Toxicokinetic Analysis of Injected Diazinon and Diazoxon in Resistant and Susceptible Houseflies, *Musca domestica* L.

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The internal dynamics of diazinon and diazoxon when administered by injection were studied in a susceptible and resistant strain of housefly. Flies which received diazinon by injection showed only a small interstrain difference in the internal amount of diazinon. In contrast, large interstrain difference was observed in the internal amount of diazoxon when the flies received diazinon or diazoxon by injection. Several kinetic models were postulated and examined to describe the internal dynamics of diazinon. Interstrain comparison of the kinetic parameter values estimated by computer simulation revealed that high degradation of diazoxon played an important role. It is also suggested that high degradation of diazinon and less activation are responsible for the decreased accumulation of diazoxon.

Key words: Toxicokinetics, diazinon, housefly, resistance, mathematical model

INTRODUCTION

A housefly strain originally collected in 1984 from sanitary landfill in Yachiyo City, Japan, had an extremely high LD₅₀ for diazinon, i.e. 50 µg per fly by topical application after several selections with the insecticide in the laboratory. *In vitro* studies identified three enzyme systems responsible for resistance, i.e. cytochrome P-450 dependent monooxygenases (MFO), glutathione transferase (GST), and phosphotriester hydrolase (OI et al., 1990). Insensitivity at the target enzyme, AChE was also a resistance factor in this strain (OI et al., 1990). A subsequent study (OI et al., 1992) on the *in vivo* dynamics of dermally applied diazinon demonstrated reduced cuticular penetration as an additional factor for the resistance. A comparison of the kinetic parameter values estimated by computer simulation suggested a remarkable interstrain difference in the ratio of diazoxon degradation compared to the activation of diazinon. This resulted in little accumulation of diazoxon in the resistant strain.

To determine the significance of these pathways responsible for diazinon resistance more accurately, this paper deals with the internal dynamics of diazinon applied by injection. Several kinetic models are postulated and examined for toxicokinetic analy-

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sis of activation and degradation of diazinon and degradation of diazoxon in resistant and susceptible houseflies.

MATERIALS AND METHODS

Houseflies. The diazinon resistant Yachiyo strain and susceptible CSMA strain of housefly *Musca domestica* L. were the same as those described previously (Ori et al., 1990, 1991 and 1992). LD₅₀ values determined by topical application were 50 µg and 0.035 µg per fly in Yachiyo and CSMA strains, respectively (Ori et al., 1990). Three- to six-d-old female flies were used throughout the study.

Chemicals. [Ethoxy-¹⁴C]diazinon, O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate (sp. act. 67 kBq/mg (>99% radiochemical purity) was the same as described previously (Ori et al., 1990). Nonradioactive diazinon was obtained from Nihonkayaku Co., Ltd., and purified before use. Bovine serum albumin fraction V (BSA) was purchased from Wako Pure Chemical Industries, Ltd. Sorpol 3005XH® surfactant was purchased from Toho Chemical Co. All other chemicals used were of the highest quality and commercially available.

Toxicity test. Diazinon solution (0.1 µl) in saline was injected into the thoracic scutum of flies using a glass capillary attached to an Arnold hand micro applicator (Kiya Manufacturing Co., Ltd.). The saline contained BSA (5%, w/v) and a surfactant, Sorpol 3005XH®. The concentration of the surfactant was adjusted to 1% (w/v) when the diazinon concentration was 1% (w/v) or below, or equal to the diazinon concentration when it was above 1%.

Fate of diazinon injected. Groups of ten flies with three replications were injected with various doses of ¹⁴C-diazinon as mentioned above. After the injection, the flies were kept in a scintillation vial (20 ml) at 25°C. At various time intervals, the flies were homogenized in a mixture of chloroform (4 ml) and water (2 ml) using an ultra-dispenser (Yamato Scientific Instruments Co., Model LK-21). The homogenate was stirred vigorously and centrifuged at 1,300 × g for 10 min. Radioactivity of the chloroform-soluble and water-soluble fractions was quantified by LSC (Aloka LSC 650) using a liquid scintillator (PATTERSON and GREEN, 1965). Unextracted radioactivity from the housefly residue should be below the detectable level as shown by the previous paper (Ori et al., 1992). Excreta in the holding vial was suspended in 1 ml of water for the determination of radioactivity. A preliminary study showed no chloroform-soluble radioactivity in the holding vial.

