Rapid evaluation of human louse susceptibility to phenothrin

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Abstract: In order to establish a simplified method of evaluating the insecticide susceptibility of the human louse, we examined the time-course knockdown of body lice (Pediculus humanus) to phenothrin, the only registered active pyrethroid ingredient of a pediculicide in Japan. Using an insecticide susceptible strain of the body louse (NIID strain), the knockdown effect of phenothrin was assessed at 1, 2, 3, 4, 18 and 24 h after the treatment by means of a continuous filter paper-contact method. The median knockdown concentration (KC50) at 3 h was 31 mg/m² and that at 24 h was 33 mg/m², showing the rapid acute-toxicity of phenothrin. Thus it was suggested that 24 h observation is not necessarily needed to evaluate the insecticidal activity of phenothrin to the body lice.

Key words: head lice, Pediculus capitis, insecticide resistance, phenothrin

Introduction

Human louse infestations used to be quite common in Japan until the middle of the 20th century. Improved quality of the sanitation status and use of insecticides such as DDT drastically decreased the number of the infestations in Japan. In 1971, the produce and sale of DDT was officially prohibited in Japan as in many industrial countries. Since the middle of the 1970’s, head louse infestations gradually increased among schoolchildren. The number of patients reported reached nearly 25,000 in 1982 (The Ministry of Health and Welfare of Japan, 1999). A new pediculicide (sumithrin powder) that contains pyrethroid insecticide (d-phenothrin) was first developed and registered in 1981, and it effectively decreased the number of head lice infestations (Agui, 1999). Afterwards, the head louse problem was successfully controlled for about ten years. However, head lice infestations unexpectedly increased again in the last decade (Agui, 1999).

Another formulation of sumithrin in the form of shampoo was registered in 1998. Despite its use, the louse infestation remains as an infectious pest in Japan. The possible factors responsible for the reappearance of head lice are the reduced efficacy of pediculicides (i.e. resistance) in addition to the recent rapid internationalization. Continued treatment with pyrethroid insecticides has resulted in the development of resistance, so the difficulty of head louse control is becoming a serious problem worldwide (Tomita, 1999), for example, in Israel (Mumcuoglu et al., 1995), in the Czech Republic (Burgess, 1995), in Argentina (Picollo et al., 2000), in France (Chosidow et al., 1994), in the UK (Downs et al., 1999) and in the USA (Pollack et al., 1999). In view of the current status of the long-term usage of a single pediculicide in Japan, and the high frequency of recent
intermingling of people from other countries with insecticide resistant head lice, it is highly possible that head lice have developed insecticide resistance in Japan as well. Therefore, it is necessary to investigate the current status of insecticidal efficacy of phenothrin against head lice in Japan. However, there are some difficulties in the bioassay using head lice. First of all, it is not easy to obtain sufficient number of head lice samples (≧200) from a human host to demonstrate the dosage-knockdown regression analysis. Second, the head lice collected from human heads are relatively weak as compared to the body lice (Mihara, 1999); their longevity is short, resulting in high control mortalities. Therefore, they are not suitable for bioassays in which mortalities are assessed at 18 or 24 h after the insecticide treatment. The longevity of head lice depends on the quantity of blood sucked before removable from their hosts. Unfortunately, an artificial blood-feeding system for human lice has not been established as in the case of other blood-sucking insects such as mosquitoes and sand flies (Rutledge et al., 1964; Hunt and McKinnon, 1990).

Herein, we examined the time-course knockdown of body lice treated with phenothrin in order to establish a rapid evaluation method of phenothrin susceptibility. A simplified estimation method of the resistance ratio with a small number of lice and possible application of these methods to head louse bioassay were also discussed.

Materials and Methods

Chemical and lice

Phenothrin (95.9%) was generously provided by Sumitomo Chemical Co., Ltd. (Osaka, Japan). An insecticide susceptible strain of body louse (NIID strain) was used for all the experiments. This strain was originally collected from Sapporo, Hokkaido, Japan in 1954 and maintained for over 40 years without exposure to insecticides (Yasutomi, 1956). The lice were fed on human blood every 2 days on the arm of one of the authors (M. Mihara).

Bioassay

The time-course phenothrin activity against body lice was examined by a continuous contact method with phenothrin-impregnated filter paper. The stock solution of phenothrin was prepared and diluted with acetone to desired concentrations. A Whatman No. 5A filter paper disk (90 mm diameter) was placed in the bottom of each petri dish and then 0.32 milliliter of the solution was spread over the paper and the solvent was evaporated. Twenty adults or 1st instar nymphs were released on the filter paper and the knockdowns were assessed at 1, 2, 3, 4, 18 and 24 h after the release of insects. All bioassays were carried out at 25°C and two replicates were completed for each insecticide concentration. The control lice were released on the filter paper treated with acetone alone. The criterion for judging if a louse was knocked down or alive was as follows: a louse was pushed with a pair of tweezers and was considered as a knocked down insect if it could not move more than 10 mm. The concentration of 1% (KC1), 50% (KC50) and 99% (KC99) knockdown values were obtained by probit analysis (Finney, 1971). Statistical tests were carried out using a computer program (SAS version 8.01) from SAS Institute (Cary, NY).

