ORIGINAL ARTICLE

EFFECT OF DOXORUBICIN AND DAUNORUBICIN ON THE ACTIVITY OF ACETYLCHOLINESTERASE IN ACUTE LYMPHOBLASTIC LEUKAMIA

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Background: Our study was based on the alteration in the Michaelis Mentin parameters Apparent Michaelis Constant (aKm) and Apparent Maximum Velocity (aVm), which reflects activity of actylcholinesterase (AChE). This activity decreases in Acute Lymphoblastic Leukaemia (ALL). This decrease in aKm and aVm values shows bad prognosis. Similarly the anticancer drugs like Daunorubicin and Doxorubicin further decreases the aKm and aVm values which worsen the prognosis. The objective of this study was to determine and compare the extent of inhibition of Acetylecholine Esterase by Daunorubicin and Doxorubicin in ALL. **Methods:** Study of 100 patients including both male and female children who's age ranged from 4 to 8 years and were advised doxorubicin and daunorubicin separately were tested by Ellman's method using acetylcholine iodide as substrate and 5,5-dithiobis 2-nitrobenzine as a colour reagent regardless of dose regimen i.e. (once in 3 week, small dose per week or a continuous infusion for 72 to 96 hours. **Results:** In this study the Michaelis Mentin parameters Apparent Michaelis Constant (aKm) and Apparent Maximum Velocity (aVm) of the enzyme were estimated both in normal individuals and in the patients and also during treatment with daunorubicin and doxorubicin. The value of Michaelis Mentin parameters, aKm, aVm and percentage activity of the enzyme in normal individual are 23, 70, and 100 respectively. The values of aKm, aVm and percentage activity of the enzyme were also estimated in the patients before and after treatment. The values of aKm and aVm in patients of acute lymphoblastic leukaemia and percentage activity of enzyme is decreased. After the treatment with daunorubicin and doxorubicin the values and activity is further decreased. **Conclusion:** We conclude that the drugs under study both decrease the enzyme activity but daunorubicin inhibits the enzyme more than doxorubicin.

Keywords: Doxorubicin, daunorubicin, acetylcholinestrase

INTRODUCTION

Actylcholinesterase is an integral part of the erythrocyte.[1] This is externally oriented on the erythrocyte membrane with its active sites exposed outward,[2,3] moreover, it is allosteric in nature and is very sensitive to both physical and chemical changes that occur in its membrane's micro environments.[4] Consequently the external orientation and sensitivity of the enzyme makes it of special interest in understanding certain disease processes at the cellular levels. For instance the change in Michaelis Menten parameters (Apparent Michaelis Constant (aKm) and Apparent Maximum Velocity (aVm)) and the change in activity of the enzyme alter in diabetes[5], autoimmune heamolytic anemia[6], menstruation[7], pregnancy[8], protein malnutrition[9], spleenomagaly[10], addiction[11], ureamia[12] and drugs[13].

The objective of this study was to determine and compare the extent of inhibition of Acetylecholine Esterase by Daunorubicin and Doxorubicin in Acute Lymphoblastic Leukaemia (ALL).

MATERIAL AND METHODS

This was an observational study on 100 people including both healthy individuals and patients of acute lymphoblastic leukaemia.

Blood samples were collected from cubital venipuncture technique into 10 ml tubes with acid citrate dextrose (ACD) used as anticoagulant and kept on ice for about 8 minutes before it was centrifuged, all samples were taken in the morning at about 10 AM, blood from the patients was collected before and after chemotherapy.

The substrate acetylthiocholine iodide and the colour reagent 5,5-dithiobis 2-nitrobenzin and ACD mixed blood was treated according to the Ellman *et al* method[14], which involves three basic steps. Acetylation, de-acetylation, and colour formation. Adsorbance was measured by electron spectrophotometer at 412 ηm wavelength. Acetylation involves the reversible formation of the enzyme (E) substrate (ATchI) complex.

De-acetylation involves the break down of acetylated enzyme to free enzyme and the product (p). In the last step of colour formation the substrate (ATchI) reacts with the colour reagent 5,5-dithiobis 2 nitrobenzine.

