In vitro inhibition of human blood cholinesterases and protection against chlorpyriphos (organophosphate) by weak anticholinesterase drugs

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ABSTRACT

In vitro inhibition of human plasma and erythrocyte cholinesterase activities by the strong cholinesterase inhibitor chlorpyriphos (organophosphate) and the weak cholinesterase inhibitors (donepezil, metoclopramide, and diphenhydramine) in different concentrations was evaluated. The protective ability of each one of the weak cholinesterase inhibitors alone and with a combination of two of them against the inhibitory action of chlorpyriphos was also detected. The results showed different patterns of inhibition depending on the inhibitor and its concentration when used alone or within a combination. When testing the protective ability, the results differed for each one of the inhibitors and for the combinations, depending on its own ability to bind the cholinesterase. Donepezil showed no ability to protect the enzyme against chlorpyriphos, but caused further increase in the inhibition when used alone or in combination. Diphenhydramine could have affinity to bind the enzyme more than metoclopramide and donepezil when used in combination against chlorpyriphos toxicity, and metoclopramide had higher affinity to inhibit erythrocyte cholinesterase in combination more than diphenhydramine and donepezil . The use of weak cholinesterase inhibitors alone or in combination may reduce the toxicity of organophosphates depending on the inhibitor used , but still not to a significant extent.

Keywords: Weak cholinesterases inhibitors, chlorpyriphos, donepezil, metoclopramide, diphenhydramine, protection against organophosphates

الخلاصة

باستخدام الأنابيب الاختبارية تمت دراسة قوة التثبيط لخميرة الكولين استراز في البلازما وكريات الدم الحمراء في الإنسان لكل من المثبط القوي للخميرة الكلوربايريفوس (مبيد حشري فسفوري) والمثبطات الضعيفة (الدونبيزيل والميتوكلوبرامايد والدايفينهايدرامين) بتراكيز مختلفة ، ومن ثم تمت دراسة قابلية حماية الخميرة من قبل المثبطات الضعيفة بصورة منفردة او ضمن خليط من المثبطات الضعيفة ضد المبيد الحشري (المثبط القوي) . وعند دراسة قابلية الحماية للخميرة ظهرت نتائج مختلفة لكل مثبط اعتمادا على قوة قابليته على الارتباط بالخميرة وكذلك اختلفت بالنسبة لكل خليط أيضا . لم يظهر الدونبيزيل اي قابلية على حماية الخميرة ضد المبيد الحشري وإنما أدت إضافته إلى زيادة التثبيط للخميرة عند استعماله لوحده أو عند استخدامه ضمن خليط من المثبطات الضعيفة . وكذلك أظهرت النتائج أن الدايفنهايدرامين ربما تكون له القابلية على الارتباط بالخميرة أكثر من الميتوكلوبراماد والدونبيزيل عند استعماله ضمن خليط ضد سمية الكلوربايريفوس . وان الميتوكلوبراميد له قابلية قوية للارتباط بخميرة كريات الدم الحمراء ضمن الخليط أكثر من الدايفنهايدرامين والدونبزيل . كخلاصة نستطيع القول ان استعمال المثبطات الضعيفة لخميرة الكولين استراز لوحدها أو ضمن خليط تظهر قابلية لتقليل سمية المثبطات القوية (المبيدات الحشرية) اعتمادا على نوعية المثبط ولكن ليس إلى حد يمكننا من استخدامها بصورة عملية .

Organophosphates (OP) are widely used as insecticides in public health, veterinary practice and agriculture [١,٢]. The single most

important mechanism of the toxic action of these insecticides in man and animals is inhibition of acetyl cholinesterase at the nerve terminals, and this causes acetylcholine accumulation that subsequently causes a series of muscarinic, nicotinic and central nervous system effects[٣,٤]. Human blood cholinesterase (ChE) are classified as acetyl cholinesterase (AChE, mainly found in erythrocytes) and butryl cholinesterase (BChE, mainly found in plasma) [٢,٥]. These enzymes differ in their sensitivity to inhibitors[٦] and therefore, the inhibitors show different toxicities.

The cardinal treatment for organophosphate poisoning includes atropine to counteract the muscarinic signs and symptoms, and oximes to reactivate the inhibited cholinesterases[٢,٣]. However, the clinical results of administering antidotal therapy for organophosphate poisoning are far from perfect[٢,٣,٤], and there are many experimental trials using other therapeutic and protective agents. Examples of such trials include antihistamine diphenhydramine[٧,٨], alpha- ٢-adrenoceptor agonists[٩] and metoclopramide,[٧,١٠], and phenothiazines [١١]

Metoclopramide is used clinically as antiemetic agent[١٢]. Other than the antidopaminergic and possible serotonergic effects, metoclopramide has been found to possess anticholinesterase properties [١٠,١٢]. The drug weakly inhibits cholinesterase activity both in vitro[١٣] and in vivo[١٠]. This protective effect of metoclopramide on the cholinesterase is thought to be of practical usefulness for the treatment of organophosphate poisoning[١٣]. However, metoclopramide which is a dopamine receptor antagonist was reported to prevent OP poisoning in man (using in vitro system)[١٠] and in chicks both in vitro and in vivo[١٤]. The protective action of metoclopramide is thought to be competition for the active site of the enzyme with the more potent OP[١٥]. Metoclopramide inhibited in vitro as well as in vivo the ChE activity in animals [١٤,١٦]. Metoclopramide reduced the toxicity by dichlorvos and daizinon by acting as a protective agent for the ChE[١٤,١٥].

