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A Preparatory Investigation
into the Student Practice Needed for Graduation
on the Obstinate Parasitic Infection
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A Preparatory Investigation into the Student Practice Needed for Graduation on the Obstinate Parasitic Infection of Hygienic Importance to International Health

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ABSTRACT

1. Introduction and Methodological Approach

The 6-year course for pharmacy in Japan has started in the year of 2006. The students of the new 6-year course called the clinical pharmacy in Matsuyama University are supposed to carry out their own research from the 4th to 6th grade year. Belonging to Department of Infectious Diseases for their seminar and research, they are expected to be familiar with the discipline of parasitic diseases of international importance.

A preparatory investigation has been made into the plans of the student practice

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required as their graduation theses on an obstinate parasitic infection of hygienic importance to international health.

One of the purposes of this paper is to prepare an English article for students to utilize in their learning the importance of parasitology in the international health and in starting their research. It is presumed to be desirable in the new curriculum for schools of pharmacy that the subject “pharmaceutical English” should be educated throughout the 6 years. Of no less importance is for them to understand the present basic and fundamental study for the eventual purpose of the development of excellent chemotherapy against intractable parasites, for instance, a nematode, *Angiostrongylus cantonensis*. A necessary basic study has been carried out in advance by the faculties affiliated to the department so that students might smoothly start the experimental studies. This communication comprehensively describes the preparatory investigation into the education and student practice on the obstinate parasitic infection in the hope that the students might begin to screen the promising candidate substances of possible efficacy in the near future.

The publications thought to have something to do with the prerequisite information on *A. cantonensis* have been collected as many as possible. They have been selected so that students might be familiar with the knowledge of the parasite, especially its distribution, the life cycle, pathogenicity, diagnosis and treatment that has so far been tried, but not established. Those related published books and papers have been chosen which students can read and understand. The basic experimental studies have already been carried out before the description of this paper (Maki et al., in press).

2. Results and Discussion

The information, papers and books selected and recommended for students are those written in Japanese (8 out of 14 references) or English (6 out of 14 references),

not in Spanish, Chinese or other languages. They are cited and listed in the results, discussion and references of this paper in the hope that participants in our seminar are sure to peruse them. This paper has been prepared so that this might be read and understood by students in a pharmaceutical-English class or those affiliated to the department of Infectious Diseases, especially those attending the English seminar on international health before their start of the screening studies.

Now that the preparation of experimental work has been finished with success, this would furnish the basis for screening tests to be put into practice in the near future by students specializing in clinical pharmacy. In thought of the contact of the intractable nematode, *A. cantonensis*, with substances in the ambient medium, their possible inhibitory effect on the glucose-1-phosphate hydrolysis by the intact worms is going to be assayed.

[Key words : experimental chemotherapy, parasites of importance to international health, obstinate nematosis, student practice for graduation]

INTRODUCTION AND METHODOLOGICAL APPROACH

1. The situation and the background for the present study

The 6-year pharmaceutical education in Japan has started since 2006. The new students are supposed to pursue experimental or non-experimental research to meet the requirements for comparatively great parts of the educational credit units before their graduation. The present research has been carried out for a preparatory investigation into the student education and practice on obstinate parasitic infections for their graduation from the schools of pharmacy, dentistry and human health, especially the new course of clinical pharmacy in Matsuyama University.

The present authors in Department of Infectious Diseases in Matsuyama

University School of Clinical Pharmacy would like to attach much importance to the studies on the parasites of international health in collaboration with other authors in the fields of dentistry and human health. In the new school of pharmacy, they have made efforts so far in the educational preparation on the parasitic infections for both the lectures and student practice.

In general, much more attention has been paid than before to the pharmaceutical education in Japan. This is evident in the 129th congress for pharmaceutical sciences held in Kyoto, March 26-28, 2009. To the present authors' opinion, how instructors in schools of pharmacy guide students so that they might be intrigued in the research on parasites of international importance seems to be one of the most pivotal matters in light of the new pharmaceutical disciplines and the international situation at present.

