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Organophosphorus compound esterase profiles as predictors of therapeutic and toxic effects

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ABSTRACT

Certain organophosphorus compounds (OPCs) inhibit various serine esterases (EOHs) via phosphorylation of their active site serines. We focused on 4 EOHs of particular toxicological interest: acetylcholinesterase (AChE: acute neurotoxicity; cognition enhancement), butyrylcholinesterase (BChE: inhibition of drug metabolism and/or stoichiometric scavenging of EOH inhibitors; cognition enhancement), carboxylesterase (CaE: inhibition of drug metabolism and/or stoichiometric scavenging of EOH inhibitors), and neuropathy target esterase (NTE: delayed neurotoxicity, OPIDN). The relative degree of inhibition of these EOHs constitutes the "esterase profile" of an OPC and serves as a major determinant of its net physiological effects. Thus, understanding and controlling the esterase profile of OPC activity and selectivity toward these 4 target enzymes is a significant undertaking. In the present study, we analyzed the inhibitor properties of 52 OPCs against the 4 EOHs, along with pairwise and multitarget selectivities between them, using 2 QSAR approaches: Hansch modeling and Molecular Field Topology Analysis (MFTA). The general formula of the OPCs was $(RO)_2P(O)X$, where R = alkyl, X = – SCH(Hal)COOEt (Hal = Cl, Br), $-SCHCl_2$, $-SCH_2Br$, $-OCH(CF_3)R^1$ ($R^1 = C_6H_5$, CF_3, COOEt, COOMe). The Hansch model showed that increasing neuropathic potential correlated with rising R hydrophobicity; moreover, OPC binding to scavenger EOHs (BChE and CaE) had different effects on potential acute and delayed neurotoxicity. Predicted protective roles of BChE and CaE against acute toxicity were enhanced with increasing hydrophobicity, but projected protection against OPIDN was decreased. Next, Molecular Field Topology Analysis (MFTA) models were built, considering atomic descriptors, e.g., effective charge, van der Waals radius of environment, and group lipophilicity. Activity/selectivity maps confirmed predictions from Hansch models and revealed other structural factors affecting activity and selectivity. Virtual screening based on multitarget selectivity MFTA models was used to design libraries of OPCs with favorable esterase profiles for potential application as selective inhibitors of CaE without untoward side effects.

1. Introduction

Certain organophosphorus compounds (OPCs) can inhibit various serine esterases (EOHs) via organophosphorylation of serine residues in their active sites. Varying degrees of adverse or therapeutic effects arise from OPC exposure depending in part on their relative inhibitory potencies against EOHs of interest – the "esterase profile" [1–4].

We studied inhibitory characteristics of OPCs against a panel of 4 EOHs whose inhibition is linked to acute neurotoxicity (acetylcholinesterase, AChE, EC 3.1.1.7) [5], delayed neurotoxicity (neuropathy target esterase, NTE, 3.1.1.5) [6,7], and drug metabolism or stoichiometric scavenging of EOH inhibitors (butyrylcholinesterase, BChE, EC 3.1.1.8; and carboxylesterase, CaE, EC 3.1.1.1) [8–10]. Inhibition of AChE and/or BChE can also exert a therapeutic effect of cognition enhancement in Alzheimer's disease [11,12]. Clearly, the particular pattern of inhibition of these 4 targets plays an important role in shaping the pharmacodynamics and pharmacokinetics of a given OPC, thereby serving as a determinant of its overall physiological influences. Accordingly, analysis of the ester-

Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; CaE, carboxylesterase; EOH(s), serine esterase(s); MFTA, Molecular Field Topology Analysis; NTE, neuropathy target esterase; OPC(s), organophosphorus compound(s); OPIDN, organophosphorus compound-induced delayed neurotoxicity; PLS, partial least squares; QSAR, quantitative structure–activity relationships.

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ase profiles of OPCs using modern QSAR methods enables us to determine the contribution of different structural elements to their potential biological effects [2–4,13,14].

The following parameters describing inhibitory activity and selectivity of the OPCs were used in our esterase profile analysis. A parameter related to inhibitory activity toward the 4 EOHs was defined as follows: $A_X = \log k_i (E_X)$, where A_X is the activity toward the enzyme E_X. These activities were named A_A for AChE, A_B for BChE, A_N for NTE and A_C for CaE. Pairwise inhibitor selectivity of an OPC between two enzymes was defined with a parameter as follows: $S_{XY} = \log k_i (E_X) - \log k_i (E_Y)$. In this way 6 selectivity parameters were defined: S_{NA} for NTE/AChE, S_{BA} for BChE/AChE, S_{CA} for CaE/AChE, S_{BN} for BuChE/NTE, S_{CN} for CaE/NTE, and S_{CB} for CaE/BChE.

Thus, we had 4 indicators of activity and 6 of selectivity for a total of 10 endpoints. Each of these parameters is based on the bimolecular rate constant(s) of inhibition (k_i) of a given OPC toward the enzyme(s) of interest, and each is important for certain aspects of the pharmacological and toxicological profile of the OPC [4]. For example, k_i(NTE)/k_i(AChE) represents the relative inhibitory potency of an OPC against targets for delayed neurotoxicity (NTE) and acute neurotoxicity (AChE). This ratio correlates with that between the LD_{50} and the neuropathic dose, thereby serving as an index of the neuropathic potential of an OPC that is subject to aging [6,15,16]. Likewise, k_i(BChE)/k_i(AChE) and k_i(CaE)/k_i(AChE) reflect the potential contributions of BChE and CaE to the attenuation of acute toxicity via stoichiometric scavenging. Similarly, k_i(BChE)/k_i(NTE) and k_i(CaE)/k_i(NTE) represent the contributions of BChE and CaE to the potential mitigation of delayed neurotoxicity [2]. Finally, k_i(CaE)/k_i(BChE) characterizes the inter-scavenger selectivity of an OPC.

