The Protozoacidal Effect of Tripropylisocyanurate (TPIC) on Symbiotic Protozoa in Japanese Subterranean Termites

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To determine the protozoacidal effect of tripropylisocyanurate (TPIC) on symbiotic protozoa in two Japanese subterranean termites, *Coptotermes formosanus* and *Reticulitermes speratus* (RHINOTERMITIDAE), we examined the movements of the protozoa by microscope. After exposing the termites to TPIC (40 mg/filter paper), the protozoa, *Pseudotriconympha grassi* in *C. formosanus* and *Trichonympha agilis* in *R. speratus*, significantly decreased their movement ($p < 0.05$) after 2 and 8 hr, respectively. These protozoa are considered to play a crucial role in cellulose digestion in the termites. TPIC was therefore considered to damage the cellulose metabolism of the termites by affecting their symbiotic protozoa. The termite *C. formosanus* did not become moribund or die within 24 hr after exposure to TPIC (40 mg/filter paper), so the effect of TPIC thus emerged in the symbiotic protozoa much earlier than in the termites themselves. *R. speratus*, however, became moribund within 2 hours after TPIC (40 mg/filter paper) treatment, and it was concluded that TPIC first exerted its effect on *R. speratus* themselves before affecting the symbiotic protozoa.

INTRODUCTION

Tripropylisocyanurate (TPIC) is a termiticide recently developed in Japan. TAKAHASHI et al. (1985) investigated the termiticidal activity of TPIC using the subterranean termite, *Coptotermes formosanus*, and described its three major effects: repellent effect, contact poisoning effect and stomach poisoning effect. They also indicated how the protozoacidal activity of TPIC affects the flagellates in the hindgut of *C. formosanus*, although they did not conduct a detailed investigation in this field. Since the discoveries of CLEVELAND (1925), the protozoa in the hindgut of termites have been considered to be symbionts. KANAI et al. (1982) indicated that the largest flagellate in *C. formosanus*, *Pseudotriconympha grassi*, played an important role in the digestion of crystallized cellulose and wood. YAGA (1985) reported that *P. grassi* was the first protozoa species to be affected by TPIC among the three flagellates in the hindgut of *C. formosanus*.

Here, we report further studies of the nature of the protozoacidal effect of TPIC in two Japanese subterranean termites, *C. formosanus* and *Reticulitermes speratus*.

MATERIALS AND METHODS

1) *Termites*

Coptotermes formosanus SHIRAKI (from a colony in the Univ. of Ryukyus)

Reticulitermes speratus (KOLBE) (from a colony in Chugai Pharmaceutical Co., Ltd.)

2) *Protozoa*. The termites harbored the following symbiotic flagellates in their hindguts:

C. formosanus

Pseudotrichonympha grassi KOIDZUMI (Fig. 1)

Holomastigotoides hartmanni KOIDZUMI

Spirotrichonympha leidy (KOIDZUMI)

R. speratus

Trichonympha agilis LEIDY var. *japonica* KOIDZUMI

Pyrsonympha spp. (Fig. 2)

Dinenympha spp.

No *Teronympha mirabilis* was detected in this colony.

Method of TPIC application. Filter papers (9 cm, Toyo No. 2) in petri dishes were treated with 1.0 ml of TPIC acetone solution (0.0625%, 0.25%, 1.0% and 4.0%). The amount of TPIC on the respective papers was 0.625 mg, 2.5 mg, 10 mg and 40 mg. We dried these filter papers for 24 hr before the experiment, then added distilled water (0.1 ml) to supply the termites with moisture during the course of the experiments. In the rest of this report the rate of TPIC applied in each experiment was 40 mg/filter paper unless otherwise specified.

Fig. 1. *Pseudotrichonympha grassi* KOIDZUMI. The arrows indicate the 'head' portion.

Fig. 2. *Pyrsonympha* sp. The arrow indicates the 'tip' portion.