Results and Discussion

In order to establish a simplified human louse bioassay method, the time-course knockdown regression analysis was conducted with the continuous filter paper-contact method using adults and 1st instar nymphs of the susceptible body louse. The log-concentration knockdown regression lines of 1, 2, 3, 18 and 24 h after the initiation of the phenothrin treatment to adult body lice are shown in Fig. 1. With adult lice, regression lines were not significantly different between 3 h and 24
h treatment (Pr=0.28 by the covariance analysis). The KC50 at 3 h treatment (33 mg/m² filter paper) was close to that obtained after 24 h (31 mg/m² filter paper), which indicates that the phenothrin rapidly knocked down the body lice and thus the phenothrin susceptibility of lice can be evaluated in a very short period (i.e. 3 h treatment). A similar result was obtained with the 1st instar nymphs although knockdown speed was relatively low (Fig. 2). For instance, the KC50 and KC90 were 52 and 97 mg/m², respectively at 3 h treatment in the 1st instar nymphs while these values decreased to 34 and 53 mg/m², respectively at 24 h after the release of the insects (Fig. 2 and Table 1). Statistical analysis showed that these two regression lines were significantly different (Pr=0.0012). This may be due to the different

![Fig. 1. Time-course knockdowns of adult body lice to phenothrin. Knockdowns were assessed at 1, 2, 3, 18 and 24 h after the phenothrin treatment started. The arrow indicates the diagnostic phenothrin dose (100 mg/m² filter paper).](image)

![Fig. 2. Time-course knockdowns of 1st instar nymphs of body lice to phenothrin. Knockdowns were assessed at 1, 2, 3, 4, 18 and 24 h after the phenothrin treatment started.](image)

<table>
<thead>
<tr>
<th>Stage of lice</th>
<th>Treatment hours</th>
<th>n²</th>
<th>Regression line</th>
<th>( \chi^2 )</th>
<th>df</th>
<th>p</th>
<th>KC² (95% CL) (mg/m²)</th>
</tr>
</thead>
</table>
| 1st instar nymphs | 3 hrs | 202 | P²=8.43X⁻¹⁴.4 | 3.13 | 3 | 0.37 | KC₁=27 (22-31)  
KC₂⁰=52 (48-55)  
KC₉₀=97 (86-117) |
| | 24 hrs | 193 | P²=12.0X⁻¹⁸.3 | 0.64 | 3 | 0.89 | KC₁=22 (18-24)  
KC₂⁰=34 (32-36)  
KC₉₀=53 (47-64) |
| Adults | 3 hrs | 203 | P²=10.8X⁻¹⁶.5 | 4.64 | 3 | 0.20 | KC₁=20 (17-22)  
KC₂⁰=33 (32-35)  
KC₉₀=54 (48-66) |
| | 24 hrs | 203 | P²=15.1X⁻²².5 | 2.16 | 3 | 0.54 | KC₁=22 (19-23)  
KC₂⁰=31 (30-32)  
KC₉₀=44 (40-51) |

a n, number of lice tested.
b P, probit.
c X, logarithm of phenothrin concentration in mg/m².
d KC, knockdown concentration, a theoretical concentration at a knockdown percentage.
area of the body part exposed to the filter paper; the relative body size to the legs of the adult body lice is obviously bigger than those in the 1st instar nymphs. The KC50 of at 24 h after the initiation of phenothrin treatment were not significantly different (PR=0.19) between adults (31 mg/m²) and 1st instar nymphs (34 mg/m², Table 1).

Since KC99 of the susceptible body lice (3 h treatment) was 54 mg/m² in adults (Table 1), we set a diagnostic concentration of phenothrin at 100 mg/m². If a sample louse is alive at 100 mg/m² after 3–5 h treatment, the louse can be regarded as resistant. Slopes of the regression lines for the susceptible body lice were relatively high and ranged from 8.4 to 15.1 (Table 1), suggesting that the susceptible body lice are genetically quite homogeneous. In the field colonies of head lice (on the human heads), each colony starts with a very limited number (probably in most cases a colony starts with a single mated female adult), the outcross of populations is limited and the genetic variation of each colony is not very abundant. Therefore, since KC5 for the susceptible body louse was 20 mg/m², the resistance ratio (RR) of the louse can be estimated to be at least 5 times as compared to the susceptible body louse (Fig. 3). Similarly, if tested lice survive at 200 or 400 mg/m², the RR is estimated to be ≥ 10- or ≥ 20-fold, respectively (Fig. 3). This simplified test method enables us to check the phenothrin susceptibility of lice with a small number of individuals.

Body lice are also important pests as they transmit some epidemic diseases including typhus, relapsing fever and trench fever (Gratz, 1997). In 2000, Bartonella quintana, the pathogen of trench fever was detected from body lice collected from homeless people in Tokyo, Japan (Kobayashi et al., 2001; Sasaki et al., 2002). This was the first report on the finding of the trench fever pathogen in this country. In proportion to the recent deterioration of Japanese economy, the number of homeless people is increasing year by year and the average infestation rate of body lice in the homeless people in Tokyo is about 6% (Makigami and Yaguchi, 1999). Currently many scientists believe that the human head and body lice are two distinct species (Schaefer, 1978; Gratz, 1985; Torrasevich et al., 1988) although it is fairly difficult to distinguish these two morphologically or even genetically (Leo et al., 2002). Therefore, some researchers still insist that body and head lice are conspecific. From this point of view, it is expected that the rapid evaluation method established in this study, although it was carried out with body louse, can be applied to head louse as well.

Epidemiologically, head lice do not transmit diseases. Their capacity as vectors of infectious diseases, however, was indirectly confirmed by the infection assay in the laboratory with the typhus pathogen, Rickettsia prowazeki (Murray and Torrey, 1975). In view of such an unsettled status of these two species and recent reemergence of head lice infestations among sch-
oolchildren in Japan, it is necessary to evaluate the insecticidal activity of phenothrin against head lice and body lice in Japan. Surveillances of their susceptibilities to phenothrin are currently in progress in our laboratory using the rapid test method with the diagnostic concentrations of phenothrin determined in this study.

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