IN VITRO EFFECT OF CERTAIN COMPOUNDS ON THE LYSOSOMAL RELEASE OF POLYMORPHONUCLEAR LEUKOCYTES

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Summary : From studies on the release of acid phosphatase and B-glucuronidase, it was observed that quercetin, a vitamin P like compound and ascorbic acid stablise while dehydroascorbate, acetoacetate and B-hydroxybutyrate labilise the leukocytic lysosomes *in vitro*. These effects were compared with chloroquine and progesterone, known stabiliser and destabiliser, respectively. The possible mode of lysosomal labilisation by ketone bodies and dehydroascorbate has been suggested.

Key words : lysosomes polymorphonuclear leukocytes

INTRODUCTION

We have recently observed an increased activity of lysosomal acid hydrolases in diabetic polymorphonuclear leukocytes (PMNL) (communicated). It was this observation that prompted us to look for factor(s) in the form of intermediary metabolites within the leukocytes which could be responsible for such an lysosomal leakage in the diabetic PMNL. The study became more relevant since earlier reports on various tissues indicated the stabilising effect of flavonoids (2), ascorbic acid (3) and labilising effects of ketone bodies (13).

MATERIAL AND METHODS

Dextran (mol. Wt. 150,000 to 200,000) was obtained from BDH Chemical Company, England and Ficoll–paque was procured from Decruz Corporation, Bombay, India; Quercetin from Fluka, Switzerland; p–nitrophenyl–B–D–glucuronide and Bovine serum albumin from Sigma Chemicals, U.S.A. All other chemicals and reagents used were of analytical grade.

Isolation of PMNL : The method was essentially that of Boyum (1), with slight modifications. Heparinized blood was drawn from normal healthy subjects and allowed

incubating the compounds for 40 min at 37°C with supernatant obtained from triton x 100 treated (final concentration 0.1%) LGF. B–glucuronidase was assayed immediately.

Enzyme assay : Acid phosphatase and B–glucuronidase were assayed by the methods of Shinowara *et al.* (11) and Kawai *et al.* (6) respectively. Protein was estimated by the method of Lowry *et al.* (8) using bovine serum albumin as standard.

RESULTS

It is evident from Figs. 1 and 2 that acetoacetate, B-hydroxybutyrate and dehydroascorbate at a concentration of 5 x 10^{-4}M labilise, whereas ascorbic acid and quercetin

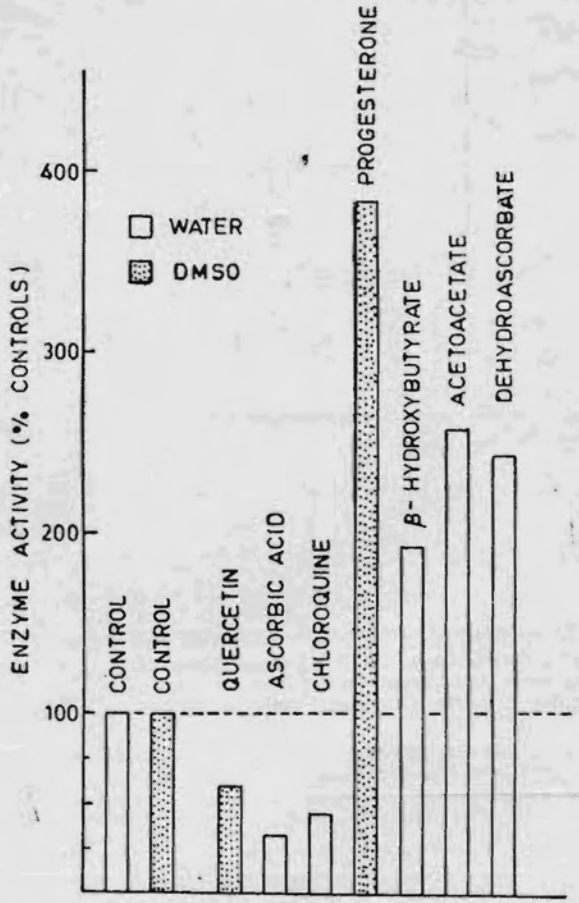

Fig. 1 : *In vitro* effect of some compounds on the release of lysosomal acid phosphatase of PMNL.
Incubation mixture consisted of 25 mM buffered sodium B-glycerophosphate (pH 5.0) and enzyme source in a final volume of 5 *ml*. Reaction was stopped by 30% TCA after 1 hr of incubation at 37°C. inorganic phosphorus liberated was estimated by the method of Fiske and Subba Row (5). Enzyme activity was expressed as μg of Pi liberated per hr per *mg* protein.
Values shown are mean of five estimations.