Diphenhydramine which is an ethanolamine derivative, nonselective histamine H١-receptor antagonist, mainly used for allergy symptoms caused by histamine release (anaphylaxis, rhinitis and dermatoses), also can be used for nausea and vertigo and motion sickness[١٧,١٨,١٩]. The efficacy of diphenhydramine in the prevention and treatment of methomyl-induced toxicosis was evaluated in female rats, the results suggest that diphenhydramine could be of therapeutic value in reducing the toxic effects of methomyl[٢٠,٢١]. Diphenhydramine inhibits in vitro pseudo cholinesterase (ChE) from several sources (human, rat, or horse plasma). This inhibition is competitive with acetylcholine (ACh) and is reversible, diphenhydramine dose not compete with either OP in inhibiting ChE. [١١,٢١,٢٢].

Donepezil is an actylchelinesteras inhibitor, mainly used in Alzheimer's disease, it reversibly inhibits actylchelinesteras[١٧,١٨,١٩]. We tried to use it in our study as a protective agent against organophosphate poisoning.

The aim of this study was to evaluate the protective effect of weak ChE inhibitors (metoclopramide, diphenhydramine and donepezil) against the strong inhibitors OP (chlorpyriphos), each one used alone and then as in combination

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Materials and methods

Subjects The subjects included in this study (male and females, age ٣٠+/- ١٠ years) apparently healthy with no history of exposure to anti-ChE insecticides or drugs. Blood samples were collected in a ٥ ml EDTA-treated test tubes then centrifuged (Centurion, UK) at ٣٠٠٠ rpm for ١٥ min. The erythrocytes and plasma were separately pooled and kept on ice for ChE assay.

Electrometric assay of ChE activity In our work we used the modified electrometric method, validated in human[٢٣,٢٤]. The reaction mixture in a ١٠ ml beaker contained ٣ ml distilled water, ٠.٢ ml plasma or erythrocytes and ٣ ml pH ٨.١ barbital-phosphate buffer[٢٥]. The pH of the mixture (pH١) was measured with glass electrode using pH meter (Hanna Instruments, Romania), then ٠.١ ml of aqueous solution of acetylthicholine (٧.٥%) was added to the reaction mixture which was incubated at ٣٧°C in water bath (Shaker bath ٥BS٣٠, UK) for ٢٠ min. At the end of the incubation period, the pH of the reaction mixture (pH٢) was measured. The enzyme activity was calculated as follows:

ChE activity (ΔpH/٢٠ min.) = (pH١-pH٢)-ΔpH of blank

The blank contained no blood aliquot. The barbital-phosphate buffer solution consist of ١.٢٤ g sodium barbital (BDH), ٠.١٦٣ g potassium dihydrogen phosphate (Merck, Germany), and ٣٥.٠٧ g sodium chloride (BDH) dissolved in one liter distilled water [٢٣,٢٥]. The pH of the buffer was adjusted to ٨.١ with ١N HCl.

In vitro ChE inhibition by the inhibitors Plasma and erythrocyte samples were collected from healthy volunteers. Different drug concentrations were prepared then they were individually added in a volume of ٠.١ ml to the reaction mixture to obtain final concentrations as follows:

chlorpyriphos: ٤ μM
metoclopramide: ٥٠, ٢٥ and ١٢.٥ μM
diphenhydramine: ١٠٠, ٥٠ and ٢٥ μM
donepezil: ١٠٠ and ٢٥ μM

the concentrations of chlorpyriphos and the drugs used in the present study were obtained from preliminary experiments to validate the experimental concentrations. The inhibitors were prepared in distilled water and individually added in a ٠.١ ml to the reaction mixture of the plasma and erythrocytes. The reaction mixture containing inhibitors was incubated at ٣٧ ºC for ١٠ min. Thereafter, the residual ChE activity in mixture was measured[١١]. The % of enzyme inhibition was calculated as follows:*% ChE inhibition= [ChE activity (without inhibitors)-ChE activity (with inhibitors)/ChE activity (without inhibitors)]X١٠٠*

In vitro ChE inhibition by the weak inhibitors and the strong inhibitor (chlorpyriphos)

Aliquots of the same plasma and erythrocyte samples used in the previous experiment were used here and the same concentrations of inhibitors were also used, chlorpyriphos was diluted with distilled water and added in a volume of ٠.١ ml to the reaction mixture to obtain a final concentration of ٤ μM, each drug concentration mentioned above was added to the reaction mixture (٠.١ ml) ١٠ min before the chlorpyriphos addition[١١], which was then incubated at ٣٧°C for ١٠ min before measuring ChE activities

and percent of enzyme inhibition as described above.

