断され，最寄りの保健所に届け出られなければ，サーベイランスが成立しないからである[1]．疾患によっては，保健所などにより積極的疫学調査が行われるが，医療機関における診療記録はその貴重な情報源である．一施設では気づかれない感染症の流行が，各医療機関からの情報提供により地域での流行が明らかになることもある[12]．受診行動に必ずしも結びつかない不顕性感染や軽症の感染症の情報収集には限界があるが，医療機関が決定的な役割を担っていることを認識する必要がある．

届出の用件として，微生物・血清診断が要求されることが多く，積極的な検査診断が望まれる．例えば，3類感染症のコレラ，細菌性赤痢は相対的に輸入例の多いことを認識し，旅行者下痢症の患者では積極的に便培養を行うようにする．検体採取とその後の処理はサーベイランスに影響することを医療機関は認識する必要がある．なお，届出の要件となる検査診断が保険診療として実施できない場合，その疾患を疑った時点で保健所に連絡をして，地方衛生研究所に検査を依頼することができる（行政検査）．

1. 積極的サーベイランスのモデルとなる麻疹の国内からの排除

感染症発生動向調査事業は医療機関からの届出を待つ受動的なものであるが，疾患によっては症例の積極的な把握が必要なものがある．2012 年に国内からの排除を目指している麻疹がその代表例である．医療機関は麻疹の疑われる症例を診療した場合は，速やかに保健所と連絡をとり，咽頭ぬぐい液・血液・尿を確保し，地方衛生研究所で微生物診断を行うことが求められている[13]．また，保健所の担当者に感染防止上必要な情報を提供することも求められている．麻疹にはワクチンという有効な予防方法があり，接触者の曝露後予防についてもある程度確立した方法がある．国内からの排除という大きな目標に向けて，医療機関の役割を認識し，保健所などとの連携を深めていく必要がある．

おわりに

本項でとりあげたように医療機関が感染症サーベイランスに果たす役割は自施設の規模や提供する医療内容などに応じて多岐にわたっている．医療機関で質の高い感染症サーベイランスを実施していくには，人材的・財政的な支援が欠かせない[3]．また，医療機関での IT 化をさらに進めるなどして，情報収集を自動化したサーベイランスなども検討される必要がある[14]．

文　献

1) Thacker SB, Choi K, Brachman PS：The surveillance of infectious diseases. *JAMA* 249：1181-1185, 1181.
2) Haley RW, Culver DH, White JW *et al.*：The efficacy of infection surveillance and control programs in preventing nosocomial infections in US hospitals. *Am J Epidemiol* 121：182-205, 1985.
3) Sydnor ERM, Perl TM：Hospital epidemiology and infection control in acute-care settings. *Clin Microbiol Rev* 24：141-173, 2011.
4) 厚生労働省：基本診療料の施設基準等及びその届出に関する手続きの取扱いについて(保医発 0305 第 2 号).
5) 厚生労働科学研究 安全性の高い療養環境及び作業環境の確立に関する研究班．中小病院/診療所を対象にした医療関連感染制御策指針(案)2006.
http://www.mhlw.go.jp/topics/bukyoku/isei/i-anzen/hourei/dl/070508-2.pdf
6) 厚生労働省：「我が国における新たな多剤耐性菌の実態調査」の結果について.
http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou19/dl/multidrug-resistant-bacteria_p.pdf
7) Ostrowsky BE, Trick WE, Sohn AH *et al.*：Control of vancomycin-resistant enterococcus in health care facilities. *N Engl J Med* 344：1427-1433, 2001.
8) 厚生労働省：院内感染対策中央会議提言について.
http://www.mhlw.go.jp/topics/bukyoku/isei/i-anzen/hourei/dl/110215_1.pdf
9) Chotani RA, Roghmann MC, Perl TM：Nosocomial infections. Infectious disease epidemiology：theory and practice, 505-574, Nelson KE, Williams CM, Johnes and Bartlett Publishers, Boston, 2007.
10) Kawana A, Teruya K, Kirikae T *et al.*："Syndromic surveillance within a hospital" for the early detection of a nosocomial outbreak of acute respiratory infection. *Jpn J Infect Dis* 59：377-379, 2006.
11) Tokars JI, Richards C, Andrus M *et al.*：The changing face of surveillance for health care-associated infections. *Clin Infect Dis* 39：134-152, 2004.
12) ヨハン・ギゼック（山本太郎，門司和彦訳）：感染症監視システム．感染症疫学，126-138，昭和堂，京都，2006.
13) 厚生労働省：麻しんの検査診断について.
http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou21/tsuuchi_101111_01.html
14) Klompas M, Yokoe DS：Automated surveillance of health care-associated infections. *Clin Infect Dis* 48：1268-1275, 2009.

