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Original Article

Insecticidal Activity and Cuticular Penetration of Indoxacarb and Its *N*-Decarbomethoxylated Metabolite in Organophosphorus Insecticide-Resistant and -Susceptible Strains of the Housefly, *Musca domestica* (L.)

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Indoxacarb, the novel oxadiazine insecticide showed potent insecticidal activities in insecticide-susceptible (SRS) and organophosphorus insecticide-resistant (R-OP) strains of the housefly, *Musca domestica* (L.). The R-OP strain showed slightly less susceptibility to indoxacarb with the tolerance ratio of 2.5. *N*-Decarbomethoxylated metabolite of indoxacarb (DCJW) also showed insecticidal activity that was more potent than indoxacarb in both SRS and R-OP strains. Similar to indoxacarb, the R-OP strain showed less susceptibility to DCJW with the tolerance ratio of 6.1. Cuticular penetration experiments revealed that the R-OP strain showed more external persistency and less internal accumulation of indoxacarb and DCJW than the susceptible strain. The reduced cuticular penetration could account for the less susceptibility of the R-OP strain to indoxacarb and DCJW. Further analyses revealed that DCJW was excreted more slowly than indoxacarb which could be one of the factors of differences in insecticidal activity between the two chemicals.

Key words: Cuticular penetration, indoxacarb, resistant mechanism, housefly, reduced penetration, DCJW.

INTRODUCTION

Indoxacarb, methyl (*S*)-7-chloro-2,3,4α,5-tetrahydro-2-[methoxycarbonyl-(4-trifluoromethoxyphenyl)carbamoyl]indeno[1,2-*e*][1,3,4]oxadiazine-4α-carboxylate, is an oxadiazine insecticide recently developed by Du-Pont Agricultural Products. Biochemical and electrophysiological studies have shown the suppressive effects of indoxacarb on voltage-gated sodium channels in insects and mammals.[1–3] These studies suggested that effects of indoxacarb on the voltage-gated sodium channel could be directly responsible for its toxic actions on animals.

Little is known about the pharmacokinetic behavior of indoxacarb including the cuticular penetration and the internal distribution in insects. In the present study, we examined the insecticidal activity and cuticular penetration of indoxacarb in the laboratory-selected organophosphorus resistant (R-OP) and standard insecticidal-susceptible (SRS) strains of housefly, *Musca domestica* (L.). It has been demonstrated that *N*-decarbomethoxylated metabolite of indoxacarb (DCJW) has more potent neural activity than indoxacarb in lepidopteran larvae.[1] Thus we also examined the cuticular penetration of DCJW in the present study. Results indicate that indoxacarb and DCJW have potent insecticidal activity to both R-OP and SRS strains. The R-OP strain showed slightly less susceptibilities to indoxacarb and DCJW.

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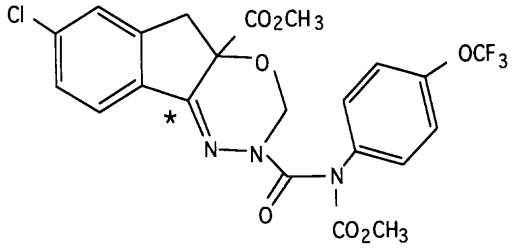
MATERIALS AND METHODS

1. *Chemicals*

Indoxacarb (>99% purity) and DCJW (>99%) were provided by Du-Pont Agricultural Products (Newark,

A. Indoxacarb



B. DCJW

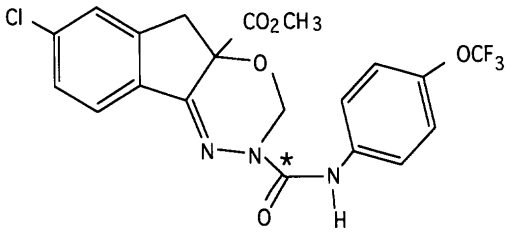


Fig. 1 Structures of indoxacarb and DCJW.
*indicates ^{14}C-labeled position.

DE, USA)(Fig. 1). [^{14}C]Indoxacarb (specific activity 116.2 MBq per mmol, radiochemical purity >99%) and [^{14}C] DCJW (specific activity 157.8 MBq per mmol, radiochemical purity >99%) were synthesized by New England Nuclear (Boston, MA, USA). All compounds used in the present study were 50 : 50 mixtures of (*R*)- and (*S*)-isomers.

