STRUCTURE-ACTIVITY RELATIONSHIP OF PYRETHROIDS AGAINST DIFFERENT GEOGRAPHICAL STRAINS OF LARVAE OF MALARIA VECTOR, ANOPHELES STEPHENSI AND ROLE OF MIXED FUNCTION OXIDASE IN RESISTANCE PHENOMENON

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Abstract- An investigation was carried out on permethrin-susceptible and resistant strain of malaria vector, *An.stephensi*. Efficacy of several pyrethroids such as permetrhrin, deltamethrin, lambdacyhalothrin, cypermethrin and cyfluthrin against this species showed that those with an α-cyano group and dibromo substitutions were more toxic to sensitive strains than the other classes like permethrin. The ranking order of toxicity of pyrethroids was different for resistant strains. Evidence of enhanced mono-oxygenase activity and the synergism shown by piperonyl butoxide suggests that each resistant strain possesses a mono-oxygenase-mediated resistance mechanism responsible, at least in part, for pyrethroid resistance.
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INTRODUCTION

The natural pyrethrins and some synthetic pyrethroids have been used for many years in the first type of environment because of their lower mammalian toxicity than those of other classes of insecticides (1). Despite outstanding insecticide efficiency and degradation to innocuous residues, their cost and instability have excluded them from many other applications. Nowadays, there is considerable scope for developing new pyrethroid insecticides with little hazard for man and mammals (low mammalian toxicity is partially important) by modifying the structures of the natural pyrethrins. Recently substantial advances have been made in improving the larvicidal activity of synthetic pyrethroids against a wide range of mosquitoes. The structure-activity approach contributes to a better understanding of the specific substituents on a permethrin analogue that will be optimal for a particular desired property. This knowledge may be useful for the development of novel compounds that could play a role in the control of insects. Structure-activity relationship studies are useful as they give an indication of the relative importance of the different parts of molecules in terms of both their activity and their metabolism. The aim of structure-activity relationship studies is to determine whether changes in the structure of the pyrethroids markedly affected their potency as insecticides against the different strains of *An.stephensi* and to examine the reaction of permethrin-resistant and susceptible strains to different classes.

An attempt was made to carry out a study by using a range of pyrethroids to assess structure-activity relationships. In our study the following pyrethroids were chosen: cypermethrin, permethrin, deltamethrin, cyfluthrin and lambdacyhalothrin. All of the

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compounds are based on the structure of permethrin but have various substitutions. Except permethrin, all have an α-cyano substitution. Cypermethrin has a similar structure to deltamethrin but with a dibromo rather than dichloro substitution. Cyfluthrin has a flouro substitution on one of the aromatic rings and lambdacyhalothrin has a triflouromethyl substitution at the end of the acid moiety.

MATERIALS AND METHODS

Insecticides

Permethrin; 3-phenoxybenzyl-(1R)-*cis, trans*-3 (2, 2-dichlorovinyl)-2, 2, dimethyl cyclopropanecarboxy-late, technical grade 96.2%, density = 1.2 and *cis/trans* ratio 40/60

lambdacyhalothrin; (S) -α-cyano -3-phenoxybenzyl (1R) -*cis*-3- (2-chloro-3, 3, 3-triflouropropenyl -2, 2-dimethylcyclopropanecarboxy-late, technical grade 98.3%

deltamethrin; (S)-α-cyano-3-phenoxybenzyl (1R)-*cis*-3-(2,2-dibromovinyl) -2, 2-dimethylcyclopropanecar-boxylate

cyfluthrin; (RS) -α-cyano- 4-flouro- 3-phenoxyben-zyll (1RS) -*cis*, *trans*-3- (2,2-dichlorovinyl)-2, 2-dimethylcyclopropanecarboxy-late cypermethrin; (RS)- α- cyano-3- phenoxybenzyl (1R) –*cis* ,*trans*-3 -(2,2-dichorovinyl) -2, 2- dimethyl cyclopropanecar-boxylate.

All the insecticides were provided by Zeneca company.

