

松 山 大 学 論 集
第 22 卷 第 6 号 抜 刷
2 0 1 1 年 2 月 発 行

A Fundamental Study for the Development
of Chemotherapeutic Agents Targeted
at One of the Intractable Nematodes
of International Importance (note)

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RESEARCH NOTE

A Fundamental Study for the Development of Chemotherapeutic Agents Targeted at One of the Intractable Nematodes of International Importance (note)

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ABSTRACT

The aim of this paper is to furnish the basis for our screening test by students specializing clinical pharmacy before their graduation. The methodology has been established. The intact worms belonging to the parasitic nematode (*Angiostrongylus cantonensis*) obtained in laboratory are incubated in buffered

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saline containing non-artificial substrate, glucose-1-phosphate at 37°C. The liberated Pi is estimated colorimetrically. At a physiological pH, the given substrate is significantly hydrolyzed by the intact worms. It has been clearly demonstrated that the substance in the surrounding medium can interact with the body of the nematode covered with the thick cuticle. In vitro screening of candidate substances against the nematode seems to be promising. Attention is paid to possible substances that inhibit the enzyme activity and/or the parasite movement. A large number of candidate substances including extracts from medicinal plants are worthy of being tested by students in an attempt to determine whether they have the potentiality to affect the nematode protected with the cuticle layer at the physiological pH.

Key words : Experimental chemotherapy, Parasites of international importance, Student practice for graduation

INTRODUCTION—the back ground and the aims of this paper

It goes without saying that a large number of problems on various diseases remain to be solved on the global level. For example, mankind on the earth has been suffering from many kinds of infectious diseases that should be treated from the viewpoint of clinical pharmaceutical sciences. Indeed, Japan has made immense progress in taking measures successfully for the domestic problems on infectious diseases. However, the country has to continue it by making greater efforts than before to overcome the problems of the diseases given the fact that they still remain to be controlled there with some species of them being no longer a hygienic problem. For example, we have to establish chemotherapy against the infectious diseases of international importance.

When we pay attention to parasitic diseases in the world, we notice that there are a number of obstinate parasitic helminthes (a large group of multi-cellular parasites) invading deeply into the host tissues, not to say the intractable parasitic protozoal diseases such as malaria with drug resistance. Not a few species of the parasites are responsible for some insoluble problems notwithstanding many research-workers' trial to eliminate them. We have to understand why they are hard to be controlled, to begin with. First, the life cycles of the parasites have been kept in the nature in spite of the hygienic measures taken there. Another reason is, no doubt, that no suitable chemotherapeutic measures have been established, though they are being imported into Japan. Thinking over the background from the viewpoint of hygienic pharmacy, the present authors are inclined to say that few pharmaceutical scientists are interested in the development of the drugs to expel the intractable parasites. The basic knowledge of the physiological and biochemical information on the parasites in question would be useful to promote the development of effective drugs. This is why the medical and pharmaceutical studies from a fundamental viewpoint is essential and the scientists in the related area are now expected to play a pivotal role in the possible elimination of their causative agents.

We always have to remember to discuss the parasite infestations of the past and today. Japan is not thought to be so much infested to our common sense nowadays, though there used to be much more patients harboring parasites. However, the kinds of parasites in and into Japan are extremely varied now. A large number of parasites that were not indigenous to our country have been imported from developing countries via vegetables and fish contaminated with eggs and larvae of parasites.

This paper pays attention to the international problems with parasites, especially to obstinate nematodes imported into Japan. *Angiostrongylus cantonensis* is one of such kinds of nematodes for the elimination of which pharmaceutical scientists

should be responsible. Though the life cycle of the parasite in the nature was clarified many years ago,¹⁾ it has been endemic in the Pacific Ocean Region, Southeast Asia and Southern Parts of Japan.²⁾ If we are infected with this nematode, it is hard for us to avoid suffering from eosinophilic meningoencephalitis,¹⁾ occasionally leading to unfortunate death. Those kinds of drugs which interact with the body of these obstinate parasites should be found *in vitro*, to begin with. Through preliminary studies, it has been clarified whether a given substance could interact with the parasite covered with the tough cuticle.

The new 6-year pharmaceutical education in Matsuyama University requires the student research practice for their graduation like that in other universities. As far as they belong to Department of Infectious Diseases for their research, they have to be familiar with the discipline of parasitic diseases of international importance. Of particular importance to them is the development of chemotherapy of intractable parasites. The basic attempts and preparations necessary for the students have been discussed in advance by the present authors so that the students might enter the experimental studies. This brief communication describes the preparatory investigations on the theme, "The contact of the intractable nematode, *A. cantonensis*, with the substances in the ambient medium" before their start to screen the candidate substances of somewhat efficacy. It deals with an example of a basis for an *in vitro* screening system to be established for the studies on the treatment of the nematode with the medical and pharmaceutical problems.