2. Insects

The SRS strain was obtained from World Health Organization (WHO). The R-OP strain was established by the selection with organophosphorus insecticides profenofos for 20 and pyrachlofos for 13 generations from the insects collected at Yumenoshima Island in Tokyo, Japan in 1987. Insects were reared under 16L-8D at 25°C conditions. LD_{50} values of topically applied profenofos in the R-OP and SRS strains were 13.3 and 0.058 μg/insect, respectively (Hanai *et al.*, unpublished data). The resistance ratio was estimated to be 229.

3. Toxicity Tests

Test chemicals diluted in 0.5 μl of acetone were topically applied on the dorsal thorax of ether-anesthetized houseflies using a repeating solution dispenser (Hamilton Co., Reno, NE, USA). Control insects were treated with acetone only. Treated insects were placed into plastic containers (100 mm in diameter, 40 mm in depth) and held at 25°C. Insects which did not respond to pencil tip prodding were considered as dead. Mortality was recorded at 48 hr after treatment. Ten insects were used at each dose level. Data were analyzed by a Probit method[4] using a computer program (SAS Probit). The experiments were replicated three times. All data were pooled to estimate the parameters.

4. Cuticular Penetration

Insects were treated topically with 0.5 μl of the test solutions containing 0.03 μg/insect of [^{14}C] indoxacarb, or 0.003 μg/insect of [^{14}C] DCJW as described above. Insects were introduced in scintillation vials (20 ml) and were maintained at 25°C. At various time intervals, insects were cooled to −18°C in a freezer for 5 min and removed from the vials. Ten milliliters of scintillator cocktail (ACS-2, Amersham Japan Co., Tokyo) were added to the vials and the radioactivity was counted as excreted quantities by a liquid scintillation counter (Aloka LSC-700, Aloka Co. Ltd., Japan). Insects were placed on a metal-screened funnel and rinsed with 1 ml of acetone three times. The acetone was collected in a scintillation vial and held in a draft chamber for 24 hr to evaporate the acetone. Ten milliliters of ACS-2 cocktail were added to the vial and the radioactivity was counted as the externally persistent quantity of insecticides. The rinsed insects were dried in the draft chamber and incinerated in a sample oxidizer (ASC-113, Aloka Co. Ltd., Japan). $^{14}CO_2$ gas released by the combustion was trapped in a mixture of Oxysorb® TM-CO_2 and Oxyprep-2® (5 : 7, v/v) scintillation cocktails (New England Nuclear Co. Ltd., UK). This radioactivity was counted to estimate the amount of internal insecticides following cuticular penetration. The amount of cuticular penetration was estimated as the sum of excreted and internal quantities of insecticides. Experiments were replicated three times.

RESULTS AND DISCUSSION

The dose-mortality relationships for indoxacarb and DCJW in two strains of housefly are shown in Fig. 2. LD_{50} values of indoxacarb in the SRS and R-OP strains were estimated to be 0.15 and 0.39 μg/insect, respectively. The R-OP strain was 2.6-fold more tolerant to indoxacarb than the SRS strain. For DCJW, LD_{50} values of SRS and R-OP were estimated to be 0.007 and 0.043 μg/insect, respectively. The R-OP strain was 6.1-fold more tolerant to DCJW than the SRS strain. Thus, indoxacarb and DCJW showed potent insecticidal activity in both R-OP and SRS strains of housefly. Although indoxacarb and DCJW are thought to have a novel mode of action, the R-OP strain showed some tolerance to indoxacarb as well as DCJW in the present experiments.

DCJW showed 21.9- and 9.0-fold greater insecticidal activity than indoxacarb in the SRS and R-OP strains, respectively (Fig. 2). Wing *et al.*[1] reported that orally applied indoxacarb and DCJW showed identical levels of insecticidal activity in *Spodoptera frugiperda* larvae.

