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The structural requirements of organophosphorus insecticides (OPI) for reducing chicken embryo NAD$^+$ content in OPI-induced teratogenesis in chickens

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ABSTRACT

The objective of this study was to determine the structural requirements of organophosphorus insecticides (OPI) for reducing chicken embryo nicotinamide adenine dinucleotide (NAD$^+$) content in OPI-induced teratogenesis and compare them with those needed for OPI inhibition of yolk sac membrane kynurenine formamidase (KFase), the proposed primary target for OPI teratogens in chicken embryos. The comparative molecular field analysis (COMFA) of three-dimensional quantitative structure–activity relationship (3D QSAR) revealed the electrostatic and steric fields as good predictors of OPI structural requirements to reduce NAD$^+$ content in chicken embryos. The dominant electrostatic interactions were localized at nitrogen-1, nitrogen-3, nitrogen of 2-amino substituent of the pyrimidinyl of pyrimidinyl phosphorothioates, and at the oxygen of crotonamide carbonyl in crotonamide phosphates. Bulkiness of the substituents at carbon-6 of the pyrimidinyls and/or N-substituents of crotonamides was the steric structural component that contributed to superiority of those OPI for reducing embryonic NAD$^+$ levels. Both electrostatic and steric requirements are similar to those defined in our previous study for OPI inhibition of chicken embryo yolk sac membrane KFase. The findings of this study provide another piece of evidence for the cause-and-effect relationship between yolk sac membrane KFase inhibition and reduced embryo NAD$^+$ content in NAD-associated OPI-induced teratogenesis in chickens.

1. Introduction

Organophosphorus insecticides (OPI) have been the largest class of insecticides used and investigated in the past 70 years. Their primary toxicity is due to their effect on the neural cholinergic system in both target and nontarget organisms. Because of OPI reactivity and structural diversity, some OPI may also produce toxic effects that do not originate from their interaction with the neural cholinergic system.

NAD-related teratogenicity induced by some OPI in chickens (reviewed in [1]) is an example of OPI toxicity not related to the disruption of the cholinergic system. Micromelia and abnormal feathering are the malformations formed in chicks following OPI administration into yolk of the fertile chicken eggs. Severity of those malformations correlates with a lowered embryo nicotinamide adenine dinucleotide (NAD$^+$) content [2,3]. The pyrimidinyl phosphorothioate and crotonamide phosphate insecticides are the most potent OPI in inducing NAD-related teratogenesis.

N-formyl-L-kynurenine formamidase (KFase) [EC. 3.5.1.9] of chicken embryo yolk sac membranes is the proposed primary target for OPI teratogens in NAD-associated teratogenesis [4]. OPI inhibition of KFase, the second enzyme in the L-tryptophan to NAD$^+$ biosynthetic pathway, would disrupt a flow of the metabolites through the L-tryptophan to NAD$^+$ pathway and result in a reduced formation of NAD$^+$. KFase inhibition correlates with both OPI teratogenicity and the reduction in embryonic NAD$^+$ content. The structural requirements for chicken embryo yolk sac membrane KFase inhibition by OPI were defined recently [5]. The comparative molecular field analysis (COMFA) of three-dimensional quantitative structure–activity relationship (3D QSAR) revealed the electrostatic and steric fields as good predictors of OPI inhibitory potency. It also identified structural elements that made pyrimidinyl phosphorothioate and crotonamide phosphate teratogens most potent KFase inhibitors.

In this project, we searched for more evidence that would further support our proposal [4] about the cause-and-effect relationship between OPI inhibition of chicken embryo yolk sac membrane KFase and the reduced embryo NAD$^+$ content. By applying the COMFA of 3D QSAR on the data obtained in our previous studies [2–4], we examined structural features that make pyrimidinyl phosphorothioates and crotonamide phosphates most effective compounds in lowering embryonic NAD$^+$ levels. The conclusions of the COMFA were then compared to those obtained for OPI inhibition of yolk sac membrane KFase [5].

