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Acetyl Cholinesterase Activity Decreases by Time after Death in Cow

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Abstract: Normal value of acetylcholinesterase (AChE) activity in brain of cattle was measured due different days after death and different period of storage to find out how its activity might influence the reliability of this biomarker in diagnosis of organophosphate and Carbamate poisoning. For this reason, 60 cows' heads were collected from Tehran abattoir to measure the activity of acetylcholine esterase enzyme in the brain at different times of sampling after slaughter and different period of storage. The enzyme activity was measured in the brain homogenate immediately, 24 and 48 h after slaughter and 40 and 60 days after freezing at -20°C. The results of this study showed that a significant ($p<0.05$) reduction in the enzyme activity at 48 h post slaughter and 60 days after freezing occurred.

Key words: Cow, acetylcholine esterase activity, brain

INTRODUCTION

ChE activity consists of both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) and certain OP insecticides are known to preferentially inhibit either AChE or BChE (Karnth *et al*., 2007). Toxicity with most of organophosphate insecticides leads to the inhibition of acetylcholine esterase in the nervous system of animals (Sturm *et al*., 2000; Stacey and Sultatos, 2002). Since the discovery of the mechanism of toxicity of organophosphorous compounds and carbamates the rate of activity of choline esterase inhibition in tissues and body fluids such as blood, plasma, RBCs and kidney, liver and brain has been recognized as an appropriate index and proper criterion to diagnose the poisoning by accidental exposure or overdosing (Car *et al*., 1997; De La Torre *et al*., 2002; Tecles and Ceron, 2001). These compounds by binding to the active site of acetylcholine esterase could block the enzyme (Gupta, 2007). Based on this mechanism study have been developed to estimate the rate of acetylcholine esterase activity in different organs. By measuring the rate of acetylcholine esterase activity in different livestock organs it is possible to diagnosis the poisonous by organ phosphorous compounds. Nowadays, Determaination of cholinesterase activity is a routine practice in many laboratories to detect the influence of ChE inhibitory drugs or Organophosphate/Carbamate pesticides. The activity of acetylcholine esterase in the brain of cattle, sheep, horse, pig and some birds has been reported and indicated that the diagnosis of toxicity with anticholiesterases could be possible by knowing the normal value of them (Blakley and Skelly, 1988). Since some times sampling of the poisoned animal have been done with delay or measuring of AChE activity is not possible at the time of delivering the samples to the lab, so the present study was performed to find out whether time of sampling of brain in unopened head and different period of storage could influences this biomarker activity in cow which is critical in diagnosis of Organophosphate/Carbamate pesticides poisoning.

MATERIALS AND METHODS

To evaluate the acetylcholine esterase activity in the brain of cows at different time after slaughter, at the summer of 2007, 60 heads were collected from Holstein cows slaughtered at Tehran

abattoir. The heads were sent to the division of toxicology of Tehran university veterinary college where they were classified in 3 groups regarding the day of slaughter: group 1, immediately after slaughter and groups 2 and 3, 24 and 40 h after slaughter respectively. Each group included 20 cow's heads. The whole brain was removed intact from the skull and splinted in 2 hemispheres. One hemisphere of each group was kept in -20°C for 40 and 60 days and another hemisphere was sent to the laboratory for the measurement of acetylcholine estrase immediately after removal from the skull. In the laboratory hemispheres of each group were homogenized using a grinder. One gram of the homogenized brain was mixed with phosphate buffered (pH 8), then 2.9 mL of chromogene solution was added to 5 mL of the homogenized brain. The samples were read by spectrophotometer at 412 nm wave length as blanks and after adding the substrates the absorbance would be read at 30 sec intervals for 4 min. Then the rate of cholinesterase activity was calculated on the base of μmol min g^{-1} of the brain (Ellman *et al*., 1961). All the brains at necropsy had no macroscopic lesions and were intact. Putrefaction was not observed in the brain at the day of slaughter and 24 h later but at 48 h post-slaughter. Although there were no changes in the appearance of the brains, some changes in color and consistency as well as their odor occurred.

RESULTS

The results of enzyme activity at different times of post slaughter are shown in Table 1 and in the Table 2 the enzyme activity is shown after freezing the brain at -20°C for 40 and 60 days.

According to results in Table 1, the mean of enzyme activity were 3.41±0.37, 3.16±0.37 and 2.76±0.45 in groups 1, 2 and 3, respectively. Meanwhile, according to Table 2, which is related to different period of storage, the mean of enzyme activity were 3.41±0.37, 2.82±0.62 and 2.68±0.50 in groups 1, 2 and 3, respectively. As it is shown in Table 1 a significant difference between acetylcholine esterase activity immediately after with 24 h after slaughter was not seen but at 48 h post slaughter a significant ($p<0.05$) reduction in acetylcholine activity was shown. The activity of acetylcholine esterase at the day of slaughter, 40 and 60 days after freezing is shown in Table 2. At the day of slaughter the activity of acetylcholine esterase was significantly ($p<0.05$) higher than the activity of the enzyme 60 days after freezing. There was a non-significant reduction at 40 days after freezing when the results were compared with the day immediately after slaughter. The results of this study showed that the time of sampling and the method of keeping samples could have effect on the enzyme activity.

