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CHANGES IN MICHAELIS PARAMETERS OF HUMAN ERYTHROCYTE ACETYLCHOLINESTERASE IN HEPATITIS AND MALARIA

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Michaelis-Menten parameters aK_m and aV_m of the erythrocyte acetylcholinesterase (AChE; EC 3.1.1.7) were assessed in adult humans whether normal (n = 80) or with clinically diagnosed viral hepatitis (n = 69) or malaria (n = 76). Huge elevations in the parameters were observed in the patients. Time courses of the parameters were characteristically different from the normal levels. The observations indicated that the parameters of the enzyme can provide with valuable diagnostic evidence of the diseases.

Key words : Kinetic studies, Acetylcholinesterase, Human erythrocyte.

Introduction

The erythrocyte AChE is an externally oriented intrinsic membrane-bound enzyme [1] whose kinetics were shown to depend on its membrane micro-environment [2]. For instance, in most chardates the aK_m and aV_m of the enzyme were found to alter with membrane [2]. Similarly, during the course of the normal menstrual cycle [3] or that of pregnancy [4] in human, the aV_m of the enzyme rose and its aK_m remained unchanged.

Recently it has been demonstrated that the aK_m and aV_m of the human erythrocyte AChE alter in diseases of the liver, spleen, pancreas, and blood [5]; (unpublished results). The present communication reports changes in the parameters of the enzyme in adults ill with hepatitis or malaria.

Materials and Methods

Selection of patients. Haematological values of the Michaelis Menten parameters aK_m and aV_m of the human erythrocyte acetylcholinesterase were estimated in randomly distributed hospitalized patient suffering from viral hepatitis and malaria with anaemia. The patients were divided in groups depending upon the duration of the disease. All the groups consisted of male as well as female patients with the ages ranging between seventeen and fifty five years.

In hepatitis the first two groups belonged to the patient in the acute stage (i.e. 1-14 days) whereas, the remaining six groups belonged to those patients in whom the duration of the disease was 1,2,3,4,5 and 6 months while in malaria the first two groups consisted of patient in whom the duration of the disease was 15 days and one month. The remaining six groups consisted of the patients who had been suffering from the disease for a duration of 2,3,4,5,6 and 10 months respectively.

Preparation of enzyme. Immediately, the blood samples, 2 to 6 obtained at a time, were mixed and then centrifuged (2000 x g, 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 coats 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 the 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 buffers (0.2 mol/1, pH 7.4).

Enzyme assay. The enzyme activity was assayed in replicate at 30° and pH 7.4, using acetylthiocholine iodide (ATChI) as substrate and 5,5- dithiobis (2-nitro benzoic acid) (DTNB) as colour reagent. To 6 ml of the haemolysate was added 100/1 of 10 mmol/1 DTNB (final concentration: 160 μ mol/1) and the, after a 10 min. pre-incubation period, 50 μl of ATChI (as concentration). The change in absorbance (ΔE) at 412 nm due to the formation of the 5-thio-2 nitrobenzoate yellow coloured anion was recorded/min. by the method of Ellman [6].

Absolute activity. The absolute activity was expressed as E_a/min per E_b, where E_b , represents the absorbance due to the haemoglobin content of the haemolysate measured at 540 nm (Dacie and Lewis, 1968).

Enzyme parameters. All the assays were run by the same observer at two concentrations of substrate, one was much lower (s_1 = 10 μmol/1) and the other much higher (s_2 = 160 μ mol/1) than a provisional estimate of K_m. The enzyme parameters aK_m and aV_m were calculated by fitting the corresponding given linear regression equations, which were derived from s/v versus s plot to the data.

$$aK_m = \{(s_1/vs_1)\,(s_2-s_1) / (s_2/vs_2) - (s_1/vs_1)\} - S_1$$

and $$aV_m = 1/\{(s_2/vs_2) - (s_1/vs_1)/(s_2-s_1)\}$$

where vs_1 and vs_2 represent absolute activities at s_1 and s_2 respectively.