Abbreviations: COMFA, comparative molecular field analysis; 3D QSAR, three-dimensional quantitative structure–activity relationship; ED_{50}, a dose of an OPI injected into the yolk of a fertile chicken egg causing a 50% reduction of the embryo NAD$^+$ content; KFase, N-formyl-L-kynurenine formamidase; NAD$^+$, nicotinamide adenine dinucleotide; OPI, organophosphorus insecticide(s).

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Table 1
The measured and COMFA-predicted OPI plnED$_{50}$'s for reducing embryo NAD$^+$ content.

	$R_1R_2P(=O$ or $=S)–O$ (or S)–X			plnED$_{50}$	
OPI	P = O (P = S)	R_1/R_2	–O–X or S–X	Measured	predicted[a]
Coumaphos	P = S	C_2H_5O/C_2H_5O	O-3-chloro-4-methyl-2-oxo-2 H-chromen-7-yl	11.42	11.42
diazinon	P = S	C_2H_5O/C_2H_5O	O-2-isopropyl-6-methyl-4-pyrimidinyl	13.95	14.04
					10.93[b]
dichlorvos	P = O	CH_3O/CH_3O	O-2,2-dichlorovinyl	8.83	8.93
Dicrotophos	P = O	CH_3O/CH_3O	O-1[E]-methyl-3- (dimethylamino)-3-oxo-1-propenyl	14.11	14.05
					10.67[c]
Dimethoate	P = S	CH_3O/CH_3O	S-(2-ethylamino)-2-oxoethyl	10.44	10.41
Ethylpirimiphos	P = S	C_2H_5O/C_2H_5O	O-2-(diethylamino)-6-methyl-4-pyrimidinyl	13.76	13.64
Etrimfos	P = S	CH_3O/CH_3O	O-2-ethyl-6-ethoxy-4-pyrimidinyl	13.85	13.86
Leptophos	P = S	phenyl/CH_3O	O-4-bromo-2,5- dichlorophenyl	8.33	8.34
					10.44[d]
Malathion	P = S	CH_3O/CH_3O	S-[1,2-bis(ethoxy- carbonyl) ethyl	10.81	10.79
Methamidophos	P = O	CH_3O/CH_3S	Amino	11.74	11.69
Methylchlorpyri-phos	P = S	CH_3O/CH_3O	O-3,5,6-trichloro-2-pyridinyl	8.09	8.00
Methylparathion	P = S	CH_3O/CH_3O	O-4-nitrophenyl	11.27	11.36
Methylpirimiphos	P = S	CH_3O/CH_3O	O-2-(diethylamino)-6-methyl-4-pyrimidinyl	13.48	13.44
MEVINPHOS	P = O	CH_3O/CH_3O	O-1[E]-methyl-3- methoxy-3-oxo-1-propenyl	10.33	10.36
Monocrotophos	P = O	CH_3O/CH_3O	O-1[E]-methyl-3- (methylamino)-3-oxo-1-propenyl	13.70	13.85
Parathion	P = S	C_2H_5O/C_2H_5O	O-4-nitrophenyl	11.69	11.61
Phorate	P = S	C_2H_5O/C_2H_5O	S-ethylthiomethyl	11.08	11.14
Phosphamidon	P = O	C_2H_5O/C_2H_5O	O-1[Z]-methyl-2-chloro-3-(diethylamino)-3-oxo-1- propenyl	12.82	12.76

[a] Correlation coefficient r^2 0.999; X — a leaving group.
[b] the torsion angle of a pyrimidinyl/P-O- fragments in diazinon modified to that of 4-nitrophenyl/P-O- fragment in parathion.
[c] Z-isomer (for KFase inhibition, plnED$_{50}$ for E-isomer was 13.68 and for Z-isomer 11.68).
[d] S-enantiomer (for KFase inhibition, plnED$_{50}$ for R-enantiomer was 11.56 and 12.23 for S-enantiomer).

