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SHORT COMMUNICATION

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CREATINE PHOSPHOKINASE LEVEL IN LIMB GIRDLE MUSCULAR DYSTROPHY: EFFECT OF DIALYSIS

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Summary

Dialysis has little influence on the elevated levels of creatine phosphokinase in cases of limb girdle muscular dystrophy. In this respect, the sera of limb girdle muscular dystrophy differ from the sera of Duchenne muscular dystrophy and dermatomyositis. The implications of the results are discussed.

Introduction

Serum factors are known to influence creatine phosphokinase (CPK) activity [1—3]. The functionally divergent nature of these serum factors in Duchenne muscular dystrophy and dermatomyositis have been demonstrated by the recent dialysis studies of Snehalatha et al. [1]. We here extend similar dialysis studies to cases of limb girdle muscular dystrophy (LGMD) and the results are discussed.

Materials and Methods

Sera from normal controls and six cases of LGMD in the age group of 11—28 years, based on clinical diagnosis were assayed in duplicate for CPK activity, before and after dialysis, following the procedure outlined earlier [1].

Routinely 0.1 ml of diluted serum (1 in 5 in distilled water) was used for the assay of CPK. The period of incubation (30 min at 37°) as well as the composition of the assay medium (assay medium I) made up of ATP-Na salt 2 μM; $MgSO_4$ 6 μM; creatine 2 μM; cysteine 1 μM; Tris buffer, pH9.0, 96 μM, were essentially that of Okinaka et al. [4].

Occasionally, the serum CPK activity was measured using an assay medium (assay medium II) of different composition containing ATP-Na salt 10 μM, cysteine 2.5 μM, and the other components the same as in assay me-

dium I detailed above. For this, 0.1 ml of serum was allowed to react for varying time intervals following which the reaction was stopped by addition of perchloric acid and inorganic phosphorus liberated was determined as detailed earlier [1]. The absorbance difference between the test and control samples was measured at 660 nm using ERMA photoelectric colorimeter Model AE II.

Usually, aliquots of serum (0.5 ml) were subjected to dialysis (overnight at 4° against isotonic saline) using cellophane dialysis tubing. The material left in the bag was used to determine the enzyme concentration after adequate dilution, as well as the protein content following Lowry's method [5].

Different diluent media namely saline, distilled water and heat-inactivated normal human serum were routinely used for serial dilution studies following which CPK was assayed as detailed [1].

Results

The CPK activity of normal controls and the study group before and after dialysis and following subsequent dilution is presented in Table I. Serum CPK activity at the usual 1 in 5 dilution is elevated in all the six cases of LGMD of the present study as compared to normal controls, and is in the order of 100—390 I.U./l. Further, there is a progressive increase in activity following dilution and this is independent of the diluent medium used (Table I and Fig. 1). On the other hand, dialysis has little influence on CPK activity in all these cases, whereas such a procedure has been shown to have a profound influence on CPK activity in cases of Duchenne muscular dystrophy and dermatomyositis [1].

The CPK activity of serum as such without any dilution in presence of the assay medium II is presented in Table II. The maximum activity is obtained following 15 min incubation and this figure is comparable to the corresponding serum CPK activity with 1 in 5 diluted serum and the usual substrate concen-

TABLE I

CPK ACTIVITY* BEFORE AND AFTER DIALYSIS AND FOLLOWING DILUTION

Dilution		Activity; dilution							
		5		10		20		100	
		Before	After	Before	After	Before	After	Before	After
Normal mean value of 10		8.0	7.8	—	—	—	—	—	—
Limb girdle	AH	225	261	232	261	—	—	—	—
	SR	369	235	402	412	680	670	—	—
	CP	100	112	189	220	340	350	1890	1880
Muscular dystrophy	RJ	150	110	275	270	—	—	—	—
	VC	240	230	500	469	—	—	—	—
	PE	156	160	260	262	310	322	551	549

* One I.U. phosphorylates 1 µmole creatine per min per litre.

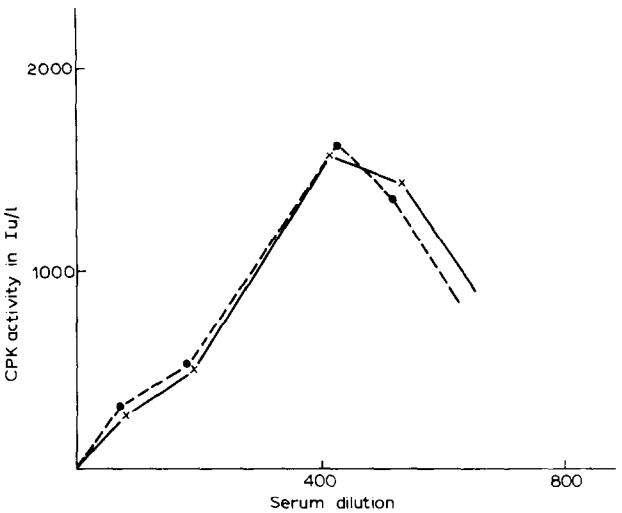

Fig. 1. Serum CPK activity following dilution. X——X, saline; ●——●, heat-inactivated normal human serum.

tration (with conventional assay medium I, CPK activity: 369 I.U./l for SR and 240 I.U./l for VC). However, increasing the period of incubation to 20 and 30 min, reveals a convincing fall in activity which may be due to the reversible nature of the enzyme reaction.

Discussion

The serum CPK activity in all six cases of LGMD in the present study is elevated as compared to normal controls (Table I). Further, there is a progressive increase in CPK activity following dilution and this is independent of the diluent medium used (Fig. 1). These results are similar to the report of Thomson [2] in his series on LGMD and he attributes such a phenomenon to some type of dissociable inhibitor to CPK in serum.

Serum factors do influence CPK [1—3] and these have been shown to be functionally divergent by recent dialysis studies [1]. Prompted by this, we have extended similar dialysis studies to cases of LGMD and it is quite surprising

TABLE II

ENZYME ACTIVITY AND TIME WITH ASSAY MEDIUM II (SEE TEXT)

Time (min)	CPK activity in I.U./l	
	SR	VC
10	200	180
15	360	240
20	220	90
30	120	90

that dialysis per se has little effect on CPK activity. In this respect, sera of LGMD differ from sera of Duchenne muscular dystrophy and dermatomyositis [1].

It is likely that the serum factors postulated as a result of dilution studies [2] may not be dialysable, possibly the CPK activity in LGMD is unrelated to these factors. The level of CPK using undiluted serum in presence of the assay medium II, containing increased concentration of ATP-Na salt and cysteine is quite in support of the latter possibility, the CPK figures by this method being fairly comparable to the corresponding figures following the conventional assay procedure using assay medium I and diluted serum samples (Table II). Dilution, as a procedure, basically helps to assess total enzyme concentration. Hence, the suggestion of serum factors influencing CPK activity in LGMD, based on dilution studies [2] alone, may possibly be speculative.

The different spectra of CPK changes [6] following dialysis, namely a drop in activity in Duchenne muscular dystrophy, an increase in dermatomyositis [1] as well as no change in LGMD as outlined in the present study are interesting. Similar detailed study in other neuromuscular disorders may possibly lead to a better understanding of the biochemical concepts of neuromuscular disorders in general.

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