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Rate constant of inhibition of cholinesterases by organophosphorus compounds: correlation between rate constant and toxicity

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A comprehensive analysis of the data on bimolecular rate constants of inhibition (BRCIs) of cholinesterases (acetylcholinesterase and cholinesterase) by organophosphorus compounds (OPCs), determined by recent methods described in the present manuscript, and LD_{50} values, reveals that there is an apparent proportionality between the values of the BRCIs of AChE and the degree of toxicity of OPCs. For strong neurotoxic agents the BRCIs exceed 10^6 M^{-1}. min^{-1}, whereas for the majority of less toxic OPCs this constant has lower values. We find it plausible to suggest the use of the bimolecular rate constant as a criterion for assessing the toxicity of OPCs, supplementing the routinely used toxicological techniques.

Key words: Inhibition constants of cholinesterases; Methods for determination of; Correlation with LD_{50}; Biological significance of.

Organophosphorus compounds have become a common element of humanity's household. The most widely distributed and easily accessible ones are those serving as pesticides. Another class of highly neurotoxic OPCs, namely the nerve gases intended for military use, has been accumulated throughout the world. Thus, OPCs have attracted a lot of attention and research efforts have been directed toward the creation of methods for estimation of their toxicity and the study of their mode of action.

The toxicity of dangerous drugs is assessed mainly by the LD_{50}-test, which is in use since 1927 [1]. During the last decade the correctness of the LD_{50} concept was reevaluated and a number of major drawbacks were pointed out: LD_{50} values obtained in animal experiments may be misleading if directly accepted for humans [1, 2]; a number of factors, such as species and strain, age, sex, diet etc., influence the LD_{50} values to an extent, which does not allow LD_{50} to be regarded as a biological constant [1]; we are now faced with important ethical considerations, which make animal experiments undesirable.

In this article we suggest the use of a biochemical constant, namely the BRCI, for the initial stages of the evaluation of the toxicity of organophosphorus compounds, prior to deployment of the traditional toxicological tests. For this purpose, we present

a concise review of methods for determination of the rate constant for the irreversible inhibition of AChE and ChE.

Methods for estimation of the bimolecular rate constant of inhibition

The bimolecular rate constant of the reaction of organophosphorus compounds with cholinesterases (AChE and ChE) has been studied intensively [see e.g. 3—14]. The experiments were carried out under conditions, where the reaction follows pseudo-first-order kinetics. The rate constant can be calculated according to Eq. (1), when the initial concentration of the strong irreversible inhibitor exceeds the initial concentration of the enzyme $[E]_o$ at least 20 fold [13. 15]:

(1) $$k_{II} = \frac{1}{t.[I]_o}.\ln(v_o/v_i),$$

where t is the time, during which the enzyme and the inhibitor have been in contact, k_{II} — the rate constant of the inhibition, $[I]_o$ — the intital (total) concentration of the inhibitor, v_o — the rate of the reaction in the absence of inhibitor, v_i — the rate of the reaction in the presence of inhibitor.

Under these conditions, the values obtained for k_{II} are numerically equal to the bimolecular rate constant [13].

The same method has been applied at an inhibitor to enzyme ratio lower than 20 [15]. However, under these conditions the calculation of k_{II} should be performed according to a different equation:

(2) $$k_{II} = \frac{1}{t([E]_o-[I]_o)}.\ln\left\{\frac{[I]_o([E]_o-[EI])}{[E]_o([I]_o-[EI])}\right\},$$

where $[EI]$ is the enzyme-inhibitor complex.

Another method is to preincubate the enzyme with several concentrations of the inhibitor, which allows the calculation of the partial inhibition (i), respectively — the estimation of the remaining enzyme activity (v_i/v_o). The inhibitor concentration and the time of preincubation have to be selected in such a way that the inhibition at the lowest inhibitor concentration (which in all cases should be 20 times higher than that of the enzyme) should be between 15 and 30%, and at the highest — from 70 up to 80% [16]. The rate constant k_{II} can be calculated with the help of Eqs. (3a) and (3b) from the value of $(-k_{II}t)$, which is equal to the slope of the straight line obtained on plotting "ln (100-i)" (which can be substituted by "$\ln(v_i/v_o)$") vs $[I]_o$ for a fixed preincubation time (in most cases 15—30 min, see e.g. [17—19]):

(3a) $$\ln(100-1) = 4{,}60517 - k_{II}t[I]_o,$$

(3b) $$\ln(v_i/v_o) = -k_{II}t[I]_o,$$

where 4,60517 = ln 100.