2. Materials and methods

2.1. COMFA of 3D QSAR for OPI and their ability to lower chicken embryo NAD$^+$ content

OPI potency to reduce chicken embryo NAD$^+$ content was expressed as a negative logarithm (plnED$_{50}$) of the ED$_{50}$'s (a dose of an OPI injected into the yolk of a fertile chicken egg causing a 50% reduction of the embryo NAD$^+$ content). ED$_{50}$'s of 18 OPI (the structures listed in Table 1) were calculated from the data obtained in [2-4] by using a formula $ED_{50} = [\%NAD^+ / (100 - \%NAD^+)] \times I$ (where $\underline{NAD}^+$ is the embryo NAD$^+$ content after egg treatment with a dose $\underline{I}$ of an OPI) [6].

The QSAR module in Sybyl 7.3 molecular modeling program package (Tripos Associates, St. Louis, MO, USA) was used for COMFA and other associated computations as reported in our previous study [6]. OPI conformers at their lowest minimal global energy were aligned manually on a common OPI substructure >P(=O or =S)-O- and superimposed on diazinon as a template molecule. The COMFA used the electrostatic and steric fields with the spacing grid 0.2 nm and sp3 hybridized carbon atom with a charge +1 as a probe atom. The data matrix was analyzed

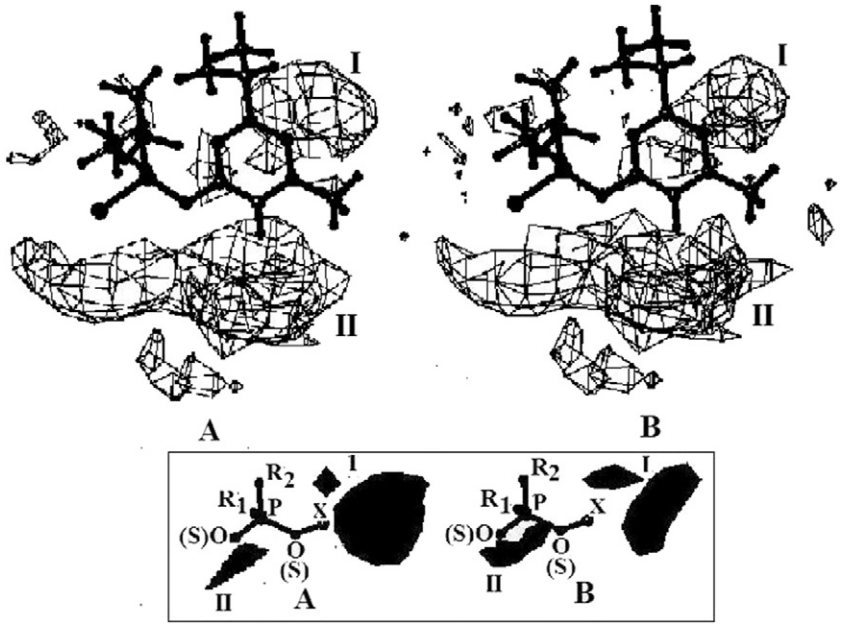

Fig. 1. The COMFA electrostatic contour plots for reducing embryo NAD$^+$ content and inhibition of yolk sac membrane KFase by OPI. The contour plots with inserted diazinon for reducing embryo NAD$^+$ content (A) and KFase inhibition (B) are shown at 20% (I) and 80% (II) of the overall COMFA field range. Polyhedrals I represent a region where an increased electronegativity would likely promote OPI reducing/inhibitory potency. Polyhedrals II represent a region where an increased electropositivity would likely promote OPI reducing/inhibitory potency. Inset are the electrostatic contour plots for a general OPI structure showing regions where the electrostatic fields would have the greatest effects. The contour plots were obtained at −0.020 (I) and 0.009 (II) kcal/mol levels for reducing embryo NAD$^+$ content (A) and at −0.009 (I) and +0.006 (II) kcal/mol for KFase inhibition (B). R_1, R_2, X (a leaving group) in OPI formula are defined in Table 1.