ATPase activities of acarid mite, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae), and their inhibition by several acaricides

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Abstract: Poisoning symptoms caused by three acaricides and one insecticide in the acarid mite, *Tyrophagus putrescentiae* (Schrank), were classified into two types, i.e. knock-down and immobilization. Since the immobilization was not preceded by hyper-excitation, convulsion or agony, an interference with the respiratory system was speculated. By analogy of certain acaricides known to produce similar poisoning symptoms in the two-spotted spider mite by inhibition of oligomycin-sensitive (=OS) Mg^{2+} ATPase involved in the mitochondrial respiratory enzyme system, ATPase activities and their inhibition by several compounds in the acarid mite were investigated *in vitro*. The mitochondrial preparation exhibited the highest activity for Na^+ – K^+ ATPase, while the activity for OS Mg^{2+} ATPase was relatively low. Among several compounds tested, the selective acaricides, dicofol and cyhexatin, exhibited the highest inhibition of both ATPase activities. Bis (2,3,3,3-tetrachloropropyl) ether (=S-421) and *pp'*-DDT also exhibited significant but less inhibition of the enzymes. However, there was no consistent trend implicating a relation between *in vitro* OS Mg^{2+} ATPase inhibition and the *in vivo* immobilization symptom, except dicofol. With dicofol, the highest inhibition of the enzyme activity coincided with the highest immobilization symptom.

INTRODUCTION

Certain acaricides, such as (1*R*, 2*R*, 4*R*)-born-2-yl 2 thiocynoacetate (=IBTA), phenyl salicylate and benzyl benzoate, exhibit mite specific toxicity against house dust mites with little insecticidal activity. In contrast, certain organophosphates and pyrethroids with good insecticidal activity exhibit low acaricidal activity (Hirakoso, 1989; Hashimoto et al., 1998, 1999). The contrasting response of the mites to these compounds suggests a possible difference in metabolism between the spe-

cific acaricides and the organophosphates and pyrethroids. A difference in target site sensitivity between mites and insects is another possible factor that may cause the contrasting response. However, only limited information is available on the exact site of action for the most specific acaricides.

Hashimoto et al. (2000) classified several acaricides into immobilizer type and knock-down type from observations on the poisoning symptoms of the house dust mites. The immobilizing effect of certain acaricides against spider mites was also observed by other workers in a phytoph-

agous mite (Carbonaro et al., 1986). These results implicate a possible interaction of the specific acaricides with the respiration system of the mites.

Certain insecticides are known as respiratory inhibitors (Fukami, 1976). Particularly, the F_0F_1 ATPase, referred to as oligomycin-sensitive (=OS) Mg^{2+} ATPase in the present paper, involved in the mitochondrial electron transport system is susceptible to inhibition by certain insecticides (Desaiah et al., 1973; Patil et al., 1979; Carbonaro et al., 1986; Cutcomp et al., 1984; Kadir and Knowles, 1991b).

In the present study, the poisoning symptoms in the acarid mite, *Tyrophagus putrescentiae* (Schrank) (= Tp), were examined following the *in vivo* application of three acaricides and one insecticide. ATPase activities and their *in vitro* inhibition by several acaricides were investigated assuming a possible effect on the mitochondrial electron transport system.

MATERIALS AND METHODS

Mites

Tp was mainly used throughout the present study. The American house dust mite, *Dermatophagoides farinae* Hughes (= Df), and the two-spotted spider mite, *Tetranychus urticae* Koch (= Tu), were also used for comparison in a part of this study. Both Df and Tp were provided by the Tokyo Women's Medical College. Rearing and harvesting methods were the same as those of Hashimoto et al. (2000). A susceptible strain of Tu was reared on kidney-bean, *Phaseolus vulgaris* L., at Chiba University. The adult mites on the bean leaves were collected by a micro-aspirator.

Chemicals

The compounds used for toxicity tests and *in vitro* ATPase inhibition studies were: cyhexatin (purity: 99.3%), dicofol (96.3%), permethrin (95.1%), benzyl benzo-

ate and phenyl salicylate (analytical grade) (Kanto Chemical); fenitrothion (96.8%) (Sumitomo Chemical); IBTA (89.0%) and bis (2,3,3,3-tetrachloropropyl) ether (=S-421, 94.1%) (Sankyo Chemical Industries); diethyltoluamid (=deet, regarded as 100%) (Showa Denko); *pp'*-DDT (= DDT, 100%) (Kureha Chemical Industry). Oligomycin was purchased from Sigma Chemical and consisted of 61% oligomycin A, 27% oligomycin B and 10% oligomycin C. ATP- Na_2 was a product of Oriental Yeast. Ouabain was purchased from Wako Pure Chemical Industries.

