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ORIGINAL ARTICLE

K-OXIME (K-27): PHOSPHYLATION-INDUCED CHANGES IN LOG*P*

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Summary

Organophosphorus compounds (organophosphates and organophosphonates) exert their toxicity by phosphylating (i.e. either phosphorylating or phosphonylating) the serine hydroxyl group of the enzyme acetylcholinesterase (AChE) in its active center, thereby inhibiting this enzyme, which inactivates the neurotransmitter acetylcholine (ACh). This results in an accumulation of ACh and an "endogenous ACh poisoning".

Oximes, which can reactivate the inhibited enzyme by dephosphylation, are used in the therapy of organophosphorus compound poisoning. During the reactivation process, oximes become themselves phosphylated. Many of these phosphylated oximes are extremely potent AChE inhibitors, which may reduce their therapeutic efficacy.

K-27 is a very promising experimental oxime. In the present study, log*P* values of phosphylated K-27 are estimated after "in-silico exposure" to a number of organophosphorus esters [ethyl-paraoxon, methyl-paraoxon, diisopropyl-fluoro-phosphate, VX, soman, tabun, sarin, cyclosarin]. These log*P* values are compared with those of the native oxime and possible therapeutic relevance is discussed.

While our previously published data regarding obidoxime and pralidoxime show that phosphylation increases their lipophilicity, facilitating penetration into the brain where they can inhibit or re-inhibit enzymes, this conclusion does not hold with respect to K-27; phosphylation of K-27 does not generally increase lipophilicity. Possible consequences with regard to blood-brain-barrier passage, toxicity and therapeutic efficacy are discussed.

Key words: cholinesterase; organophosphorus esters; oxime, K-27; phosphorylation; phosphonylation; logP

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INTRODUCTION

Organophosphorus esters (organophosphates and organophosphonates) which are used as pesticides and parasiticides are amongst the most frequent causes of accidental and suicidal intoxications [1-3]. Their acute toxicity is due to the inhibition

of the enzyme acetylcholinesterase (AChE), which inactivates the neurotransmitter acetylcholine (ACh) at cholinergic synapses. Esterase inhibition results from phosphylation (i.e. either phosphorylation or phosphonylation) of the serine hydroxyl group in the active center of the enzyme and translates into an "endogenous acetylcholine poisoning". The therapy of poisoning with organophosphorus compounds, which can be memorized by the acronym **A FLOP** = **A**tropine, **FL**uids, **O**xygen, **P**ralidoxime [4], is generally disappointing.

The history of the synthesis of organophosphorus compounds and of their applications has recently been investigated [5-7]. One therapeutic option is reactivation of phosphylated AChE by pyridinium oximes [8]. Working at Columbia in the laboratory of David Nachmansohn, Wilson and Ginsburg synthesized a number of pyridine oximes, one of which was **pralidoxime**, the first aldoxime cholinesterase reactivator of clinical relevance. In Great Britain, Davies and Green independently performed similar research [9].

Pyridinium oximes reactivate phosphylated AChE by interacting with the anionic site of the enzyme. An optimal orientation of the reactivator at the catalytic site of the enzyme is facilitated by the pyridinium moiety, which thus increases efficacy [10, 11]. It is generally accepted that nerve gas exposure can be treated with oximes; however, the therapeutic value of oximes in human organophosphate pesticide poisoning is controversial [1, 12].

One possible reason for the disappointing efficacy may be the generation of phosphylated oximes during AChE reactivation [12-14]. When interpreting kinetic studies of the reactivation and aging of organophosphate-AChE conjugates, physico-chemical properties of phosphylated oximes therefore need to be considered [15]. The octanol-water partition coefficient [log*P*], a concept introduced over a century ago by Berthelot (according to ref. [16]) which is based on the partition of substances between oil and water, is often correlated with their biological activities [3, 17-19]. We have recently been able to demonstrate that phosphylation of two oximes in clinical use, pralidoxime and obidoxime, results in a significant reduction in the absolute value of log*P*, corresponding to an increase in lipophilicity [14]. K-27 is an experimental oxime with very promising *in vivo* and *in vitro* characteristics [20-27].

PURPOSE OF THE STUDY

To estimate log*P* values of phosphylated K-27 after "in-silico exposure" to a number of organo-phosphorus esters [ethyl-paraoxon, methyl-paraoxon, diisopropyl-fluoro-phosphate (DFP), VX, soman, tabun, sarin, cyclosarin], to compare them with the log*P* of the native oxime and with those of phosphylated pralidoxime and obidoxime and to discuss possible therapeutic relevance.

