Kinetic Alterations in Michaelis - Menten Parameters of Human Erythrocyte Acetylcho linesterase in Splenomegally

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Summary: A huge elevation in the Michaelis-Menten parameters (aKm, avm) of the human erythrocyte acetylcholine esterase (AChE.Ec.3.1.1.7) in splenomegally were observed. 76 blood samples were assayed from the hospitalized patients compared with the normal blood samples as control. Time course effect of the disease upto six months were also observed. Attempts have been made to suggest possible interpretations for the change observed.

Introduction

AChE is an externally oriented intrinsic membrane bound enzyme and we wish to study accurately the kinetics of the enzyme in pathological cells. Recently there have been, *in vivo* reports of biological oscillations notably of carcadian or semicarcadian nature, in the activities of several enzymes of human erythrocytes. They described about 30 to 60% rise in activity between the first and the maximum activity of the enzymes. The above reports naturally aroused our interest, because much huge oscillations if existed in the activity of AChE could confound our attempts to characterise the enzyme as a marker of memberane changes in disease states. A systematic attempt has been made to evaluate the correlation between the enzyme activity of AChE with the time course of the disease. A study of the association between these two factors were of great interest, and a clear correlation were observed. Further progress in this important field will depend on the extent of enzyme kinetics on the basis of models and also on the basis of clinical experiences.

Methods

Selection of Patients

Michaelis-Menten Parameters a Km and aVm were determined in patients of malaria with splenomegally in all the nine groups. The patients consisted male as well as female with the ages ranging between seventeen and fifty five years. The patients were diagnosed and referred for investigation by the physicians of the Medical C ward of Khyber Hospital Peshawar. The first two groups consisted of patients whom the duration of disease was 7 and 15 days. The remaining 7 groups belong to the patients who had been suffering from the disease for a duration of 1,2,2,5,3,4,5 and 6 months.

Blood Samples

The doners gave blood at their own consent. Blood samples were drawn from the patients prior to the drugs given to them. In general, blood samples were collected by sterile venipuncture and added to freshly prepared acid-citrate-dextrose anticoagulant solution in the ratio of 1:4.

Preparation of Enzyme

Immediately, the blood samples 2 to 6 ml obtained at the time, were mixed and then centrifuged (200 xg, 5 min) at room temperature. The plasma, the top buffy coat and one-third upper portion of the packed cells were sucked off and the remaining packed cells were washed 3 times with 10 volume of ice-cold 0.9% (W/V) NaCl.

The enzyme (haemolysate) was prepared by adding 0.4 ml of cells to 1000 ml of ice-cold distilled water. After about 15 min. this preparation was diluted with an equal volume of ice-cold potassium phosphate buffer (0.02 mol/l, PH 7.4).

Enzyme Assay

The enzyme activity was assayed in replicate at 30°C and PH 7.4, using acetyl-thiocholine iodide(ATChI) as substrate and 5,5- dithiobis(2-nitro benzoic acid) DTNB as colour reagent. To 6 ml of haemolysate was added 100 μl of 10 mmole/1 DTNB (final concentration 160u mol/1) and then after 10 min. pre-incubation period, 50 μl of ATChI was added. The change in absorbance (E) at 412 nm due to the formation of 5-thio-2-nitrobenzoate, yellow coloured anion was recorded per min. by the method of Ellman *et. al.*. [2], 1961.

Absolute activity

The absolute activity was expressed as Ea/min per Eb, where Eb represent the absorbance due to haemoglobin content of haemolysate measured at 420 nm[6]?

Enzyme Parameters

All the assays were run by the same observor at two concentrations of substrate. One was much lower (S_1 = 10 u mole/1) and the other was much higher (S_2 = 160 u mole/1). The enzyme parameter aKm and aVm were calculated by fitting the corresponding given linear regression equations., which were derived from S/V verses S plot [6] to the date.

$$aKm = (S_1/Vs_1)\,(S_2-S_1)/(S_2/V_{s2})-(S_1/V_{s1}) - S_1$$

and

$$aVm = 1/\,(S_2/V_{s2})/-(S_1/V_{s1})-(S_1/V_{s1})/(S_2-S_1)$$

where Vs and Vs_2 represent absolute activities at S_2 and S_2, respectively.