2. The objectives of the preparation of this research paper

Many Japanese people visit foreign countries nowadays. Some of them stay for a long period there. The tendency is that they are more or less careless taking raw food even in the poor hygienic areas as if they were in Japan. It is unfortunate, however, that they are sometimes infected with parasites which are not so much familiar even to clinicians in Japan now. Students in schools of pharmacy, not to say those of medicine, are expected to be versed in the knowledge on parasites in and out of Japan during the undergraduate studies. They should learn that some parasitic diseases are intractable and challenge more successful chemotherapeutic studies. In other words, they are advised to recognize the increasing social demands for chemotherapeutic agents targeted at the parasites of international importance.

For this purpose, if they choose it, it is advisable that the students before their graduation are given the opportunity to maintain the life cycles of parasites in

laboratory and to study the interaction of the parasites with the surrounding substances including chemotherapeutic candidates. Among a number of parasitic helminthes, the rat lungworm (*Angiostrongylus cantonensis*), a nematode of international-health importance, is chosen as one of the candidates for their study partly because the maintenance of the life cycle of *A. cantonensis*¹⁾ is not so difficult for them and partly because this is one of the intractable nematodes with the chemotherapy to be established.²⁾

A bibliographical approach for the education of the students has been thought over. Faculties should guide the students specializing clinical pharmacy into the recognition of the social necessity to establish chemotherapy against the obstinate parasitic diseases of hygienic importance to international health. This recognition should be accompanied with some experimental work. Experimental prerequisites have been checked for the preliminary preparation necessary for the undergraduate students to understand before their carrying out the screening test.

All of the publications thought to have something to do with *A. cantonensis* has been collected with the aid of Internet and other accumulated information such as references in papers. The information, papers and books expressed in Japanese or English, not in Spanish, Chinese or other languages have been chosen for students. We have to take consideration of the fact that very few students have learned Chinese with no opportunity for them to choose Spanish as the second foreign language in Matsuyama University School of Clinical Pharmacy.

This English paper is intended to be handed out to the students in Matsuyama University so that they might read this English "text" and learn medical and pharmaceutical English at the same time. The importance of the English education throughout the 6-year pharmaceutical course period cannot be overestimated nowadays. The public evaluation cannot be avoided from the nationwide viewpoint. The students reading the English texts are advised to understand pivotal facts related

to international health, given lectures and educated before entering our laboratory. Related papers have, thus, been reviewed and cited.

We have already carried out experimental work on *A. cantonensis* preliminarily in the hope that they might enter the studies on the parasites of international importance as far as readily and continuously. The present communication describes the preparation for their practice and the items to which undergraduate students should pay attention for their safety. The aims of this paper are to make the students understand them fundamentally and deeply through some laboratory work. The introductory guidance for them has been prepared as mentioned in the result and discussion of this paper. Now they are ready to start the studies for their graduation.

RESULTS AND DISCUSSION

1. The instructive description for the students to practice the research

The information, papers and books chosen for students are all expressed in Japanese or English, not in Spanish, Chinese or other languages.¹⁻¹⁴⁾ The 8 out of 14 references are written in Japanese while the other ones are in English. They are cited and listed in the results, discussion and references of this paper in the hope that participants in our seminar are sure to peruse them. The following introductory guidance has been prepared so that students might understand it before their experimental work.

Needless to say, the world has been confronting a large number of yet-unsolved infectious diseases to be solved as always has been discussed in the congress for International Health. In the world, for example, mankind has been threatened to fatal parasitic diseases, though Japan has made great efforts to overcome these diseases.

Some of them are no longer a hygienic problem in our country, but others still remain biohazardous and occasionally lethal to be controlled. Medical and pharmaceutical scientists, and the related faculties and staffs are expected to play a pivotal role in the possible elimination of their causative agents. Attention is paid to an international infestation with parasites. Parasites in the present Japan are not so ubiquitously distributed, as compared with those after the World War II. It is true that there used to be much more parasites there. Nowadays the number of patients infected with parasites is not as large as it used to be. However, the kinds of parasites are extremely varied in Japan now. A large number of parasites, which were not indigenous to Japan, have been imported from developing countries *via* contaminated foods and vegetables. Not a few species of these parasites are responsible for some unsolved problems in spite of our efforts to eliminate them. Why are they so obstinate ?

