Organophosphate and Ageing Induced Decline in Activity of Acetylcholinesterase Enzyme: Potential Implication on Cognitive Function

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ABSTRACT

Nigerian local farmers are occupationally exposed to organophosphate pesticides with a resultant side effect on the cholinergic pathway. Impairment of acetylcholinesterase (AchE) enzyme activity is characteristic of this defect, leading to accumulation of the neurotransmitter acetylcholine at the neuromuscular junction. The main objective of this study was therefore to determine the plasma levels of AchE activity and their correlation with age of the subjects.

A total of 52 farmers occupationally exposed to OP pesticides were recruited at Idi-Ayune farm settlements, Ibadan, South-Western Nigeria. 52 control subjects were also recruited. All participants were apparently healthy with none on any medication. Mean AchE level of OP-exposed farmers (7.22 ± 1.99)kIU/L was significantly lower (p<0.05) than that of unexposed controls (12.23 ± 1.67)kIU/L. Correlation of AchE activity with age in the control subjects was -0.325 (p=0.019).

Low AchE activity level of the farmers indicates a persistent accumulation of acetylcholine at the neuromuscular junction, which thus predisposes them to intermediate syndrome. Significant negative correlation between AchE activity and age of the controls indicates a steady decline in AchE activity as a result of normal ageing, and this could also possibly mediate some changes in cognitive function.

KEYWORDS: Acetylcholinesterase, Cholinergic Pathway, Defects, Ageing, Cognitive Function

INTRODUCTION

The enzyme acetylcholinesterase (AchE; E.C. 3.1.1.7) found in the cholinergic terminal is a specific choline esterase, hydrolyzing predominantly choline esters (acetylcholine- Ach) and characterized by high levels in brain, nerve and red blood cells [1]. AchE plays a key role in cholinergic metabolism as it hydrolyzes the neurotransmitter acetylcholine into acetate and choline, thus terminating nerve impulse transmission. The distribution of the enzyme in the central and peripheral nerve tissues of different vertebrates demonstrates a high range of variation [2, 3].

The importance of AchE in the body homeostasis is underscored by the fact that they are the targets of some of the most potent toxins including insecticides (or pesticides), snake venom and chemical weapons [4]. Inhibition of AchE by these compounds leads to accumulation of acetylcholine in the synaptic cleft and results in impeded neurotransmission [5]. Acetylcholine is considered as the most important neurotransmitter involved in regulation of cognitive functions [6], and accumulation within the nervous system leads to continuous stimulation of cholinergic receptors, resulting in symptoms of toxicity such as salivation, tremors, and miosis and in severe cases, respiratory paralysis and death [7].

Organophosphates, esters of phosphoric acid, are a class of irreversible AchE inhibitors [5]. Thus, the inhibition of AchE activity has been used widely as a biomarker of exposure to organophosphate pesticides [8]. In our local environment, farm workers are the ones directly at the receiving end of the hazards associated with chronic exposure to organophosphate pesticides, and are therefore vulnerable to defects in the cholinergic system attendant on accumulation of acetylcholine at the neuromuscular junction.

Similarly, a marked decrease in AchE activity with increasing age has been reported by Jha and Rizvi [9], which also suggests the vulnerability of those with advanced age to impaired neurotransmission. Normal ageing is reported to be associated with a slow decline in brain functions such as sensory and motor performance and at times, this decline is accompanied by progressive memory loss, dementia and cognitive dysfunctions, ultimately resulting in limited functionality [10].
A neurochemical hypothesis has been proposed in which brain ageing is related to changes in cerebral neurotransmission, and the initial focus has been on cholinergic neurotransmission [11]. Considering a body of animal literature [12, 13, 14, 15, 16, 17, 18], Trollor and Valenzuela [11] further suggested that disruption to the cholinergic system could be one possible mediating factor in age-related cognitive change in humans. Changes in cholinergic function have been characterized and a strong correlation has been observed with cognitive decline associated with aging [9].

Extensive evidence, ranging from experiments assessing the effects of cortical cholinergic inputs on cognitive performance to studies assessing cortical acetylcholine release or changes in Ach-mediated neuronal activity in task-performing animals, has substantiated the general hypothesis that cortical cholinergic inputs primarily mediate attentional processes and capacities [19, 20, 21, 22, 23]. The aim of this study therefore, was to determine the plasma levels of AchE activity in organophosphate-exposed farm workers and control subjects, and correlation with age of the subjects.

II. MATERIALS AND METHODS

The study was approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee and informed consent was obtained from each of the participants prior to specimen collection.

2.1 STUDY PARTICIPANTS

A total of 52 occupationally exposed farmers (41 males, 11 females; aged 30-62 years) were recruited for this case-control study at Idi-Ayunre farm settlements, Oluyole Local Government Area located on the outskirts of Ibadan metropolis, Southwestern Nigeria. The 52 control subjects (41 males, 11 females; aged 38-60 years) were recruited from the premises of UCH Ibadan and environs. All participants were apparently healthy and none was on any medication.

2.2 METHODOLOGY

2.2.1 Questionnaire Administration: At enrollment, the participants completed a short structured questionnaire designed to obtain information on their demographic characteristics, life style, number of years in the farming profession, duration of exposure to organophosphate pesticides per day, medical histories and dietary habits.