Their biochemical experiments revealed that indoxacarb was metabolized to DCJW. In electrophysiological studies using lepidopteran larval nerve preparations, DCJW showed a potent blocking effect to action potentials, whereas indoxacarb was weakly active in these blocking actions.[1] From these results, Wing *et al.* concluded that indoxacarb was bioactivated in insects. The difference of insecticidal activities between indoxacarb and DCJW may be due to lower bioactivation of indoxacarb in housefly than *S. frugiperda*. Further studies including the detailed metabolism of indoxacarb and DCJW are warranted.

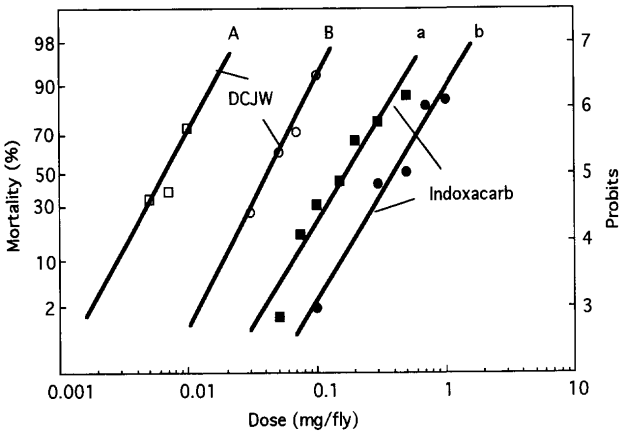


Fig. 2 Dose-mortality relationships for indoxacarb and DCJW in SRS and R-OP strains of housefly.
Mortality was determined at 48 hr after treatment. The experiments were replicated three times. Three replicated data were pooled for the analyses. A and a, SRS stain ; B and b, R-OP strain.

Fig. 3 shows the time courses of external persistency, internal accumulation, and excretion of [^{14}C] indoxacarb and [^{14}C] DCJW in two strains of housefly. The [^{14}C] indoxacarb penetration was faster in the SRS strain than in the R-OP strain (Fig. 3A). About 50% of indoxacarb was penetrated at 12 hr after treatment in the SRS strain, while it was required 24 hr to reach at the same level of penetration in the R-OP strain. Internal accumulation of [^{14}C] indoxacarb in the SRS strain was more than in the R-OP strain. However, there was little or no difference in the amount of excretion of [^{14}C] indoxacarb between these two strains. Overall, the total amount of [^{14}C] indoxacarb in the body was more in the SRS strain than in the R-OP strain. A similar tendency was observed for DCJW (Fig. 3B). Thus, there are clear differences in cuticular penetration and internal accumulation between these two strains. It is well known that the reduced cuticular penetration of insecticides is one of the resistance mechanisms in housefly.[5] Because of continuous selection by organophosphorus insecticides in the laboratory, the R-OP strain tested in the present study has obtained the capability to reduce cuticular penetration of organophosphorus insecticides (Hanai *et al.*, unpublished data). Although the detailed mechanisms of reduced cuticular penetration remains to be seen by molecular biological approaches, the capability to reduce cuticular penetration in the R-OP strain appeared to be effective even with indoxacarb and DCJW, though the strain has not yet been exposed to these two insecticides. Thus, the reduced cuticular penetration in the R-OP strain would not specify organophosphorus insecti-