Fate of diazoxon following the injection of diazinon or diazoxon. After the injection of nonradioactive diazinon or diazoxon, the internal diazoxon was extracted with chloroform as described above at various time intervals. The amount of internal diazoxon was determined biochemically by inhibiting head AChE from the CSMA strain with a portion of the chloroform according to the method of Ori et al. (1992).

Estimation of kinetic parameters by computer simulation. Kinetic parameters from various proposed mathematical models were estimated by computer simulation through a commercially available program (Nankoudou, Inc., Tokyo) which was made on the basis of the paper of YAMAOKA et al. (1981). Details of the simulation were described in the previous paper (Ori et al., 1992). When a time-course obtained with radioactive diazinon was simulated, the weight value 1 was used so that larger values could fit (smaller values were easily subjected to experimental error). On the other hand, when

a time-course obtained biochemically was simulated, the weight value $1/C_i^2$ was used so that all values could fit since accuracy of smaller values were the same as larger values, where C_i is measured value at time t_i .

Selection of kinetic models. Kinetic models were examined based upon AIC (An Information Criterion), which is incorporated in the above program. The AIC can be determined using the following equation:

$$AIC = n \ln SS + 2m$$

where n , SS and m denote number of data, sum of squares and number of parameters, respectively, in the kinetic model. A model which gives minimum AIC is judged as the most suitable model.

RESULTS

Toxicity test

The LC_{50} (95% C.L.) of the CSMA and the Yachiyo strain by injection of diazinon were 0.012 (0.009–0.016) and 0.76 (0.67–0.87) $\mu\text{g}/\text{female}$, respectively.

Dynamics of organo-soluble radioactivity after the injection of radioactive diazinon

Five doses, i.e., 0.02, 0.05, 0.2, 1 and 5 $\mu\text{g}/\text{female}$ were used. Results are shown in Fig. 1. The radioactivity recovery was greater than 80% throughout the experiments. A decrease in organo-soluble radioactivity, consisting mostly of diazinon and a small amount of diazoxon, was more rapid with the Yachiyo strain than with the CSMA strain at all the doses examined.

Dose dependency was suggested because the relative rate of decrease at the higher two doses were obviously slower than at the lower three doses.

Fate of diazoxon after the injection of diazinon

Since no diazoxon was detected in the Yachiyo strain at the lower three doses used in the previous experiment, two higher doses were used. As shown in Fig. 2, there was a remarkable interstrain difference. In the CSMA strain, a large amount of diazoxon was found and the concentration was constant or gradually decreased with time, while in the Yachiyo strain a smaller amount of diazoxon was found and decreased rapidly.

Fate of diazoxon after the injection of diazoxon

To investigate the interstrain difference in the degradation of diazoxon, the amount of diazoxon was determined at various time intervals after the injection of diazoxon (Fig. 3). Two doses, i.e. 0.01 and 0.1 $\mu\text{g}/\text{female}$ were used. The result again showed the interstrain difference and dose dependency of this process.

Kinetic analysis of the injected diazinon

Two mathematical models were assumed to describe the overall detoxification of organo-soluble diazinon and diazoxon (Fig. 4). The following equations were derived from the Type I model:

$$dX_G/dt = -k_{CW}X_G \quad (1)$$

$$dX_W/dt = k_{CW}X_G \quad (2)$$

Fig. 5 presents the experimental data in the CSMA strain (Fig. 1, 0.02 $\mu\text{g}/\text{female}$) and

Fig. 1 Degradation of organo-soluble internal radioactivity following injection of various doses of diazinon in CSMA (●) and Yachiyo (○) strains. The curves were obtained by computer simulation, based on the rate constants listed in Table 2. The ratio of radioactivity was expressed based upon the total radioactivity recovered.

a corresponding curve predicted by computer simulation using equation 1. The k_{ow} values are listed in Table 1. There is a considerable deviation between the experimental and predicted results.