Piperonyl butoxide (PB); 5-[2-(2-butoxyethoxy) ethoxymethyl)-6-propyl-1, 3-benzodioxole, technical grade 90%.

Insecticide testing method: Larvae were tested at the early 4th instar at 25±1°C according to the method of WHO (2). Larvae were exposed to different concentrations of insecticides followed by a 24-hour recovery period. After 24 h mortality was counted. For synergist studies, larvae were pre-treated with PB at 5 mg/l, 4 hr before exposure to insecticides. Dosage mortality regression lines were determined by the probit analysis method of Finney (3).

Mosquito strains:

DUB-S strain, from Dubai, United Arab Emirates (U.A.E), colonized in insectarium.

DUB-LPR strain, from DUB-S, selected with permethrin at the larval stage.

DUB-APR strain, from DUB-S, selected with permethrin at the adult stage.

IND-S strain, originating in New Delhi, India

IRAQ strain, originating in Iraq.

TEH strain, from Tehran, colonized for more than 25 years.

BAN strain, from Bandar Abbas, Iran.

Place of work: This work was carried out jointly by School of Public Health and Institute of Health Research, Tehran University and Liverpool School of Tropical Medicine, UK.

RESULTS

Structure-activity relationship of pyrethroids to larvae of susceptible strains

a) IND-S strain

Deltamethrin was by far the most toxic pyrethroid with LC_{50} value of 0.025 mg/l. Cypermethrin and cyfluthrin with LC_{50}s of 0.57 and 0.32 mg/l, respectively, were the least toxic, with over 23- and 13-fold difference in toxicity compared to deltamethrin (Tables 1 and 2). Permethrin with LC_{50} value of 0.13 mg/l had a moderate effect. Lambdacyhalothrin with LC_{50} value of 0.04 showed 1.6 times less toxicity than that of deltamethrin.

b) IRAQ strain

The potency of 5 pyrethroids, as toxicant in larvae of IRAQ strain decreased in order of cypermethrin> cyfluthrin> lambdacyhalothrin> permethrin> deltame-thrin. Cyfluthrin and cypermethrin with LC_{50} values of 0.49 and 0.55 mg/l showed 110 and 98-fold difference in toxicity compared to deltamethrin respectively (Table 2). Lack of trifuoromethyl substitution in permethrin structure caused this compound to be more active than lambdacyhalothrin, an α-cyano group with trifluo-romethyl substitution to IRAQ strain with 3-fold difference in toxicity.

c) BAN strain

The pyrethroid sensitivity assay with BAN strain revealed that among pyrethroids again deltamethrin is

the most toxic compound with LC_{50} value of 0.006mg/l) (Table 1). Deltamethrin showed 78, 72, 11 and 3-fold difference in toxicity compared to cypermethrin, cyfluthrin, permethrin and lambdacyhalothrin, respectively (Table 2).

d) TEH strain

Bioassay for TEH strain is shown in table 2. Deltamethrin was most toxic with LC_{50} value of 0.0045 mg/l and cypermethrin the least toxic with LC_{50} value of 0.16 mg/l, 36 times difference in terms of toxicity (Table 3). The remaining pyrethroids showed moderate toxicity with order of lambdacyhalothrin> permethrin> cyfluthrin (see Fig.1).

Structure-activity of pyrethroids in larvae of permethrin-resistant strains

a) DUB-APR

In DUB-APR larvae lambdacyhalothrin was the most toxic pyrethroid with an LC_{50} value of 3.6 mg/l (Table 2). Cyfluthrin with an LC_{50} of 13.06 mg/l was the least toxic with 3.6 fold difference in toxicity compared to lambdacyhalothrin. The other pyrethroids had different LC_{50} values with order of toxicity deltamethrin> permethrin> cypermethrin.

