Serum and Urinary β -galactosidase and β -glucuronidase Activity in Patients with Acute Myocardial Infarction

***Zainul ABEDIN, M.D., F.R.C.P. (C), F.A.C.C.,**
****William DANIEL, Ph.D.,**
and *Daniel K. BLOOMFIELD, M.D., F.A.C.P.**

SUMMARY

To determine the effect of acute myocardial infarction (AMI) on serum and urinary activity of β -galactosidase and β -glucuronidase (lysosomal enzymes) 40 patients were studied. Eighteen patients had acute myocardial infarction and 22 were assigned as controls. Three of the 18 patients with acute myocardial infarction died within 5 to 10 days after hospitalization. Although the serum and urinary β -glucuronidase and serum β -galactosidase activity was higher in patients with acute myocardial infarction when compared with the control subjects these differences did not achieve statistical significance. However, the mean values of urinary β -galactosidase in the control and acute MI groups were 158.68 and 333.3 nmol/mg creatinine/hr, respectively ($p < 0.046$). These findings indicate that there is a significant increase in the urine β -galactosidase activity during the early phases of acute myocardial infarction.

Additional Indexing Words:

Acute myocardial infarction Lysosomal enzymes

IT has been shown that ischemic injury and consequent intracellular acidosis permit lysosomal hydrolases to gain access to the cytosol and extracel-

From the Departments of Medicine and Genetics, University of Illinois, School of Basic Medical Sciences and Clinical Medicine, Urbana, Illinois 61801, U.S.A.

Supported by University of Illinois Research Grant.

* Assistant Professor of Medicine (Cardiology), Texas Tech University Health Sciences Center.

** Associate Professor, Department of Genetic & Development, University of Illinois, Urbana, Illinois.

*** Dean, School of Basic Medical Sciences & Clinical Medicine, University of Illinois, Urbana, Illinois.

Address for reprint: Zainul Abedin, M. D., Department of Medicine, Texas Tech University Health Sciences Center, Regional Academic Health Center-El Paso, 4800 Alberta Avenue, El Paso, Texas 79905, U.S.A.

Received for publication September 2, 1981.

Manuscript revised January 12, 1982.

lular milieu.¹⁾⁻³⁾ This leakage of hydrolytic enzymes begins early during ischemia, and progresses as necrotic lesions enlarge. Lysosomes are known to contain potent proteolytic and lipolytic enzymes which are capable of degrading macromolecules to biologically active peptides.⁴⁾ Intracellular release of lysosomal enzymes following myocardial ischemia may directly,⁵⁾ or through activation of the complement pathway, result in cell injury and death. These mechanisms may also be responsible for the extension of the myocardial infarction.

This study was undertaken to evaluate the activity of serum and urinary β -galactosidase and β -glucuronidase activity during the acute phase of myocardial infarction.

MATERIALS AND METHODS

All patients admitted to the Coronary Care Unit of Mercy Hospital, Urbana, within 6 hours after the onset of chest pain, were evaluated. Appropriate consent from the patients was obtained. There were 18 patients who had clinical, electrocardiographic and serum enzyme evidence (CK-MB elevation of >5 units) of acute myocardial infarction. Twenty-two patients who had noncardiac chest pain and no electrocardiographic or enzyme evidence of ischemia or infarction were assigned as controls. Subjects who had clinical and laboratory evidence of hepatic and renal disease were excluded from the study.

These patients were managed according to CCU protocol which included bed rest, continuous monitoring for cardiac arrhythmia, I. V. morphine sulfate for chest pain, and treatment of ventricular arrhythmias by lidocaine infusion. None of these patients had clinical evidence of congestive heart failure, cardiac rhythm disturbances requiring cardioversion, intramuscular injections, noncardiac disease, or had received steroid preparations. None of these patients had clinical or laboratory evidence of hepatic or renal dysfunction.

Twelve-lead electrocardiogram and blood samples for serum CK, CK-MB levels were obtained at the time of admission and every 12 hours on four consecutive occasions. Although the collection of urine and blood samples for β -galactosidase and β -glucuronidase was stopped 24 hours after admission, the blood samples for CK-MB were continued to be collected if the patient had recurrent episodes of chest pains on the second and third days after admission.

SMA-12, hemoglobin and white blood cell count were done daily for the first 2 days. Blood and urine samples were obtained for estimation of β -galactosidase and β -glucuronidase at the time of admission and every 6 hours for

the next 2 days. The collection of the urine samples was delayed for 2 to 3 hours if the patients were unable to void at the scheduled time. The samples were immediately refrigerated. These samples were analyzed within 4–6 weeks after collection.

CK estimation was performed by enzymatic method using Dupont's Automated Clinical Analyzer (normal-male 50–180 IU/L, female 50–160 IU/L). CK-MB estimation was performed by electrophoretic method (normal 0). CK and CK-MB estimations were performed within 24 hours after collection of blood samples.

Serum and urinary β -galactosidase were estimated fluorimetrically. Urine and serum were dialyzed overnight at 4°C against 0.02 M acetate buffer, pH 5.2, to remove dialyzable inhibitors. β -galactosidase activity was determined on aliquots of dialyzed serum and urine using 4-methylumbelliferyl-beta-D-galactoside as a substrate.⁶⁾ β -glucuronidase activity was determined on aliquots of dialyzed serum and urine using 4-methylumbelliferyl-beta-D-glucuronide as a substrate. Urinary creatinine levels were determined with an appropriate automated method and serum protein was estimated by the method of Lowry et al.⁷⁾ Serum and urinary acid hydrolase activities are expressed as nmol/mg protein/hr, and nmol/mg creatinine/hr, respectively. The absolute activities of enzyme during AMI were compared to those of the control samples.