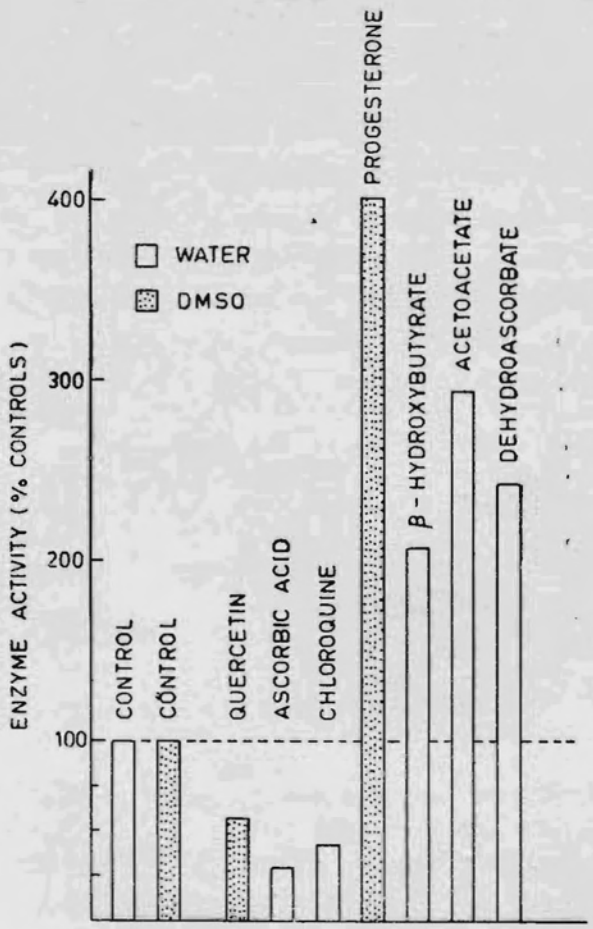

Fig. 2 : *In vitro* effect of some compounds on the release of lysosomal B-glucuronidase of PMNL. Incubation mixture of a final volume of 0.5 *ml* contained 100 μg of each substrate (p-nitrophenol B-glucuronide) and enzyme. After incubation for 30 mins. 3 *ml* of Na_2CO_3 was added and the absorbance at 400 *nm* was measured as μg of p-nitrophenol per hr per *mg* protein.

TABLE I : *In vitro* effect of some compounds at various concentrations on the release of B-glucuronidase from large granular fraction (LGF) of PMNL.

	Additions	*Enzyme Activity (Percent release from contol)*			
		0.001M	0.0005M	0.00025M	0.0001M
1.	Nil (control)	100.0	100.0	100.0	100.0
2.	Quercetin	60.0	62.5	78.9	86.8
3.	Ascorbic acid	30.0	33.3	48.4	67.1
4.	B-hydroxybutyrate	201.4	191.6	175.0	150.6
5.	Acetoacetate	271.3	258.3	208.2	184.5
6.	Dehydroascorbic-acid	256.1	244.4	200.4	171.3

Values are mean of five estimations in each case.

at the same concentration stabilise the leukocytic lysosomes *in vitro*, as indicated by the percent release of lysosomal acid hydrolase from the leukocytic LGF compared to controls. The above compounds were compared with progesterone, a known destabiliser and chloroquine, a stabiliser. The effects were, however, found to be proportional to the concentrations of the compounds (Table I).

The labilisation effect of acetoacetate, B--hydroxybutyrate and dehydroascorbate was found to be significantly reduced in combination with either reduced glutathione directly or with glutathione treated lysosomes. PCMB was found to enhance the release of lysosomal enzymes, *in vitro* (Fig. 3).