Table 1. LC_{50}, 95% Confidence interval with various pyrethroids for larvae of different strains of *An.stephensi*

Pyrethroids Strain	Deltamethrin	Lambdacyhalothrin	Permethrin	Cyfluthrin	Cypermethrin
	0.018	0.033	0.11	0.25	0.47
IND-S	0.025	0.04	0.13	0.32	0.57
	0.04	0.05	0.15	0.39	0.68
	0.004	0.2	0.078	0.47	0.41
IRAQ	0.005	0.23	0.08	0.55	0.49
	0.0055	0.27	0.082	0.65	0.58
	0.005	0.015	0.063	0.36	0.39
BAN	0.006	0.018	0.064	0.43	0.47
	0.007	0.02	0.066	0.51	0.56
	0.004	0.023	0.064	0.09	0.13
TEH	0.0045	0.027	0.07	0.12	0.16
	0.005	0.032	0.073	0.15	0.2
	4.6	3.1	6.11	11.58	9.51
DUB-APR	5.58	3.6	7.33	13.05	10.53
	6.88	4.12	8.99	14.85	11.7
	6	3.33	12.14	1.14	1.52
DUB-LPR	6.65	4.16	13.06	1.31	1.74
	7.3	5.25	14.07	1.5	2.0

Table 2. Relative activity of pyrethroids in comparison with deltamethrin for larvae of different strains of *An.stephensi*

Pyrethroid Strain	Lambdacyhalothrin	Permethrin	Cyfluthrin	Cypermethrin
IRAQ	+16	+48	+98	+110*
BAN	+3	+11	+72	+78
TEH	+6	+16	+27	+36
IND-S	+1.6	+5	+13	+23
DUB-APR	-0.6	+1.3	+1.9	+2.3
DUB-LPR	-1.6	+2	-5	-3.8

* At the $LC_{50,}$ deltamethrin is 110 times more toxic than cypermethrin to IRAQ strain.

Table 3. Effect of synergist piperonyl butoxide (PB) on the status of pyrethroid resistance in larvae of DUB-LPR strain of *An.stephensi*

Pyrethroids	With/out PB	LC_{50},95% C.I	SR*
Permethrin	+	0.09 0.1 0.13	130.6
	-	12.14 13.06 14.07	
Deltamethrin	+	0.066 0.078 0.093	85.3
	-	6.0 6.65 7.3	
Lambdacyhalothrin	+	0.041 0.048 0.56	86.67
	-	3.33 4.16 5.25	
Cypermethrin	+	0.074 0.092 0.12	18.9
	-	1.52 1.72 2.0	
Cyfluthrin	+	0.052 0.062 0.075	21.13
	-	1.14 1.31 1.5	

* SR= LC_{50} of insecticide alone/ LC_{50} of insecticide+synergist

At the LC_{50} level deltamethrin was 0.6 fold less toxic than lambdacyhalothrin and 2.3, 1.9 and 1.3-fold more toxic than cypermethrin, cyfluthrin and permethrin, respectively (Table 3).

b) DUB-LPR

Larvae of DUB-LPR strain have become highly resistant to their selecting chemical by the high insecticide concentration needed for mortality (LC_{50}=13.06 mg/l) (Table 2). Cyfluthrin was the most toxic with LC_{50} value of 1.31 mg/l. Results from bioassay data in table 2 indicated that resistance affected by presence of α-cyano group. Cyfluhrin, cypermethrin, lambdacyhalothrin and deltamethrin had more potency against larvae of DUB-LPR in comparison with permethrin with potency of 10, 7.5, 3 and 2-fold exceeded than permethrin, respectively (see Fig.2).

Synergist study

Because DUB-LPR and DUB-APR larvae have developed resistance to permethrin and this resistance is partially P450-dependent, it is important to consider the biochemical mechanism of resistance to other classes of pyrethroids to determine whether similar mechanism(s) are involved in each resistant strain. To do such, the effect of the mono-oxygenase synergist, piperonyl butoxide (PB) were examined. The results of the synergist study are summarized in table 4.

