Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 9, Issue 2, June 2014 , p.p(29-41)
ISSN 1992 - 0849

Animal hygiene assessment by spontaneous reactivation and aging kinetics of pesticide compounds

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Received date : 19/ 1 / 2014 **Accepted date : 30 / 3 / 2014**

ABSTRACT

The main aims of this paper were to investigate the rate of spontaneous reactivation of butyrylcholinesterase from plasma and serum of farm animals inhibited by the organophosphorus, chlorfenvinphos and trichlorfon. Organophosphorus are among the most toxic of all substances that cause poisoning in farm animals and are the most frequently encountered insecticides, commonly detected in agricultural products, animal-derived foodstuffs, environmental samples and home use and represent a significant potential health risk.The first-order rate constants obtained for spontaneous reactivation (k_s) for chlorfenvinphosand trichlorfon for plasma and serum was ranged between 0.012 to 0.811h^{-1}, while aging of phosphorylated butyrylcholinesterase follows the kinetics of a first-order reaction with rate constants aging (k_a) ranged between 0.004to 0.653 h^{-1}. Half-time ($t_{1/2}$) for spontaneous reactivation and aging are higher in trichlorfon compared to chlorfenvinphos and ranged from 1.1 to 28.9h (sheep), 2.1 to 63 h (cattle) and 2.8 to 53.3 h (goat), respectively.

Keywords: Spontaneous reactivation, Aging, Farm animals, animal hygiene, pesticide compounds.

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Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 9, Issue 2, June 2014 , p.p(29-41)
ISSN 1992 - 0849

تقييم صحة الحيوان بواسطة اعادة التنشيط الذاتي وديناميكية الهرم للمركبات المبيدة للحشرات

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تاريخ استلام البحث : 19 / 1/ 2014 تاريخ قبول البحث : 30 / 3 / 2014

الملخص

ان الهدف الرئيسي من هذه الدراسة هو ايجاد معدل التنشيط الذاتي لانزيم butyrylcholinesterase في بلازما ومصل الدم في الحيوانات الحقلية والمثبطة بواسطة المركبات الفسفورية العضوية، chlorfenvinphos و trichlorfon.والمعروف ان المركبات الفسفورية العضوية من بين اكثر المركبات السامة المسببة للتسمم في الحيوانات الحقلية ، ومن اغلب المبيدات الحشرية المالوفة حيث نجدها اكثر شيوعا في المحاصيل الزراعية، والمواد الغذائية المشتقة من الحيوانات وفي عينات البيئة والمنزل كما يمثل المخاطر الكامنة الموءثرة على الصحة .

تم الحصول على ثابت معدل الرتبة الاولى لاعادة التنشيط الذاتي (ks) لمركب chlorfenvinphos و trichlorfon, في بلازما ومصل الدم تراوحت بين 0.012الى 0.811 h−1 ، بينما هرم الانزيم butyrylcholinesterase المفسفر يسلك تفاعل ديناميكية الرتبة الاولى مع معدل ثابت الهرم (ka) تراوحت مابين 0.004 الى 0.653 h−1 . نصف العمر(t1/2) لاعادة التنشيط الذاتي والهرم كانت اعلى في trichlorfon, بالمقارنة مع chlorfenvinphos وتراوحت من 1.1الى 28.9 h (الاغنام) ، 2.1 الى 63 h (الابقار) و 2.8 الى 53.3 h (الماعز) ، على التوالي .

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 9, Issue 2, June 2014 , p.p(29-41)
ISSN 1992 - 0849

نستنتج من هذه الدراسه ، بان معدل الهرم كانت بطيئه جدا من اعادة التنشيط الذاتى لانزيم butyrylcholinesterase فى بلازما دم الحيوانات الحقليه (الابقار ، الاغنام ،والماعز) والمثبطه مع كل من chlorfenvinphos و trichlorfon, وهذه تنطبق ايضا لمصل الدم مع المركبين المذكورين على الرغم من وجود فرق ضئيل .

الكلمات الدالة : اعادة التنشيط الذاتي، الهرم، صحة الحيوان، الحيوانات الحقلية، المركبات المبيدة للحشرات.

1. INTRODUCTION

Organophosphorus insecticides still pose a main problem in animal hygiene. The use of organophosphorus compounds for pest control and endeavoured suicide reasons huge numbers of intoxications and several hundreds of thousands of fatalities per year mainly in developing countries [1-3]. In the current study, we have chosen chlorfenvinphos and trichlorfon. Both of them are routinely used in veterinary medicine. Organophosphorus compounds products are prevalent in animals destined for human consumption in the world with serious public health implications. Animal handlers are at risk of pollution and can serve as source of contamination to vulnerable hosts. Targeted pest control on farm animals, concerted veterinary efforts, professional health instruction, active attachment of farm animal careers and good health care systems are necessary for effective control [1, 4, 5].

