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Esterase profiles of organophosphorus compounds *in vitro* predict their behavior *in vivo*

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ABSTRACT

We studied 4 serine esterases (EOHs) that are associated with the following consequences from their inhibition by organophosphorus compounds (OPCs): 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, which we hypothesize can serve as a predictor of its overall physiological effects. To test this hypothesis, we selected 3 OPCs known from previous work on reference enzymes to span a wide range of esterase profiles, neuropathic potential, and acute cholinergic toxicity. For each compound, we determined *in vitro* IC_{50} and *in vivo* ED_{50} values for inhibition of AChE, BChE, CaE, and NTE in mouse brain and blood. The results showed good correlations between *in vitro* and *in vivo* measures of potency and selectivity except for brain CaE, a tissue-specific isoform of the enzyme that was less sensitive to the test compounds than expected. Thus, this synthesis of new and previously published results indicates that the concept of the esterase profile of OPCs is useful for the prediction of therapeutic and toxic effects *in vivo*.

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 selectivities against EOHs of interest – the "esterase profile" of a given OPC [1–4].

We chose to study a panel of 4 EOHs whose inhibition is involved in acute neurotoxicity (acetylcholinesterase, AChE, EC 3.1.1.7) [5], delayed neurotoxicity (neuropathy target esterase, NTE, 3.1.1.5, target of OPC-induced delayed neuropathy, OPIDN) [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 (AD) [11,12].

Analysis of esterase profiles helps to identify the main potential pharmacological effect of the compound and its possible side and toxic effects [2–4,13]. For example, effective inhibitors of AChE and BChE can be used for AD treatment. However, inhibition of CaE by such anticholinesterase compounds leads to adverse drug-drug interactions [14] because CaE is known to hydrolyze numerous drugs or prodrugs containing ester, amide, and carbamate groups such as angiotensin-converting enzyme inhibitors, antiplatelet

Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; CaE, carboxylesterase; EOH(s), serine esterase(s); NTE, neuropathy target esterase; OPC(s), organophosphorus compound(s); RIP(s), relative inhibitory potency/potencies; OPIDN, organophosphorus compound-induced delayed neurotoxicity; AD, Alzheimer's disease; diEt-PFP, *O,O*-diethyl-*O*-(1-trifluoromethyl-2,2,2-trifluoroethyl) phosphate; diBu-PFP, *O,O*-dibutyl-*O*-(1-trifluoromethyl-2,2,2-trifluoroethyl) phosphate; PrDChVP, *O,O*-di-1-propyl-*O*-2,2-dichlorvinyl phosphate.

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drugs, statins, antivirals, anti-asthmatic agents, central nervous system drugs, and anticancer agents [15]. On the other hand, potent and selective inhibitors of CaE can be used for the regulation of metabolism and pharmacokinetics of ester-containing drugs [10,16]. For such CaE inhibitors, anticholinesterase activity can be an undesirable side effect.

Inhibitory potency of progressive (time-dependent) inhibition of an OPC against an EOH is best assessed kinetically by the bimolecular rate constant of inhibition, k_i. Alternatively, potency can be expressed as the less-preferred fixed-time IC_{50} (inhibitor concentration required to inhibit 50% of the enzyme's activity after a fixed time of incubation (t) of the OPC with the EOH). When pseudo-first-order kinetics of inhibition are obtained, it is valid to calculate the IC_{50} from the k_i via the equation, $IC_{50} \approx 0.693/(k_i \times t)$.

In addition to the inhibitory potency of an OPC against a single EOH, it is useful to determine the inhibitory selectivity of an OPC in relation to different EOH targets [3,4,13]. These selectivities consist of ratios of inhibitory potencies of a given OPC against a pair of EOHs. For example, as defined here, k_i(NTE)/k_i(AChE) or IC_{50}(AChE)/IC_{50}(NTE) represents the relative inhibitory potency (RIP) 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 a convenient index of the neuropathic potential of an OPC that is subject to undergoing the aging reaction [6,17–20]. An OPC is neuropathic when the RIP >1 and is predominantly cholinergic when the RIP <1 [6,7,17,18,21]. 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 [3,4,13]. Selectivity against BChE compared to AChE is also an important characteristic of the pharmacological profile of the anticholinesterase compound as a potential anti-AD agent [22–24]. Finally, the selectivity ratio k_i(CaE)/k_i(BChE) characterizes probable adverse drug-drug interactions [14]. For a given OPC and 4 target enzymes, there would be 4 measures of inhibitory potency (either the IC_{50} or the k_i against each of the 4 EOHs) and 6 unique ratios of inhibitory potencies (eliminating reciprocals and ratios for the same EOH) [3,13]. This collection of 4 measures of inhibitory potency and 6 unique ratios (selectivities) constitutes the complete esterase profile.