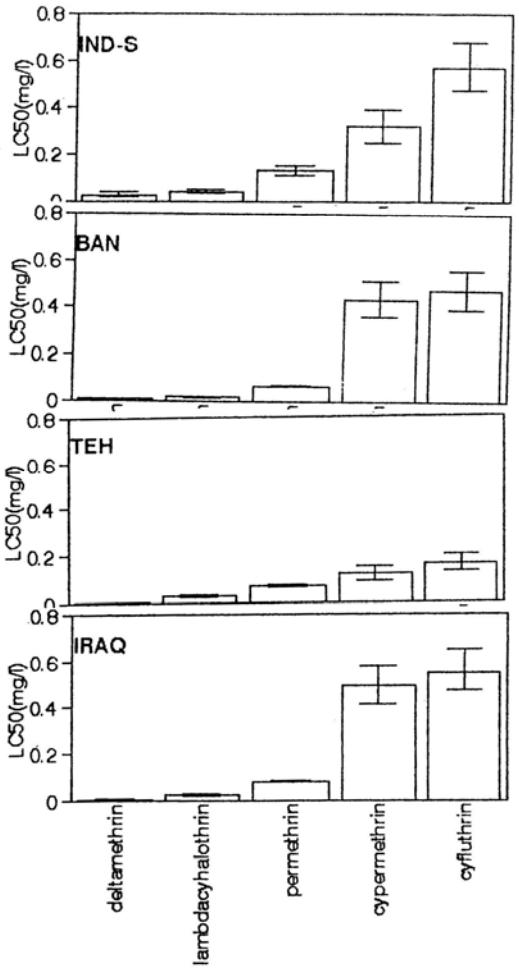

Fig. 1. Relative toxicity of pyrethroids to larvae of susceptible strains of An. Stephensi. vertical bars= 95% CI

Table 4. Effect of synergist piperonyl butoxide (PB) on the status of pyrethroid resistance in larvae of DUB-APR strain of *An.stephensi*

Pyrethroids	with/out PB	LC_{50},95% C.I	SR*
Permethrin	+	0.076 0.1 0.14	73.3
	-	6.14 7.33 8.99	
Deltamethrin	+	0.09 0.11 0.135	50.72
	-	4.6 5.58 6.88	
Lambdacyhalothrin	+	0.85 1.0 1.22	3.6
	-	3.16 3.6 4.12	
Cypermethrin	+	0.74 0.92 1.14	11.45
	-	9.51 10.53 11.7	
Cyfluthrin	+	0.8 0.99 1.23	13.2
	-	11.58 13.05 14.84	

SR= LC_{50} of insecticide alone/ LC_{50} of insecticide+synergist

In larvae of DUB-LPR and DUB-APR strains, permethrin was strongly synergized by PB with synergist ratios of 130.6 and 73.3-fold. PB significantly reduced the resistance factor (RF), causing it to drop from 100.5 to 0.8-fold and from 56 to 0.8 fold for DUB-LPR and DUB-APR, respectively. In DUB-LPR strain PB produced a high synergist (85.3-fold) of deltamethrin and 86.67-fold of lambdacyhalothrin.

The application of synergist with cypermethrin and cyfluthrin gave SRs of 18.9 and 21.13-fold at the LC_{50} level (Table 4).

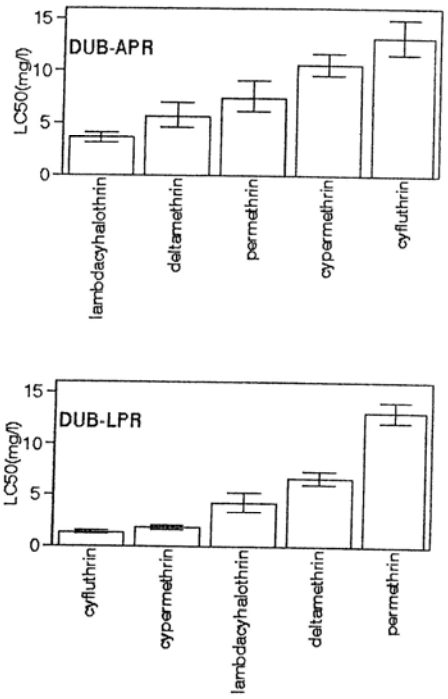

Fig. 2. Relative toxicity of pyrethroids to larvae of permethrin-resistant strains of An. Stephensi. vertical bars= 95% CI