MATERIAL & METHOD

Chemical structures of all compounds were drawn using ChemDraw Ultra 12.0 (CambridgeSoft Software, PerkinElmer Inc. Waltham, Massachusetts). Log*P* values of organophosphorus esters, K-27 and phosphylated K-27 were estimated using the PrologP module of the Pallas 3413 software (CompuDrug Inc., Sedona, AZ, USA). Details of the algorithm used for calculations are given by [28]. The program takes into account all lipophilic and hydrophilic fragments of a specific compound and makes minor corrections based on octanol-water partition data, as available from the literature. The authors emphasize that their neural network-based method (pseudo-linear algorithms) combines the precision of non-linear approaches with the transparency of the early linear methods. The log*P* value of a substance is most relevant for neutral substances and is also useful as a general reference point to help compare overall hydrophobicity trends of compounds.

RESULTS

Table 1 lists the structures and log*P* values of organophosphorus pesticides (ethyl-paraoxon, methyl-paraoxon), DFP and nerve gases (sarin, cyclosarin, soman, tabun, VX). Log*P* values are lowest for tabun (-0.02) and highest for ethyl-paraoxon (2.18), implying that all compounds except tabun are lipophilic, reflected by their positive log*P* values.

The chemical formulas and log*P* values of K-27 phosphylated by the same organophosphorus compounds (ethyl-paraoxon, methyl-paraoxon, DFP, sarin, cyclosarin, soman, tabun, VX) are shown in table 2. Native K-27 has a log*P* value of -3.03 ± -0.39, reflecting its hydrophilicity. Phosphylation of K-27

barely influences hydrophilicity: log*P* values range from -3.48 ± -0.44 (slight increase in hydrophilicity after phosphylation by soman) to -2.38 ± -0.46 (decrease in hydrophilicity after phosphylation by cyclosarin). With the exception of K-27 phosphylated by cyclosarin, phosphylated K-27 derivatives are thus not less hydrophilic than unphosphylated K-27.

Table 1. Log*P* values of organophosphorus esters [phosphylating agents]. All compounds with the exception of tabun are lipophilic (positive log*P* values).

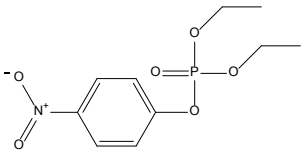
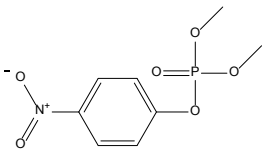
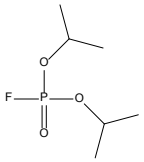
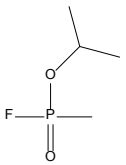
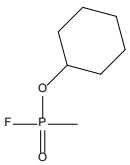
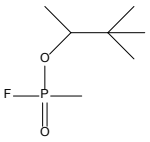
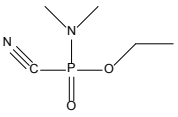
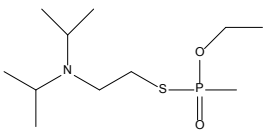
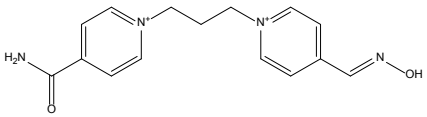
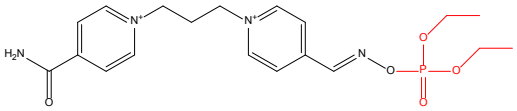
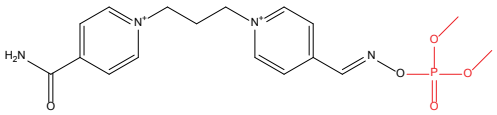
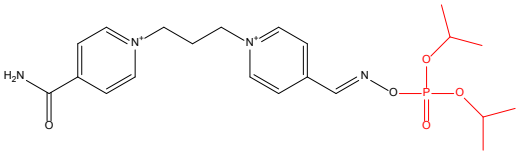
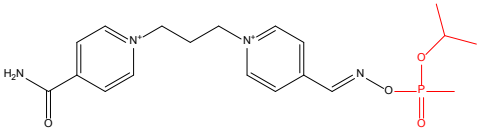
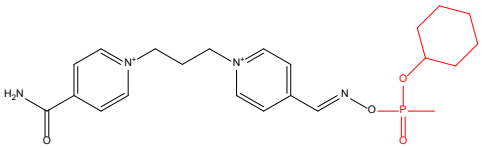
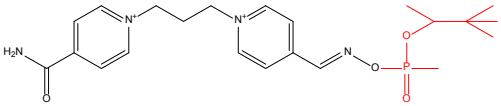
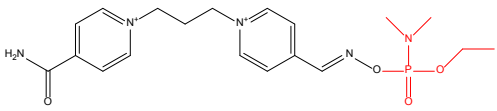
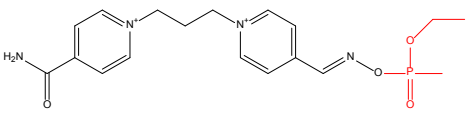
Ethyl-paraoxon		log*P* = 2.18
Methyl-paraoxon		log*P* = 1.51
Di-isopropyl-fluoro-phosphate		log*P* = 1.08
Sarin		log*P* = 0.84
Cyclosarin		log*P* = 1.78
Soman		log*P* = 1.89
Tabun		log*P* = -0.02
VX		log*P* = 2.10

Table 2. Log*P* values of K-27 and of phosphylated K-27. Phosphylation by cyclosarin is the only reaction somewhat reducing hydrophilicity.