Measurement of protozoal activity. Observations of the protozoa in the hindgut of termites were made under a light microscope. Preparations for microscopic examination were made in 0.35% NaCl aqueous solution. To monitor their activities, we measured the following movements of each protozoan three times for 1 min each.

1) *C. formosanus*

P. grassi: frequency (times/sec) of the movement of the 'head' portion (Fig. 1)

S. leidy: frequency (times/sec) of whole body movement. The movement of *H. hartmanni* was not measured in this experiment.

2) *R. speratus*

T. agilis and *Dinenympha* spp.: frequency (times/sec) of whole body movement

Pyrsonympha spp.: frequency (times/sec) of movement of the 'tip' portion (Fig. 2)

RESULTS

The termites, *C. formosanus*, exposed to TPIC for 24 hr did not die and less than 10% of them became moribund. This was similar to the result obtained by TAKAHASHI et al. (1985). The effects of TPIC on the movement of protozoa during this period are shown in Figs. 3–5.

As shown in Fig. 3, the frequency of the movement of the 'head' portion of *P. grassi* was 2.65 times/sec before TPIC exposure. Two hours after the treatment, it dropped to 1.45 times/sec and dropped further to 0.61 times/sec after 24 hr. The frequency was significantly lower ($p < 0.05$) than the control level after 2 hr. After 48 hr none of the *P. grassi* were moving their 'head' portions, and significant morphological changes had occurred (Fig. 4).

The frequency of the movement of the whole body of *S. leidy* was 2.15 times/sec before the exposure to TPIC (Fig. 5). One hour after the treatment, it increased significantly ($p < 0.05$) and remained at the increased level for 5 hr. At 8 hr it had

Fig. 3. Effects of TPIC (40 mg/filter paper) on *P. grassi*. The vertical lines show S.D. ($N=5$). * Significantly different from the initial at $p < 0.05$ (t -test) (----- Control).

Fig. 4. Morphological change in *P. grassi* 48 hr after exposure to TPIC (40 mg/filter paper).

Fig. 5. The effect of TPIC (40 mg/filter paper) on *S. leydyi*. The vertical lines show S.D. ($N=5$). * Significantly different from the initial at $p<0.05$ (t -test) (----- Control).

Fig. 6. The effect of TPIC on *R. speratus*. Twenty worker termites were used for each concentration. No knockdown was observed in the control.

dropped to 1.74 times/sec and remained at this significantly lower-than-control frequency ($p<0.05$) until 16 hr later. The frequency at the 24 hr stage was not significantly lower than the control.

Figure 6 shows the knockdown effect of TPIC on the termite, *R. speratus*. Unlike

C. formosanus, the knockdown rate was 100% (TPIC: 40 mg/filter paper and 10 mg/filter paper) and 95% (TPIC: 2.5 mg/filter paper) in 3 hr, clearly showing that *R. speratus* was much more susceptible to TPIC than *C. formosanus*. The effects of TPIC in protozoa of *R. speratus* are shown in Figs. 7–9. As illustrated in Fig. 7, the frequency of the whole body movement of *T. agilis* was 1.23 times/sec before TPIC exposure. It remained at the same level for a period of 8 hr after the treatment, after which the

Fig. 7. The effects of TPIC (40 mg/filter paper) on *T. agilis*. The vertical lines show S.D. ($N=5$). * Significantly different from the initial at $p < 0.05$ (t -test) (---- Control).

Fig. 8. The effects of TPIC (40 mg/filter paper) on *Dynenympha* spp. The vertical lines show S.D. ($N=5$). * Significantly different from the initial at $p < 0.05$ (t -test) (---- Control).

Fig. 9. The effects of TPIC on *Pyrsonympha* spp. The vertical lines show S.D. ($N=5$). * Significantly different from the initial at $p < 0.05$ (t -test) (---- Control).

termites became moribund. The frequency significantly decreased 16 to 24 hr after exposure. *Dinenympha* spp. increased its whole body movement significantly ($p < 0.05$) at 4 and 8 hr after the treatment (Fig. 8). It decreased to the initial level 16 hr later, then further dropped to a significantly lower frequency ($p < 0.05$) after 24 hr. The frequency of the 'tip' movement of *Pyrsonympha* spp. also increased significantly ($p < 0.05$) in the first 8 hr following TPIC treatment, and then dropped back to the control level after 16 hr (Fig. 9).