Table 1: Cholinesterase activity in the brain of cattle, sampling in different days after slaughter (μmol min^{-1} g^{-1})

Groups	No. of cases	Mean	Min-Max
1	20	3.41±0.37[a]	2.87-3.95
2	20	3.16±0.37[a]	2.48-3.91
3	20	2.76±0.45[b]	2.17-3.82

Group 1: 20 brains of cattle immediately after slaughter, Group 2: 20 brains of cattle 24 h after slaughter, Group 3: 20 brains of cattle 48 h after slaughter. [a]: Non-significant ($p \geq 0.05$), [b]: Significant ($p \leq 0.05$)

Table 2: Cholinesterase activity in the brain of cattle at different time after freezing (μmol min^{-1} g^{-1})

Groups	No. of cases	Mean	Min-Max
1	20	3.41±0.37[a]	2.87-3.95
2	20	2.82±0.62[a]	1.56-3.28
3	20	2.68±0.50[b]	1.89-3.55

Group 1: 20 brains of cattle immediately after slaughter, Group 2: 20 brains of cattle 30 days after freezing, Group 3: 20 brains of cattle 60 days after freezing. [a]: non-significant ($p \geq 0.05$), [b]: Significant ($p<0.05$)

DISCUSSION

Cholinesterase is a neurotransmitter with an important role in the nervous system and is hydrolyzed by acetylcholine esterase shortly after its release. There are many anticholinesterases among which organophosphorous and carbamate insecticides are the most important. These compounds exert their toxic effects through inhibiting the cholinesterase enzyme (Ecobicon, 2001). According to the latest reports assessing of cholinesterase activity is the most important index in anticholinesterases poisoning diagnosis (Gupta, 2007; Brahmi *et al*., 2006). There are many reports indicating that body fluids and the brain could be used to assess acetylcholine esterase activity (Blakley and Yole, 2002; Abdelsalam and Ford, 1985). Although some studies revealed that acetylcholine esterase activity in blood is not a sensitive parameter in cases that were against with Organophosphate pesticides (Worek *et al*., 2005). The normal value for cholinesterase activity has been reported to be at the range of 2.06±30 μmol min^{-1} g^{-1}. Harlin *et al*. (1989) showed the value for cholinesterase activity in the bovine retina before and after exposure to the anticholiesterase insecticide Tebufos was 2.68±0.61 and 0.73±0.17, respectively. In another study Abdelsalam and Ford (1985) measured the cholinesterase activity in plasma, whole blood and tissues in cows and reported that the minimum activity of the enzyme to be in the kidney. They showed the enzyme activity in the brain was 3.01±0.19 μmol min^{-1} g^{-1}. An interspecies difference regarding the enzyme activity has also been reported by Halbrook *et al*. (1992) and Mount (1981). These investigators showed that the enzyme activity in cattle was lower than sheep. In sheep interbreed differences in acetyl cholinesterase activity in the brain were observed; therefore the brain is not a suitable site of sampling for the enzyme assessment in this species. The cholinesterase activity in various parts of the brain does show regional differences (Bajgar *et al*., 2007; Mount, 1981). Although these differences appear to be substantially less than much of the interspecies variation observed in some studies. Since the enzyme activity measurement in the whole brain is time consuming and difficult therefore it has been suggested that a certain area such as brain hemispheres should be used for the enzyme activity assessment (Blakley and Skelly, 1988). In the present study a few hours after slaughter the amount of acetyl cholinesterase was 3.39±0.42 μmol mim^{-1} g^{-1} and significantly reduced 48 h post-slaughter. Our finding is in line with the study of Harlin *et al*. (1989) who showed that acetylcholinesterase activity in bovine retina is continuously decreased after death. According to Mount (1981) brain ChE activity in animals dead for 1 to 3 days would show little decrease, but a significant decrease could occur in animals dead for several days in warm condition, as observed in brain samples stored at 37°C. We also noticed that long time freezing of the brain can significantly reduce the enzyme activity either. Other study also suggested differences from frozen brains of birds. Zinkle *et al*. (1980) revealed that temperature of -18 to -22°C caused deterioration in brain ChE activity where is -40 to -76°C did not produce deterioration in brain ChE activity during 1 month. Although Mount (1981), had reported no differences between cholinesterase activity for cattle, swine and sheep brains held at refrigerated (4°C) and freezing temperatures (-22°C) a 3 months. In conclusion, combination with (Mount, 1981) it is suggested that at the time of poisoning with anticholiesterase insecticides the best time for sampling to assess the brain's cholinesterase activity would be 24 h after death in dead animal and also according to our results, keeping the samples for a long time more than 30 days in a freezing condition (-20°C) may interfere in the results of assessment.

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