Observations of poisoning

To compare the poisoning symptoms produced by exposure to four compounds including dicofol, DDT, cyhexatin and IBTA, the conditions of the mite were observed under binoculars as described by Hashimoto et al. (2000). Twenty female adults of Tp were allowed to wander on the chemical residue in a glass vial (2 mg ai/16 cm²) for 24 h. The numbers of knocked down and immobilized mites were counted periodically up to 3 h after the release. The mortality was observed at 24 h. Acetone served as a control.

Preparation of ATPase source

Mitochondrial fractions were prepared by the procedure of Kadir and Knowles (1991b) and used as ATPase source. Whole mites were homogenized in ice-cold buffer (pH 7.4) (ca. 40 mg wet weight/ml) consisting of 10 mM MOPS, 10 mM Tris, 0.01 mM EDTA and 0.32 M sucrose. Homogenates were centrifuged at $1,000 \times g$ for 10 min at 5°C. Supernatants were centrifuged at $13,000 \times g$ for 30 min at 5°C, and the sediments were resuspended in the buffer and used as the ATPase sources. The ATPase sources in the buffer were stored at -20°C until used. Protein concentration of the ATPase sources was measured using Coomassie protein assay reagent (Pierce).

Determination of ATPase activity

The ATPase activity was usually measured according to the method of Kadir and Knowles (1991b) with some modifications. To compare the activity of Mg^{2+} , Na^+-K^+ and $\text{Ca}^{2+}-\text{Mg}^{2+}$ ATPase among Tp, Df and Tu, the assays were carried out under the same conditions in terms of protein concentration, ATP concentration, incubation time and temperature. The Mg^{2+} ATPase activity was measured with the reaction mixture (final volume 1.0 ml, pH 7.0) containing the ATPase source (100 μg protein/ml), 30 mM MES, 30 mM Tris, 160 mM KCl, 5 mM MgCl_2 , 0.1 mM ouabain and 1 mM ATP. The Mg^{2+} ATPase activity was further separated into oligomycin-sensitive (=OS) and -insensitive (=OIS) parts by adding 10 ng oligomycin/ml to the reaction mixture. The Na^+-K^+ ATPase activity was measured using the reaction mixture (final volume 1.0 ml, pH 7.0) containing the ATPase source as above, 30 mM MES, 30 mM Tris, 120 mM NaCl, 20 mM KCl, 5 mM MgCl_2 , and 1 mM ATP in the presence or absence of 0.1 mM ouabain. The Na^+-K^+ ATPase activity was an ouabain-sensitive component. The $\text{Ca}^{2+}-\text{Mg}^{2+}$ ATPase activity was measured with the reaction mixture (final volume 1.0 ml, pH 7.4) containing the ATPase source as above, 30 mM MES, 30 mM Tris, 100 mM KCl, 5 mM MgCl_2 , 0.1 mM CaCl_2 , 0.1 mM ouabain and 1 mM ATP. Oligomycin for the Mg^{2+} ATPase and ouabain for the Na^+-K^+ ATPase were preincubated for 15 min at 25°C before adding ATP. Following the addition of ATP the reaction mixture was incubated for additional 60 min at 25°C. The ATPase activity was determined by monitoring the production of inorganic phosphate (Pi). The Pi was measured by the modified procedure of Goldenberg and Fernandez (1966). To stop the reaction, a 200 μl of the reaction mixture was mixed with 2 ml of iron-TCA reagent containing 10% (w/v) trichloroacetic acid (TCA) and 3% ammonium iron (II) sulfate at room temperature for 20 min. The color was developed

by adding 250 μl of molybdate reagent containing 4.4% ammonium molybdate tetrahydrate and 9% H_2SO_4 . After the 20 min-reaction, the absorbance at 660 nm was measured.