In vitro ChE inhibition by a combination of weak ChE inhibitorsnd then by the same combination with the strong inhibitor (chlorpyriphos)

In another set of experiment other plasma and erythrocyte samples used and the drugs were used here as a combination of inhibitors in different concentrations as follows:

donepezil ١٠٠ μM with metoclopramide ٥٠ μM

donepezil ٢٥ μM with metoclopramide ٢٥ μM

donepezil ١٠٠ μM with diphenhydramine ١٠٠ μM

donepezil ٢٥ μM with diphenhydramine ٥٠ μM

Then we continued with the following inhibitors and concentrations

metoclopramide ٥٠ μM with diphenhydramine ١٠٠ μM

metoclopramide ٢٥ μM with diphenhydramine ٥٠ μM

metoclopramide ٢٥ μM with diphenhydramine ٢٥ μM

metoclopramide ١٢.٥ μM with diphenhydramine ٥٠ μM

metoclopramide ١٢.٥ μM with diphenhydramine ٢٥ μM

Each combination mentioned above was added to the reaction mixture (٠.١ ml) (both weak inhibitors added at the same time), which was then incubated at ٣٧ °C for ١٠ min before measuring ChE activities and percent of enzyme inhibition as described in the previous experiment.

Then the same plasma and erythrocyte samples used in this experiment and the same combination of inhibitors mentioned above were also used in the same way of addition and incubation. Chlorpyriphos was then added in a volume of ٠.١ ml to the reaction mixture to obtain a final concentration of ٤ μM (chlorpyriphos was diluted and used as described in the previous experiment), which was then incubated at ٣٧ °C for ١٠ min before measuring ChE activities and percent of enzyme inhibition as described in the previous experiment.

Statistical Analysis

The data were subjected to analysis of variance followed by the least of significant difference test[٢٦]. Paired Student's t-test was used for comparing the means of ٢ groups[٢٧]. The level of significance was at $P<٠.٠٥$.

Results

The in vitro inhibition of plasma and erythrocyte ChE activities by the strong ChE inhibitor (chlorpyriphos) ٤ μM and weak ChE inhibitors (donepezil, metoclopramide, and diphenhydramine) in different concentrations, and then each one of the weak ChE inhibitors with the strong one was detected and the results are shown in Table ١.

Tables ٢ and ٣ represent in vitro inhibition of plasma and erythrocyte cholinesterase activities by a combination of weak ChE inhibitors (donepezil ١٠٠, ٢٥μM with metoclopramide ٥٠, ٢٥μM and then donepezil ١٠٠, ٢٥μM with diphenhydramine ١٠٠, ٥٠μM) each combination alone and then with the strong ChE inhibitor (chlorpyriphos) (Table ٢), and the combination of (metoclopramide ٥٠, ٢٥μM and ١٢.٥μM with diphenhydramine ١٠٠, ٥٠μM and ٢٥μM) each combination as alone and then with the strong ChE inhibitor (chlorpyriphos) (Table ٣).

In order to compare the combinations, Figure ١ and ٢ represent % inhibition of plasma and erythrocyte cholinesterase of each combination used in this experiment as alone and then with the strong ChE inhibitor (chlorpyriph)

Table ١: In vitro inhibition of human plasma and erythrocyte cholinesterase activities by numbers of ChE inhibitors and their effect on ChEs inhibition by the strong inhibitor (chlorpyriphos)

Inhibitors (μM)	Plasma ChE		Erythrocyte ChE	
	Δ pH/٢٠ min	% Inhibition	Δ pH/٢٠ min	% Inhibition
٠ μM	١.١٦±٠.٠٩٣	٠	٠.٧٤±٠.٢٠٧ a	٠
chlorpyriphos ٤ μM*	٠.٠٦±٠.٠١٢ a	٩٤.٧٠	٠.٩٥±٠.٠٢١ a	٢١.٤٠
donepezil ١٠٠ μM*	٠.٣٥±٠.٢١٣ a b	٦٨.٦١	٠.٠١±٠.٠١٤ a b	٩٧.٣٨
donepezil ١٠٠ μM before ‡ chlorpyriphos ٤ μM	٠.٠٢±٠.٢١٢ a	٩٨.٢١	٠.٠٠±٠.٠٠ a b	١٠٠
donepezil ٢٥ μM*	٠.٦٢±٠.٠١٤ a b	٤٤.٨٤	٠.٠٦±٠.٢١٢ a b	٨٠.٣٣
donepezil ٢٥ μM before ‡ chlorpyriphos ٤ μM	٠.٠٠٣±٠.٠١ a	٩٩.٧٣	٠.٠٠±٠.٠٠ a b	١٠٠
metoclopramidee ٥٠ μM*	٠.٠٧±٠.٠٠٧ a	٩٤.٣٠	٠.٣٠±٠.٠٥٥ a b	٧٧.٧٠
metoclopramide ٥٠ μM‡ before chlorpyriphos ٤ μM	٠.٠٢±٠.٠٢١ a	٩٨.٧٦	٠.٣٢±٠.١٣٤ b	٥٤.٩٩
metoclopramide ٢٥ μM*	٠.٢١±٠.٠١٠ a b	٨٢.٩٠	١.٢٦±٠.٠١٢ a b	٦.٢٠
metoclopramide ٢٥ μM‡ before chlorpyriphos ٤ μM	٠.٠٥±٠.٠٣٥ a	٩٦.٢٨	٠.٥٦±٠.٠٣٥ a	٢٠.٧١
diphenhydramine ١٠٠ μM*	٠.٣٤±٠.٠٢١ a b	٦٧.٧٩	١.٠٣±٠.٠٩٨ b	٧.٢١
diphenhydramine ١٠٠ μM‡ before chlorpyriphos ٤ μM	٠.٠٠±٠.٠٠ a	١٠٠	٠.٥١±٠.٠٠٧ a	٢٧.٨٦
diphenhydramine ٥٠ μM*	٠.٥٢±٠.٠٥٦ a b	٥٠.٠٠	٠.٣٦±٠.٢٦١ a b	٤٩.٢٩
diphenhydramine ٥٠ μM‡ before chlorpyriphos ٤ μM	٠.٠٠±٠.٠٠ a	١٠٠	٠.٢٣±٠.٠١٤ a b	٦٧.١٤

N=٢ / concentration groups.

