

TOXICOLOGICAL HIGHLIGHT

Organophosphates, Serine Esterase Inhibition, and Modeling of Organophosphate Toxicity

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The highlighted article in this issue (Ashani and Pistinner, "Estimation of the Upper Limit of Human Butyrylcholinesterase Dose Required for Protection against Organophosphates Toxicity: A Mathematically Based Toxicokinetic Model") is an innovative approach to modeling the amount of protective enzyme, human butyrylcholinesterase, that could be administered to humans to protect them from the lethal effects of organophosphate nerve agents. The threat of nerve agent exposures at lethal levels regrettably remains a threat to military as well as civilian populations, and the authors of this article have used their previous experimental data along with new *in vitro* data to devise and calibrate a mathematical model that could have practical utility in the prophylaxis of military personnel against chemical warfare agents.

Organophosphorus compounds are frequently called organophosphates for convenience even though the term is sometimes chemically incorrect; these compounds have been known since World War II as neurotoxic agents with potential use as chemical warfare agents and agents of chemical terrorism. These chemicals, the most widely known being sarin, soman, tabun, and VX, are potent inhibitors of serine esterases, the most critical of which is the widely distributed nervous system enzyme acetylcholinesterase (AChE). AChE is involved in the rapid hydrolysis of the neurotransmitter acetylcholine, a neurotransmitter involved in the very numerous cholinergic pathways in the body, i.e., the central nervous system as well as several aspects of the peripheral nervous system: the somatic nervous system innervating skeletal muscles and both the sympathetic and the parasympathetic divisions of the autonomic nervous system. Inhibition of AChE leads to the accumulation of acetylcholine in synapses and neuromuscular junctions in these cholinergic pathways, leading to hyperstimulation of these ubiquitous cholinergic pathways. Quite obviously, then, impact on these cholinergic pathways will cause widespread

and potentially life-threatening signs. Some of the most obvious signs in a severe poisoning by an anticholinesterase are the signs associated with the clinical syndrome termed SLUD (i.e., salivation, lacrimation, urination, and defecation) arising from impacts on the autonomic nervous system, tremors arising from the impact on the somatic nervous system, and potentially convulsions from impacts on the central nervous system. Death is usually attributed to respiratory failure resulting from a combination of central and peripheral effects, specifically bronchiolar constriction, enhanced bronchiolar secretions, paralysis of the respiratory muscles, and shut-down of the respiratory control center in the brain. Because of the severity of these effects, the critical importance of these target systems to life, and the rapidity with which many of these agents act, incapacitation and death can occur quickly, and perhaps too quickly to depend upon the administration of therapeutic antidotes for survival. A typical antidote is the acetylcholine receptor agonist atropine (which has the potential to antagonize the action of the excess acetylcholine at muscarinic acetylcholine receptors, the receptors that are most critical to maintaining normal respiration). In addition, there is an oxime reactivator (pralidoxime or 2-PAM) approved for clinical use in poisonings with the insecticidal organophosphates that facilitates the reactivation of phosphorylated AChE back to normal activity; however, 2-PAM is not an effective reactivator of AChE inhibited with the nerve agents. For reviews of organophosphate chemistry, toxicity, biochemical considerations, and therapy, see Chambers and Levi, 1992; Ecobichon, 2001; Eto, 1961; and Taylor, 2001.

The anticholinesterase organophosphates are pentavalent, tetracoordinate compounds having three singly bonded substituents (often but not always organic) and an oxygen atom, in the case of the nerve agents, coordinately bonded to the central phosphorus atom. These organophosphate compounds are inhibitors of serine esterases and serine proteases, and have a high affinity for AChE. The group among the singly bonded substituents that is connected to the phosphorus by the least stable bond is considered the "leaving group," and it is the labile group eliminated during the reaction in which the organophosphate creates a covalent bond with the catalytic serine

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group of the esterase or protease. Thus during this phosphorylation reaction, the organophosphate molecule is destroyed through stoichiometric reaction with the enzyme. The inhibition from organophosphates is persistent, lasting hours to days, and potentially may not be reversible if a nonenzymatically mediated dealkylation, termed "aging," occurs. The phosphorylated aged AChE is refractory to reactivation.

