Application of an electrometric method for measurement of *in vitro* inhibition of blood cholinesterases from sheep, goats and cattle by dichlorvos and carbaryl

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ABSTRACT: A modified electrometric method was described in sheep, goats and cattle and used to demonstrate *in vitro* inhibition of plasma and erythrocyte cholinesterase activities by the organophosphate and carbamate insecticides dichlorvos and carbaryl, respectively. A typical reaction mixture for the measurement of cholinesterase activity contained 3 ml distilled water, 3 ml barbital-phosphate buffer (pH 8.1), 0.2 ml plasma or erythrocytes and 0.1 ml acetylthiocholine iodide (7.5%) as a substrate. The mixture was incubated at 37°C for 30 min in sheep, 40 min in goats and 20 min in cattle. The pH of the reaction mixture was determined by a pH meter before and after the incubation. The initial pH was measured before the substrate addition. The enzyme activity was expressed as ΔpH/incubation time = (pH1 – pH2) – ΔpH of blank. The method of inhibitor-cholinesterase incubation was used to measure the *in vitro* inhibition of plasma and erythrocyte cholinesterase activities. Dichlorvos in concentrations of 0.5 and 1 μm inhibited plasma and erythrocyte cholinesterase activities by 24–85%, whereas carbaryl in concentrations of 5 and 10 μm inhibited them by 50–89%. The results suggest that the described electrometric method could be efficiently used for detecting cholinesterase inhibition in ruminants, and further point to the value of the present experimental protocol of *in vitro* cholinesterase inhibition in preliminary toxicological examinations of anticholinesterase compounds.

Keywords: cholinesterase; organophosphate; carbamate; electrometric method; *in vitro*; ruminant

Determination of blood (plasma or erythrocyte) cholinesterase (ChE) activities is a non-invasive and useful tool for monitoring the exposure of animals to organophosphate and carbamate insecticides (Fairbrother et al., 1991; Wilson, 1998, 1999). Various colourimetric and electrometric methods are available for measurement of ChE activity (Witter, 1963; Wills, 1972; Fairbrother et al., 1991; Wilson, 1999). One of the principle methods for measuring blood ChE activity is the electrometric method which is based on the hydrolysis of acetylcholine and production of acetic acid, which in turn decreases the pH of the reaction mixture (Witter, 1963; Wills, 1972; Mohammad and St. Omer, 1982).

The original electrometric method of Michel (1949) is most commonly used in humans (Wills, 1972; Fairbrother et al., 1991; Wilson, 1999). However, the method is not directly applicable to blood of different animal species (Witter, 1963; Wills, 1972; Fairbrother et al., 1991; Wilson, 1999). This is due to the inherent variations of blood ChE activities of different animal species (Mohammad and St. Omer, 1982; Wilson, 1999, Al-Qarawi and Ali, 2003), and the special need for different buffer compositions, reaction temperatures, incu-

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bation times and sample volumes (Callahan and Kruckenberg, 1967; Silvestri, 1977; Mohammad and St. Omer, 1982; Mohammad et al., 1997). In addition, the original electrometric method is not used in cases of carbamate poisoning, since carbamylated ChE is unstable in the reaction mixture due to sample dilution and long incubation time (60 min) (Williams and Casterline Jr., 1969; Wills, 1972; Nostrandt et al., 1993).

Various modifications of the electrometric method have been advocated for measuring blood ChE activity in animals (Wills, 1972; Silvestri, 1977; Mohammad and St. Omer, 1982; Mohammad et al., 1997; Wilson, 1999). One of these modifications of the electrometric method is that of Mohammad et al. (1997) which was introduced for rapid measurement of erythrocyte and plasma ChE activities in sheep. The method is characterized by its simplicity, reproducibility, accuracy and one-step short incubation time (Mohammad et al., 1997; Abass and Mohammad, 2004; Al-Jobory and Mohammad, 2004).