❋ Cholinesterase inhibition was detected after ١٠ min incubation of the sample with ChE inhibitors.

‡ weak ChE inhibitors was added to the reaction mixture ١٠ min before the strong ChE inhibitors (chlorpyriphos) addition.

a Significantly different from their respective control (٠) group, P<٠.٠٥

b Significantly different from the strong ChE inhibitors (chlorpyriphos) (٤ μM), P<٠.٠٥

Table ٢: In vitro inhibition of human plasma and erythrocyte cholinesterase activities by a combination of weak ChE inhibitors (donepezil with metoclopramide and then donepezil with diphenhydramine) and their effect on ChEs inhibition by the strong inhibitor (chlorpyriphos)

Inhibitors (μM)	Plasma ChE		Erythrocyte ChE	
	Δ pH/٢٠ min	% Inhibition	Δ pH/٢٠ min	% Inhibition
٠ μM	١.١٣±٠.٠٨٢	٠	٠.٩٤±٠.٢١٥	٠
donepezil ١٠٠ μM + ❋ metoclopramide ٥٠ μM	٠.١٧±٠.٠١٤ a b	٨٤.٠٤	٠.٠٦±٠.٠٠٢ a b	٨٨.٨٩
donepezil ١٠٠ μM + ‡ metoclopramide ٥٠ μM before chlorpyriphos ٤ μM	٠.٠٨±٠.٠٢١ a	٩٢.٩٦	٠.٠٤±٠.٠١٤ a b	٩١.٩٢
donepezil ٢٥ μM + ❋ metoclopramide ٢٥ μM	٠.٢٤±٠.٠٠ a b	٧٧.٤٧	٠.٠٦±٠.٠٢٨ a b	٨٧.٨٨
donepezil ٢٥ μM + ‡ metoclopramide ٢٥ μM before chlorpyriphos ٤ μM	٠.١٠±٠.٠١٤ a	٩٠.٦١	٠.٠٤±٠.٠٠٧ a b	٩٢.٩٣
donepezil ١٠٠ μM + ❋ diphenhydramine ١٠٠ μM	٠.٢٣±٠.٠٢٨ a b	٧٩.٩١	٠.٠١±٠.٠٠٧ a b	٩٨.٥١
donepezil ١٠٠ μM + ‡ diphenhydramine ١٠٠ μM before chlorpyriphos ٤ μM	٠.٠٢±٠.٠٢١ a	٩٨.٦٩	٠.٠٠±٠.٠٠ a b	١٠٠
donepezil ٢٥ μM + ❋ diphenhydramine ٥٠ μM	٠.٤٣±٠.٠٥٧ a b	٦٢.٤٥	٠.٠٢±٠.٠١٤ a b	٩٤.٠٣
donepezil ٢٥ μM + ‡ diphenhydramine ٥٠ μM before chlorpyriphos ٤ μM	٠.٠٤±٠.٠١٤ a	٩٦.٥١	٠.٠٠±٠.٠٠ a b	١٠٠

N=٢ / concentration groups.

❋ Cholinesterase inhibition was detected after ١٠ min incubation of the sample with the mixture of weak ChE inhibitors.

‡ The mixture of weak ChE inhibitors was added to the reaction mixture ١٠ min before the strong ChE inhibitors (chlorpyriphos) addition.

a Significantly different from their respective control (٠) group, P<٠.٠٥

b Significantly different from the strong ChE inhibitors (chlorpyriphos) (٤ μM), P<٠.٠٥

Table ٣: In vitro inhibition of human plasma and erythrocyte cholinesterase activities by a combination of weak ChE inhibitors (metoclopramide with diphenhydramine) and their effect on ChEs inhibition by the strong inhibitor (chlorpyriphos)