The chemical technology of the Second World War that developed these nerve agents also allowed the development of the insecticidal organophosphorus compounds, but these latter compounds are generally either weaker anticholinesterases or they require bioactivation (usually mediated by cytochromes P450) to the active anticholinesterase metabolites. Therefore the insecticidal organophosphates are less toxic than the nerve agents because they are weaker and/or they require time for bioactivation, thereby allowing the body to utilize its defenses more effectively. The rapidity of action for the nerve agents, which do not require bioactivation, does not allow efficient protection from the endogenous protective mechanisms.

One such protective biochemical mechanism arises from the family of nontarget serine esterases, which include the carboxylesterases, nontarget AChE (such as occurs in erythrocytes), and butyrylcholinesterase. These protective esterases apparently do not have critical life-sustaining functions; elimination of their activities has little, if any, negative impact on physiology and health. These protective esterases are frequently highly sensitive to inhibition by organophosphates, sometimes even more sensitive than the target AChE, suggesting that they will be inhibited at lower organophosphate concentrations than AChE would be. Because of their sensitivity and their apparently rather inconsequential natural function, inhibition of these esterases serves as protection because the event of phosphorylation of the active site serine destroys the organophosphate molecule, thus accomplishing a stoichiometric detoxication. Since the inhibition is persistent, this detoxication is noncatalytic. Therefore the amount of protection that the nontarget esterases can afford is obviously limited by the number of nontarget esterase molecules present in accessible locations.

A suggested strategy for affording a vulnerable military person with protection from nerve agent intoxication is the prophylactic administration of one of the protective esterases; the exogenous nontarget esterases could be phosphorylated prior to the nerve agent reaching neural (target) AChE, thus stoichiometrically destroying a fraction of the absorbed nerve agent and making that fraction unavailable for inhibiting AChE. As indicated above, the degree of protection will be limited by the number of available active sites on the exogenous protective esterases. Therefore it is critical to the success of such a military strategy to be able to predict the amount of esterase to administer in order to provide the individual with a sufficient number of inhibitable active site serines. One very likely serine esterase to be utilized in this prophylactic strategy would be human butyrylcholinesterase, which has been produced in purified form. To be practical, a sufficient amount of enzyme would need to be administered to allow survival of

lethal doses, but caution would need to be exercised to avoid excessive amounts to prevent any untoward physiological reactions such as potential immunological effects.

Drs. Ashani and Pistinner, in the highlighted article, have built upon information and/or modeling approaches that they and others have previously developed to create a mathematical model capable of predicting the amount of purified human butyrylcholinesterase that would need to be administered in order to allow survival of a hypothetical individual poisoned with various amounts of soman, sarin, or VX. The experimental data upon which the model is built were derived from straightforward *in vitro* methods of AChE and butyrylcholinesterase inhibition and calculation of bimolecular rate constants. The model developed has two parts. The first part is a first order model predicting the exponential decay in the concentration of the exogenously administered butyrylcholinesterase. The second part is a mass action model with a source for the simultaneous interactions between the organophosphate and butyrylcholinesterase and also the organophosphate and erythrocyte AChE (which is viewed as a surrogate for the neural AChE). The model is cleverly used to predict the minimum required dose of exogenous butyrylcholinesterase required to effect prophylaxis against a specified dose of the organophosphate. The goal of this model as presented in the highlighted article was to estimate the protection of erythrocyte AChE above 30% inhibition following a challenge of one LD₅₀ in which the predicted exogenous butyrylcholinesterase would reduce the level of nerve agent by half within 10 s; the authors assume that such prophylaxis would protect the individual from severe toxic signs. The model allows predictions on individual compounds, and will also allow prediction for individual humans who have different levels of butyrylcholinesterase because of specific polymorphisms. Such modeling is clearly an important step forward in allowing prediction, based on empirical data using the bimolecular rate constants for the appropriate target and protective enzymes, of the amount of prophylactic enzyme to administer based on anticipated time and level of nerve agent exposure.

While a useful mathematical model is necessary for developing this strategy into an actual battlefield procedure, it should be borne in mind that those using it would need to be able to anticipate with some level of precision the amount of nerve agent that would be absorbed as well as the time of exposure (i.e., administration of the prophylaxis too early might render it ineffective by the time of exposure or too late might render the individual incapacitated before he/she receives the prophylaxis and distributes it through his/her circulation). Whether such precision in predicting military attacks is feasible is not within our realm of academic experience and the authors of this highlight will leave answering this question to military strategists. An additional caveat about the utility of this prophylactic strategy would be regarding multiple uses of the prophylaxis, which would probably be unwise. Even though the proposed enzyme is a human enzyme, there is still the possibility of immunological reactions to it, so repeated use of this enzyme in the same individual probably should not be considered.