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SHORT COMMUNICATION

A novel C-type lectin identified by EST analysis in tissue migratory larvae of *Ascaris suum*

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Abstract C-type lectins (CTLs) are a group of proteins which bind to carbohydrate epitopes in the presence of Ca^{2+}, which have been described in a wide range of species. In this study, a cDNA sequence coding a putative CTL has been identified from the cDNA library constructed from the pig round worm *Ascaris suum* lung L3 (LL3) larvae, which was designated as *A. suum* C-type lectin-1 (As-CTL-1). The 510 nucleotide open reading frame of As-CTL-1 cDNA encoded the predicted 169 amino acid protein including a putative signal peptide of 23 residues and C-type lectin/C-type lectin-like domain (CLECT) at residue 26 to 167. As-CTL-1 was most similar to *Toxocara canis* C-type lectin-1 and 4 (*Tc*-CTL-1 and 4), and highly homologous to namatode CTLs and mammalian CTLs as well, such as human C-type lectin domain family 4 member G (CLECG4). In addition, As-CTL-1 was strongly expressed in tissue migrating LL3 and the L4 larvae, which were developmental larvae stages within the mammalian host. These results suggest that *A. suum* larvae might utilize As-CTL-1 to avoid pathogen recognition mechanisms in mammalian hosts due to it is similarity to host immune cell receptors.

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Introduction

C-type lectins (CTLs) constitute a large family of proteins that binds carbohydrate moieties in a Ca^{2+}-dependent manner (Drickamer 1988, 1996). They are characterized by a conserved C-type lectin/C-type lectin-like domain (CLECT) which shares Ca^{2+}- and carbohydrate-binding motifs. CLECT also contains at least four critical cysteine residues which form a two-loop structure by disulphide bounds. It is well known that CTLs are widely expressed among metazoan organisms (Drickamer and Fadden 2002; Zelensky and Gready 2005). In vertebrates, CTL represents a very large family that is classified into 17 groups (Drickamer and Fadden 2002), many of which are known as pattern-recognition receptors implicated in the recognition of pathogens by innate immunity (Weis et al. 1998). In addition, some evidence have indicated that CTLs play an important role in immune homeostasis by endogenous 'self' ligand recognition (García-Vallejo and van Kooyk 2009), and they themselves have a bactericidal activation (Cash et al. 2006).

Ascaris suum, a common round worm in pigs, is infective to a wide range of hosts, including humans, mice, cattle and chickens. When embryonated eggs are ingested by a definitive swine host, larvae hatch in the small intestine, penetrate the intestinal mucosa, and migrate through the liver and lungs, before finally reaching the intestine, where they sexually mature and produce eggs (Dold and Holland 2011). In contrast, it is generally considered that larvae, which are reached the lungs following liver migration, disperse into various tissues and

organs without further development in non-swine host (Slotved et al. 1998; Crompton 2001), although *A. suum* has been reported to develop into adult stage infrequently in human hosts (Anderson 1995; Nejsum et al. 2005; Arizono et al. 2010). However, it has not been fully explained how they discriminate pigs and other animals and what kind of interaction is involved between host and parasite during the lung phase of migration.

Expressed sequencing tag (EST) analysis is a powerful tool for profiling the gene expression pattern in a particular parasite population. Although publicly available EST databases of *A. suum* already exist in NAMBASE4 (http://www.nematodes.org/nembase4/index.shtml), they were constructed from adult worm, intestinal L4 larvae, newly hatched infective larvae (iL3) and egg embryos. EST analysis of tissue-migrating larvae has not yet been performed. Therefore, we explored cDNA of *A. suum* lung L3 (LL3) collected from infected rabbit lungs in order to examine what kind of biological processes were activated in tissue-migrating larvae. As one of the most frequently occurring clones, we identified a cDNA sequence for a putative *A. suum* CTL that showed specific expression during internal larval stages in mammalian hosts.

Materials and methods

Parasites and infection

Adult *A. suum* were collected from infected pigs at a local abattoir in Japan. Eggs were freed from the uterine tissue by incubating uteri in 0.1 N NaOH. After washing with distilled water, eggs were suspended and stirred in 0.1 N H_2SO_4 and cultured at 27°C for 3–4 weeks. Infective L3 larvae (iL3) were mechanically hatched from eggs and isolated free from egg shell contaminants (Takamiya et al. 1993). For the preparation of lung L3 larvae, male Japanese white rabbits (Kyudo, Kumamoto, Japan) were orally inoculated with 1.5×10^5 embryonated eggs. Six days after infection, the lungs were removed and cut into 5-mm cubes using scissors. The cubes were wrapped with Kimwipe papers and incubated in phosphate-buffered saline (PBS) at 37°C for 1.5 h, and then emerging worms were collected. Culture driven-L4 larvae (cL4) were obtained from cultures of LL3 in vitro (Islam et al. 2006).