Results and Discussion

A comparison of the aK_m and aV_m values of the enzyme between the adult patients of hepatitis and malaria in all the eight groups are given in Table 1. It seems that in hepatitis there is a rise of 130% in the aK_m and 48% in the aV_m, whereas in malaria the increases in the aK_m were 284% and 100%, respectively.

In addition, the magnitudes of the values of aK_m and aV_m of the enzyme in healthy adults and their time courses in the diseases, all arising from the same origin, are shown in Fig. 1 and 2. It appears that the value of the parameters in the diseases vary characteristically with the duration of the diseases. For example, in hepatitis the aK_m rose for upto 1.2 months and then it declined first rapidly and the slowly, whereas the aV_m rose rapidly on the 2nd week after the onset of disease. Then it declined by the first month and again increased on the 2nd month. The aV_m attained a normal level on 5th month and again showed a slight increase on the 6 th month. Nonetheless an increase in a aV_m was found on the 10th day and 4th month of the onset of disease, while a low level was observed on the 1st month on the onset of disease and then it declined progressively, whereas the aV_m tended to decline first for upto 2-3 months and then it rose to the same level and thereafter it declined slowly over rest of the eight month period.

TABLE 1. ESTIMATES OF APPARENT MICHAELIS MENTEN PARAMETERS OF HUMAN ERYTHROCYTE AChE IN ADULTS ILL WITH VIRAL HEPATITIS AND MALARIA WITH ANAEMIA. VALUES ARE MEANS ± S.E. FOR aK_m (μ MOL/l) AND aV_m ($E_a \times 10^{-3}/E_b$ PER MIN) IN LYSED RED BLOOD CELLS. FIGURES IN PARENTHESIS INDICATE THE NUMBER OF PATIENTS IN A GROUP ASSESSED.

Group No.	Hepatitis		Malaria	
	aK_m	aV_m	aK_m	aV_m
1.	36±2.04 (11)	100±1.73 (11)	56±2.59 (16)	150±1.60 (16)
2.	58±2.30 (5)	126±1.90 (5)	70±2.73 (12)	90±1.73 (12)
3.	60±1.91 (10)	96±1.04 (10)	80±2.15 (13)	110±1.45 (13)
4.	46±2.05 (8)	118±2.11 (8)	100±2.35 (9)	120±1.56 (9)
5.	44±1.74 (9)	100±1.67 (9)	86±2.43 (6)	170±1.48 (6)
6.	44±1.53 (7)	94±1.75 (7)	72±2.71 (10)	140±1.78 (10)
7.	43±2.31 (10)	80±1.81 (10)	62±2.35 (7)	130±1.65 (7)
8.	44±2.05 (9)	88±1.73 (9)	58±2.14 (3)	140±1.79 (3)

The parameters aK_m and aV_m of the enzyme were estimated by a two-substrate concentration design, because it gives more rapid, precise and accurate estimates of the parameters than K_m and V_m. Another advantage of this method is that the use of these empirical parameters is independent of