There are certain differences between the two methods, which have to be considered by the experimenter prior to making his choice. While the first method relies on the use of a single inhibitor concentration, the second one requires the use of several different concentrations. The first method is faster to perform, it still leaves some uncertainty whether the initial inhibitor and the initial enzyme concentrations have been properly selected to satisfy the requirement that the inhibitor concentration exceeds that of the enzyme by at least 20 times. On the other hand, with the second method one can be sure that the experimental conditions have been

chosen correctly if a straight line is obtained on plotting of ln(100-i) vs $[I]_o$ (see e.g. [20]).

The third method for calculating the value of the rate constant requires that the inhibitor concentration is far below the value of the dissociation constant. In this case the overall inhibition rate is determined by the bimolecular rate constant [21, 22]. This can be illustrated by the reaction between AChE (E) and an OPC (PX):

(4) $$E + PX \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EPX \xrightarrow{k_p} EP + X, \qquad k_{II}$$

where k_p is the phosphorylation constant, EP — the phosphorylated enzyme.

The dissociation constant (K_d) can be obtained from:

(5) $$K_d = k_{-1}/k_1.$$

Knowing the phosphorylation and dissociation constants, the bimolecular rate constant is calculated from:

(6) $$k_{II} = k_p/K_d.$$

This method is made less attractive by the fact that it requires the application of additional time consuming techniques, however, it allows the calculation of the values of further constants, characterizing the enzyme-inhibitor interactions.

Evaluation of the resistance of animals to organophosphorus compounds with the help of the rate constant

In order to characterize the apparent correlation between the rate constants of inhibition of cholinesterases by certain OPC and their LD_{50} values we summarized available published data [5-13, 17-19, 21, 23-27, 30, 32, 33, 37, 39, 41-46]. Since most of the authors work under compatible conditions we accept that a comparison of the reported data is viable.

A good example to illustrate this correlation are the two strongly toxic isomers of soman, namely $C_{(+)}P_{(-)}$- soman and $C_{(-)}P_{(-)}$-soman, characterized by low LD_{50}-values of 0,099 and 0,038 mg/kg (subcutaneous application, rat) respectively [23]. The rates of inhibition of electric eel AChE by these two soman isomers are $2{,}8.10^8$ and $1{,}8.10^8$ $M^{-1}.\ min^{-1}$ respectively [23]. The other two soman isomers are of much lower toxicity, which is reflected by their high LD_{50}-values exceeding 5 or 2 mg/kg (subcutaneous, rat) respectively, and by considerably lower $k_{II} \leq 5.10^3$ $M^{-1}.\ min^{-1}$ for both of them [23]. Similar results were reported by K e i j e r and W o l r i n g [24].

The values reported for the bimolecular rate constant of inhibition of electric eel AChE by certain potent neurotoxic agents, destined for military use [25] are: V_x — $2{,}54.10^7$ $M^{-1}.\ min^{-1}$; sarin — $1{,}55.10^7$ $M^{-1}.\ min^{-1}$; a racemic mixture of soman — $5{,}58.10^7$ $M^{-1}.\ min^{-1}$; $C_{(-)}$-soman — $4{,}08.10^7$ $M^{-1}.\ min^{-1}$; and $C_{(+)}$-soman — $6{,}32.10^7$ $M^{-1}.\ min^{-1}$. All of these compounds have correspondingly low LD_{50} -values. The inhibition of the same enzyme by the less toxic DFP (LD_{50} = 7,7-13,5 mg/kg, per os, rat) and paraoxon (LD_{50} = 3 mg/kg, per os, rat), is characterized by correspondingly low k_{II} -values of $9{,}48.10^3$ $M^{-1}.\ min^{-1}$ and $1{,}16.10^5$ $M^{-1}.\ min^{-1}$ [see e.g. 39].

