Journal of Animal and Veterinary Advances 5 (11): 878-881, 2006

Butyrylcholinesterase Levels in Nubian and Saanen Goats in the Highlands of Mexico: Effect of the Organophosphate Fenthion after Topical Aplication

[1]V.O. Fuentes and [2]P.I. Fuentes
[1]Centro Universitario de los Altos, Universidad de Guadalajara, Km 7.5 Carretera a Yahualica, Tepatitlan, Jalisco 47600 Mexico
[2]Hospital PEMEX SUR de alta especialidad, Mexico DF Mexico

Abstract: The main objective of this study was first to study the normal concentration of blood Butirylcholinesterase in Nubian and Saanen Goats. To determine serum cholinesterase (ChE) a kinetic method was used. Normal mean values of ChE in 40 Nubian non lactating goats was found to be 0.031±0.008 U mL^{-1} in 4 lactating Nubian goats butirylcholinesterase values was 0.031±0.008 U mL^{-1} and in four Saanen goats 0.036±0.008 U mL^{-1}. When 10 mg kg^{-1} of Fenthion was applied topically in the dorsal lumbar area, it was observed that serum ChE plummeted presenting a decrease of 64.8% during the first 3 to six days post application 64.5%. Thereafter, normal values similar to those observed before medication, were recorded until 18 and 24 days post application. None of the animals medicated showed signs of toxicity.

Key words: Butyrylcholinesterase, goat, organophosphate, fenthion

INTRODUCTION

Butyrylcholinesterase (BuChE) is an enzyme synthesized in many tissues, including the liver, lungs, heart and brain. Roles for BuChE have been suggested, for example, in lipoprotein metabolism[1], myelin maintenance[2], cellular adhesion, neurogenesis, neurite growth[3], as a scavenger of toxic molecules such ad the organophosphates[4] and in the processing of the amyloid precursor protein[5,6]. In human brain, the enzyme is found in neurones[7], glia[8] and in the plaques and tangles of AD[9]. Furthermore, the activity of BuChE in the brain increases with age (>60 years) and is raised in Alzheimer's Disease (AD)[10]. Butyrylcholinesterase it is sometimes referred to as serum cholinesterase as opposed to red cell cholinesterase (AChE). It hydrolyzes butyrylcholine 4 times more rapidly than acetylcholine. ChE does not hydrolyze D-β-methyl acetylcholine whereas AchE does. Both are inhibited by 10^{-5} M physostigmine. Because the enzyme is so markedly inhibited by organophosphate compounds used as insecticides and neurotoxins, it is widely used in monitoring systems. An immobilized cholinesterase detection device has been described by Goodson *et al.*[11].

Since the 70´s Fenthion has been used as an insecticide for the control of ectoparasites such as flies[12] and lice[13]. Fenthion is not used in Europe and other first world country, however in third world countries it is still used, recommended for the treatment of endoparasites such as Dirofilaria immitis. In Mexico the organophosphate Fenthion (Tiguvon Spot On, Bayer Mexico) is at present used in range managed cattle due to easy of application.

The mechanism of action of this phosphorous organic compound is due to the inactivation of the enzyme Butyrylcholinesterase; responsible for the termination of the physiological action of acetylcholine. Therefore this drug is classified as an anticholinesterase inhibitor. Also called indirect parasympathomimetic drug.

Fenthion is used extensibly in Mexico in different animal species such as: cows, sheep, deer, pigs, dogs, cats and some birds. Birds are very sensitive to fenthion[14]. The topical application of this drug, is followed by the onset of side effects due to parasympathetic stimulation; therefore this organophosphate derivative can produce severe toxic effects if the dose is not computed with certainty[15].

Up to date information related to normal values of blood cholinesterase in goats and also on the effect of this drug in goats is not readily available. For this reason, it was considered of interest first to study the concentration of normal serum cholinesterase in goats and there after the effect of the topical application of a commercially availabe organophosphate is many developing countries such as Fenthion in serum BuChe levels in goats.

