Original Article

α -Tocopherol Antagonizes the Toxicity of the Pyrethroid Insecticide Permethrin in Susceptible and *kdr*-Resistant Insects

Jeffrey G. SCOTT

Department of Entomology, Comstock Hall, Cornell University, Ithaca, New York 14853, USA

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Pyrethroid insecticides exert their toxic actions by prolonging the open time of individual neural sodium channels. α -Tocopherol (vitamin E) has recently been shown, via a patch clamp analysis of rat cells, to antagonize this action. To determine if α -tocopherol could provide protection against pyrethroids *in vivo* we examined the toxicity of a common pyrethroid (permethrin) against house flies and German cockroaches. Tocopherol antagonized the toxicity of permethrin by 4.3- and 6.6-fold in house flies and German cockroaches, respectively. α -Tocopherol also antagonized permethrin toxicity by 6.2-fold in a *kdr*-type resistant strain of German cockroach (*i.e.* strain having an altered form of the *para*-homologous sodium channel). These studies indicate α -tocopherol is an effective antagonist of permethrin *in vivo*, protecting insects that have either normal or *kdr*-type sodium channels.

INTRODUCTION

Pyrethroid insecticides are widely used and effective against numerous pests. Pyrethroid insecticides generally have low mammalian toxicity, but under conditions of high exposure may produce an abnormal skin sensation or paresthesia due to stimulation of the sensory nerve endings.¹⁾ In insects, a common type of resistance that develops in response to selection with pyrethroid insecticides is known as knockdown resistance or *kdr*.^{2,3)} This resistance mechanism appears to be due to a change in the sodium channels resulting in insensitivity.^{4–7)}

α -Tocopherol (vitamin E) was recently shown to antagonize pyrethroid-induced sodium channel modification *in situ* in rat dorsal root ganglion neurons and cerebellar Purkinje cells.⁸⁾ To determine if α -tocopherol could provide protection against pyrethroids *in vivo*, the toxicity of a common pyrethroid (permethrin), with and without α -tocopherol, was evaluated against susceptible house flies and German cockroaches and a *kdr*-type resistant strain of German cockroaches.

MATERIALS AND METHODS

Three strains of German cockroach were used: CSMA is an insecticide susceptible strain obtained from F. Matsumura in 1986, Cochran-S⁹⁾ is an insecticide susceptible strain obtained from D. Cochran in 1991 and Ectiban-R¹⁰⁾ is a *kdr*-type (*i.e.* pyrethroid) resistant strain selected with permethrin from the DDT-selected VPIDLS strain. Cockroaches were reared as described earlier.¹¹⁾

The CS (insecticide-susceptible) strain of house flies¹²⁾ was reared as described previously.¹³⁾ Permethrin (25 : 75 *cis* : *trans*), a Type I pyrethroid insecticide,^{14,15)} was from AgrEvo (Wilmington, DE) and $\pm$ - α -tocopherol was from Sigma (St. Louis, MO).

Permethrin or α -tocopherol (30% wt : vol) was delivered in 0.5 μ l acetone to the abdominal sternum of male cockroaches¹⁰⁾ or to the thoracic notum of female house flies.¹⁶⁾ α -Tocopherol (or acetone for the controls) was delivered 1 hr before the insecticide application. Ten adult cockroaches or twenty 3–5-day-old house flies were treated for each dose. A minimum of 3 doses giving > 0% and < 100% mortality at 72 hr after permethrin treatment was used for each experiment. Each experiment was replicated at least 3 times. The treated insects were put in 200 ml Sweetheart ice cream cups covered with cheese cloth and held at 25°C. Each cup contained a piece of 4 cm dental wick soaked in 15% sugar water and the dental wicks were kept wet during the experiment. Mortality was assessed 24 hr after insecticide application. Insects were considered dead if they were on their backs and unable to right themselves when disturbed. Bioassay data were pooled and analyzed based on standard probit analysis¹⁷⁾ as adapted to personal computer use,¹⁸⁾ using Abbott's correction for control¹⁹⁾ mortality.