First, the life cycles of the parasites have been kept in the nature despite the hygienic measures taken there. This is one of the subjects for the international health and hygiene deeply associated with socio-medical and socio-pharmaceutical sciences. Another reason is, no doubt, that suitable chemotherapeutic measures have not been established notwithstanding the fact that they are being imported into Japan. Thinking over the background medically and pharmaceutically, the present authors feel inclined to say that few pharmaceutical scientists are interested in the development of the drugs that can expel such intractable parasites.

The basic knowledge of the biological and biochemical information on the parasite in question would promote the development of effective drugs for diagnosis and treatment. Even if the establishment of an ideal chemotherapy is theoretically possible, pharmaceutical companies tend to be rather indifferent to its development because of the enormous expenditure and little profit. This is why the basic studies should be carried out in universities.

In the world, there are a number of obstinate parasitic protozoa and helminthes. The nematode academically called *A. cantonensis* is one of such helminths. Although the life cycle of this parasite in the nature was clarified many years ago by Australian reseachers,¹⁾ it has been endemic in the Pacific Ocean Region, Southeast Asia and Japan,^{2,6)} hazardous to inhabitants. Once infected with this nematode, for instance, through eating contaminated vegetables, we have to be prepared for the worst. We cannot avoid suffering from eosinophilic meningoencephalitis, severe nervous and eye problems. Occasionally or rarely the unfortunate infection will lead to coma and unfortunate death.

Thus, students are going to be educated through this English article so that they might be interested in one of the fields of international health. However, they cannot enter the experimental studies in the next step until they understand the present authors' preparatory investigation.

2. The new domestic problems to be discussed with students from the viewpoint of international health and their education

The students are hopefully interested in the bibliographical and experimental studies with the background of the present social situation and environments. Of particular importance is the urgent necessity for the establishment of experimental chemotherapy of obstinate parasitic diseases from the viewpoint of social pharmacy and international health. This is thought to be exemplified in our student research based on the methodology in this paper. The experimental data will be shown as one of the basic studies for the eventual purpose of internationally social welfare.

Travelers from Japan to the countries in poor hygienic conditions should be careful not to be infected with parasites there. The number of visitors to Japan carrying parasites has increased in recent years.⁶⁾ The development of suitable drugs against various obstinate parasitoses should be promoted with the changes in

international circumstances being kept in mind. This seems, in some cases, to belong to a kind of the neglected tropical diseases (NTD) mentioned by Ohta.⁷⁾

The present authors should add something important. We have to be cautious enough not to be infected with international parasites. Even when we do not visit foreign countries, parasites will “visit” or invade into Japan and inhabit. Our students are informed of the enzootic areas of *A. cantonensis* in Japan.²⁾ It goes without saying that they should be educated in the present situation in the field. For instance, rats infected with *A. cantonensis* are found in Kobe area, Hyogo Prefecture.⁸⁾ According to the presentation,⁸⁾ rats in the Kobe Port Area infected with *A. cantonensis* are subsequently problematic, being responsible for the infection of the slugs and snails there. These intermediate mollusks, when they are infected and harbors the larval *A. cantonensis*, are hazardous to people who eat the vegetables possibly contaminated by the intermediate hosts in the surrounding area or have contact with the mollusks (especially among children).

Another enzootic area where land snails were found to harbor larval *A. cantonensis* is Chichi-jima, one of the Ogasawara Islands (English name: Bonin Islands),^{2,6)} belonging to Tokyo Metropolis and located far to the south of Tokyo. The published book⁹⁾ written by Prof. A. Ishii “Mankind and Parasites (in Japanese)” is highly recommendable for students. The author himself found larval *A. cantonensis* in the intermediate hosts from the Ogasawara Islands.⁹⁾ It was in the 1970’s that the invasion by *A. cantonensis* was first confirmed on Chichi-jima. The present enzootic situation on the islands is presumed to be quite different from that in former days. It is rather hard to find land snails on the main island, Chichi-jima (the direct translation: “Father Island”). The first author of this paper, J. Maki could not find a single land snail, *Achatina fulica* on the island in the beginning of 2005, remembering an island, Ishigaki-jima (belonging to Okinawa Prefecture, most south-western parts of Japan), another enzootic area with *A. cantonensis* where *A.*