Corresponding Author: V.O. Fuentes, Centro Universitario de los Altos, Universidad de Guadalajara, Km 7.5 Carretera a Yahualica, Tepatitlan, Jalisco 47600 Mexico

MATERIALS AND METHODS

The goats for this study belong to a local goat milk producer, they are housed in open barns in the high planes of Mexico (19° Lat. 13° N). Fed with chopped hay and supplemented with 250 g of a commercial pelleted food (Purina Goat mix, Mexico) and water ad libitum.

The determination of butrylcholinesterase (BuChe): For the determination of normal values of BuChe 40 non lactating Nubian goats were used, age fluctuated from 2 to 5 years and a mean weight of 45 ±8 kg. Four adult Saanen goats (two 3 year old non-lactating does of 40 and 45 kg body weight and two 4 years old bucks of 57 and 60 kg of body weight) and 4 lactating Nubian goats with a mean body weight of 50±4.5 kg were also chosen to study their serum cholinesterase activity. Goats were housed in open paddocks with water ad libitum, they were range fed from 08:00 to 17:00 h and on their return they were supplemented with 250 g/head of a commercial concentrate (Small Ruminants, Purina, Mexico).

Blood samples were taken twice a week for 40 days. Blood samples were centrifuged and cholinesterase levels immediately determined. There after Blood BuChE levels were determined using a commercial colorimetric kit (SIGMA, Mexico) following known standard methods[16-18]. In this procedure, the hydrolysis of propionylthiocholine liberates thiocholine, that reacts with 6-6'-Dithiodinicotinic Acid (DTNA) to yield thionicotinic acid, which has an optimal a bsorption wavelength at 340 nm. The increase in absorbance at 340 nm is proportional to enzyme activity[19,20].

The effect of fenthion on blood cholinesterase levels: The dose of Fenthion (Tiguvon Spot On, Bayer Mexico) used was 10 mg kg^{-1} applied topically in the withers. The vehicle for the drug was dimethyl sulphoxide

Blood samples (5 mL) were taken at the moment of drug administration and 90 min later. Thereafter samples were taken every third day for 30 days.

To evaluate the results a simple Student T test was used.

RESULTS

The values for serum BuChe in 40 Nubian non lactating and 4 lactating goats, was found to be 0.031±0.0057 U mL^{-1}. In 4 Saanen goats Buche levels were in the order of 0.0037 U mL^{-1} Table 1.

In Table 2, it can be observed that 90 min after the topical application of Fenthion (10 mg kg^{-1}) in four Nubian goats, serum cholinesterase levels decreased to <50%. At three days post administration, there was a further reduction in the levels of the enzyme 67.8%. On day six, slight recovery 64.4% was detected. From this point Che levels showed a continuous recuperation Table 2. The graphic representation of these results can be observed in Fig. 1.

In Table 3, we can observe the effect of fenthion 1.5 h after topical application in Saanen goats, in which there was a 43.3% inhibition in serum Che levels; At 3 days a

Table 1: Normal Values of serum cholinesterase for the non lactating nubian goat U mL^{-1} or KU mL^{-1}

Number of goats	Standard error	Calculated error	Average	Range	Variance
40 Nubian	0.031	0.021	0.0057	0.00090	0.0000328
4 Nubian lact.	0.031	0.020	0.0055	0.00089	.0000320
4 Saanen	0.037	0.027	0.0059	0.00091	0.000319

Table 2: The effect of topical fenthion (10 mg kg^{-1}) on the serum cholinesterase levels of 4 lactating nubian goats U mL^{-1} or KU mL^{-1}

Time h or days	Value average	% Inhibition
0	0.031±0.008	
1.5 h	0.015±0.009	51.6
3 d	0.010±0.007	67.8
6 d	0.011±0.009	64.5
9 d	0.014±0.007	54.8
12 d	0.017±0.008	45.2
15 d	0.019±0.009	38.7
18 d	0.021±0.008	32.3
21 d	0.024±0.007	22.6
24 d	0.026±0.009	16.1
27 d	0.026±0.008	16.1
30 d	0.029±0.009	6.5