RNA isolation and cDNA library construction

Total RNA of LL3 was isolated with TRIzol Reagent (Invitrogen, Carlsbad, CA), followed by purification of poly $(A)^+$ RNA with GenElute™ mRNA Miniprep Kit (Sigma, St. Louis, MO). A cDNA library was constructed using the SMART cDNA Library Construction Kit (Clontech, Mountain View, CA). The reverse transcription step was performed using MMLV Reverse Transcriptase with the SMART IV oligonucleotide primer and the CDS III/3′ PCR primer provided in the kit. The double-stranded cDNA (ds-cDNA) was synthesized by long distance PCR with the 5′ PCR primer and the CDS III/3′ PCR primer using the Advantage 2 PCR kit (Clontech). The ds-cDNA was treated with proteinase K and then digested by *Sfi*I. After size fractionation, cDNA was cloned into pDNR-LIB vector, and transformed into *Escherichia coli* ElectroMAX™ DH10B™ cells (Invitrogen, Carlsbad, CA).

EST sequencing, processing and analysis

Plasmid DNA of the 2,024 randomly selected clones was extracted and single-pass sequenced from the 5′-end using sequencing primer (5′-GCATACATTATACGAAGTTAT CAGTCG-3′). The sequencing was conducted on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA), using ABI Prism Big-Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). EST sequences were clustered using SEQUENCHER (Gene Codes Corporation, Ann Arbor, MI), with a minimum sequence overlap length cut-off of 30 bases and an identity threshold of 90%, for the removal of flaking vector and adaptor sequences, followed by assembly. These contigs and singletons were subjected to BLASTN and BLASTX programs (E value of $\leq1\times10^{-5}$) at the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A protein sequence motif was identified using the InterProScan at The European Bioinformatics Institute (Zdobnov and Apweiler 2001). Alignment of ESTs was conducted by using GENETYX-WIN software (Genetyx Corporation, Tokyo, Japan).

Real-time PCR analysis

Total RNA from iL3, LL3, cL4 and adult worm tissues (head, muscle, intestine, uterus, ovary and testis) was extracted with TRIzol reagent. After treatment with DNaseI (Ambion Inc., Austin, TX), cDNA was generated from 250 ng of total RNA using PrimeScript® 1st strand cDNA Synthesis Kit (Takara Bio Inc., Shiga, Japan). Primer sets for amplification were as follows: As CTL-1 (sense, 5′-CCACCATGTTCTCGACCGTTGCT-3′; antisense, 5′-ATTTCCTCCTACTGGCGCTCCT-3′) and 18S ribosomal RNA gene (sense, 5′-ATCGGTCGCGTAGGGTGGCT-3′; antisense, 5′-AAGCCGCAGGCTCCACTCCT-3′). Real-time PCR was then performed with an ABI Prism 7000 Sequence Detection Systems (Applied Biosystems) and a GoTaq® qPCR Master Mix (Promega, Madison, WI). Relative quantification was assessed by normalizing the amount of the target transcript to the 18S ribosomal RNA gene.

Results and discussion

In paratenic hosts such as humans, larvae of *A. suum* penetrate the mucosal epithelium but thereafter remain developmentally arrested in the migratory tissue phase (Crompton 2001). To understand biological events taking place in the arrested larvae, we carried out EST analysis in a cDNA library of migrating L3 larvae (LL3) collected from infected rabbit lungs (LL3). As a result from 5′ ends single-pass sequencing of 2,024 clones, 1,650 ESTs were yielded. Upon clustering, these ESTs were represented by 279 distinct gene products, which consist of 78 contigs and 201 singletons.