Table 5. Resistance Factor (RF) of pyrethroids for larvae of *An.stephensi*

strain	Pyrethroids	(RF*)±95%C.I
DUB-LPR	Permethrin	119, 101, 94
	Deltamethrin	333, 266, 182
	Lambdacyhalothrin	105, 104, 101
	Cypermethrin	5, 4, 3.8
	Cyfluthrin	3.3, 3, 2.9
DUB-APR	Permethrin	60, 56, 55
	Deltamethrin	256, 223, 172
	Lambdacyhalothrin	94, 90, 82
	Cypermethrin	46, 41, 38
	Cyfluthrin	20, 18, 17

*RF=LC_{50} of permethrin resistant strain/LC_{50} of susceptible strain (IND-S)

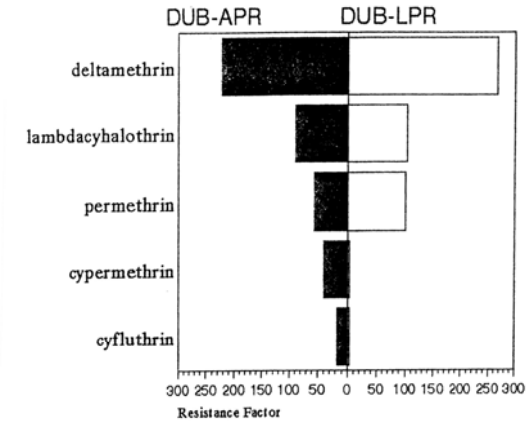

Fig. 3. Resistance factor (RF) of pyrethroids for larvae of permethrin-resistant strains (DUB-APR and DUB-LPR) in comparison with IND-S strain.
RF= LC_{50} resistant strain/ LC_{50} susceptible strain

Synergist study revealed that although a slight change (3.6-fold) occurred in the DUB-APR strain response to lambdacyhalothrin when PB was added, PB synergism was relatively high where it was recorded 73.3-fold for permethrin, 50.71-fold for deltamethrin, 11.45-fold for cypermethrin and 13.2- fold for cyfluthrin at the LC_{50} level (Table 5). This work clearly showed that with most pyrethroids tested, the synergist made a difference in the response of resistant larvae, hence after synergism by PB, resistance factor for all of pyrethroids declined (see Fig.3).

DISCUSSION

The order of toxicity among the pyrethroids depends on the target strain but in general, deltamethrin, an α-cyano pyrethroid with dibromo substitution, is the most potent compound against the susceptible larvae. Cypermethrin and cyfuthrin were the least toxic, lambdacyhalothrin and permethrin had moderate toxicity. The range of pyrethroid toxicity at the LC_{50} between the two extremes (deltamethrin and cypermethrin) was greatest for the susceptible strains. For example deltamethrin was up to 98, 78, 36 and 23 times more potent than cypermethrin for larvae of IRAQ, BAN, TEH and IND-S strains and the pattern of sensitivity to pyrethroids was unique for susceptible strains. In contrast, with the DUB-APR strain the difference in the highest and the lowest potency (lambdacyhalothrin and cyfluthrin) did not exceed 3.6-fold. The ranking order in toxicity of pyrethroids to DUB-LPR strain was different from DUB-APR larvae. Permethrin as a selected agent had the least potency and cyfluthrin the most. Similarly another α-cyano pyrethroids (deltemethrin, lambdacyhalothrin, cypermethrin) were more toxic than permethrin. The range of toxicity at the LC_{50} between the two extremes (cyfluthrin and permethrin) was around 10-fold for DUB-LPR strain. In DUB-LPR strain resistance was affected by α-cyano substitution. In the resistant strains (DUB-APR and DUB-LPR) the compression in LC50 values was most marked for all the pyrethroids.