The technique of *in vitro* ChE inhibition by organophosphates and carbamates has various toxicological implications, and can be used to assess the potential toxicity of antiChE compounds (Iyaniwura, 1990; Karanth and Pope, 2003; Long et al., 2003). The purpose of the present study was to further validate the electrometric method described earlier in sheep (Mohammad et al., 1997), and to determine its ability in detecting *in vitro* ChE inhibition by the organophosphate insecticide dichlorvos and the carbamate insecticide carbaryl. For this purpose, the blood of sheep, goats and cattle were used as the enzyme source; plasma and erythrocytes contain mainly pseudoChE and trueChE, respectively (Wilson, 1999).

MATERIAL AND METHODS

Domestic 1–2 year old goats, sheep and cattle of both sexes were used in the study. The animals were apparently healthy, and were not exposed to any insecticide for at least one month prior to blood sampling.

Blood samples were collected from the jugular vein using heparinized test tubes (Coles, 1986). Plasma was separated from erythrocytes by centrifugation at 3 000 rpm (Centurion, U.K.) for 15 minutes. The plasma and erythrocyte samples of 4–5 animals from each species were pooled for ChE inhibition experiments.

Preliminary experiments were conducted to optimize the conditions of the ChE assay for sheep, goats and cattle, depending on previous studies in sheep (Mohammad et al., 1997) and goats (Al-Jobory and Mohammad, 2004). The modified electrometric method of Mohammad et al. (1997) was used to measure plasma and erythrocyte ChE activities of the animals. For a typical assay, the reaction mixture in a 10-ml beaker contained 3 ml distilled water, 0.2 ml plasma or erythrocytes and 3 ml, pH 8.1 buffer. The pH of the mixture (pH1) was measured with a glass electrode using a pH meter (Hanna Instruments, Romania), and then 0.1 ml of 7.5% aqueous solution of acetylthiocholine iodide (BDH, U.K) was added to the mixture, which was then incubated at 37°C for 30 min in sheep, 40 min in goats and 20 min in cattle. At the end of the incubation period, the pH of the reaction mixture (pH2) was measured. The enzyme activity was calculated as follows:

$$\text{ChE activity } (\Delta\text{pH/incubation time}) = (\text{pH1} - \text{pH2}) - \Delta\text{pH of blank}$$

All measurements were done in duplicate, and the blank was without plasma or erythrocytes. The pH 8.1 buffer consisted of 1.237 g sodium barbital (BDH), 0.63 g potassium dihydrogen phosphate (E-Merck, Dermstadt, Germany) and 35.07 g sodium chloride (BDH)/1 l of distilled water (Mohammad et al., 1997). The pH of the buffer was adjusted to 8.1 with 1 N HCl.

The method of inhibitor-ChE incubation was used to measure the *in vitro* inhibition of plasma and erythrocyte ChE activities (Mohammad et al., 1997; Karanth and Pope, 2003) by dichlorvos (Al-Tariq Co., Iraq) and carbaryl (Sociedad Anonima De Agroquimicos, Spain). The insecticides were separately added to the reaction mixtures of the plasma or erythrocytes, and the final concentrations obtained for each insecticide in the reaction mixtures were as follows:

Dichlorvos: 0 (base-line control), 0.5 and 1 μm

Carbaryl: 0 (base-line control), 5 and 10 μm

The reaction mixtures containing the insecticides were incubated at 37°C for 10 minutes. Thereafter, the residual ChE activity in the mixture was measured as described above. The percentage of enzyme inhibition was calculated as follows:

% ChE inhibition = ChE activity (without insecticide) – ChE activity (with insecticide)/ChE activity (without insecticide) × 100

RESULTS

Tables 1, 2 and 3 show the *in vitro* inhibition of plasma and erythrocyte ChE activities in sheep, goats and cattle, respectively by dichlorvos and carbaryl.

Dichlorvos in a concentration-dependent manner inhibited plasma and erythrocyte ChE activities *in vitro* in the three animal species by 24 to 85% (Tables 1–3). Similarly, carbaryl inhibited plasma and erythrocyte ChE activities in a concentration-dependent manner by 50 to 89% (Tables 1–3).