For inhibition of the OS Mg^{2+} ATPase and Na^+-K^+ ATPase of Tp by acaricides, the reaction mixture was preincubated with an acaricide for 15 min at 25°C before the addition of ATP. The protein content in the reaction mixture was 200 μg for the OS Mg^{2+} ATPase and 100 μg for the Na^+-K^+ ATPase. Cyhexatin was dissolved in ethanol and the other inhibitors were dissolved in acetone. The final concentration of ethanol or acetone was 2.0% for the Mg^{2+} ATPase and 1.0% for the Na^+-K^+ and $\text{Ca}^{2+}-\text{Mg}^{2+}$ ATPase assay. The same amount of the organic solvents was used for control. The optimum ATP concentration for the OS Mg^{2+} and Na^+-K^+ ATPase assay was selected based upon preliminary experiments without acaricides, i.e. 8 mM and 1 mM for the OS Mg^{2+} and Na^+-K^+ ATPase, respectively.

RESULTS

Table 1 shows poisoning symptoms in the progress of time and the mortality at 24 h when Tp was exposed to a dry film of three acaricides and one insecticide. Dicofol immobilized 92.5% at 3 h exposure. IBTA also immobilized 31.6% of the mites. Both acaricides produced similar symptoms without preceding excitation, convulsion, or agony. A clearly different symptom of poisoning was observed with DDT, which knocked down the mites with hyper-excitation. Cyhexatin resulted in 9.5% immobilization and 11.2% knock-down by 3 h exposure. Only dicofol completely killed all the mites tested after 24 h. The lethal activity of DDT was the lowest against Tp.

Table 2 shows a comparison of various ATPase activities among 3 mite species. The Na^+-K^+ ATPase activity was the highest in Tp, while $\text{Ca}^{2+}-\text{Mg}^{2+}$ ATPase activity was the highest in the Df and Tu. The

Table 1. Poisoning symptoms in progress of time following the application of four compounds in Tp.

Compounds applied (2 mg/16 cm ²)	Symptom	% Knocked down and immobilized mites								% Mortality after 24 h
		10	20	30	45	60	90	120	180 min	
IBTA	KD	0	2.6	2.6	5.3	5.3	5.3	5.3	5.3	55.3
	IM	0	18.4	26.3	23.7	31.6	34.2	31.6	31.6	
	Total	0	21.1	28.9	28.9	36.8	39.5	36.8	36.8	
Dicofol	KD	0	0	0	0	2.5	2.5	0	0	100
	IM	0	7.5	20.0	57.5	65.0	82.5	82.5	92.5	
	Total	0	7.5	20.0	57.5	67.5	85.0	82.5	92.5	
DDT	KD	0	0	7.7	7.7	10.3	23.1	28.2	28.2	17.9
	IM	0	0	0	0	0	0	0	0	
	Total	0	0	7.7	7.7	10.3	23.1	28.2	28.2	
Cyhexatin	KD	0	0	4.8	4.8	4.8	11.9	14.3	11.9	52.4
	IM	0	0	2.4	2.4	0	0	4.8	9.5	
	Total	0	0	7.1	7.1	4.8	11.9	19.0	21.4	

Values are the mean of 2 replicates.

KD, knock down; IM, immobilization; Mean mortality of control was 7.5%.

Table 2. Comparison of various ATPase activities among three mite species.

ATPase	Activity in μ mole Pi/h/mg protein		
	Tp	Df	Tu
OS-Mg ²⁺	1.57 (0.21)	1.09 (0.06)	1.34 (0.15)
OIS-Mg ²⁺	2.34 (0.17)	1.67 (0.16)	5.95 (0.23)
Na ⁺ -K ⁺	5.95 (0.64)	1.99 (0.11)	1.14 (0.20)
Ca ²⁺ -Mg ²⁺	3.50 (0.29)	2.41 (0.41)	6.77 (0.52)

Values are the mean with standard deviation (SD) of 3 replicates.

ATPase preparation containing 100 μ g protein/ml were incubated with 1 mM ATP for 60 min at 25°C.

OS-Mg²⁺, oligomycin-sensitive Mg²⁺ ATPase; OIS-Mg²⁺, oligomycin-insensitive Mg²⁺ ATPase, Tp, *Tyrophagus putrescentiae*; Df, *Dermatophagoides farinae*; Tu, *Tetranychus urticae*.