METHODOLOGY ESTABLISHED AND DISCUSSION

(I) Establishment of the methodology-A trial to furnish a basis for the screening system

The life cycle of this nematode is readily maintained in laboratory using Wistar

rats and fresh-water snails, *Biomphalaria glabrata*, as the final and experimental intermediate hosts.³⁾ Intact adult females of *A. cantonensis* are recovered from the pulmonary artery and heart of male Wistar rats infected with the nematode 4 months previously. They are washed in saline copiously to remove occasional blood clots and blotted on the filter paper. Five intact females were incubated for 1 hour at 37°C in Veronal-buffered saline containing 10 mM glucose-1-phosphate of analytical grade in a stoppered, cylindrical glass vessel. Control vessels are free from the worms. The experimental and control vessels are each duplicated. The pH range examined is pH 3.5-7. The pH of the incubation medium is checked before and after the incubation. At the end of a one-hour-period of shaking incubation (amplitude : 5.5 cm ; shaking rate : 100 cycles/min), the vessels is transferred into iced water, and the worms are removed without delay. After being blotted on the filter paper, they are dried at 90°C for 3 hours, and weighed to the nearest mg. Pi liberated is quantified by the method of Fiske and Subbarow. The phosphatase activity is expressed with the microgram of Pi liberated /hour/mg dry weight.

Whether a given substance such as glucose-1-phosphate in the ambient medium can interact with the nematode greatly interested the present authors before the trial. Nematodes are generally covered with a cuticle layer on the surface, thus being protected from the substances present in the ambient medium. However, it has been demonstrated that the substances in the surrounding medium interacted with the nematode as shown below.

Glucose-1-phosphate in the ambient medium is demonstrated to be hydrolyzed releasing phosphate due to the phosphatase activity of the intact parasites at the optimal pH ranging pH 5-6 (Fig. 1). The pH profile is as follows. At pH 3.5, the activity is about 20 Pi microgram/hour/mg dry weight. Comparable activity is observed at the neutral pH range. At the optimal pH the activity is more than twice as much high as that at pH 3.5. Thus it has been clearly demonstrated that a given

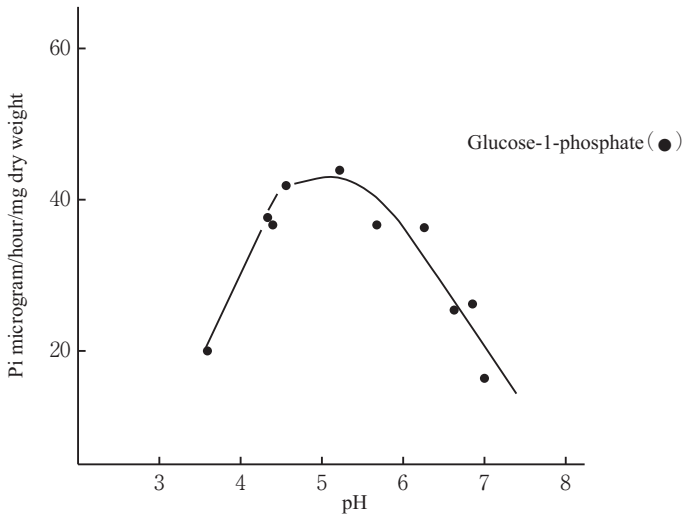


Fig. 1. Effect of pH on the hydrolysis of glucose-1-phosphate by intact *A. cantonensis*

substance in the ambient medium can interact with the body of the nematode.

(II) The basis to be furnished for the development of screening systems and the conclusion in the present communication

A large number of other test substances are going to be tested to know whether they have the potentiality to affect the nematode covered and protected with the cuticle. The present authors are interested in the possible efficacy of the extract from various kinds of medicinal plants as well as synthesized drugs against tissue-parasitic adult nematodes that are difficult to eliminate. Hopefully there seem to be some useful plants, the extracts of which were found to be effective against some filariae in vitro. A possible antifilarial effect of the extract from a medicinal plant against the dog heartworm has been studied with the positive result.⁴⁾ The extracts from the African and Latin-American plants have been tested so far in their

effectiveness on the movement of filariae *in vitro* by other researchers. For instance, the extract from *Neurolaena lobata*,⁵⁾ a plant indigenous to Central America and harvested in Guatemala, has been found to be effective *in vitro* against the movement of a kind of the filariae, *Brugia pahangi*. This extract effective on *B. pahangi* is worthy of being examined for the possible effectiveness on *A. cantonensis* because both of *B. pahangi* and *A. cantonensis* belong to the group of what is called the tissue-parasitic nematodes.