fulica were found readily on a street a few years before (unpublished). Reportedly^{10,11)} on Chichi-jima, the main island of the Ogasawara Archipelago, they have been eaten by a natural enemy, *Platyemus manokwari*, one of the species belonging to planarians (one of the flatworms or the turbellaria) introduced about 20 years ago and having multiplied. According to the reports,^{10,11)} approximately 70% of the number of the snails have been reduced on the main island, Chichi-jima with the surviving snails being mostly restricted in some parts of the island now. Contrarily, almost no invasion by the natural enemy has been observed so far on Haha-jima (the direct translation: "Mother Island"), a part of the Ogasawara group of islands. Education from a viewpoint of ecology is thus important for the students. Incidental to this, students should be taught in the classification of helminthes.^{2,6)} They are instructed in understanding and memorizing the following facts. "Human parasites are classified into two main groups, protozoa (for examples, malaria and cryptosporidium) and helminthes which contain nematodes, and platyhelminths (flatworms) like trematodes (e. g. schistsomes) and cestodes (so-called "tapeworms"). Though turbellians are classified in the group of platyhelminths, they are mostly free-living (not parasitic)."

3. The plans for the safe and significant experimental work by students for their graduation

① Safe handling of the infectious agent by undergraduate students

The preparatory experimental studies have been pursued so far and mostly completed to be useful for students carrying out their own graduation research.^{3~5,12)} The methodology has been mentioned in the previous communications,^{5,12)} being reported somewhat in detail. However, when it is briefly summarized here, the important pointers are as follows. This description on safety, together with the report,¹²⁾ should be thoroughly understood by the students for their safety.

It is true that the life cycle of this nematode could be maintained rather readily in laboratory even by undergraduate students using rats and experimental fresh-water snails, *Biomphalaria glabrata*, as the final and experimental intermediate hosts.³⁾ The snails are fed on lettuce in an aquarium for the 1st-stage larvae in them to be raised to the 3rd-stage ones. However, a special care should be taken for researchers, especially students so as to avoid the infection with the larvae because the 3rd-stage ones from the snails and about to orally be inoculated to rats are biohazardous and highly infectious to humans. We should not immerse our hands directly in the water because the larvae will go out into the water. These should be protected with plastic disposable gloves worn and the mouth covered with a mask. Rats anesthetized previously with ether should be orally inoculated with the 3rd-stage larvae very carefully without any leakage.

The intact adult worms harvested in laboratory and washed in saline are not infectious to humans, the reason of which should be understood and explained by students.

Careful manners for protection are also necessary during the process when they are incubated using a shaking incubator at 37°C in a glass vessel containing buffered saline adjusted to physiological pH, the substrate (glucose-1-phosphate), and the test substances such as crude drugs mentioned below. After the incubation, Pi liberated in the medium is quantified colorimetrically for the detection of the phosphatase activity. Students should also learn the method by Fiske-Subbarow for Pi detection.

An in vivo study is also possible in regard to the effect of benzimidazoles on larval and adult *Angiostrongylus cantonensis* for comparison with that of the extracts from medicinal plants when the infected rats above mentioned are given the test drugs.

The preliminary trial has been done by the group of Maki and Itou (unpublished) using the infected rats. In the trial, infected rats have been administered with

benzimidazoles. The results are going to be shown elsewhere.

②Pointers for the biological and physiological understanding by students

The recognition by students of the thick cuticle covering the nematode is very important in the first place. Generally speaking, nematodes are covered with the cuticle layer in contrast to trematodes and cestodes. Therefore it is thought to be hard for substances surrounding the nematodes to penetrate into them.

The undergraduate students with international health in mind need the knowledge of biochemistry and enzymology related as the basis for the screening. Whether a given substance such as glucose-1-phosphate in the ambient medium can interact directly and/or indirectly with the nematode activity will hopefully interest the students. Because, like other nematodes, *A. cantonensis* is covered with a thick cuticle layer on the surface, thus being protected from the substances present in the ambient medium.

Surprisingly, however, it has been demonstrated that a given substance in the ambient medium at the physiological pH can interact with the worms.