Inhibitors (μM)	Plasma ChE		Erythrocyte ChE	
	Δ pH/٢٠ min	% Inhibition	Δ pH/٢٠ min	% Inhibition
٠ μM	١.١٧±٠.١٣٤	٠	٠.٥٤±٠.٣٢٤	٠
metoclopramide ٥٠ μM + ❋ diphenhydramine ١٠٠ μM	٠.٠٩±٠.٠٠ a	٩٢.١٤	٠.٢٩±٠.٠٩١ b	١٣.٤٣
metoclopramide ٥٠ μM + ‡ diphenhydramine ١٠٠ μM before chlorpyriphos ٤ μM	٠.٠١±٠.٠١٤ a	٩٩.١٣	٠.١٣±٠.٠٤٩ a b	٦١.٤٩
metoclopramide ٢٥ μM + ❋ diphenhydramine ٥٠ μM	٠.١٢±٠.٠٢١ a	٨٩.٩٦	٠.٢٣±٠.٠٠٧ a b	٣٢.٨٤
metoclopramide ٢٥ μM + ‡ diphenhydramine ٥٠ μM before chlorpyriphos ٤ μM	٠.٠٤±٠.٠٠٧ a	٩٦.٩٤	٠.١٧±٠.٠٢٨ a b	٤٩.٢٥
metoclopramide ٢٥ μM + ❋ diphenhydramine ٢٥ μM	٠.٢١±٠.٠١٤ a b	٨٢.٦٤	٠.٥٠±٠.٠٢٨	٢٨.٥٧
metoclopramide ٢٥ μM + ‡ diphenhydramine ٢٥ μM before chlorpyriphos ٤ μM	٠.٠٩±٠.٠٩٢ a	٩٢.٩٨	٠.٥٥±٠.٠١٢	٢٢.١٤
metoclopramide ١٢.٥ μM + ❋ diphenhydramine ٥٠ μM	٠.٥٨±٠.٠٠٧ a b	٥٢.٤٨	٠.٦٧±٠.١٢٠ a b	٤.٩٩
metoclopramide ١٢.٥ μM + ‡ diphenhydramine ٥٠ μM before chlorpyriphos ٤ μM	٠.٠٣±٠.٠٠٧ a	٩٧.٩٣	٠.٣٨±٠.٢٧٥ b	٤٦.٤٣
metoclopramide ١٢.٥ μM + ❋ diphenhydramine ٢٥ μM	٠.٢٨±٠.٠١٤ a b	٧٦.٨٦	٠.٥٥±٠.٠١٤ a	١٦.٦٧
metoclopramide ١٢.٥ μM + ‡ diphenhydramine ٢٥ μM before chlorpyriphos ٤ μM	٠.٠٤±٠.٠٠ a	٩٦.٦٩	٠.٤٧±٠.٠٢١ a	٢٩.٥٥

N=٢ / concentration groups.

❋ Cholinesterase inhibition was detected after ١٠ min incubation of the sample with the mixture of weak ChE inhibitors.

‡ The mixture of weak ChE inhibitors was added to the reaction mixture ١٠ min before the strong ChE inhibitors (chlorpyriphos) addition.

a Significantly different from their respective control (٠) group, P<٠.٠٥

b Significantly different from the strong ChE inhibitors (chlorpyriphos) (٤ μM), P<٠.٠٥

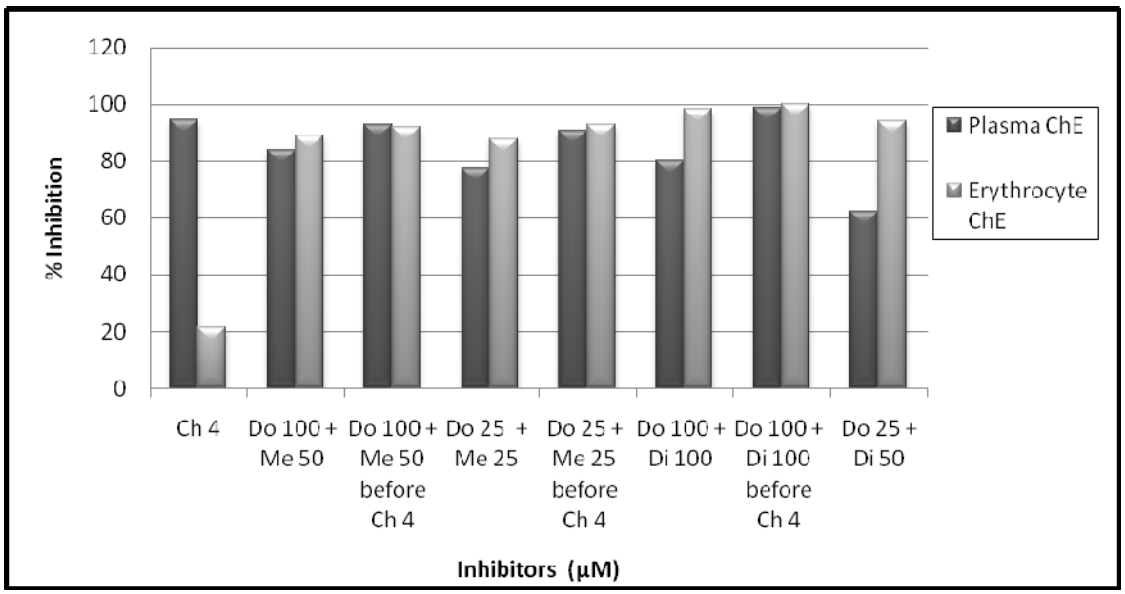

Figure ١ : % Inhibition of human plasma and erythrocyte cholinesterase by a combination of weak ChE inhibitors donepezil (Do) with metoclopramide (Me) and then donepezil (Do) with diphenhydramine (Di) and then each combination with the strong ChE inhibitor chlorpyriphos (Ch).