OS Mg²⁺ ATPase activity was relatively low regardless of the mite species. The ratio of OS Mg²⁺ ATPase activity in the total Mg²⁺ ATPase activity was 40%, 39% and 18% for Tp, Df and Tu, respectively.

Figure 1 shows the effect of ATP concentration on the ATPase activities in Tp. The OS Mg²⁺ ATPase activity increased with increase of the ATP concentration up to 8 mM. In contrast, the Na⁺-K⁺ ATPase activity reached a plateau at 1 mM ATP showing inhibition by excess ATP.

Figure 2 shows percent inhibition of the OS Mg²⁺ and Na⁺-K⁺ ATPase by several compounds in Tp. The most potent inhibitor was dicofol against both OS Mg²⁺ and Na⁺-K⁺ ATPase. Cyhexatin also yielded higher inhibition against the OS Mg²⁺ ATPase, though the S-421 and DDT produced less but significant inhibition for both ATPase.

DISCUSSION

Hashimoto et al. (2000) reported that the poisoning symptoms in Tp and Df by acaricides were classified into immobilization and knock-down types. According to the study, IBTA, benzyl benzoate and deet were considered to be immobilizer-type compounds against Tp. In the current study, dicofol also demonstrated the immobilizing effect. Because of the similarity in the symptom, these compounds seemed to have a common nature in the mode of action.

The mites treated with these compounds gradually lost their movement and reached a quiet death without hyperexcitation, convulsion, or agony. The latter symptoms are characteristic of organophosphates and pyrethroids which are

Fig 1. Effect of ATP concentration on OS Mg²⁺ ATPase and Na⁺-K⁺ ATPase activities in Tp. Values are the mean with standard deviation (SD) of 3 replicates. ATPase preparations (containing 200 μg and 100 μg protein/ml for OS Mg²⁺ and Na⁺-K⁺ ATPase, respectively) were incubated for 60 min at 25°C.

Fig 2. *In vitro* inhibition of OS Mg²⁺ ATPase and Na⁺-K⁺ ATPase in Tp by several compounds including acaricides, insecticides, synergists and repellent. Values are the mean with standard deviation (SD) of 3 replicates. ATPase preparations (containing 200 μg and 100 μg protein/ml for OS Mg²⁺ and Na⁺-K⁺ ATPase, respectively) were preincubated with respective compounds at a final concentration of 100 μM for 15 min. OS Mg²⁺ and Na⁺-K⁺ ATPase were incubated for additional 60 min at 25°C with 8 mM and 1 mM ATP, respectively.

known to interfere with the nervous transmission mechanisms in mites and insects. The immobilization and subsequent death produced by these acaricides in the present study suggested a possible interference with the respiratory system (Fukami, 1976). On the other hand, dicofol, cyhexatin, DDT, carbodiimides and oligomycin are known inhibitors of mitochondrial OS Mg²⁺ and Na⁺-K⁺ ATPase (Desaiah et al., 1973; Patil et al., 1979; Chetty et al., 1984; Carbonaro et al., 1986; Kadir and Knowles, 1991a, b). Based upon these observations, a relation between the inhibition of ATPase activities and the im-

mobilization symptom was postulated.

Dicofol and cyhexatin were found to be the most potent inhibitors against the OS Mg²⁺ and Na⁺-K⁺ ATPase of Tp preparation, while S-421 and DDT also affected the ATPase activities to some extent. The ATPase inhibition by S-421 coincided with the relatively high immobilizing effect observed *in vivo* against Tp (Hashimoto et al., 2000). However, the ratios of immobilized mites by DDT and cyhexatin were relatively low despite their high *in vitro* inhibitory potencies against ATPase activities. Furthermore, a high immobilizing effect was observed with benzyl ben-

zoate and deet (Hashimoto et al., 2000), which showed only low ATPase inhibition rates in the present study. The inconsistent relation between the ATPase inhibition and the immobilization symptom may rule out the above postulation. In support of this conjecture, Kadir and Knowles (1991b) also observed the lack of the characteristic symptoms in the diamond back moth treated with two carbodiimides, both having high ATPase inhibition activity.

In the case of dicofol which showed both high ATPase inhibition and high immobilizing effect, however, further studies are required to examine the relation of the ATPase inhibition with the immobilization symptom.

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