2.2.2 Blood Collection: Five milliliters of blood was collected into lithium heparin bottles, and centrifugation of the blood samples was done at 500g for 5 minutes, followed by freezing of the plasma samples at -20°C until they were analyzed.

2.2.3 Procedure for Acetylcholinesterase Activity Assay in Plasma: Using the method of Ellman et al. [24], the measurement of AChE activity in plasma samples was carried out as follows: 10 μl of the plasma sample was added to 3 mL of solution containing 25 mM DTNB (5, 5'-dithiobis-2-nitrobenzoic acid) in 75 mM phosphate buffer. 10 μl of 3 mM acetylcholine iodide was added, and changes in absorbance were measured at 412 nm in a double beam spectrophotometer.

2.2.4 Statistical Analysis: The statistical analysis of the data was carried out using SPSS version 17 for windows. Results were presented as mean ± SD and the level of significance was set at p< 0.05. Student t-test was used to examine the differences in mean AchE activity between the farmers and controls. Pearson correlation coefficient (r) was used to test the relationship between variables.

III. RESULTS

Some demographic and clinical indices of the subjects are shown in Table 1. As depicted in this table, there was a significantly (p< 0.05) higher systolic blood pressure in the farmers (132.88mmHg) compared with the controls (123.37mmHg). However, their mean blood pressures fall below the normal threshold of 140/90mmHg as specified by the World Health Organization [25].

Table 2 depicts the correlation of AchE activity with age of the farmers (r= 0.189, p= 0.179) and the Controls (r= -0.325, p= 0.019), while figure 1 shows a graphical representation of the mean plasma AchE activity observed in the subjects.
Table 1. Comparison of Some Demographic and Clinical Indices of the Subjects

<table>
<thead>
<tr>
<th>INDEX</th>
<th>FARMERS (n=52)</th>
<th>CONTROLS (n=52)</th>
<th>p-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEARS OF FARMING EXPERIENCE</td>
<td>19.79 ± 13.97</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DURATION (HOURS) OF EXPOSURE/DAY</td>
<td>2.53 ± 1.30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SYSTOLIC BLOOD PRESSURE (mmHg)</td>
<td>132.88 ± 8.25</td>
<td>123.37 ± 10.88</td>
<td>0.000*</td>
</tr>
<tr>
<td>DIASTOLIC BLOOD PRESSURE (mmHg)</td>
<td>82.12 ± 7.76</td>
<td>9.52 ± 8.30</td>
<td>0.102</td>
</tr>
</tbody>
</table>

*significant at p< 0.05, n= number of subjects

Fig. 1 Mean AchE Activity (kIU/L) of the Subjects

Table 2. Correlation of AchE Activity with Age and Years of Farming Experience of the Subjects

<table>
<thead>
<tr>
<th>INDEX</th>
<th>FARMERS (n=52) r-value; p-value</th>
<th>CONTROLS (n=52) r-value; p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AchE (kIU/L)</td>
<td>0.189; 0.179</td>
<td>-0.325; 0.019*</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*significant at p< 0.05, n= number of subjects, r= Spearman correlation coefficient

IV. DISCUSSION

Occupational exposure to organophosphate pesticides has been shown to be characterized by a marked inhibition of acetylcholinesterase activity. The findings reported in this study clearly indicate a high level of exposure to organophosphate pesticides among the local farmers around Ibadan, Southwest Nigeria, hence their significantly lower AchE activity (Fig. 1), compared with that of the Controls. This further corroborates reports from previous studies [26, 27] in which significantly inhibited AChE activity was found in organophosphate-exposed workers. Inhibition of AChE leads to accumulation of the neurotransmitter, acetylcholine at the neuromuscular junction, thus rendering these farmers vulnerable to defects in cholinergic neurotransmission.

This study also reveals that the normal ageing process is associated with a progressive decline in AChE activity as indicated by the significant negative correlation observed between age and AchE activity in the control subjects (p< 0.05) (Table 2). This finding supports previous reports of Jha and Rizvi [9] in which there was a marked decrease in AchE activity with increasing age in normal healthy subjects. Similar findings were also reported separately by Das et al. [28] and Skau & Triplett [29] in studies in which the relationship between ageing and AchE activity in different brain regions were examined.
Decline in cholinergic indices (choline acetyltransferase, AchE, and muscarinic acetylcholine receptors) has already been reported during normal ageing process [30]. Ageing is an inevitable biological process and has been defined as the progressive accumulation of diverse deleterious changes with time that increases the chance of disease and death [9], Trollor and Valenzuela [11] have suggested that disruption to the cholinergic system could be one possible mediating factor in age-related cognitive change in humans. Therefore, it is presumed that increasing cholinergic transmission may enhance cognitive function [31] in aged individuals. Cells in all regions of the nervous system are affected by ageing, as indicated by the decline in sensory, motor and cognitive functions with time [32]. However, there is considerable variability among individuals in the apparent rate of ageing, the neural systems most affected, and whether and how age-related deficits are compensated [33].

V. CONCLUSION

The marked inhibition of AchE activity by organophosphates is well established and further demonstrated by this study. Based on the findings in this study, it is concluded that normal ageing is accompanied by a gradual decline in AchE activity. However, the correlation of changes in cholinergic function (occasioned by a marked decrease in AchE activity) with cognitive decline associated with ageing is needed, thus necessitating further studies.

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REFERENCES