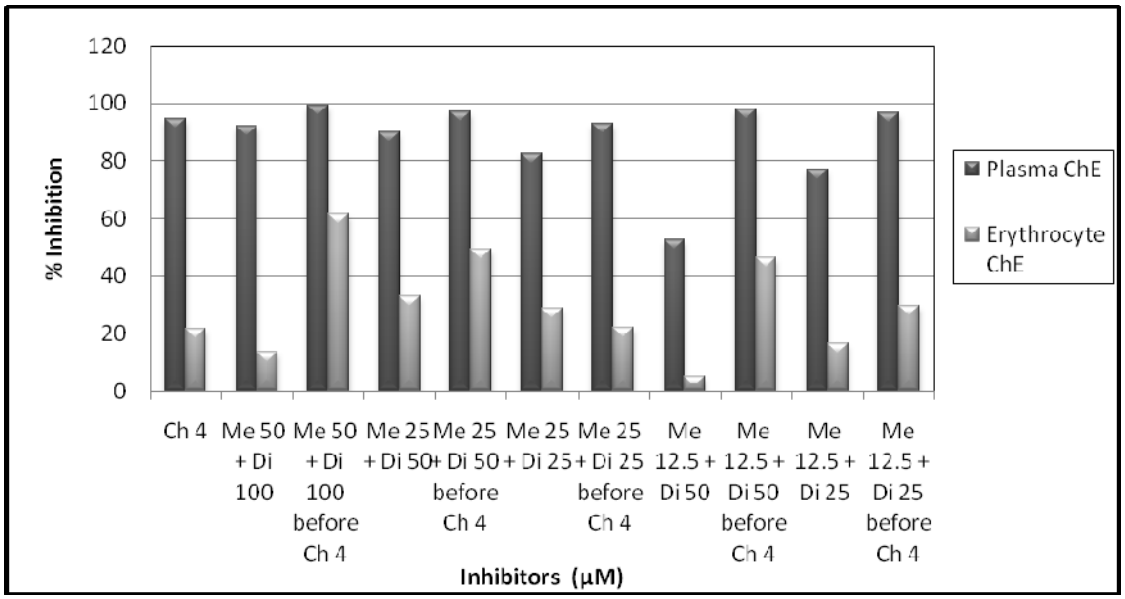

Figure ٢ : % Inhibition of human plasma and erythrocyte cholinesterase by a combination of weak ChE inhibitors metoclopramide (Me), diphenhydramine (Di) and then each combination with the strong ChE inhibitor chlorpyriphos (Ch).

Discussion

In vitro ChE inhibition system is used to evaluate ChE inhibiting potential of chemicals with binding affinities to ChE [٢٨,٢٩]. It was found that there was a good correlation between ChE inhibitions in vitro and in vivo[٣٠], and usually in vitro ChE inhibition assists in the clinical interpretation of pesticide intoxication [٦,٣١].

As expected, chlorpyriphos , which is an OP, caused ChE inhibition in the plasma and erythrocyte (in vitro). This is in correlation with previous in vitro studies in human[٢٣,٢٤]. In the present study, chlorpyriphos inhibited plasma ChE activity more than erythrocyte ChE. This effect has been reported by others[٢٥]. It was attributed to the inherent activity of the particular OP; it is well known that OP may vary in their inhibition potentials of tissue ChE [٣١,٣٢].

Weak or reversible ChE inhibitors can reduce or prevent toxicity of OP[١٠], and also prevent further increase in ChE inhibition caused by OP[٢١,٣٤]. However, weak ChE inhibitors given after OP, might increase the toxicity of the latter compound[٢٨], this is why we use the weak inhibitor before the strong one rather than after it. These weak inhibitors increased ChE inhibition in subjects already exposed to anti ChE, the weak inhibitor may inhibit a small fraction of residual ChE activity or they do not bind the same sites that bind with OP resulting in maximum toxicity [٢٢].

All the drugs used in this study are of known ability to inhibit the ChE in varying degrees, metoclopramide and diphenhydramine (as a weak inhibitors) both have been tested as protective agent against OP poisoning both in vitro and in vivo [١١,٢٠,٣٤]. Donepezil, a known anticholenesterase drug [٣٥], no previous study poses its ability to protect the ChE against OP poisoning. In this study we use each of them as ChE inhibitor and as a protector against OP, and then we combined both of them to find whether the combination will protect or further increase the OP ChE inhibition.

We found that all the weak ChE inhibitors showed different levels of inhibition of plasma ChE but all of them increased OP ChE inhibition when given before it (Table ١). The erythrocyte ChE showed a similar result (Table ١) except that metoclopramide ٥٠μM which reduced the ability of its toxicity from (٧٧.٧%) to (٥٤.٩%) when given before the OP (chlorpyriphos), but still higher than OP ChE inhibition (Table ١). Also metoclopramide ٢٥μM showed further decrease in OP ChE inhibition to lower than the normal inhibitory level (٢١.٤%) reduced to (٢٠.٧١%) (not statistically significant), but it is still higher than its normal inhibitory level (٧.٢١%) (i.e. Increase its toxicity and decrease OP ChE inhibition) (Table ١). Diphenhydramine ١٠٠μM showed low level of erythrocyte ChE inhibition alone but still increased OP ChE inhibition (Table ١), whereas diphenhydramine ٥٠μM show high level of increasing ChE inhibition as alone and with OP but still lower than the other weak ChE inhibitors (Table ١).

The combinations showed slightly different results, donepezil combination with other weak ChE inhibitor show maximal increase in the toxicity for both plasma and erythrocyte ChE (increase both its own alone and the OP % of inhibition) (Table ٢, Figure ١). Although the two combinations (donepezil ١٠٠ μM with metoclopramide ٥٠ μM, donepezil ٢٥ μM with metoclopramide ٢٥ μM) showed increase in their own toxicity alone when used with the OP but still lower than (yet not statistically significant) the % of plasma ChE

inhibition of OP alone (Table ٢, Figure ١). This result probably refers to the effect of metoclopramide because no such effect have been seen with the same donepezil concentration alone or with the same concentration when used as combination with the diphenhydramine (Table ٢, Figure ١). However, metoclopramide affect the erythrocyte ChE more than the plasma ChE (Table ١, Table ٣, Figure ٢)[١١,٣٤], so such effect could be referred to the effect of the two weak ChE inhibitors (donepezil and metoclopramide) as a combination when used together in this concentrations.