The contamination with organophosphorus causes a generalized cholinergic crisis due to an irreversible inhibition of butyrylcholinesterase (acylcholineacylhydrolase, EC 3.1.1.8) by phosphylation of their active site serine [1, 6] and successive accumulation of the neurotransmitter butyrylcholine.Phosphorylated butyrylcholinesterase is spontaneously hydrolysed, liberating phosphoric acid and the original active butyrylcholinesterase enzyme. This phenomena spontaneous reactivation, which proceeds very slowly and depends on the leaving group of the original organophosphorus inhibitor, but on the residual substituted groups on the phosphorus atom and the source of butyrylcholinesterase enzyme [1, 7].

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 9, Issue 2, June 2014 , p.p(29-41)
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However, organophosphorus-inhibited butyrylcholinesterase changes gradually into a non-reactivatable form on storage, then this phenomena is called aging. It was assumed that the aging might be caused by a migration of the phosphoryl group from an initial position to form more stable bond or by the elimination of serine phosphate to lose serine hydroxyl group. Generally is accepted that spontaneous reactivation and aging mechanism for alkoxyl group of organophosphorus residue bound to butyrylcholinesterase[8, 9]. Recently, the standard treatment for organophosphorus poisoning comprises a mixture of atropine, an oxime, e.g. obidoxime. The oxime compounds that can reactivate organophosphorus-inhibited butyrylcholinesterase by attaching to the phosphorus atom of the organophosphorus compounds forming an oxime-phosphonate, which ruptures away from the butyrylcholinesterase molecule [1, 10].The main aims of this paper were to investigate the rate of spontaneous reactivation of butyrylcholinesterase from plasma and serum of farm animals inhibited by the chlorfenvinphos and trichlorfon. As well as, to investigate the time course of aging of organophosphorus-inhibited butyrylcholinesterase from plasma and serum of farm animals.

2. MATERIALS AND METHODS

Samples were collected from healthy farm animals (sheep, cattle and goats) from local abattoirs were used in this paper. The samples (plasma and serum) were transported on ice to the laboratory for immediate processing. To obtain serum blood samples were allowed to clot for at least 2 h at 30 °C, after which they were centrifuged at 6000 *g* for 15 min. To obtain plasma 4 ml blood samples were added to anticoagulant heparin, 3.7 mg, 0.1% in 10 ml centrifuge tubes. Plasma was separated by centrifugation at 6000 *g* for 10 min [11-13].Butyrylcholinesterase activity was determined by the Ellman method [14], adapted for use with microtitre plates as described by Haigh et al. [15] using butyrylthiocholine iodideas substrate (2 mM final concentration) for measuring butyrylcholinesterase activity. Organophosphorus-inhibited butyrylcholinesterase was prepared by incubating samples with

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 9, Issue 2, June 2014 , p.p(29-41)
ISSN 1992 - 0849

appropriate organophosphorus concentrations for 45 min at 30 °C resulting in an inhibition of 90–98% of control activity. Organophosphorus-treated samples were stored in aliquots at -60 °C until use.In order to remove excess organophosphorus after affecting inhibition the samples by chlorfenvinphos and trichlorfon, the samples were filtrated and the absence of inhibitory activity was tested by incubation of organophosphorus-treated and control butyrylcholinesterase (20 min, 30 °C). Aliquots were taken after various time intervals for determination of butyrylcholinesterase activity (k_s) and of the decrease of oxime-induced reactivation (k_a).Organophosphorus-treated samples were incubated with 250µM obidoxime (45 min.)[16, 17].

Statistical analysis

The method was used to calculate the means and standard errors (SE). Differences with $P<0.05$ were regarded to have statistical significance. The pseudo-first-order rate for constants k_s and k_a were calculated by a non-linear regression analysis[1, 17].