The concept of using a protective esterase as a prophylactic strategy has some advantages over the strategy of using a more transient carbamate anticholinesterase, e.g., pyridostigmine bromide as was prescribed during the Persian Gulf conflict. The theory of using a carbamate is to transiently inhibit a select fraction of the AChE to “protect” it during an organophosphate nerve agent exposure; the phosphorylation is persistent whereas the carbamylation is transient, allowing the “protected” AChE to recover in a relatively short period of time after the organophosphate is eliminated from the body by normal clearance mechanisms. This strategy is clearly dangerous since the carbamate is a known toxic compound. A “one-size-fits-all” dose of the carbamate might leave smaller individuals more likely to be incapacitated at the time of the nerve agent attack and therefore unable to escape or to operate machinery; conversely, this standard dose might leave larger individuals insufficiently protected.

It might be tempting to think that this exogenous butyrylcholinesterase strategy would be helpful in accidental poisonings with the insecticidal organophosphates. However, as indicated above, many of the insecticidal organophosphates require bioactivation in order to appreciably inhibit AChE. This bioactivation delay coupled with the general weaker potency of the insecticidal organophosphates and the overall efficacy of 2-PAM therapy makes the typical clinical therapy of supportive therapy plus atropine/2-PAM administration sufficient for most accidental poisonings. In addition, the accidental poisonings cannot be anticipated in advance, since they are, indeed, accidental, and therefore any administration of protective esterases would necessarily be therapeutic, not prophylactic; any prophylactic administration would be scientifically unjustified.

Lastly, it is worthwhile to discuss the modeling approach. Clearly it will be impossible to conduct all of the laboratory experiments on toxicant effects and possible prophylaxis or therapy on all possibilities of compounds, dosages, time frames, and routes of administration, not only for nerve agents but for all toxicants. Mathematical modeling, when based on logical approaches and solid data, can help provide useful predictions on effects, preventatives, and remedies. Sometimes these models must be developed with an absence of some of the important data sets; when this occurs certain assumptions must be made from similar data or certain simplifications must be placed into the modeling. Drs. Ashani and Pistinner recognize that certain simplifications were necessary in their modeling. For example, they indicate that they did not know what the rate of diffusion of the organophosphates to the target cells would be, so they limited the use of their model at this time to a bolus administration only and not to continuous exposures. Another simplification was the use of erythrocyte AChE as a surrogate for neural AChE; while the inherent sensitivity of the erythrocyte and neural enzymes is probably the same, the membrane environment of the neural AChE might render its theoretical sensitivity to inhibition different than the erythrocyte enzyme, so the assumptions made in this model might

render it inaccurate. Nevertheless the model is potentially valuable in the estimation of doses of butyrylcholinesterase that may titrate specific absorbed doses of the nerve agents using the protection of erythrocyte AChE as an endpoint. The endpoint of easily measured cholinesterase inhibition does make the modeling of organophosphate-molecular interactions easier than the modeling of many toxicants that do not covalently bind to targets or that exert as-yet-undefined molecular actions. However, it should be borne in mind that survival alone may not be the ideal ultimate goal; high dose poisonings, even with the avoidance of severe signs of toxicity, may still induce long-term physiological consequences, as has been observed in the aftermath of high dose accidental poisonings with insecticidal organophosphates (Savage *et al.*, 1988). Ultimately the goal may be to also prevent long-term consequences of nerve agent exposure in addition to assurance of survival. The article's authors indicate that they intend to expand the model with greater complexity in the future, such as to a multicompartment model. The authors have constructed their model guided by the modeling of Sweeny and Maxwell (1999, 2003) of nerve agent interactions with endogenous and exogenous protective enzymes in rodents. The authors are also aware of physiologically based pharmacokinetic models of insecticidal organophosphorus compounds (Timchalk *et al.*, 2002). The authors of the highlighted article have expanded beyond these approaches to project to humans, and apparently plan on future modeling efforts being even more relevant to human exposure possibilities. While it is most regrettable that scientists must consider strategies to combat acts of warfare or terrorism, the development of predictive models such as that presented in the highlighted article may well lead to the saving of valuable human lives.

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