In the present work, inhibitor properties of 52 OPCs – *O*,*O*-dialkylphosphates of general formula $(RO)_2P(O)X$ (Fig. 1) – against the 4 EOHs of interest, along with pairwise and multitarget selectivities between them, were analyzed using two QSAR approaches: (1) Hansch's analysis in certain homologous series; and (2) Molecular Field Topology Analysis (MFTA). In addition, because CaE inhibition can result in reducing hydrolytic metabolism of many therapeutically important drugs [10,17,18], we applied MFTA to design a library of inhibitors as potential modulators of the pharmacokinetics of drugs containing ester or amide bonds.

2. Methods

2.1. Kinetic data on EOH inhibition

The bimolecular rate constants of inhibition (k_i) of the OPCs were determined using human erythrocyte (RBC) AChE, equine serum BChE, and porcine liver CaE (Sigma, St. Louis, MO), as well as avian (female Gallus domesticus) brain NTE (prepared in our laboratory as described previously [19]). The use of enzymes from different tissues and species is a potential limitation of the study. However, regarding tissues, it is known that the catalytic domain of human AChE is a single gene product that is identical in RBCs and brain AChE [20], BChE is a single gene product secreted into the serum from the liver [21], hen brain is commonly used as the source for NTE [22], and CaE, considered as liver carboxylesterase 1 (CES1), is produced in the liver, which may export it to plasma in many animal species [23], but not in humans [24]. With respect to species, protein sequence identities compared to the human sequence (determined using NCBI protein BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) were human AChE, 100%; horse BChE, 90%; porcine CaE, 77%; and hen NTE, 63%. Despite the species differences, QSAR predictions based on these inhibition constants have been confirmed in other studies using enzymes from the same species [15,16,25–28].

Detailed descriptions of the inhibition kinetics have been presented elsewhere [29]. In brief, enzyme samples were incubated for different time intervals with an inhibitor such that $[I]_o >> [E]_o$, and the residual enzymatic activity was determined. The k_i values were calculated according to [30] by linear regression using OriginPro 6.1 software and published earlier along with details of the synthesis and chemical characterization of the OPCs [2,3,31–37].

2.2. QSAR modeling using Hansch's approach

The relationship between structure of the OPCs and their inhibitor selectivity was analyzed by polynomial regression analysis using Origin 6.1 software, OriginLab Corp., (Northampton, MA, USA). Hansch constants for hydrophobicity of substituents, π, were used additively to yield values of $\sum\pi$ for the R-groups in the OPCs [38], and QSAR models for inhibitor selectivity of OPCs were developed. The significance of the equations obtained for N data points was estimated with values of R (correlation coefficient), S (standard deviation of the fit), and P (probability that R^2 is zero).

2.3. QSAR modeling using Molecular Field Topology Analysis (MFTA)

The bioactivity model in MFTA was constructed from values of local molecular descriptors (e.g., atomic properties) [39,40]. Two-dimensional structures of compounds in the training set (structural formulas) were topologically superimposed to construct a molecular supergraph to provide a common frame of reference for meaningful comparison and analysis of local properties in different structures. In addition to the predictive model, constructed

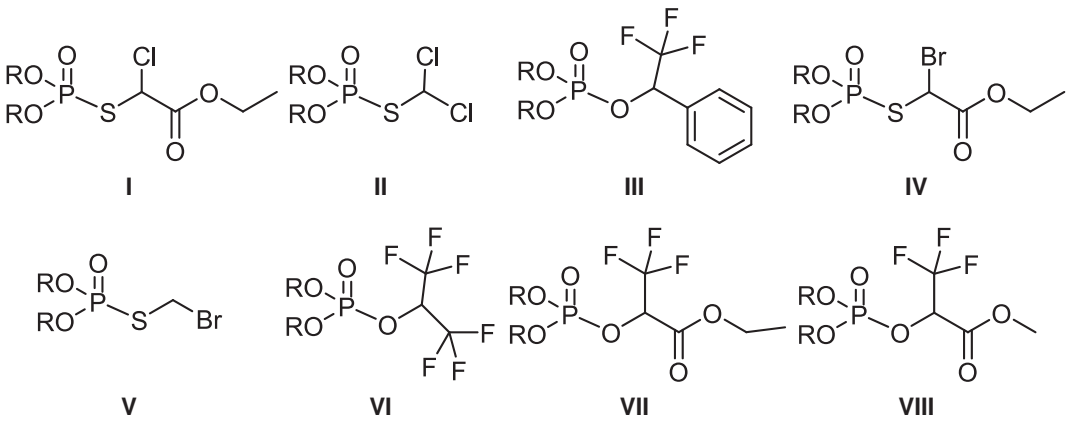

Fig. 1. General structures (**I**–**VIII**) of the OPCs in this study.