Structure-activity relationships for 10 pyrethroids against susceptible, *kdr* and super-*kdr* strains of houseflies were investigated by Farnham et al. (4). They found that deltamethrin was more potent than cypermethrin and permethrin to susceptible strains. The range of pyrethroid toxicity at the LD50 between the two extremes (deltamethrin and pyrethrin) was greatest for the susceptible strains, intermediate for *kdr* strains, and least for strains with super-*kdr*. Among the *kdr* strains there was no uniform and consistent pattern in structure-activity relationship. The same pattern of inconsistent pattern in structure-activity relationship of pyrethroids can be seen in DUB-APR and DUB-LPR strains, where cyfluthrin was the most potent for DUB-LPR and least for DUB-APR. Davies (5) pointed out that pyrethoids act at many different sites and different compounds can produce different symptoms of toxicity.

The results from resistance factor (RF): DUB-LPR LC_{50}/IND-S LC_{50} and DUB-APR LC_{50}/IND-S LC_{50}, shows and it can be concluded that the relative activities of different pyrethroids are not the same. Range of RF for DUB-LPR varied. he highest RF was obtained with deltamethrin, (266-fold) and the lowest was by cyfluthrin with RF of 3-fold. A similar trend can be seen with DUB-APR strain . In general the RF can be categorized into 3 groups; deltamethrin with high RF, permethrin and lambdacyhalothin with moderate RF, cypermethrin and cyfluthrin with low RF for both resistant strains. Rate of penetration, transport to the site of action, susceptibility to detoxication and fit of the

molecular structure at the site of action are possible reasons for such differences in observed resistance factor. It is important to determine whether such differences arise through selective penetration, transport and/or metabolism, or through preferential action in the nervous system.

The trend for high toxicity of α-cyano compounds has been well documented in house flies (6,7) in *Cx.quinque-fasciatus*, and in a predatory mite *Amblyseins fallacis* (8). Soderlund & Casida (9) pointed out that the addition of a cyanide group to the α-carbon of the pyrethroids derived from 3-phenoxybenzyl alcohol reduces the susceptibility of the molecule to both hydrolytic and oxidative metabolism. Structure-activity relationships of pyrethroids against larvae and adults of *Cx.quinquefasciatus* depended on the nature and position of the substitutent in the phenyl ring of the acid moiety and also the type of alcohol moiety. The larvicide activity was found to increase in the following order: bromo< choloro< flouro, whereas the adulticide activity was found vice versa in comparison to larvae (10). Studies of Clements and May (11) and Nishimura and Narahashi (12) using a variety of pyrethroids have failed to show a clear correlation between the structure-activity relationship found in neurophysiological preparations in *Preplaneta americana* and crayfish. Toxicity of six larvicides including deltamethrin and permethrin was evaluated against *An. stephensi*, *Culex quinquefasciatus, Aedes aegypti* and *Toxoryhnchites splendens* larvae in India by Amalraj and Das (13). They found different LC50s for different species. Mukhopadhyay et al (14) found that larvae of *An. stephensi* were susceptible to deltamethrin. In another study Chunina et al (15) showed that deltemethrin and cypermethrin at sub-lethal doses, at the larval stage had no effect on malaria refractory at the adult stage. Dorta et al (16) tested alphamethrin and deltamethrin against *An. stephensi* larvae and adults. They found that pyrehtroids could be effectively employed for integrated vector control program.

In our study permethrin had the least activity against DUB-LPR strain of all the pyrethroids.

Evidence of enhanced mono-oxygenase activity and the synergism shown by PB suggest that each resistant strain possesses mono-oxygenase-mediated resistance mechanisms responsible at least in part for the pyrethroid resistance. It appears likely, therefore, that permethrin selection may have led to an increase in cytochrome P-450 specific for detoxication of these compounds and leading to the low, moderate and high cross-resistance observed. This would suggest that there is at least a common resistance mechanism for all the pyrethroids via P-450. Moreover, results indicate that relative activities of different pyrethroids are not the same, suggesting thereby that another resistance mechanism(s) may play a role. Although the ability to metabolize pyrethroids varies greatly with compound and insect species, but the extent to which this affects insecticidal potency is not yet precisely defined. The situation in resistant strains is further complicated by interaction between the various factors of resistance, therefore further work is needed to clarify the possibility of quantitative differences in the mono-oxygenase systems in each selected strain. An understanding of such differences between strains will be an important part of developing insecticide use strategy to prevent further resistance problem with synthetic pyrethroids.

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