The consensus sequences of contigs and singletons were compared against NCBI BLAST databases in BLASTX analysis, revealing a novel CTL sequence referred to as *A. suum* C-type lectin-1 (As-CTL-1). We focused on this molecule, because CTL might contribute to the establishment of successful parasitism in nematodes (Loukas et al. 1999; Urwin et al. 2002). Using RT-PCR, the full-length cDNA corresponding to As-CTL-1 was successfully amplified from LL3 RNA (data not shown). As-CTL-1 is 710 nucleotides (GenBank accession no. HQ025087), which encoded the protein of 169 amino acids including the putative signal peptide of 23 residues and C-type lectin/C-type lectin-like domain (CLECT) at residue 26 to 167. The four cysteine residues at positions 62, 136, 154 and 166, which are required to form the CLECT internal disulfide bridge formations (Zelensky and Gready 2003), were completely conserved in As-CTL-1, but the WIGL and WND motifs conserved in the classical CTLs (Zelensky and Gready 2003) were replaced by WLAL and WDD. According to carbohydrate specificity, CTLs are categorized into mannose/GlcNAc - or galactose/GalNAc -recognizing lectins (Weis et al. 1992; Iobst and Drickamer 1994). These differences suggest substitutions at the key substrate binding residues. As-CTL-1 had QPD, which was found in galactose/GalNAc-binding CTLs. Thus, although the Ca^{2+}-dependent carbohydrate binding activity of As-CTL-1 was not assessed in this study, it is most likely a galactose-binding CTL from its amino acid motifs.

Subsequent sequence analysis showed that the amino acid sequence of CLECT in As-CTL-1 was found to have 33% identity to canine roundworm *Toxocara canis* C-type lectin-1 (*Tc*-CTL-1) and 38% identity to *Tc*-CTL-4. It has been reported that the free-living nematode *Caenorhabditis elegans* has more than 270 genes encoding CLECT of CTLs in their genome (Schulenburg et al. 2008). Interestingly, As-CTL-1 showed greater identity with human CTL domain family 4 member G (CLECG4; 28% identity) than with the *C. elegans* homologue (clec-149; 24% identity). Considering the eukaryotic phylogeny, it is reasonable that several CTLs from parasitic nematodes, such as *Ancylostoma ceylanicum* (AceCTL-1), *Necator americanus* (NaCTL-2), *Heligmosomoides polygyrus* (*Hp*-CTL-1) and *Nippostrongylus brasiliensis* (*Nb*-CTL-1 and 2) CTLs, share greater identity with *C. elegans* CTLs than mammalian CTLs (Brown et al. 2006; Daub et al. 2000; Harcus et al. 2009). On the other hand, *Tc*-CTL-1, *Tc*-CTL-4 and NaCTL-1, as well as As-CTL-1, appear to be much closer to homologues in mammalian CTLs than those in *C. elegans* (Loukas et al. 1999, 2000; Daub et al. 2000), raising speculations about the role they might play in the adaptation to parasitism.

The expression of As-CTL-1 was evaluated by real-time PCR in different developmental stages, including mechanically hatched iL3, LL3, cL4 and adult (Fig. 1). The mRNA for As-CTL-1 was scarcely detected in iL3 and tissues from adult worms, including the head, muscle, intestine, uterus, ovary and testis, whereas LL3 and cL4 larvae showed strong expression of As-CTL-1 transcript. After arriving at the jejunum, L3 larvae developed to L4 stage larvae in definitive swine host in vivo. These results indicate that the expression of As-CTL-1 is up-regulated through the tissue migrating stage and intestinal larval stage.

Although the physiological function of CTLs remains unclear, a number of nematode CTLs identified so far act as a pathogen recognition molecule or an antibacterial protein in immune responses to protect the worm itself against microbial infection (O'Rourke et al. 2006; Schulenburg et al. 2008). However, considering that the expression of As-CTL-1 is confined during tissue migration, it would not be very likely that As-CTL-1 is employed for the recognition of microbes in *A. suum*, because the worms may not encounter hazardous bacteria on the migrating route, which would be maintained clean by the host immunity. Instead, greater sequence identity that As-CTL-1 shares with mammalian CTLs than *C. elegans* proteins, seems to suggest that As-CTL-1 acts as a

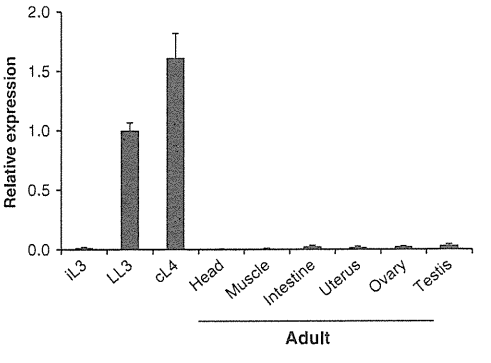

Fig. 1 Comparison of As-CTL-1 mRNA expression in developmental stages. Real-time PCR was performed with mechanically hatched iL3, LL3, cL4 and various adult worm tissues (head, muscle, intestine, uterus, ovary and testis). Relative expression of the As-CTL-1 mRNA was assessed by normalizing to 18S rRNA expression. Data were expressed as a ratio to As-CTL-1 gene expression in LL3