RESULTS AND DISCUSSION

The toxicity of permethrin, with and without α -tocopherol pretreatment, to susceptible house flies and German cockroaches is shown in Table 1. α -Tocopherol

Table 1 Protection from permethrin toxicity by tocopherol.

Species	Strain	α -Tocopherol	LD ₅₀ ^{a)} (95% CI)	n	Slope (SE)	AR ^{b)}
House fly	CS	—	0.0094 (0.0072–0.0112)	300	3.0 (0.6)	—
House fly	CS	+	0.040 (0.035–0.045)	360	4.6 (0.6)	4.3
German cockroach	CSMA	—	0.20 (0.18–0.23)	280	6.2 (1.0)	—
German cockroach	CSMA	+	1.32 (1.08–1.67)	255	2.9 (0.6)	6.6
German cockroach	Ectiban-R	—	4.25 (3.71–4.87)	280	5.1 (0.8)	—
German cockroach	Ectiban-R	+	26.3 (22.6–30.5)	310	4.9 (0.9)	6.2

^{a)} LD₅₀ in units of $\mu\text{g}/\text{insect}$. ^{b)} Antagonism ratio: permethrin LD₅₀ with α -tocopherol (0.5 μl of a 30% solution)/permethrin LD₅₀ without α -tocopherol.

antagonized the toxicity by 4.3- and 6.6-fold in the susceptible strains of house fly (CS) and cockroach (CSMA), respectively. This indicates that the protective effect of α -tocopherol on voltage dependent sodium channels, which was first demonstrated *in situ*,⁸⁾ can also be observed as a reduction in toxicity *in vivo*.

Given that *kdr*-type resistance to pyrethroids is associated with a change in the *para*-homologous sodium channel, the effect of α -tocopherol was investigated in a strain of cockroach having this resistance mechanism. α -Tocopherol antagonized permethrin toxicity by 6.2-fold in this strain, similar to the level of antagonism observed in the susceptible strain (Table 1). Thus, the α -tocopherol is effective at protecting insects having either normal or *kdr*-type of sodium channels.

To test if the antagonism of permethrin by α -tocopherol that was observed by topical application could be observed by other assay methods, the toxicity of permethrin (with or without α -tocopherol) in susceptible (Cochran-S) German cockroaches was investigated via injection. Injection of 16 ng of permethrin, either in 0.5 μl of acetone or in 0.5 μl of 10% α -tocopherol (wt : vol) in acetone, gave 47% and 0% kill after 24 hr, respectively. Cockroaches injected with 0.5 μl acetone had 3.3% kill, and those injected with 0.5 μl of 10% α -tocopherol (wt : vol) in acetone had 0% kill. Thus, the α -tocopherol antagonism was observable both by topical application and injection.

The above results indicate that α -tocopherol is effective at antagonizing the toxicity of a Type I pyrethroid in susceptible and *kdr*-type resistant insects. This supports the conclusion of Song and Narahashi that “ α -tocopherol opens the door for development of antidotes for pyrethroid intoxication”.⁸⁾

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要 約

感受性および *kdr* 抵抗性昆虫における α -トコフェロールとペルメトリンとの拮抗作用

Jeffrey G. Scott

ピレスロイド殺虫剤は神経におけるナトリウムチャンネルのオープンタイムを延長することにより、その生理作用を示す。最近、 α -トコフェロール（ビタミン E）は、ラット細胞を用いたパッチクランプ法により、ピレスロイドの毒性と拮抗することが報告された。そこで α -トコフェロールが、*in vivo* でイエバエやチャバネゴキブリに対するピレスロイド（ペルメトリン）の殺虫力を低下させることが出来るかどうか検討した。 α -トコフェロール処理は、イエバエおよびチャバネゴキブリにおいて、ペルメトリンの殺虫

力をそれぞれ 1/4.3 及び 1/6.6 に低下させた。 α -トコフェロールは変異型 *kdr* を持つ抵抗性チャバネゴキブリにおいても、ペルメトリンの殺虫力を 1/6.2 に低下させた。これらのことから、 α -トコフェロールは正常型あるいは *kdr* 型

のいずれのナトリウムチャンネルを持つ昆虫に対しても、*in vivo* においてペルメトリンの拮抗剤として有効であることを示した。