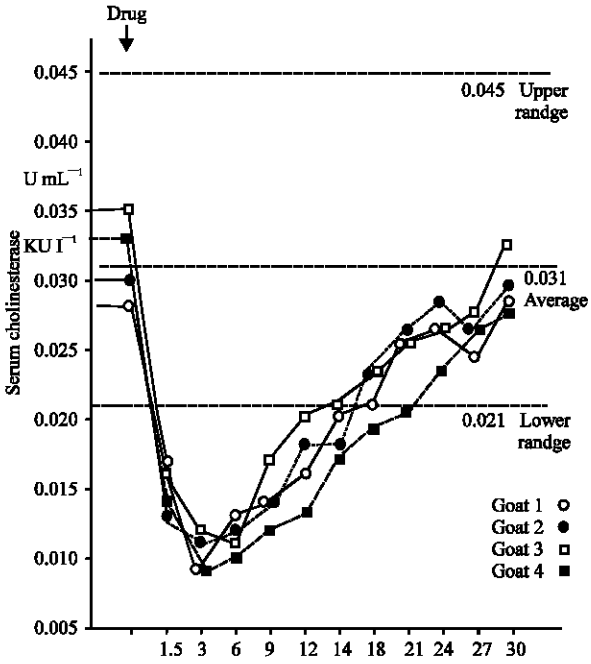

Fig. 1: The effect of the organophosphate Fenthion (10 mg kg^{-1}) on the serum Butyryl Cholinesterase levels of the non lactating Nubian goat

Table 3: The effect of topical fenthion (10 mg kg^{-1}) on the serum cholinesterase levels of saanen goats (n = 4) U mL^{-1} or KU mL^{-1}

Time h or days	Value average	% Inhibition
- 3 d	0.036±0.008	
-2 d	0.038±0.009	
-1 d	0.037±o.008	
0 h	0.037±0.007	
1.5 h	0.021±0.009	43.3
3 d	0.015±0.009	59.5
6 d	0.017±0.008	54.1
9 d	0.015±0.009	58.4
18 d	0.014±0.008	61.2
21 d	0.017±0.009	52.8
24 d	0.020±0.008	44.5
27 d	0.028±0.008	22.2
30 d	0.029±	19.4

59.9% inhibition was observed, The effect of the organophosphate drug on serum cholinesterase activity was similar to the one observed in Nubian does.

DISCUSSION

In the preliminary experiment using 40 Nubian goats it can be noted that the serum cholinesterase levels are lower than the ones reported for other species[21].

The topical administration of Fenthion at a dose level of 10 mg kg^{-1} in goats depressed rapidly serum cholinesterase and one hour serum cholinesterase was depressed up to 67.8%.

It is surprising to observe that signs of toxicity were absent, probably because the concentration of serum cholinesterase after inactivation with Fenthion, is sufficient to permit normal function of the Cholinergic Autonomic System.

The effect of the drug shows two events:

- That the drug is readily absorbed from or after topical application and
- The drug produces a rapid inactivation of the enzyme.

When Saanen does and bucks were medicated with fenthion Fig. 1, Table 2 the degree of enzyme inhibition was not as marked as the one observed in the lactating does. This might be due to the fact that the Saanen animals were in a better physiological condition because they were not lactating and this latter status can be considered as a physiological burden.

Recuperation, of the enzyme towards basal levels, was very slow. When figure one is studied, it can be observed that cholinesterase levels were diminished for further 15 days after application of the drug. This is probably due to the time necessary to replace the enzyme inactivated by Fenthion, as we know that this pharmacological agents inactivate the enzyme irreversibly. Therefore, for the purpose of replenishing normal enzyme activity, we could consider that the lower range of normal cholinesterase activity is 0.021±0.005 U mL^{-1}. Therefore, when cholinesterase levels of treated animals returned to the levels observed in normal animals, it was then considered that the effect of the drug was terminated. However, it can be observed in Fig. 1 that basal levels (range 0.031 U mL^{-1}) were not attained until after 3O days of medication, time when serum cholinesterase levels reached 0.029 U mL^{-1} in Fenthion treated goats.

From this study we can conclude that the use of these drugs should be directed towards healthy animals, because when used in animals suffering of disease they are more susceptible to the toxic effects of the drug, as has been observed to happen in other species[14]. At an international level mandatory regulations are needed in all countries (spetially in third world countries) for the appropriate disposal of animal wastes and residuals of these organo phosphate drug.