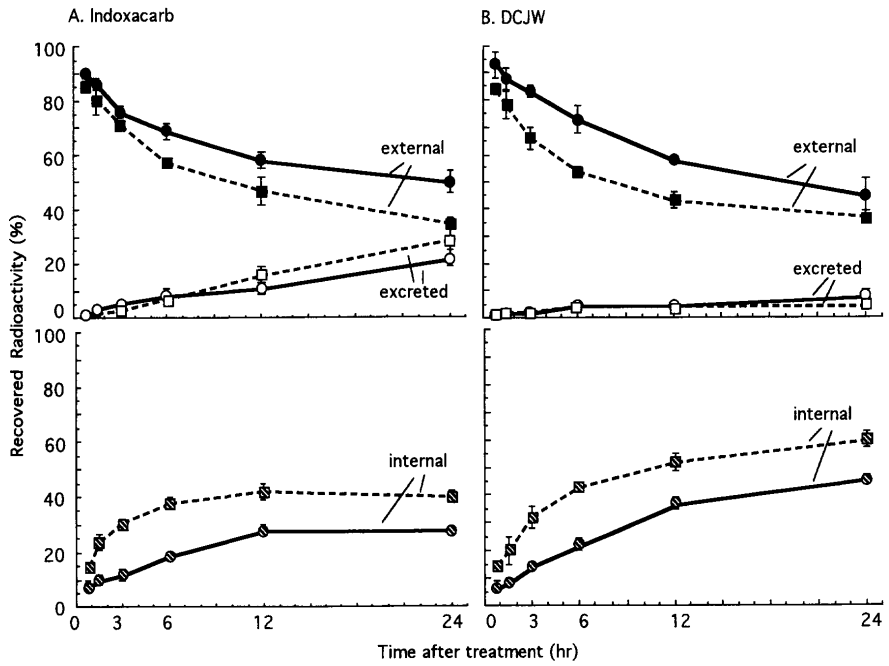


Fig. 3 Time courses of external persistency, internal accumulation, and excretion of [^{14}C] indoxacarb and [^{14}C] DCJW in two strains of housefly.
See text for further explanation. Mean±S.D. (n=3). Dotted line, SRS strain ; solid line, R-OP strain.

cides only, and may contribute to developing multiple resistance against various insecticides. The low susceptibility to indoxacarb and DCJW in the resistant strain seemed to be caused by the reduced cuticular penetration rather than the insensitivity of target site of action.

Although external persistency rates of [^{14}C] DCJW were not significantly different from those of [^{14}C] indoxacarb in both R-OP and SRS strains, excretion rates were much lower for [^{14}C] DCJW than [^{14}C] indoxacarb in both strains, resulting in a higher internal accumulation of [^{14}C] DCJW (Fig. 3). Since inherent insecticidal activities in housefly are different between indoxacarb and DCJW, it is difficult to directly argue the relationship between the insecticidal activity and internal accumulation of DCJW. However, it would be possible that the higher internal accumulation of DCJW in insects also contribute to the higher insecticidal activity compared to indoxacarb. The differences in pharmacokinetic behavior between indoxacarb and DCJW in housefly could be one of the mechanisms of differential insecticidal activities.

Wing *et al.*[1] reported that DCJW blocked action potentials in a dose-dependent manner, whereas indoxacarb had only a weak effect. Indoxacarb weakly inhibited sodium currents in rat dorsal root ganglion neurons.[3] Dihydropyrazoles, analog insecticides of oxadiazines, potently inhibited the sodium channel currents in crayfish giant axons.[6] It appears that the blocking effect on sodium channels is an important action mechanism of indoxacarb and DCJW. We have shown that indoxacarb and DCJW also act on nicotinic acetylcholine receptors.[7] Due to the lack of sufficient data, controversies remain over which ion channels are the most important target site for indoxacarb and DCJW. More detailed studies at ion channel level are warranted to clarify the mode of action of indoxacarb and DCJW.

In conclusion, the differences in insecticidal activity between indoxacarb and DCJW may be due to the differential action at the ion channel level as well as the differential pharmacokinetic behavior.

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要　　約

インドキサカルブおよび脱メトキシカルボニル代謝物の有機リン剤抵抗性および感受性イエバエに対する殺虫活性と皮膚透過性

杉山茂勲，鶴淵裕治，柄澤　朗，永田啓一
河野義明，正野俊夫

新規殺虫剤であるインドキサカルブは，感受性系統（SRS）および有機リン剤抵抗性（R-OP）系統イエバエに対し高い殺虫活性を示したが，R-OP系統のほうがSRS系統と比較して感受性が低下しており，抵抗性比は2.5倍だった．同様の傾向は代謝物であるDCJWにおいても見られ，抵抗性比は6.1倍だった．系統間における殺虫活性の違いの原因を明らかにするために，標識化合物を用いて皮膚透過性を調べた．その結果，SRS系統に比較してR-OP系統では両化合物の皮膚透過量の減少，体内蓄積量の減少が認められた．以上のことからR-OP系統における殺虫剤に対する感受性低下は，皮膚透過性の低下が主な要因であると考えられた．また，インドキサカルブとDCJWの間で排泄量に大きな違いが認められたので，このことも化合物間で見られる殺虫活性の違いのひとつの要因となっていることが考えられた．