Further analysis of the same set of data yielded support to the notion, that there is a clearly expressed correlation between the LD_{50}-values and k_{II} [see also 13, 17, 19,

26, 27]. A plot of LD_{50}-values vs k_{II} (Fig. 1) reveals that the strongly toxic nerve agents are characterized by inhibition constants exceeding 10^6 M^{-1}. min^{-1}, while less toxic compounds are characterized by inhibition constants with lower values. Therefore, we find it feasible to suggest the value of 10^5 M^{-1}. min^{-1} as a conventional criterion for the toxicity of organophosphorus compounds.

Furthermore the plot on Fig. 2 shows that the same correlation is found for compounds, known to be of low toxicity, and the only compound for which the k_{II} is higher than 10^6 M^{-1}. min^{-1} is tetraethyl pyrophosphate, previously used as a pesticide, however, because of its high toxicity its application has been limited [28].

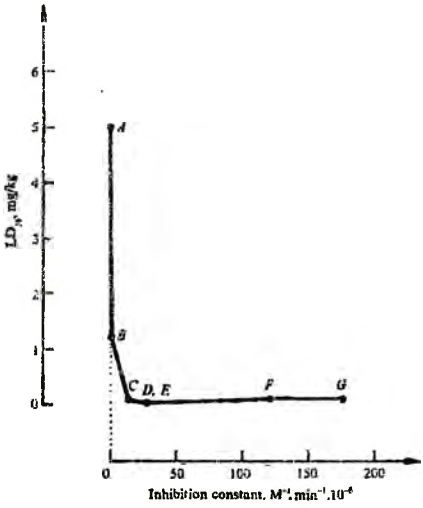

Fig. 1. Corellation between the toxicity and the inhibition constant of AChE inhibitors (subcutaneous application)
Capital letters on right of the points of the curve denote the organophosphorus compound studied: *A* — DFP; *B* — *S*-butyl-*o*-(4-nitrophenyl) methylthiophosphonate; *C* — $C_{(-)}$ $P_{(-)}$-soman (ref. 24); *D* — $C_{(-)}$ $P_{(-)}$ soman (ref. 23); *E* — $C_{(+)}$ $P_{(-)}$-soman (ref. 24); *F* — $C_{(+)}$ $P_{(-)}$-soman (ref. 23); *G* — Sarin. The vertical dotted line shows the position of k_{II}-value of 10^6 M^{-1}. min^{-1}

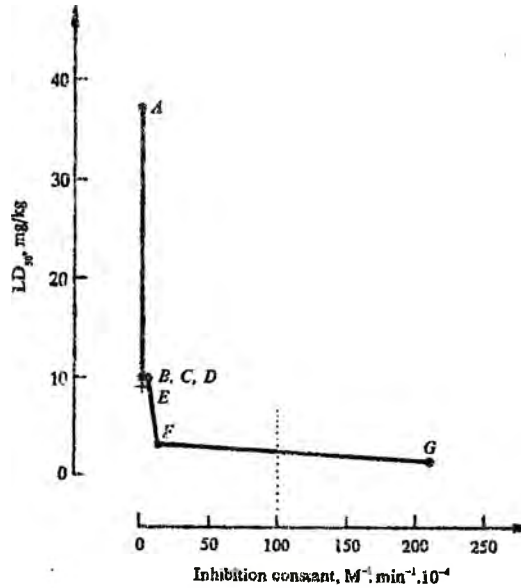

Fig. 2. Corellation between the toxicity and the inhibition constant of AChE inhibitors (per os intake)
Capital letters on right of the points of the curve denote the organophosphorus compound studied: *A* — Parathion-methyl; *B* — DFP (ref. 25); *C* — DFP (ref. 13); *D* — DFP (ref. 33); *E* — Parathion; *F* — Paraoxon; *G* — TEPP. The vertical dotted line shows the position of k_{II}-value of 10^6 M^{-1}. min^{-1}

We suggest that this criterion can be used to forecast the toxicity during early stages of investigations on OPCs. It can be expected that substances for which the k_{II} exceeds 10^6 M^{-1}. min^{-1} should be extremely toxic. If the expected high toxicity precludes the use of these substances in the household or in the agriculture, the tests for determining the LD_{50} of such compounds can be avoided, or in cases of great interest in a given OPC, one could at least lower considerably the number of animals used for LD_{50} tests.

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