In vitro addition of ATE offsets the allethrin induced biochemical and biophysical changes in biomembranes

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Citation

M Narendra, G Kavitha, A Kiranmai, N Varadacharyulu. *In vitro addition of ATE offsets the allethrin induced biochemical and biophysical changes in biomembranes*. The Internet Journal of Toxicology. 2007 Volume 5 Number 2.

Abstract

Allethrin is a widely used insecticide in India and other countries to get protection from mosquitoes and other insects, exposing the population to the risk of toxicity. Earlier studies demonstrated that allethrin is toxic at 6-10µM concentrations affecting voltage gated sodium and calcium channels. Since membranes are the chief targets for the action of allethrin, present study is aimed at evaluating the biochemical changes and physico-chemical properties of erythrocyte membrane at 7µM concentration which inhibited calcium channels. Altered cholesterol and phospholipids contents and the consequent cholesterol/phospholipids (C/P) ratio, lipid peroxidation (LPO) and osmotic fragility (OF) were observed in the present study. In vitro addition of 0.5 ml aqueous ATE (Aqueous Tea Extract 2.5%) to red cells resulted in restoration of the above membrane parameters to normal levels. Probably antioxidant and therapeutic properties of ATE might have contributed for the same which needs further study.

INTRODUCTION

The widespread use of allethrin and also accumulation of its metabolites in humans have made it essential to determine the molecular targets of this pyrethroid to evaluate the risks of its use [1]. Membranes are chiefly responsible for the characteristic effects of allethrin and certain membrane channels, pumps and receptors that are affected by allethrin have been identified [2,3,4,5,6]. Due to its lipophilicity allethrin interacts with lipid domains of cells and membranes [7]. However the precise molecular events and interactions related to membrane lipid components are not clear. Allethrin (6-10µM) appears to be toxic affecting various physiological functions [8]. Hence the present study is aimed at studying the structural as well as functional changes and integration at 7µM where it affects calcium channels and other pumps [9,10,11,12,13,14]. In the present paper haemolytic behaviour of red cells which reflects the functional and structural integrity and physicochemical properties of membranes of erythrocytes that are pre incubated with allethrin when exposed to different concentrations of NaCl were investigated and were compared with erythrocytes that were pre incubated in a medium containing allethrin and ATE (Aqueous Tea Extract) [15]. Besides biochemical changes in membrane key lipid profile chiefly cholesterol and phospholipids concentrations were studied and comparison has also been made with that of controls.

MATERIALS AND METHODS

COLLECTION OF BLOOD AND ANALYSIS

Blood was drawn from volunteers between aged 40-45 (Mean age 42±2 years) at 7-10 am into heparinized test tubes. Plasma and red cells were separated and used for analysis. In an experiment the red cells were incubated for 30 min in a medium containing allethrin 7µM concentration (0.5 ml) and another set of red cells were incubated for 30 min in a medium containing allethrin + ATE (0.5 ml) for 30 min and to see osmotic fragility and lipid composition similarly a control set was run. Erythrocyte membrane proteins were estimated by the method of Lowry et al., [16]. Membrane cholesterol was estimated as outlined by Zlatkis et al., [17]. Membrane phospholipids were estimated by the method of Connerty et al., [18], and membrane lipid peroxidation extent was measured by thiobarbituric acid (TBA) reaction with the formation of malondialdehyde (MDA) following the method of Buege and Aust [19].

All the volunteers in the present study were free from any chronic disease or illness, and, teetotallers with no smoking habit and free from use of any tranquillizers, drugs and anaesthetics. Controls (age, sex and diet matched) who did not use any mosquito repellent were selected for the study. All the volunteers were explained about the experimentation and their written consent was obtained. This study was approved by institutional ethical committee. Blood samples

from over night fasted subjects were used for the study.

OSMOTIC HAEMOLYSIS OF RED BLOOD CELLS

Isolated red blood cells were incubated in different concentrations of NaCl ranging from 0.1 to 0.9% for 30 min with gentle stirring. Then RBC suspensions were centrifuged at 700 X g 5 min and the optical density of the supernatant was determined at 540 nm [20].

ERYTHROCYTE MEMBRANE PREPARATION

Erythrocyte membranes were prepared using the method adopted by Dodge et al., [21]. Erythrocyte suspension was washed with phosphate buffered saline (pH 7.2), and then cells were lysed with 5 mM phosphate buffer (pH 8.0) and spun at 15000 X g for 30 min. The supernatant was removed carefully and by using the same buffer the latter step was repeated to obtain haemoglobin-free ghosts for further analysis.