Therefore, the Type II model in Fig. 4 was proposed, which includes a distribution factor such as partitioning of diazinon/diazoxon between the hemolymph and internal tissues. The following equations were derived from the model:

Fig. 2. Formation of diazoxon following injection of diazinon with time in CSMA (S) and Yachiyo (R) strains. Applied doses are $1 \mu\text{g}/\text{♀}$ ($\bullet$) and $5 \mu\text{g}/\text{♀}$ ($\circ$). The curves were obtained by computer simulation, based on the rate constants listed in Table 4.

Fig. 3. Percent diazoxon remaining following injection of diazoxon in CSMA (S) and Yachiyo (R) strains. Applied doses are $0.01 \mu\text{g}/\text{♀}$ ($\bullet$, $\circ$), and $0.1 \mu\text{g}/\text{♀}$ ($\blacktriangle$, $\triangle$). The lines were obtained graphically from solid points, based on the rate constants listed in Table 5.

$$dX_{\text{H}}/dt = -(k_{\text{HW}} + k_{\text{HT}})X_{\text{H}} + k_{\text{TH}}X_{\text{T}} \quad (3)$$

$$dX_{\text{T}}/dt = k_{\text{HT}}X_{\text{H}} - k_{\text{TH}}X_{\text{T}} \quad (4)$$

$$X_{\text{C}} = X_{\text{H}} + X_{\text{T}} \quad (5)$$

The parameter values were estimated by computer simulation using the experimental

Fig. 4. Kinetic models describing the detoxification of injected diazinon with (Type II) or without (Type I) distribution process. X_C : Internal diazinon and diazoxon; X_H : diazinon and diazoxon in hemolymph; X_W : water-soluble metabolites in the internal extract and excreta; X_T : diazinon and diazoxon distributed to internal tissues; k_{HT} and k_{TH} : rate parameters for reversible exchange of diazinon and diazoxon between X_H and X_T .

Table 1. Kinetic parameter (k_{CW}) for the Type I model given in Fig. 4

Dose ($\mu\text{g}/\varphi$)	$k_{CW} (\text{h}^{-1}) \pm \text{S.D. (AIC)}$	
	S	R
0.02	2.18 ± 0.17 (126)	3.08 ± 0.32 (132)
0.05	1.63 ± 0.17 (136)	2.98 ± 0.20 (117)
0.2	0.59 ± 0.15 (133)	2.47 ± 0.22 (128)
1	0.385 ± 0.029 (131)	0.851 ± 0.097 (144)
5	0.164 ± 0.009 (106)	0.224 ± 0.015 (123)

Table 2. Kinetic parameter (k_{HW})^a for the Type II model given in Fig. 4

Dose ($\mu\text{g}/\varphi$)	$k_{HW} (\text{h}^{-1}) \pm \text{S.D. (AIC)}$	
	S	R
0.02	3.83 ± 0.18 (106)	5.27 ± 0.31 (109)
0.05	3.10 ± 0.12 (101)	5.06 ± 0.25 (104)
0.2	2.98 ± 0.11 (98.8)	4.37 ± 0.27 (114)
1	0.987 ± 0.037 (105)	1.94 ± 0.06 (96.6)
5	0.448 ± 0.014 (87.2)	0.601 ± 0.015 (87.8)

^a The values 1.75 and 0.806 were used for k_{HT} and k_{TH} , respectively, to estimate k_{HW} .

data shown in Fig. 1 and equations 3–5. With regard to k_{HT} and k_{TH} , 1.75 and 0.806 h^{-1} , respectively, were assumed in both strains obtained by preliminary stimulation for the estimation of the k_{HW} values, which are listed in Table 2. Judging from the smaller AIC values as compared to the values obtained for the Type I model (Table 1), the Type II model appears more appropriate. Predicted curves obtained by the Type II model shows good agreement with the experimental data (Fig. 1). The Type II model assumes that diazinon and diazoxon partitioned into the internal tissues and thus was not available for detoxification reactions. Lack of this process in the Type I model will explain the deviation in the experimental data from the predicted data (Fig. 5).