The increase in absorbance at 412 ηm due to the coloured anions is a measure of the rate of enzymatic hydrolysis of the substrate (ATchI).

RESULTS

The value of Apparent Michaelis Constant (aKm), Apparent Maximum Velocity (aVm) and percentage activity of the enzyme in normal individual were 23, 70, and 100 respectively. The values of aKm and aVm, and percentage activity of the enzyme were also estimated in the patients before and after treatment.

The values of aKm, aVm, and percentage activity before and after the treatment *in vitro* are given in Table-1 and Figure-1.

Table-1: Estimation of Michaelis Mentin parameters aKm, and aVm in acute lymphoblastic leukaemia with doxorubicin and daunorubicin (*in vitro* study)

	aKm µm	aVm unit	Percentage Activity	Percentage activity lost
Control normal	23±1.2	70±1.8	100	Nil
Patient before treatment	18±2.5	48±2.9	70	30
After treatment with doxorubicin	14±3.3	40±3.9	60	40
Treatment with daunorubicin	10±1.9	28±2.1	40	60

DISCUSSION

Human erythrocye acetylcholine esterase (AchE) is an externally oriented membrane bound protein[3,4], whose kinetics were studied spectrophotometrically in acute lymphoblastic leukaemia.

The apparent Michaelis Mentin paramerters Apparent Michaelis Constant (aKm) and Apparent Maximum Velocity (aVm) of the enzyme were calculated by fitting the given linear regression equation to the data (S_1 V_1 and S_2 V_2)

$$(aKm)= \{(S_1/V_1)\ (S_2-V_2)\ (S_2-S_1)/\ (S_2/V_2)-(S_1/V_1)\}-S_1$$

$$(aVm)=1/\{(S_2/V_2)-(S_1/V_1)/\ (S_2-S_1)\}$$

The daunorubicin and doxorubicin used showed substantial changes in the parameters Apparent Michaelis Constant (aKm) and Apparent Maximum Velocity (aVm) of the enzyme and showed non competitive inhibition of the enzyme, i.e., both the Apparent Michaelis Constant (aKm) and Apparent Maximum Velocity (aVm) of the enzyme declined substantially and bind at substrate enzyme (SE) complex only.

The values of aKm and aVm in patients of acute lymphoblastic leukaemia, and percentage activity of enzyme was decreased and due to the treatment with daunorubicin and doxorubicin the values and activity is further decreased.

The present study of alteration in the parameters of the enzyme may be of special importance in developing a pharmaceutical method for assessing the active ingredients in the commercial products of the same formulated drugs as well as in the prognosis of pathological disorders that are related to red cell membrane changes by diseases or drugs. Mabood in 1981 showed that the kinetic properties of the enzyme depend upon its structural organisation[5] and its membrane micro-environment. Also Steck in 1974 showed that enzyme is externally oriented membrane bound protein.[4] So any change in the environment of AchE whether due to disease or drugs will alter its activity.

The major objective was to establish whether the observed alteration in kinetic parameters aKm and aVm of the erythrocyte AchE could follow the changes in the underlying biochemical prognostic profile and if so whether the former parameters could serve as better indicators for the prognosis of the underlying diseases.

The result obtained indicated identical trends as expected in the disease studied. The proposed enzyme kinetic method developed so for may be use full for certain clinical investigation as well as sensitive tools to distinguish between different commercial products of the same formulated drugs. Present results showed that daunorubicin and doxorubicin give non competitive inhibition of AchE activity in the patients of acute lymphoblastic leukaemia.

The parameters were found most precise, accurate and they did not vary in healthy individuals, although in abnormal situations, i.e., (diseases or under drug influence) there had been characteristic oscillatory trends in the parameters of the enzyme and these parameters can be used for distinguishing between different anti cancer drugs of different pharmaceutical companies judging their efficacy and toxicities.

CONCLUSION

If Apparent Michaelis Constant (aKm) is maintained in the disease state or if any drug which causes less decrease in the enzyme activity will be helpful in the good prognosis or will be showing less toxicity. The probe kinetic of the enzyme aKm and aVm can be useful for the doctor to assess the efficacy and toxicity of a drug. So in the present study daunorubicin inhibits the activity more than doxorubicin.

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