The fact of the physiological activity (or activities) of the nematode covered with the thick cuticle suggests the possibility that the cuticle is not impermeable to the surrounding substances including anthelmintics. This observation is consistent with the data.⁴⁾ The nematode hydrolyzes an artificial and convenient substrate, *p*-nitrophenyl phosphate in the ambient medium.

③Provision with medicinal-plant extracts to be assayed for our laboratory

A large number of candidate substances including extracts from medicinal plants are worthy of being tested by students to know whether they have the potentiality to affect the nematode protected with the cuticle layer at the physiological pH. The methodology for the in vitro screening of candidate

substances against the nematode should be confirmed by students.

Those kinds of drugs which interact with the body of these obstinate parasites should be found *in vitro*, to begin with. For a preliminary study, we have clarified whether a given substance could interact with the body of the parasite covered with the tough cuticle and/or its activity. An example of the basis is unequivocally demonstrated for the future use of an *in vitro* screening system. This would be useful to find drugs for the treatment of the nematode with the medical and pharmaceutical problems.

The candidate crude drugs have hitherto been supplied to us so as to be examined in the possible efficacy against the nematode. They are from Central American countries such as Guatemala and Mexico. The test substances have been supplied by Prof. A. Caceres (Pharmacy "Farmaya" and Department of Immunopathology, School of Biological Sciences and Pharmacy, San Carlos University, Republic of Guatemala) to be examined. They are extracts from traditional medicinal plants grown in the Republic of Guatemala, Central America. Plant extracts which have been prepared in Mexico⁵⁾ are worthy of our assay. Some of the extracts have been preliminarily tested in their efficacy against the movements of the nematode.⁵⁾ Candidate substances are going to be supplied from Meikai University and Hachinohe University, too.

Students are advised to be familiar with the drug information which is going to be given to them. The students with the help of the present authors are sure to be interested in the possible efficacy of the extracted substances from various kinds of medicinal plants as well as synthesized drugs against the tissue-parasitic nematodes that are difficult to eliminate.

CONCLUSION

The necessary review and most of experimental preparations have been completed as above mentioned. Much importance has been attached to the safety in handling of the infectious agent by students. The interaction of the substance, or ester, in the ambient medium with the nematode covered and protected by the cuticle layer has been fundamentally but very clearly demonstrated.

The preliminary data should be obtained for their experimental studies by themselves. The given substrate is significantly hydrolyzed by the intact worms at a physiological pH. Students are advised to understand that the substance (substrate) in the surrounding medium can interact with the body of the nematode despite the coverage of the thick cuticle.

The system for the hydrolysis of esters by the intact obstinate parasitic nematode, especially that at neutral pH values where the activity is unequivocally recognized will warrant the successful *in vitro* screening of candidate substances against such parasites. This will be made the best of together with the test substances donated. Attention should be paid to their possible inhibition of the phosphatase activity and/or the movement of the parasite in the hope that some of them might be of chemotherapeutic value.

The present work in this paper has been accomplished now under the collaboration by the present authors as follows. Though it has been written mainly by the first author, J. Maki having performed experimental trials and collected information and findings, the authors, H. Sakagami and M. Kuwada have overviewed and criticized the manuscript as supervisors for the revision of this paper. And Y. Sekiya and E. Tamai have been cooperative enough to put the data and information in order, discussing the essential problems. Thus, the present authors are all hard working faculties and staffs. They have prepared and expanded the

facilities in their laboratory successfully, and will continue to implement strategies which meet the challenges of Matsuyama University in the beginning of the 21st century, with the exemplification of Department of Infectious Diseases, School of Clinical Pharmacy opened in 2006. This fact is similar to the one suggested by Prof. P. T. Reynolds¹³⁾ in the university. Encouraged by and responding to him, we are confident that it will continue to provide the standard educational opportunities in our Department of Infectious Diseases supported also by the pharmaceutical English instructions for decades to come in order to create excellent clinical pharmacists truly in an international sense.

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Last but not least, this brief note is dedicated to the late Professor Hiroshi MIYAOKI in Matsuyama University where he devoted himself to the future of many a student and another seed on the field of the new pharmaceutical research, such as this graduation study, has been sprouted on the same campus.

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