Fig. 5. Comparison between predicted time course of organo-soluble internal radio-activity determined by computer simulation for the Type I model using the rate constants listed in Table 1 and the experimental data. The CSMA strain was subjected to injection of $0.02 \mu\text{g}$ diazinon at zero time.

Type III

Type IV

Fig. 6. Kinetic models describing the dynamics of injected diazinon. The type III model consists of first-order processes. The type IV model consists of diazinon activation under bimolecular reaction, and apparent inactivation of the activation enzyme and degradation of diazoxon under first-order reactions. X_4 : internal diazinon; X_5 : metabolites of diazinon; X_6 : internal diazoxon; X_7 : metabolites of diazoxon; E_p : residual P-450 activity.

Kinetic analysis of diazinon activation and diazoxon degradation

Two models are postulated to describe the activation of injected diazinon and the subsequent degradation of diazoxon (Fig. 6). The following equations are derived from the Type III model:

Fig. 7. Disappearance of injected diazinon in CSMA (●) and Yachiyo (▲) strains. Applied doses were $1 \mu\text{g}/\text{♀}$ and $5 \mu\text{g}/\text{♀}$. The curves were obtained by computer simulation using the rate constants listed in Table 3.

Table 3. Kinetic parameters for diazinon disappearance determined by the method of residuals [$X_4 = A e^{-at} + B e^{-bt}$]

Parameter	Parameter values			
	$1 \mu\text{g}/\text{♀}$		$5 \mu\text{g}/\text{♀}$	
	S	R	S	R
A^a	74.4	59.3	64.0	83.6
a^b	0.262	0.361	0.0896	0.157
B^a	25.6	40.8	33.6	16.5
b^b	5.02	5.77	0.586	3.26

^a A and B : % diazinon.

^b a and b : rate constant (h^{-1}).

$$X_4 = A e^{-at} + B e^{-bt} \quad (6)$$

$$dX_6/dt = k_{46}X_4 - k_{67}X_6 \quad (7)$$

For the estimation of k_{46} and k_{67} , Eq. 6 was employed to describe a disappearance of diazinon caused by degradation of diazinon and activation to diazoxon. A comparison of predicted data and the experimental data on diazinon disappearance shows good agreement (Fig. 7). The parameter values for the process are listed in Table 3.

However, the experimental data on internal amount of diazoxon X_6 and the predicted data did not show good agreement, especially with the Yachiyo strain (Fig. 8). The AIC values obtained from these computations were -17.9 and 15.0 for the CSMA strain and 35.7 and 52.4 for the Yachiyo strain at doses of 1 and $5 \mu\text{g}/\text{female}$, respectively. Two reasons are possible for this disagreement: a decrease in activation over time or an increase in degradation of diazoxon over time. The former appears more reasonable since little increase in degradation potential of diazoxon was actually observed following the injection of diazoxon (Fig. 3).

Activation of a phosphorothioate to a phosphate is catalyzed by MFO system where cytochrome P-450 plays an important role (NAKATSUGAWA and MORELLI, 1976). It is unlikely that the cytochrome P-450 involved in the activation of diazinon may be competitively inhibited by diazoxon, because *in vitro* activation of diazinon was not inhibited by the addition of diazoxon (OI et al., 1990).

Fig. 8. Time-course of diazoxon present in CSMA (S) and Yachiyo (R) strains. Applied doses were 1 $\mu\text{g}/\text{♀}$ (●) and 5 $\mu\text{g}/\text{♀}$ (○). The curves, showing deviation from the experimental data (particularly in the Yachiyo strain), were obtained by computer simulation using the Type III model.