PREPARATION OF ATE (AQUEOUS TEA EXTRACT)

Preparation of aqueous extract of tea was done following the method of Wei et al., [22] 1.25 g powder of tea leaves was added to 25 ml of boiling water and was steeped for 15 min. The infusion was cooled to room temperature and then filtered. The powder of tea leaves was extracted for second time with 25 ml boiling water and filtered, and the two filtrates were combined to obtain a 2.5% aqueous tea extract. The ATE was added to red blood cells.

STATISTICAL DATA ANALYSIS

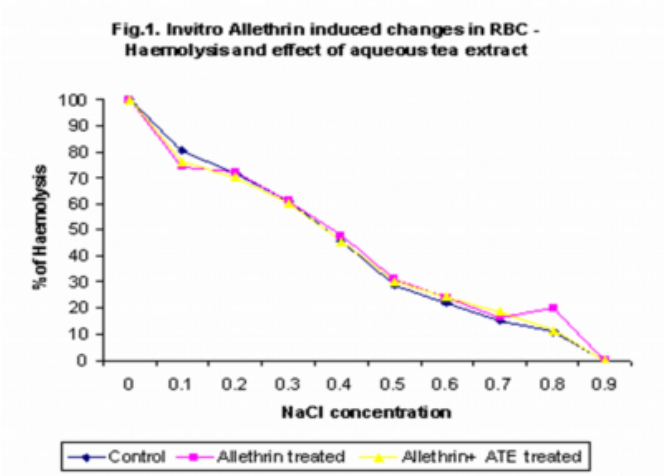
The data values were expressed as mean ± SEM. Statistical analysis was performed using Duncan's Multiple Range (DMR) test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Data presented in Fig.1 reveals that incubation of red cells with allethrin 7µM concentration altered the red cell membrane osmotic property behaviour when those red cells were exposed to different concentrations of NaCl with maximal haemolysis at 0.45% NaCl concentration. Inclusion of ATE in the medium could prevent the haemolysis.

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test, n=12.

Figure 1



Besides a decrease in membrane cholesterol and phospholipid moieties were observed and membrane lipid peroxidation decreased observation in red cells that were pre incubated with allethrin for 30 min. However the consequent C/P ratio was not altered (Table.1). Membrane proteins were not altered in the present study. Surprisingly inclusion of ATE in the medium could restore the contents of membrane cholesterol and phospholipids to normal by rectifying the observed above discrepancy (Table.1).

Figure 2

Table 1: Effect of allethrin and ATE on membrane lipid profile

Parameter	Groups		
	Controls (n=12)	Allethrin treated (n=12)	Allethrin + ATE treated (n=12)
Membrane proteins (mg/dl)	241.98±1.52[a]	248.68±2.52[a]	245.34±1.92[a]
Membrane cholesterol (µg/mg protein)	100.35±0.74[a]	75.48±2.17[b]	84.43±0.71[a]
Membrane phospholipids (µg/mg protein)	112.98±1.17[a]	88.04±2.32[b]	104.69±0.88[a]
Membrane lipid peroxidation (µmoles/mg protein)	0.59±0.03[a]	0.49±0.01[b]	0.54±0.01[a]
C/P ratio	0.88	0.85	0.86

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test, n=12.

Our findings corroborate with that of earlier studies by Moya-Quiles et al., [23] who reported that allethrin treatment caused changes in native and artificial membranes and erythrocyte membrane lipid packing by the interaction of allethrin forming leaky cells and liposomes [24]. Observed changes in osmotic fragility and lipid moieties in

present study can be attributed to the formation of leaky cells due to alterations in lipid packing as observed by [$_{23}$, $_{25}$]. Probably addition of ATE along with allethrin might have prevented these changes and also the formation of leaky cells and thereby preventing haemolysis.

CONCLUSIONS

Pre incubation of red cells in allethrin causes alterations in membrane lipid moieties and lipids packing which inturn lead to haemolysis. Inclusion of ATE in the medium prevents the said haemolysis. Besides allethrin induced changes in lipid moieties can be restored by ATE.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal and authorities of Govt. Medical College Anantapur & General Hospital for providing blood samples and authors are thankful to CSIR Senior Research Fellow for financial assistance to M. Narendra.

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