K-oxime (K-27)		log*P* = -3.03 ± -0.39
Phosphylated Oxime	**Structure**	**logP phosphylated Oxime**
K-27 phosphylated by ethyl-paraoxon		log*P* = -3.16 ± -0.45 Δlog*P* = - 0.13
K-27 phosphylated by methyl-paraoxon		log*P* = -3.19 ± -0.45 Δlog*P* = - 0.16
K-27 phosphylated by di-isopropyl-fluoro-phosphate		log*P* = -3.10 ± -0.43 Δlog*P* = - 0.07
K-27 phosphylated by sarin		log*P* = -3.48 ± -0.44 Δlog*P* = - 0.45
K-27 phosphylated by cyclosarin		log*P* = -2.38 ± -0.46 Δlog*P* = 0.65
K-27 phosphylated by soman		log*P* = -3.11 ± -0.46 Δlog*P* = - 0.08
K-27 phosphylated by tabun		log*P* = -3.33 ± -0.51 Δlog*P* = - 0.30
K-27 phosphylated by VX		log*P* = -3.45 ± -0.45 Δlog*P* = -0.42

DISCUSSION

Although the action mechanism of oximes is relatively well characterized in theory, their practical value remains uncertain and oximes have disappointed clinically [1, 29]. Due to the presence of a positively charged nitrogen atom, oxime molecules are polar, have a negative log*P* and are hydrophilic. Since only small lipophilic compounds easily penetrate the blood brain barrier, oximes barely enter the brain [30]. The brain concentration of the monopyridinium aldoxime pralidoxime is only 10% of its blood concentration, and penetration of bis-pyridinium aldoximes, such as obidoxime and K-27 is even lower [31, 32].

The relationship between oxime efficacy and their entry into the brain is still a contentious issue. Is the restricted brain penetration of oximes the reason for their limited efficacy? Could superior efficacy be achieved by an increase in brain penetration? Apparently, the initial answer to these questions has been "yes", as demonstrated by the attempt to develop a less hydrophilic pro-drug of pralidoxime [33]. This dihydropyridine derivative of pralidoxime, pro-2-PAM, was supposed to enter the brain more easily, and 2-PAM brain levels were indeed considerably higher when using this brain penetrating pro-drug. However, overall results were disappointing [34], leading to the following conclusion in a recent review: "*Increasing the BBB penetration by oximes does not actually lead to significant benefits of survival rate, but certainly amplifies the neurotoxic risks*" [35].

After correlating the *in vivo* toxicity of various oximes with different *in vitro* parameters [3, 19], our own experimental studies indicate that a very negative log*P* (strong hydrophilicity) is a good predictor for low oxime toxicity (as assessed by survival). In addition, oximes with very negative log*P* (strong hydrophilicity) were significantly more efficacious in reducing DFP-induced mortality than more lipophilic ones. This suggests that limited brain penetration is actually desirable. One possible explanation for this unexpected conclusion is the formation of phosphylated oximes, which are generated by the reaction of oximes with organophosphorus-inhibited enzymes and which are highly toxic [13]. Phosphylation is the umbrella term used to describe phosphorylation (occurring during the reactivation of an enzyme inhibited by an organophosphate insecticide, such as paraoxon) or phosphonilation (occurring during the reactivation of an enzyme inhibited by an organophosphonate "nerve gas"). Organophosphates do not contain a direct phosphorus-carbon link, while organo-phosphonates do contain one. Phosphylated oximes are themselves potent inhibitors of AChE, sometimes much more potent than the initial offending organophosphate or organophosphonate, which generally translates into very high toxicity.

Log*P*: Pyridinium oximes are hydrophilic compounds (large negative value of log*P*) with very limited CNS penetration [30-32]. We have previously been able to demonstrate that phosphylation of two established oximes, obidoxime and pralidoxime, results in a significant decrease in the absolute log*P* value, corresponding to a reduction in hydrophilicity, i.e. an increase in lipophilicity [14]. This decrease in hydrophilicity favors penetration into the brain, where phosphylated oximes might phosphylate AChE, thereby defeating their therapeutic purpose and resulting in limited efficacy. In the case of K-27, the present study demonstrates that phosphylation by the majority of toxic organophosphates and –phosphonates increases hydrophilicity. Phosphylated K-27 therefore barely enter the brain. Our own animal work indicates that K-27 is much more efficacious than obidoxime or pralidoxime, when given in the same equitoxic dosage [23, 25, 26]. This superior efficacy may be related to the limited passage of phosphylated K-27 into the brain.

CONCLUSION

We conclude that an "ideal" oxime must not only be non-toxic itself, but should also yield non- toxic products after phosphylation. Moreover, these phosphylation products should ideally be very hydrophilic, thus barely entering the brain. K-27 might come closer to this ideal than the established oximes.

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