Table 4. Kinetic parameters for diazinon activation, diazoxon detoxification, and apparent inactivation for the Type IV model given in Fig. 6

Parameter	Dose ($\mu\text{g}/\text{♀}$)	Parameter value (h^{-1}) $\pm$ S.D.	
		S	R
k_{46} (activation)	1	0.319 ± 0.024	0.120 ± 0.022
	5	0.115 ± 0.010	0.0875 ± 0.0230
k_{67} (detoxification)	1	5.62 ± 0.63	31.7 ± 1.9
	5	4.06 ± 0.52	32.1 ± 1.9
k_P (apparent inactivation)	1	-0.0942 ± 0.0259	0.544 ± 0.128
	5	-0.137 ± 0.016	0.641 ± 0.116
AIC	1	-25.6	14.9
	5	-10.3	33.5

Therefore, the Type IV model which includes an apparent inactivation process of the cytochrome P-450 responsible for activation of diazinon was postulated (Fig. 6). By assuming a linear equation for the apparent decrease in the P-450 activity and a bimolecular reaction of the P-450 and diazinon, the following equations were derived from the model:

$$dX_6/dt = k_{46}X_4E_P - k_{67}X_6 \quad (8)$$

$$dE_P/dt = -k_P E_P \quad (\text{initial condition of } E_P \text{ is } 1) \quad (9)$$

Table 5. Kinetic parameters for diazoxon detoxication obtained graphically from solid points in Fig. 3

Dose ($\mu\text{g}/\varphi$)	Parameter value (h^{-1}) $\pm$ S.D.	
	S	R
0.01	5.60 ± 0.19	24.5 ± 4.2^a
0.1	4.57 ± 0.38	9.75 ± 0.46

^a The weighted value of X_7^{-1} was used for the computation.

Parameter values estimated by computer simulation with Eqs. 6, 8 and 9, time-course of diazoxon formation (Fig. 2) and the parameter values listed in Table 3 are listed in Table 4. The AIC values for the Type IV model are smaller than those for the Type III model. Predicted data also showed good agreement with the experimental data (Fig. 2), indicating that the Type IV model describes the internal event of diazinon more accurately. An interstrain comparison shows that the k_{46} , k_{67} and k_P , all of which are determinants of the internal amount of diazoxon, are in favor of affording resistance to the Yachiyo strain.

DISCUSSION

A remarkable interstrain difference was observed in the amount of diazoxon found following the injection of diazinon (Fig. 2). This result is similar to those obtained previously when diazinon was applied dermally by topical application. This difference was quantified by k_{HW} in Table 2, and k_{46} , k_{67} and k_P in Table 4. Values for k_{46} and k_{67} were not obtained correctly when diazinon was applied dermally (Ori et al., 1992). In the present study, the values were obtained more accurately, as indicated by standard deviations of the parameters.

The k_{HW} value should be regarded, approximately, as the sum of k_{45} and k_{46} values which indicate disappearance of diazinon, because organo-soluble radioactivity was almost all diazinon. Therefore k_{45} is calculated by subtracting k_{46} (Table 4) from k_{HW} (Table 2). The k_{45} values thus obtained were 0.668 and 1.82 at $1 \mu\text{g}/\varphi$ in the CSMA and the Yachiyo strain, respectively, and similarly 0.333 and 0.514 at $5 \mu\text{g}/\varphi$, indicating that the interstrain difference of diazinon degradation was smaller than that of diazoxon degradation (Table 4).

The greater degradation of diazoxon was confirmed by the injection of diazoxon (Fig. 3) and the parameter values for diazoxon degradation were estimated graphically from the slope of the regression lines obtained from the data illustrated with the solid symbols (Table 5). The increased degradation of diazoxon indicated by the higher k_{67} values (Tables 4 and 5) is probably due to phosphotriester hydrolase activity which was present in all the subcellular fractions of the Yachiyo strain (Ori et al., 1990), and this factor was supported by the synergistic effect of DEF, a phosphotriesterase inhibitor (Ori and Motoyama, 1991).

Kinetic analysis of the fate of injected diazinon suggested less activation of diazinon in the Yachiyo strain (Table 4), although this factor was not shown through *in vitro* study (Ori et al., 1990). The presence of a cytochrome P-450 isozyme which is responsible for less activation of phosphorothionate to phosphate is not surprising. A cytochrome P-450, which has less specificity for activation, was recently reported for methyl

parathion resistance in the tobacco budworm (KONNO et al., 1989).

In conclusion, the presence of an extremely low level of diazoxon in the Yachiyo strain (Fig. 2) should be the result of three factors: greater degradation of diazoxon (the main factor), greater degradation of diazinon, and less activation of diazinon.

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