The system in the present communication for the hydrolysis of esters by the intact obstinate parasite, especially that at neutral pH values where the activity is still recognized³⁾ is thought to be useful for the *in vitro* screening of candidate substances against such parasites if attention is paid to the possible inhibitory effect of the substances on the phosphatase activity and/or the movement of the parasite in the hope that some of them might be of eventually chemotherapeutic value.

Travelers from Japan to countries of poor hygienic conditions should be careful not to be infected with parasites there.⁶⁾ The number of people carrying parasites and visiting our country has increased in recent years. Such changes in international circumstances have to be kept in mind, and the development of suitable drugs against obstinate parasitoses should go hand in hand with this.

The present authors have been interested both in medical and pharmaceutical studies on the parasite. It is of particular importance for the experimental chemotherapy of obstinate parasitic diseases to be pursued successfully from the viewpoint of international medicine and pharmacy. This is thought to have been exemplified in this research note and review. Our experimental data have been shown as one of the basic studies for the eventual purpose of internationally social welfare.

The series of these studies were originally started about 40 years ago.^{3,7)} In those days our interest was confined rather on the physiological aspects of the

nematode than on its chemotherapy. It has been unequivocally demonstrated that the nematode has the potentiality to hydrolyze the surrounding substrates such as glucose-1-phosphate with the pH profile as shown in this brief note.^{3,7)} The contact of one of the substances in the ambient medium to the nematode has clearly been concluded. The system has been found to be useful in demonstrating the modes of action of anthelmintic such as benzimidazoles.⁸⁾

Now, we are ready to screen many kinds of candidate extracts from various plants in their possible anthelmintic effect against the nematode, using the proposed system. The introduction and detailed data on it have been described in this communication as an extension of those in the previous review.⁹⁾ The methodology has been established now in this paper under the harmonious collaboration by the present authors as follows. Although it has been written mainly by the first author, J. Maki having performed experimental trials and collected information and findings, the senior authors, M. Kuwada and H. Sakagami have reviewed and criticized the manuscript as supervisors for the revision of this paper. And Y. Sekiya, R. Nishioka and E. Tamai have been cooperative enough to put the data and information in order, discussing the essential problems.

Last but not least, this brief paper would hopefully be dedicated to Dr. T. Yanagisawa, Emeritus Professor of School of Medicine, Kitasato University, who introduced *A. cantonensis* for parasitological studies into Japan, and attached much importance to the study on the physiology of parasites as a basis for the development of suitable chemotherapy.

REFERENCES

- 1) Mackerras, M. J. and Sandars, D. F. : The life history of the rat lungworm, *Angiostrongylus cantonensis* (Chen) (Nematoda : Metastrongylidae). Australian Journal of Zoology, Vol. 3, 1-25, (1955).

- 2) Yoshida, Y. : "Medical Zoology", *Angiostrongylus cantonensis*, 71, Nanzando(Tokyo), 4th ed. (2006).
- 3) Maki, J. and Yanagisawa, T. : Acid phosphatase activity demonstrated in the nematodes, *Dirofilaria immitis* and *Angiostrongylus cantonensis* with special reference to the characters and distribution. *Parasitology*, Vol. 80, 23-38, (1980).
- 4) Ghosh, N. K., Babu, S. P. S. and Sukul, N. C. : Antifilarial effect of a plant *Carica papaya*, *Japanese Journal of Tropical Medicine and Hygiene*, Vol. 26, 117-119, (1998).
- 5) Fujimaki, Y., Kamachi, T., Yanagi, T., Caceres, A., Maki, J. and Aoki, Y. : Macrofilaricidal and microfilaricidal effects of *Neurolaena lobata*, a Guatemalan medicinal plant, on *Brugia pahangi*, *Journal of Helminthology* Vol. 79, 23-28, (2005).
- 6) Yoshida, Y. and Arizono, N. : "Illustrated Parasitology", Nanzando(Tokyo), 7th edition, (2006).
- 7) Maki, J. and Yanagisawa, T. : "Phosphatase activity in *Angiostrongylus cantonensis* ", The 44th Annual Meeting of the Japanese Society of Parasitology held in Kyoto Prefectural Medical University (Kyoto), April, (1975).
- 8) Maki, J., Tongu, Y. and Ishii, A. : Studies on alterations in acid phosphatase activity, body weight and ultrastructure of adult *Angiostrongylus cantonensis* in rats treated with flubendazole at a subcurative dose, *Tropical Medicine*, 39, 95-100, (1997).
- 9) Maki, J., Sekiya, H., Sakagami, H., Kuwada, M., E. Tamai and Caceres, A. : A growing need for international cooperative studies to establish medicinal-plant therapy against obstinate and biohazardous nematodes in the tropical and subtropical areas and in Japan, *Japanese Journal of Social Pharmacy*, 28, 11-21, (2010).