DISCUSSION

The present electrometric method described for the measurement of blood ChE activities in sheep, goats and cattle depended mainly on a procedure introduced earlier (Mohammad et al., 1997). The method has been applied successfully for the determination of blood or tissue ChE activities in se-

Table 1. *In vitro* inhibition of sheep plasma and erythrocyte cholinesterase (ChE) activities by dichlorvos and carbaryl

Inhibitor concentration (μM)	Plasma ChE		Erythrocyte ChE	
	ΔpH/30 min	inhibition (%)	ΔpH/30 min	inhibition (%)
Dichlorvos				
0	0.195		0.585	
0.5	0.105	46	0.340	42
1.0	0.085	56	0.225	62
Carbaryl				
0	0.200		0.480	
5	0.100	50	0.180	63
10	0.040	80	0.100	80

Measurements were done in duplicate

Table 2. *In vitro* inhibition of goat plasma and erythrocyte cholinesterase (ChE) activities by dichlorvos and carbaryl

Inhibitor concentration (μM)	Plasma ChE		Erythrocyte ChE	
	ΔpH/40 min	inhibition (%)	ΔpH/40 min	inhibition (%)
Dichlorvos				
0	0.205		0.525	
0.5	0.155	24	0.270	49
1.0	0.090	56	0.190	64
Carbaryl				
0	0.260		0.490	
5	0.080	69	0.145	70
10	0.060	77	0.055	89

Measurements were done in duplicate

Table 3. *In vitro* inhibition of cattle plasma and erythrocyte cholinesterase (ChE) activities by dichlorvos and carbaryl

Inhibitor concentration (μM)	Plasma ChE		Erythrocyte ChE	
	ΔpH/20 min	inhibition (%)	ΔpH/20 min	inhibition (%)
Dichlorvos				
0	0.195		1.000	
0.5	0.070	64	0.470	53
1.0	0.030	85	0.330	67
Carbaryl				
0	0.190		0.865	
5	0.085	55	0.220	75
10	0.030	84	0.145	83

Measurements were done in duplicate

veral animal species (Faris et al., 1999; Al-Baggou' and Mohammad, 1999; Mohammad et al., 1999, 2002; Abass and Mohammad, 2004; Mohammad and Al-Baggou', 2005). The present study is the first attempt to validate the described electrometric procedure in detecting *in vitro* ChE inhibition by dichlorvos and carbaryl collectively in three ruminant species.

In vitro inhibition of plasma and erythrocyte ChE by dichlorvos and carbaryl in sheep, goats and cattle is in agreement with the reported antiChE effects of these insecticides (Anonymous, 1986a,b; Abdelsalam, 1987; Khan et al., 1990; Mohammad et al., 1997; Wilson, 1998). The present findings suggest that the sensitivity of the described method in detecting ChE inhibition is caused by organophosphates or carbamates. Further ChE inhibition should not be excluded from this *in vitro* system during the 20 to 40 min incubation time. However, the original electrometric method cannot be recommended for the detection of ChE inhibition induced by carbamates (Witter, 1963; Williams and Casterline Jr., 1969; Wills, 1972). Carbamylated ChE is unstable in the reaction mixture of the electrometric method of Michel because of the considerable sample dilution, and long incubation time (>60 min) (Williams and Casterline Jr., 1969; Wills, 1972; Nostrandt et al., 1993). Therefore, an additional advantage of the present experimental protocol lies in its ability to detect ChE inhibition caused by carbamates.

In vitro ChE inhibition is a useful technique for detecting the potential antiChE activity of chemicals such as organophosphates and crabamates (Iyaniwura, 1990; Karanth and Pope, 2003; Long et al., 2003). The results of the present study indicate the efficiency of the described electrometric method in detecting ChE inhibition, and extends its value to use the present experimental protocol for *in vitro* ChE inhibition in preliminary toxicological examinations of antiChE compounds.

Previous reports from our laboratory also indicated the efficiency of the method in detecting ChE inhibition induced by organophosphates or carbamates *in vivo* in other animal species (Al-Baggou' and Mohammad, 1999; Aldabagh and Mohammad, 1999; Faris et al., 1999; Mohammad et al., 1999; 2002; Abass and Mohammad, 2004; Mohammad and Al-Baggou', 2005). Further studies are needed on the application of the described method on ruminants exposed to organophosphate or carbamate insecticides.

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