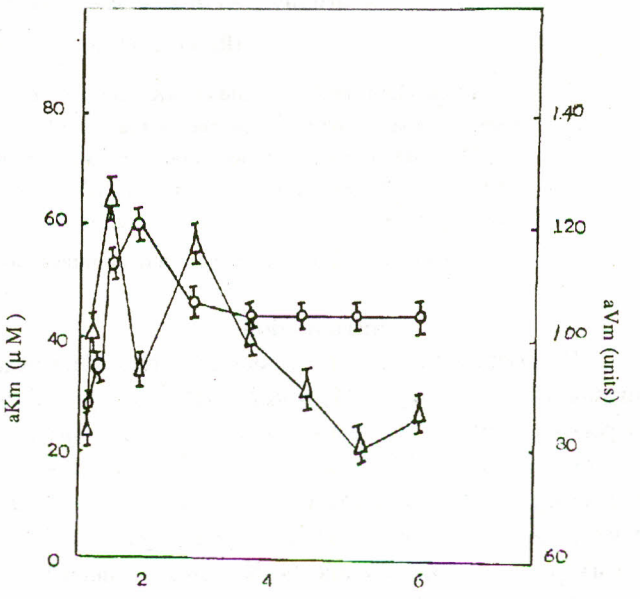

Fig. 1. Time course of AChE activities in viral hepatitis, based on 69 patients. Relationship between aK_m (Δ) and aV_m (0).

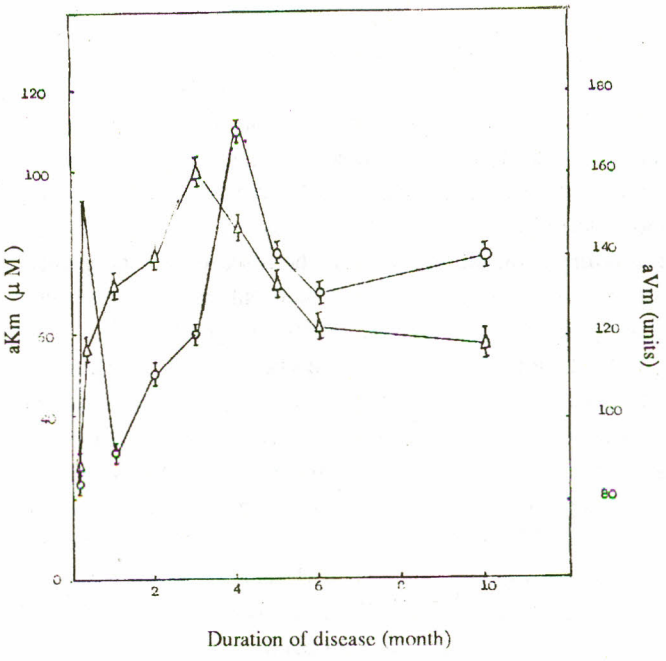

Fig. 2. Time course of AChE activities in Malaria, based on 76 patients. Relationship between a K_m (Δ) and aVm (0).

whether or not the kinetics actually follow the Michaelis Menten equation [8].

The study was undertaken because we were interested to characterize mainly the aK_m and aV_m of the enzyme as membrane markers for clinical diagnosis of diseases. The kinetic properties of the enzyme in the red cell membrane are immutable, i.e. they depend on several things such as: (1) Osmotic haemolysis; (2) *in vivo* ageing of the cell; (3) thermal stability of the cells; (4) erythropoietic activity; (5) ionic concentrations; (6) cell intactness; (7) malnutrition; and (8) the membrane lipid fluidity. For instance, on osmotic haemolysis, both the aK_m of the enzyme are increased [2] and during the *in vivo* ageing of the erythrocytes, the aK_m declines and the aV_m rises [8]. Similarly, thermal inactivation of the enzyme in isolated membranes or intact erythrocytes causes an increase in the aK_m and a decrease in the aV_m [8], whereas the reversal of the changes are encountered when the lipid fluidity of the membrane is decreased. Moreover, there is evidence that the enzyme is allosteric in nature, very sensitive to ions, i.e. a rise in ionic strength of salts increases only the K_m[6] and the orientation is such that part of the molecule containing the active site(s) is exposed [9] and the rest is presumed to be embedded in the outer-lipid bilayer of the membrane [10]. Moreover, in children with malnutrition, there is a high K_m and low V_m of the enzyme [11].

In summary, the enzyme is characteristically very sensitive to changes in the organization, composition and lipid fluidity of the erythrocyte membrane, and these changes in the parameters of the enzyme can be employed as indicators of the particular disease provided the accurate mean values and ranges of the parameters in that disease are known.

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