Results

Michaelis-Menten parameters aKm and aV/m of the human erythrocyte acetylcholinesterase (AChE; Ec. 3.1.1.7) were estimated in randomly distributed hospitalized patients suffering from malaria with splenomegally. These patients were divided in nine different groups depending upon the duration of disease. (Table I). The individual values in each groups were pooled together and the mean value $\pm$ S.E. were calculated for each group. The values were tabulated for general comparison, special graph (Fig. 1) was prepared to depict the

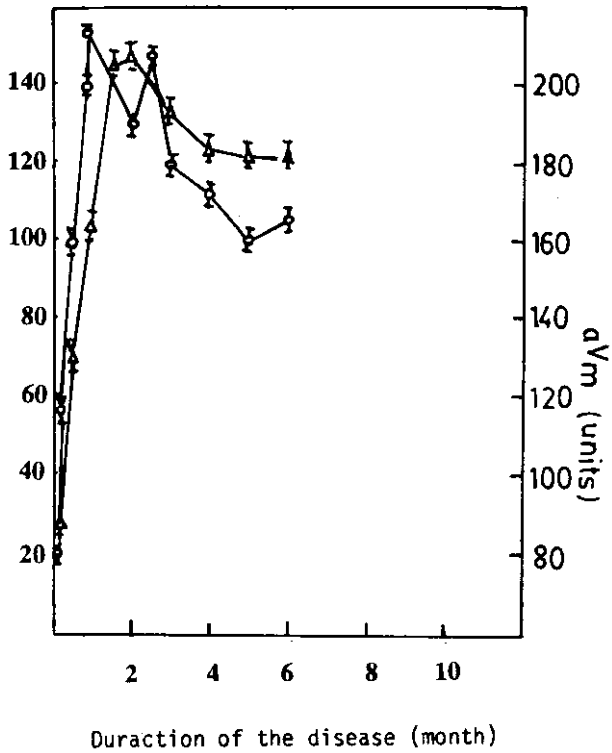

Fig.1: Time course of AChE activities with Splenomegaly, based on 79 patients. Relationship between aKm ⊕ and aVm ⊕.

oscillation of these values with duration of the disease. A huge increase in both the parameters were observed. A maximum peak for aKm was demonstrated on the second month of the onset of disease. Then it declined and the same value was found on 4th, 5th and 6th month. However an abrupt increase in aVm was observed on the first month of the onset of disease. The aVm declined and then again increase followed by decline up to the 5th month. A slight increase in aVm was found on the 6th month.

Discussion

In the present study, huge elevation in the parameters akm and aVm were observed in the patients suffering from splenomegally. The values of the parameters of the enzyme were found much higher than the values of the parameter determined in patients suffering from diabetes millitus however the same pattern of fluctuation were exibited [8]. Nonetheless, 246.66% increase in aKm and 155%

Estimate of apparent Michaelis - Menten Parameters of Human erythrocyte AchE in adults ill with splenomegally.

Group No.	aKm	aVm
1.	70 ± 1.76 (14)	118 + 1.60 (14)
2.	106 ± 2.56 (9)	160 ± 2.56 (9)
3.	145 ± 2.75 (10)	214 ± 2.46 (10)
4.	148 + 2.68 (11)	190 + 1.30 (11)
5.	140 ±2.58 (9)	208 ± 1.76 (9)
6.	132 ± 2.43 (5)	180 ± 2.58 (5)
7.	125 ± 2.16 (7)	172 ± 2.62 (7)
8.	122 ± 2.38 (8)	160 ± 2.52 (8)
9.	122 ± 2.90 (6)	166 ± 2.48 (6)

Figures in parenthesis indicate the number of patients in a group assessed.

increase in aVm were observed as compared with the diabetes mellitus patients [9]. The observation indicated that the parameters of the enzyme can provide valuable diagnostic evidence of the diseases. The variations in aKm is due to the changes occuring in the affinity of the enzyme to its substrate and/or coenzyme. The oscillations in aVm on the other hand, reflect a real change in the soluble concentration of the enzyme. These oscillations could be explained by different levels of association and dissociation, due to the structural organization of the fluidity of membrane. The oscillations in the biological activity are also related to changes in ion transport system in the membrane [10].

It is concluded that the oscillation in both the enzyme activity and in memberane properties are strongly correlated and that the changes in membrane properties may be associated with the oscillation of the enzyme activity [8]. Certain diseases may be responsible for producing various changes in the membrane, which then lead to a progressive decrease in the level of the enzyme, as there is no evidence indicating that these alterations in activity are related to a progressive denaturation of the enzyme with an actual loss [9]. We do not know at present wether the observed fluctuation in AChE activity are due to an enzyme induction or to a conformational change of the enzyme. However, more work should be carried out to clarify these and other question on the kinetic variation in diseases.

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