The esterase profile has served as a convenient approach for quantitative analysis of structure-inhibitory activity and structure-selectivity of anticholinesterase compounds, investigation of structural determinants of inhibitory activity/selectivity in several series of OPCs, and performing molecular design of compounds with a desirable esterase profile – for example, selective inhibitors of BChE or CaE without unwanted side effects [2–4,13,25–27]. However, successful application of the esterase profile is based on the assumption that the particular pattern of inhibition of the 4 selected EOH targets plays an important role in shaping the pharmacodynamics and pharmacokinetics of a given OPC, thereby serving as a determinant of its overall physiological effects.

With the foregoing in mind, we carried out the studies presented in the present paper to test the hypothesis that the esterase profile of an OPC determined *in vitro* serves as a major determinant of its biological effects *in vivo*. To do this, we selected 3 OPCs that span a wide range of esterase profiles, neuropathic potential, and acute cholinergic toxicity. The structures of the OP compounds are shown in Fig. 1: *O*,*O*-diethyl-*O*-(1-trifluoromethyl-2,2,2-trifluoroethyl) phosphate (diEt-PFP), *O*,*O*-dibutyl-*O*-(1-trifluoromethyl-2,2,2-trifluoroethyl) phosphate (diBu-PFP) and *O*,*O*-di-1-propyl-*O*-2,2-dichlorvinyl phosphate (PrDChVP).

Regarding our choice of animal model, we recognize that the most widely accepted *in vivo* model for the study of OPIDN and assessment of the neuropathic potential of OP compounds has been the adult hen [28]. Moreover, standard laboratory rodents, mice and rats, have been thought to be resistant to OPIDN, because they do not readily display clinical signs of hind limb paralysis despite the administration of high doses of neuropathic compounds [29,30]. However, in our recent work we demonstrated that mouse brain and blood NTE can be used as biochemical markers of neuropathic OPC exposure [31,32]. Given these findings, and in view of the convenience afforded by the use of a mouse model, we used this species to investigate the relationship between the esterase profile of the OPCs *in vitro* and their biological effects *in vivo*.

For each of the selected OPCs, we studied inhibition of 4 EOHs in mouse brain and blood preparations *in vitro* and *in vivo* 1 h after i.p. dosing of mice with the compounds. The results were compared with esterase profiles of the OPCs determined *in vitro* on a set of reference EOHs including human erythrocyte AChE, equine serum BChE, and porcine liver CaE from commercial sources along with hen brain NTE prepared in our laboratory. Portions of the data presented here have been published previously; in particular, inhibition of mouse brain NTE and AChE for all 3 OPCs [31], inhibition of mouse blood NTE and AChE by PrDChVP and diBu-PFP [32], and inhibition of mouse brain EOHs by diEt-PFP and diBu-PFP [33]. Here, we present a synthesis of new and previously published data with the goal of illustrating the utility of the esterase profile concept for predicting the biological effects of OPCs.

2. Material and methods

2.1. Chemicals and enzymes

2.1.1. Chemicals

Acetylthiocholine iodide, ethopropazine hydrochloride; 5,5′-dithiobis(2-nitrobenzoic acid), (4-NPA), (1-NA), paraoxon (*O*,*O*-diethyl-4-nitrophenyl phosphate) and protein standard (BSA) were purchased from Sigma-Aldrich Co. (Steinheim, Germany). Phenyl valerate (PV), *N*,*N*′-di-2-propylphosphorodiamidofluoridate (mipafox, MIP), *O*,*O*-di-1-propyl-*O*-2,2-dichlorovinyl phosphate (PrDChVP), *O*,*O*-diethyl-O-(1-trifluoromethyl-2,2,2-trifluoroethyl) phosphate (diEt-PFP) and *O*,*O*-dibutyl-*O*-(1-trifluoromethyl-2,2,2-trifluoroethyl) phosphate (diBu-PFP) were synthesized and characterized in the Institute of Physiologically Active Compounds, Russian Academy of Sciences (IPAC RAS, Chernogolovka, Russia). Synthesis of diEt-PFP and diBu-PFP is described in Refs. [2,34]. The purity of all substances produced by IPAC RAS was >99% (by spectral and chromatographic analysis data). All other chemicals were analytical grade and used without further purification. Aqueous solutions were prepared using deionized water.

2.1.2. Enzymes

The following enzymes were purchased from Sigma-Aldrich Co. (Steinheim, Germany): acetylcholinesterase (AChE, EC 3.1.1.7, from human erythrocyte (RBC), C0663 Sigma), butyrylcholinesterase (BChE, EC 3.1.1.8, from equine serum, C4290 Sigma), and carboxylesterase (CaE, EC 3.1.1.1, from porcine liver, E2884 Sigma). Neuropathy target esterase (NTE, EC 3.1.1.5) was a stable lyophilized paraoxon-pretreated membrane fraction ($P_2 + P_3$) prepared from hen brain homogenates as previously described in detail [35]. The lyophilized NTE preparation was stored about two months before use.

2.2. EOHs activity and inhibition kinetics for esterase profile

The bimolecular rate constants of inhibition (k_i) of the OPCs that characterize their esterase profile were determined using the