3. RESULTS AND DISCUSSION

This is the first study, which compares the ks and ka of organophosphorus inhibited butyrylcholinesterase in the plasma and serums of farm animals. Phosphorylated butyrylcholinesterase is susceptible to spontaneous hydrolysis of an alkyl-ester bond, resulting in a negatively charged residue which is resistant towards nucleophilic attack [18, 19]. The rate of ksto inhibit erythrocyte butyrylcholinesterase by chlorfenvinphos has been observed to be 0.92 min-1 for cattle [17, 20], 0.347 h-1 for rat [21], while ks values to trichlorfon was 0.012 h-1 for human [21]. The ks and ka kinetics of butyrylcholinesterase inhibited by chlorfenvinphos and trichlorfonwere determined in plasma and serumfor sheep, cattle and goat using Ellman method as described in the section of Materials and Methods **(Tables1 and 3) .** Plasma ks and ka kinetic parameters for reaction between butyrylcholinesterase and two organophosphorus (chlorfenvinphos and trichlorfon) are shown in Table 1. The ks for the three animals was decreased according to the rank order of sheep >cattle >goat for chlorfenvinphos andtrichlorfon.

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 9, Issue 2, June 2014 , p.p(29-41)
ISSN 1992 - 0849

However, ka values in plasma for animals was reduced in order of cattle >goat >sheep for chlorfenvinphos and trichlorfon.The relative activity between chlorfenvinphos and trichlorfon in cattle found highest ks(4) and lowest in ka (0.5). Kinetic ks and ka of all tested animals in plasma gave no correlation between ka and ks (R2 < 0.24) **(Table 1)**.

Table 1. Rate constants for the spontaneous reactivation (ks) and aging (ka) of butyrylcholinesterase inhibited by chlorfenvinphos and trichlorfon from plasma of sheep, cattle and goat.

Inhibitor	Sheep	Cattle	Goat
ks(h-1)			
Chlorfenvinphos	0.645±0.19	0.324±0.05a	0.244±0.04a
Trichlorfon	0.188±0.06	0.081±0.021b	0.068±0.006b
Ratioa	3.4	4	3.7
ka(h-1)			
Chlorfenvinphos	0.028±0.013a	0.053±0.006a	0.045±0.014
Trichlorfon	0.004±0.002 b	0.150±0.007 b	0.067±0.014
Ratioa	7	0.5	0.67

Values in the table are mean±SE obtained from nonlinear regression analysis, each performed in triplicate (n=4 in each animal).Different letters in column is significantly different (ANOVA, P<0.05). aRatio (chlorfenvinphos vs. trichlorfon).

The half time (t1/2) from plasma for ks and ka kinetic parameters for reaction among butyrylcholinesterase and (chlorfenvinphos and trichlorfon) are shown in Table 2.

All farm animals studied in this research, the half time for trichlorfon was higher than chlorfenvinphos for spontaneous reactivation, in contrast with the values of aging **(Table 2).**

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 9, Issue 2, June 2014 , p.p(29-41)
ISSN 1992 - 0849

Table 2. Half times for the ks and ka of butyrylcholinesterase inhibited by chlorfenvinphos and trichlorfon from plasma of sheep, cattle and goat.

Inhibitor	Sheep	Cattle	Goat
ks(t1/2)			
Chlorfenvinphos	1.07 ± 0.16	2.14 ± 0.75a	2.83 ± 0.84a
Trichlorfon	3.69 ± 1.57	8.56 ± 0.98b	10.5 ± 2.87b
ka(t1/2)			
Chlorfenvinphos	24.75 ± 4.55	13.08 ± 1.675a	15.40 ± 1.36
Trichlorfon	28.88 ± 3.54	4.62 ± 0.346b	10.34 ± 3.87

The letters between the chlorfenvinphos and trichlorfon is significantly different (ANOVA, P< 0.05).

Serum ks and ka kinetics for reaction among butyrylcholinesterase and two organ phosphorus (chlorfenvinphos and trichlorfon). The values of ks for animals were decreased according to the rank order of goat >sheep > cattle for chlorfenvinphos while in range sheep >goat >cattle for trichlorfon. Instead, first-order rate constants for ka values was decreased according to the rank order of goat >cattle >sheep for chlorfenvinphos while decreased: sheep >goat> cattle for trichlorfon. Chlorfenvinphos/trichlorfonwere1.9, 8.9 and 18.9, respectively for ks and 0.8, 3 and 50.8, respectively for ka. The comparison between the ks and ka kinetics in the serums of all tested animals gave also poor correlation between ka and ks (R2 < 0.22) **(Table 3).**

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 9, Issue 2, June 2014 , p.p(29-41)
ISSN 1992 - 0849

Table 3. Rate constants for the spontaneous reactivation (ks) and aging (ka) of butyrylcholinesterase inhibited by chlorfenvinphos and trichlorfon from serum of sheep, cattle and goat.