REFERENCES

1. Kutty, K.M. and R.H. Payne, 1994. Serum pseudocholinesterase and very-low-density lipoprotein metabolism. J. Clin. Lab Anal, 8: 247-250.
2. Earl, C.J. and R.H. Thompson, 1952. Cholinesterase levels in the nervous system in tri-ortho-cresyl phosphate poisoning. Br. J. Pharmacol, 7: 685-694.
3. Layer, P.G., 1995. Novel functions of cholinesterases in development, physiology and disease. Prog Histochem Cytochem, 29: 1-94.
4. Schwarz, M., D. Glick, Y. Lowenstein and H. Soreq, 1995. Engineering of human cholinesterases explains and predicts diverse consequences of administration of various drugs and poisons. Pharmacol Ther., 67: 283-322.
5. Balasubramanian, A.S., 2001. Amyloid beta peptide processing, insulin degrading enzyme and butyrylcholinesterase. Neurochem Res., 26: 453-456.
6. Darvesh, S., R. Kumar, S. Roberts and R. Wash, E. Martin, 2001. Butyrylcholinesterase-mediated enhancement of the enzymatic activity of trypsin. Cell. Mol. Neurobiol, 21: 285-296.
7. Darvesh, S., D.L. Grantham and D.A. Hopkins, 1998. Distribution of butyrylcholinesterase in the human amygdala and hippocampal formation. J. Comp. Neurol, 393: 374-390.
8. Wright, C.I., C. Geula and M.M. Mesulam, 1993. Neuroglial cholinesterases in the normal brain and in Alzheimer's disease: Relationship to plaques, tangles and patterns of selective vulnerability. Ann. Neurol., 34: 373-384.

9. Geula, C. and M.M. Mesulam, 1995. Cholinesterases and the pathology of Alzheimer disease. Alzheimer Dis Assoc Disord., 9(Suppl. 2): 23-28.
10. Perry, E.K., 1978. Changes in brain cholinesterases in senile dementia of Alzheimer type. Neuropathol Appl. Neurobiol, 4: 273-277.
12. Andrews, A.H., D.R. Melrose and W.H. Lewis, 1976. Efficacy of organophosphorus warble fly dressing Vet. Record, 99: 187-188.
13. Kettle, P.R., 1972. Pour-on insecticides for the control of Linognathus vituli New Zealand Vet. J., pp: 20-167.
14. Goodson, L., W. Jacobs and A. Davis, 1973. An Immobilized Cholinesterase Product for Use in the Rapid Detection of Enzyme Inhibitors in Air or Water, Anal Biochem., pp: 51-362.
14. Zinkl, J.G., D.A. Jessup, A.I. Bischoff, T.E. Lew and E.B. Wheeldon, 1981. Fenthion poisoning of wading birds J. Wildlife Dis., 17: 117-119.
15. Andrews, A.H., 1981. Abnormal reactions and their frequency in cattle following the use of organophosphorus warble fly dressings, Vet. Record, 109: 171-175.
16. Ellman, G.L., K.D. Courtney, V. Andres and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.
17. Garry, P.J. and J.I. Routh, 1965. A Micro Method for Serum Cholinesterase. Clinical Chemistry, 11: 91-96.
18. Garry, P., I. Schreibman and J. MacQueen, 1971. Cholinesterase Determination, Clinical Chemistry, pp: 17-1145.
19. Jiménez, D.M. and M.V. Martínez, 2000. Validación de la determinación de colinesterasa plasmática humana a 340 nM (Validation of the determination of plasma cholinesterase at 340 nM). Rev Biomed, 11: 91-98.
20. Tecles, F. and J.J. Cerpm, 2001. Determination of whole blood cholinesterase in different animal species using specific substrates Res. Vet. Sci., 70: 181-293.
21. Mohammad, F.K., G.A.M. Faris, A.S. Alias and B.K.H. Al-baggou, 2005. Blood Cholinesterase activities in Cattle, sheep and gotas measured with a modified electrometic method. J. Anim. Vet. Adv., pp: 4-9.