The combinations containing metoclopramide and diphenhydramine showed different results, % of inhibition of plasma ChE showed the same result of the previous combinations (i.e. Increase in % of its inhibition alone and that of OP plasma ChE) (Table ٣, Figure ٢). However, in case of erythrocyte ChE showed different way of inhibition, all of the combinations showed reduction of % of inhibition of one of its component when used alone (i.e. the weak ChE inhibitors protect erythrocyte ChE from each other to produce what we call it a mean of % of inhibition which is between the % of erythrocyte inhibition of the two weak inhibitors when used alone in different degree of inhibition regarding each combination) (Table ٣, Figure ٢). Such protection can be caused by the competition of the two ChE inhibitors on the enzyme molecule but in different degrees of affinity for each concentration, this is why we found different abilities of lowering the % of inhibition for each concentration used alone and when used as a combination with other concentrations of the other weak ChE inhibitors (Table ٣, Figure٢).

When all the combinations were used with the strong ChE inhibitor (chlorpyriphos) they showed increase in their own toxicity compared to its use alone and higher than that the % of inhibition of OP alone , but still not very high like those in the combinations containing the donepezil (Table ٣, Figure ٢). This could be caused by their abilities of inhibition of the enzyme being lower than that of donepezil (i.e. the selectivity of the donepezil is higher). Although the two combinations (that containing diphenhydramine ٢٥μM) show the lowest % of erythrocyte ChE inhibition when given before the OP (chlorpyriphos) (slightly above the % of inhibition of OP alone), but still not considered as protective since these could not reduce the % of inhibition below the that of OP alone.

Suggesting that the diphenhydramine could have affinity to bind the enzyme more than metoclopramide and donepezil when used within a combination with other weak ChE against the OP toxicity (chlorpyriphos), and the metoclopramide have high affinity than diphenhydramine to bind the erythrocyte ChE enzyme in a combination containing no OP. This is seen in all combinations with different concentrations of both metoclopramide and diphenhydramine (Table ٣, Figure ٢).

Conclusion

The use of weak ChE inhibitors may reduce the toxicity of strong one OP , but still not to significant degree. The use of a combination of such weak ChE inhibitor as a protector to reduce the toxicity of strong one (OP) show slightly different way of inhibition and reduction of OP toxicity than when used as alone depending on their different abilities to compete with each other and different affinities to bind the

enzyme, and this phenomenon differs from one concentration to other for each one of the weak ChE inhibitor.

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References

١- Coggon D. Work with pesticides and organophosphate sheep dips. Occup Med,٢٠٠٢;٥٢:٤٦٧٤٧٠.
٢- Wilson BW. Cholinesterase inhibition. In: Wexler P (ed.). Encyclopedia of Toxicology. Vol. ١ Academic Press, San Diego ١٩٩٨;٣٢٦-٣٤٠.
٣- Bajgar J. Organophosphates/nerve agent poisoning: mechanism of action, diagnosis, prophylaxis, and treatment. Adv Clin Chem ٢٠٠٤;٣٨:١٥١-٢١٦.
٤- Rusyniak DE, Nanagas KA. Organophosphate poisoning. Semin Neurol ٢٠٠٤;٢٤:١٩٧-٢٠٤.
٥- Wilson BW. Clinical enzymology. In: Loeb WF,Quimby FW (eds.). The clinical chemistry of laboratory animals. Philadelphia, PA: Taylor and Francis ١٩٩٩;٣٩٩-٤٥٤.
٦- Karanth S, Pope C. In vitro inhibition of blood cholinesterase activities from horse, cow, and rat by tetrachlorvinphos. Int J Toxicol ٢٠٠٣;٢٢(٦):٤٢٩-٣٣.
٧- Bird SB, Gaspari RJ, Lee WJ, et al. Diphenhydramine as a protective agent in a rat model of acute, lethal organophosphate poisoning. Acad Emerg Med ٢٠٠٢;٩:١٣٦٩-١٣٧٢.
٨- Faris GAM, Mohammad FK. Prevention and treatment of dichlorvos-induced toxicosis in mice by diphenhydramine. Vet Hum Toxicol ١٩٩٧;٣٩:٢٢-٢٥.
٩- Yakoub LK, Mohammad FK. .Medetomidine protection against diazinon-induced toxicosis in mice. Toxicol Lett ١٩٩٧;٩٣:١-٨.
١٠- Petroianu GA, Hasan MY, Nurulain SM, Arafat K, Sheen R, Saleh A, Schmitt A.,. Protective drugs in acute large-dose exposure to organophosphates: A com parison of metoclopramidee and tiapride with pralidoxime in rats. Anesth Analg ٢٠٠٥;١٠٠:٣٨٢-٣٨٦.
١١- Mahmood AAJ. Interaction of Phenothiazines with Dichlorvos (Cholinesterase Inhibitors) in Vitro Study. M.Sc. Thesis, College of Pharmacy, University of Mosul, Mosul. ٢٠٠٥.
١٢- Rang HP, Dale MM, Ritter JM, Moore PK.. Pharmacology. ٥th ed, Churchill Livingstone, Edinburgh. ٢٠٠٣;٣٧٤.
١٣- Petroianu GA, Hasan MY, Kosanovic M, Vijayasarathy C, Saleh AM.,. Metoclopramidee protection of cholinesterase from paraoxon inhibition. Vet Hum Toxicol ٢٠٠٣;٤٥:٢٥١-٢٥٣.
١٤- Al-Zubaidy, MHI. Interaction of metoclopramidee with cholinesterase Inhibitors in chicks. M.Sc. Thesis, College of Veterinary Medicine, University of Mosul, Mosul, ٢٠٠٤
١٥- Petroainu, GA, Kuhn,F, Thyes C, et al. In vitro protection of plasma cholinesterases by metoclopramidee from inhibition by paraxon. J. Appl. Toxicol ٢٠٠٣;٢٣:٧٥-٧٩.
١٦- Shinner, HJ, Girling, KJ, Whitehurst A, Nathanson, MH. Influence of metoclopramidee on plasma cholinesterase and duration of action of mivacurium. Br J Anesth ١٩٩٩;٨٢(٤):٥٤٢-٥٤٥.
١٧- BNF. British National Formulary ٥٦, British Medical Association, Royal Pharmaceutical Society of Great Britin. UK ٢٠٠٨.
١٨- Laurence DR, Bennett PN, Brown MJ.. Clinical Pharmacology. ٩th