Inhibitor	Sheep	Cattle	Goat
ks(h-1)			
Chlorfenvinphos	0.231±0.046	0.107±0.05a	0.811±0.012a
Trichlorfon	0.123±0.219	0.012±0.03b	0.043±0.05b
Ratioa	1.9	8.9	18.9
ka(h-1)			
Chlorfenvinphos	0.027±0.003	0.034±0.007a	0.653±0.004a
Trichlorfon	0.033±0.015	0.011±0.009b	0.013±0.02b
Ratioa	0.8	3	50.2

Values in the table are mean ± SE obtained from nonlinear regression analysis, each performed in triplicate (n = 4 in each animals).Different letters in column is significantly different (ANOVA, $P < 0.05$). aRatio (chlorfenvinphos vs. trichlorfon).

Half time (t1/2) from serum for ks and ka kinetic parameters for reaction among butyrylcholinesterase and two organophosphorus (chlorfenvinphos and trichlorfon) are shown in Table 4. Again the half time in all farm animals, the trichlorfon was higher than chlorfenvinphos for ks, while chlorfenvinphos for aging was much higher (5-times) than that of trichlorfon **(Table4)**. Other than t1/2for aging was nearly similar in trichlorfon than chlorfenvinphos in goat **(Table4).**

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 9, Issue 2, June 2014 , p.p(29-41)
ISSN 1992 - 0849

Table 4. Half times for ks and kaof butyrylcholinesterase inhibited by chlorfenvinphos and trichlorfon from serum of sheep, cattle and goat.

Inhibitor	Sheep	Cattle	Goat
ks(t1/2)			
Chlorfenvinphos	3.0 ± 0.654	6.48 ± 0.309a	6.24 ± 0.665a
Trichlorfon	5.64 ± 1.232	57.75 ± 5.87b	16.12 ± 1.765b
ka(t1/2)			
Chlorfenvinphos	25.67 ± 3.87	20.38 ± 1.24a	231 ± 9.46a
Trichlorfon	21.0 ± 1.82	63.0 ± 5.77b	53.31 ± 2.76b

The letters between the chlorfenvinphos and trichlorfon is significantly different (ANOVA, P< 0.05).

Comparable to our work the chlorfenvinphos and trichlorfon was higher than above results.However, ks in the plasma proceeded substantially faster with goat chlorfenvinphos and trichlorfon compared with the sheep and cattle. While serum ks was faster with cattle exposed to chlorfenvinphos and trichlorfon compared with sheep and goat (Tables1 and 3). Literature values of the half time (t1/2) of ks for butyrylcholinesterase are about 2 and 58 h for organophosphorus in human erythrocyte [21, 22]. The recovery of rate ka of reserved erythrocyte butyrylcholinesterase by organophosphorus was 2.62 x 104 min-1 and 7.77 x 104 min-1 for cattle and horse, respectively [16]. The serum trichlorfon-inhibited butyrylcholinesterase ka proceeded markedly later than ka of sheep, cattle and goats. In addition, t1/2 of ka for human erythrocyte butyrylcholinesterase is about 41 h for organophosphorus[21], concurring with our results which ranged between 21to 231 h in serum and ranged between 4.62 to 24.75 in plasma **(Table 4).** This indicates that the reduction of chlorfenvinphost1/2 in the plasma and serum might alter the use of oximes. In clinical research, the level and time course of ka is important, because it is the factor that limits the period for useful oximes administration after affecting farm animals with organophosphorus pesticides [1, 16, 23]. Finally,this paper establishes that values

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 9, Issue 2, June 2014 , p.p(29-41)
ISSN 1992 - 0849

of ka and/or ks could play a role on the administration of oximes in the plasma and serums of sheep, cattle and goats.

4. CONCLUSIONS

This research is provided original data concerning an enzymological characterization of these inhibitors in these farm animals. As well as in this research, we surveyed recent developments in our understanding of the kinetic properties of k_s and k_a for sheep, cattle and goat butyrylcholinesterase is comparable in view of interactions with chlorfenvinphos and trichlorfon.In plasma from all three animals studied (sheep, cattle and goats), the rate of the aging process is much slower than spontaneous reactivation of butyrylcholinesterase inhibited with both of them (chlorfenvinphos and trichlorfon). This was also true in serum with chlorfenvinphos and trichlorfon, although the difference was less. Hence, in the case of $t_{1/2}$ butyrylcholinesterase inhibited with trichlorfon the enzyme will tends to become much higher than chlorfenvinphos. The determination of reactivation and aging constants of chlorfenvinphos and trichlorfon organophosphorus pesticides with farm animals butyrylcholinesterase indicates that a structure activity relationship can be derived for inhibition as well as for spontaneous reactivation but not for aging and oxime-induced reactivation.

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Volume 9, Issue 2, June 2014 , p.p(29-41)
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