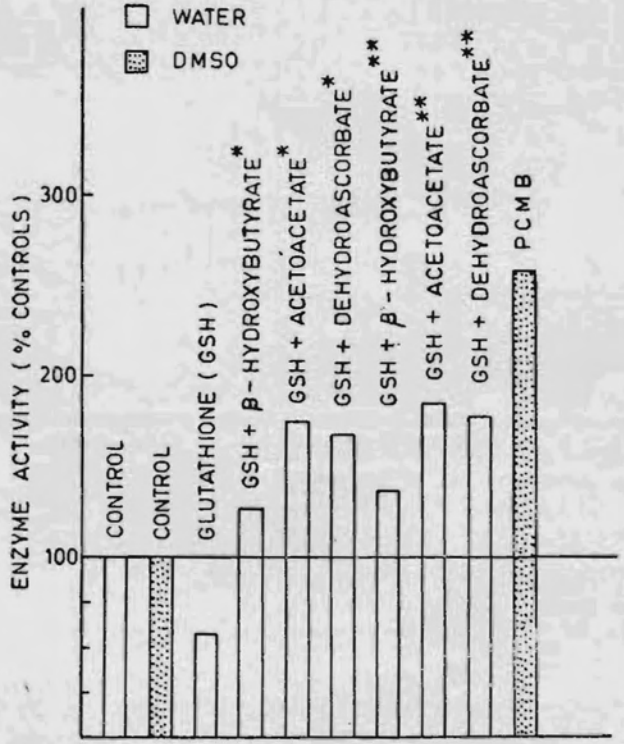

Fig. 3 : Protection of lysosomal conformation by reduced glutathione against ketone bodies and dehydroascorbate, *in vitro*.

*Both components preincubated simultaneously for 40 mins.

**1st component preincubated for 20 mins, centrifuged at 16,000 x g and 2nd component incubated with LGF for further 20 mins.

Fig. represents changes in B-glucuronidase.

Values shown are mean of five estimations.

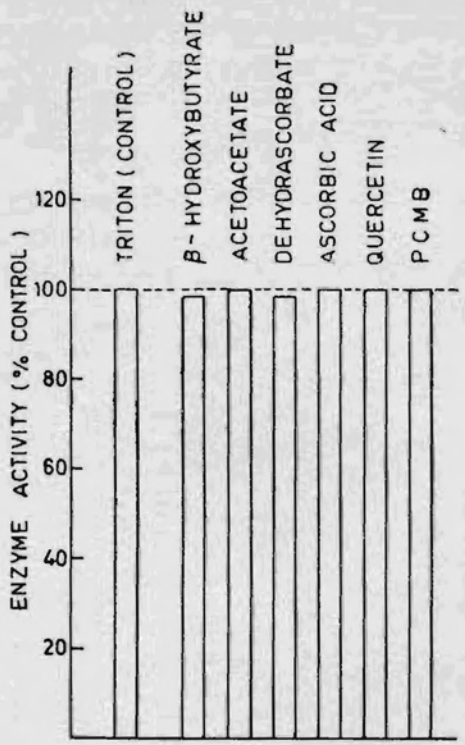

Fig. 4 : Action of ketone bodies, dehydroascorbate, ascorbic acid, quercetin and PCMB on lysosomal B-glucuronidase, of PMNL, *in vitro*.

Values shown are mean of five estimations.

It is demonstrated in Fig. 4 that the above substances have no apparent effect on the lysosomal enzymes as such.

DISCUSSION

Earlier work indicated an increased lysosomal enzyme activity either by acetoacetate administration (10) or in ketosis (13). Ketone bodies and dehydroascorbate could lead to lysis of lysosomal membrane and/or activation of the lysosomal enzymes. The second possibility is ruled out since no effect of these compounds on the activity of the enzymes as such could be observed.

Verity and Reith (12) indicated the importance of thiol groups of membrane proteins in maintaining both the integrity of the lysosomal membrane and the latent properties of the lysosomal enzymes. This contention is further verified by the enhanced release

of the lysosomal enzymes by thiol inhibitor, p–chloromercury benzoate in the present study. Ketone bodies (9) and dehydroascorbate (7) being potent thiol inhibitors may exert their effect by deactivating the –SH conformation of the lysosomal membrane thus releasing the lysosomal enzymes.

The stability effect of ascorbic acid could only be explained by its reducing property which is reported to maintain the —SH conformation (3) and thus probably the lysosomal membrane stability.

Flavonoids such as (+) –catechin, rutin and tri (hydroxyethyl) rutoside were shown to stabilise the lysosomes in *vitro* (2). It is, therefore, tempting to suggest that the stabilising effect of quercetin observed in the present study could be due to the phenolic groups in ortho position (C3' & C4' and the hydroxyl group in position C5, which is common in all the above mentioned flavonoids. However, the discrete manner in which quercetin might act as a stabiliser of lysosomes is not understood.

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