ed., Churchill Livingstone, New York ٢٠٠٣;٢٦٧-٤٠٤.

١٩- Potter WZ, Hollister LE. Antihistaminic agents. In. Katzung, BG. (editor). Basic and Clinical Pharmacology. ٩th edition, Lange Medical Books / Mc Graw Hill. New York. ٢٠٠٧;٥١١-٥٦٤.

٢٠- Al-Baggou' B.Kh, Mohammad F. K. Antagonism of methomyl-induced toxicosis by diphenhydramine in rats; Environ Toxicol Pharmacol, ١٩٩٩;٧:١١٩-١٢٥

٢١- Al-Baggou BKh, Mohammad F.K. Effects of histamine H١ antagonists on the acute toxicity of physostigmine and neostigmine in mice. Iraqi J Vet. Sci ١٩٩٨;١١:

٢٢- Fernandez G, Diaz Gomez MI, et al. Cholinesterase inhibition by phenothiazine antihistaminic: analysis of its postulated toxicity. Toxicol Appl Pharmacol ١٩٧٥;٣١:١٧٩-١٩٠.

٢٣- Ahmed OAH, Mohammad FK.,.A simplified eleclrometric technique for rapid measurement of human blood cholinesterase activity. Internet J Toxicol ٢٠٠٥;٢(١).

٢٤- Mohammad FK, Alias AS, Ahmed OAH.,. Electrometric measurement of plasma, erythrocyte and whole blood cholinesterase activities in healthy human volunteers. J Med Toxicol ٢٠٠٧;٣:٢٥-٣٠.

٢٥- Mohammad FK, Faris GA-M, Al-Kassim NA. A modified electrometric method for measurement of erythrocyte acetylcholinesterase activity in sheep. Vet Hum Toxicol ١٩٩٧; ٣٩:٣٣٧-٣٣٩.

٢٦- Bruning JL, Kintaz, BL. Computational Handbook of Statistics. Scott, Foresman and Co., Glenview, Illinois, ١٩٧٧:١٨.

٢٧- Petrie A. Lecture Notes on Medical Statistics. Black Well Scientific Publications, Oxford. ١٩٧٨

٢٨- Long PW. Phenothiazine tranquilizers. Internet Mental Health. J Appl Toxicol ٢٠٠٥;١٥(٨): ٥٠-٧.

٢٩- Nasello AG, Gidali, D, Felicio LF. A comparative study of the anticholinestrase activity of several antipsychotic agents. Pharmacol Biochem Behav ٢٠٠٣;٧٥(٤):٨٩٥-٩٠١.

٣٠- Iyaniwura TT. In vitro toxicology of organophosphorus pesticide combinations. In Vitro Toxicol ١٩٩٠;٣:٣٧٣-٣٧٧.

٣١- WHO. Methomyl. World Health Organization, Geneva ١٩٩٦;٧٤-٧٦.

٣٢- Fikes JD. Organophosphorous and carbamate insecticides. Vet. Clin. North Am: Small Anim. Prac ١٩٩٠;٢٠:٣٥٣-٣٦٧.

٣٣- Somani SM, Dube SN. Physostigmine: An overview as pretreatment drug for organophosphate intoxication. Int. J. Clin Pharmacol Therap Toxicol ١٩٨٩;٢٧:٣٦٧-٣٨٧.

٣٤- Al-Zubaidy MHI, Mohammad FK, Metoclopramidee protection of diazinon-induced toxicosis in chickens J Vet Sci ٢٠٠٧;٨:٢٤٩-٢٥٤

٣٥- Inglis F.The tolerability and safety of cholinesterase inhibitors in the treatment of dementia.Int J Clin Pract Suppl ٢٠٠٢;١٢٧:٤٥-٦٣.