DISCUSSION

Cellulose, constituting approximately 40 to 50% of wood, is the main source of energy for lower termites dependent on wood. MAULDIN et al. (1972) reported that *C. formosanus* artificially deprived of *P. grassi* could metabolize only 5.5% as much [U-¹⁴C]-cellulose as it could with this symbiont, thus indicating the importance of *P. grassi* in cellulose metabolism. KANAI et al. (1982) also indicated the important role of *P. grassi* in cellulose digestion by demonstrating that *P. grassi* was maintained for the 8-week test period only when termites were fed with such substances as wood, pure cellulose powder and Avicel. When the termites were fed such non-cellulose substances as amylose, cellobiose, sucrose, maltose, glucose and fructose, they lost their *P. grassi* after 1 week although the termites maintained their normal activities for the 8 weeks of the experiment. KANAI (1982) also indicated the importance of *T. agilis* in the cellulose metabolism of *R. speratus* by an experiment similar to that on *C. formosanus*. YAMAOKA (1982) showed that *T. agilis* in *R. speratus* selectively ingested cellulose particles in culture media, again demonstrating the usual role of symbiotic protozoa in the hindgut of termites. The frequency of the movement of protozoa measured in this experiment was not directly related to their cellulose metabolism. However, a decrease in the movement of protozoa does imply reduction in the cellulose metabolic activity of the host termites, because, as reported by YAMAOKA (1982), symbiotic protozoa need to move around in the hindgut of termites in order to take wood or cellulose particles for digestion.

Apparently the decreased movement of the two major protozoa, *P. grassi* in *C. formosanus* and *T. agilis* in *R. speratus*, greatly affects cellulose metabolism in the termites. Therefore, the data in Figs. 3 and 7 indicate that *C. formosanus* and *R. speratus* significantly reduced their cellulose metabolic activity based on protozoa, 2 and 8 hr after TPIC treatment respectively. In contrast, protozoa other than *P. grassi* and *T. agilis*, which are generally considered to play a supplementary role in the cellulose metabolism of termites, increased the frequency of their movement temporarily within 2 to 8 hr after the TPIC treatment in both *C. formosanus* and *R. speratus*. These supplementary protozoa may have increased their activities to compensate for the decreased activities of the major protozoa, or they may simply have shown abnormal hypermovement with or without a change in their cellulose metabolic activity.

TAKAHASHI et al. (1985) reported that *C. formosanus* became moribund 4 days after TPIC exposure. However, as is shown in Fig. 7, the effect of TPIC on this termite appeared first in *P. grassi* as early as 2 hr after the treatment. Thus we conclude that in *C. formosanus* TPIC exerted its effect first on *P. grassi* rather than on the termites themselves. It appears that TPIC is more protozoacidal than termiticidal. In *R. speratus*, the movement of *T. agilis* was maintained at the same level until 8 hr after TPIC exposure when all termites became moribund (Figs. 6 and 7). This suggests that the

protozoa retained their cellulose digestive activity for a few hours after the host termites become moribund. However, for the digestion of wood, protozoa depend on the termites to grind the wood into particles small enough for the protozoa to ingest. Thus *R. speratus* may have lost its ability to digest cellulose when the termites themselves become moribund. In this sense, TPIC is more termiticidal in *R. speratus* than protozoacidal. Finally, we conclude that TPIC affects the termites *C. formosanus* and *R. speratus* in different ways. In *C. formosanus*, the protozoacidal effect of TPIC on *P. grassi* occurred before the termiticidal effect. In *R. speratus*, however, the reverse was true. The termiticidal effect appeared before the protozoacidal effect on *T. agilis*.

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