RESULTS

The distribution of age, sex, race, and site of myocardial infarction among 18 patients is shown in Table I. Three of the 18 patients died in the hospital 5 to 10 days later. The mean value of CK-MB among the 18 patients was 430 units (range 10–1,802 units). Age, sex, and race distribution among the control subjects was similar to that of the patients with acute myocardial infarction (Table I).

Table I. Clinical Data in Control and MI Group

	Age yrs. mean	Sex		Race		Site of M.I.
		M	F	W	B	
Control 22 pts.	53	12	10	19	3	
M. I. 18 pts.	58	12	6	17	1	9 Anterior 9 Inferior

W=white; B=black.

Fig. 1. Mean urinary β -galactosidase activity after acute myocardial infarction in 18 patients.

Table II. β -galactosidase and β -glucuronidase Data in Control and MI Group

	Urine β -galactosidase*	Serum β -galactosidase**	Urine β -glucuronidase*	Serum β -glucuronidase**
Control 22 pts.	Mean=158.68 SEM= 34.3	Mean=0.66 SEM=0.12	Mean=315.95 SEM= 55.9	Mean=3.68 SEM=0.52
M. I. 18 pts.	Mean=333.3 SEM= 71.7	Mean=0.46 SEM=0.06	Mean=336.9 SEM= 45.5	Mean=3.57 SEM=0.65
p	<0.046	<0.31	0.5	0.5

SEM=standard error of the mean.

* nmol/mg creatinine/hr, ** nmol/mg protein/hr.

The mean value of urine β -galactosidase in the control group (66 urine samples from 22 subjects) was 158.68 nmol/mg creatinine/hr (standard error of mean=34.3). The mean value of urinary β -galactosidase in the myocardial infarction group (69 urine samples from 18 patients) (Fig. 1) was 333.3 nmol/mg creatinine/hr (standard error of mean=71.7). This difference was statistically significant ($p=0.046$) (Student's t -test 38 degrees of freedom=2.32). The difference between the mean value of serum β -galactosidase and serum and urine β -glucuronidase in 22 control subjects and 18 patients with myocardial infarction was not statistically significant (Table II).

DISCUSSION

Our results indicate that the activity of urinary β -galactosidase increases during the acute phase of myocardial infarction. The peak activities of urine β -galactosidase and serum CK-MB occurred at different times. This may have been due to the fact that urinary β -galactosidase was measured every 6

hours and serum CK-MB was measured every 12 hours. Welman et al²⁰⁾ have reported that the peak activity of serum CK-MB and N-acetyl β -glucosaminidase (another lysosomal enzyme) occurred at the same time following acute myocardial infarction.

Since the enunciation of the lysosomal concept by De Duve,⁸⁾ considerable attention has been focused on the possible role of lysosomes in the development of cell injury. Experimental evidence suggests that, with the onset of ischemic injury, there is an increase in intracellular acidosis, which perhaps results in release of lysosomal enzymes.^{1)-3),5),9)-16)} Leakage of these enzymes may also occur through a morphologically intact lysosomal membrane.¹¹⁾ It is possible that the release of these enzymes is an early and a triggering event in cell damage.¹⁷⁾

In the present study, the collection of blood and urine samples for the estimation of β -galactosidase and β -glucuronidase was completed within the first 24 hours. None of the 18 patients with acute myocardial infarction demonstrated leukocytosis during this phase. In this regard, it was felt that a rise in urine β -galactosidase activity could not have been derived from the leukocytes. Moreover, it has been demonstrated that an important source of lysosomal enzymes in the myocardium is myocytes, not fibroblasts, macrophages, or leukocytes.^{5),13)}

The absolute levels of β -galactosidase in the urine vary during the day and from one day to the next. However, there are no sexual differences. The lack of correlation between plasma and urine enzyme levels, together with the high molecular weight of this enzyme indicates that it is not derived by glomerular filtration. Lack of correlation with lactic dehydrogenase, as reported by Piagen et al,⁶⁾ indicates that β -galactosidase is not derived from exfoliated cells. It is possible that this urinary enzyme is derived by extrusion from serum into the proximal tubules.

We did not do urinary β -galactosidase clearance studies. In the absence of confirmatory data, one can only speculate that the other reason for discrepancy between serum and urinary β -galactosidase levels may be due to the rapid clearance of this enzyme from the plasma.

Enzyme stability in the bladder before collection does not seem to be a problem. It has been shown that there is no difference in enzyme excretion in short (2 hours) and long (overnight) term urine collection. Refrigeration of samples does not produce any significant change in enzyme activity.⁶⁾

There is no evidence for the presence of inhibitors of β -galactosidase or β -glucuronidase in dialyzed urine samples. However, an increase in urinary pH can result in the destruction of the enzymes before or during the first hour of dialysis. Persistent highly alkaline urine excretion is uncommon in sub-

jects without renal disorders (cystectomy and urethral implantation).

As lysosomal enzyme activation appears to be critical, lysosomal membrane stabilization by pharmacological agents, such as methylprednisolone, might effectively protect the ischemic myocardium. Conceivably, these and other measures could limit or delay myocardial damage. A simple, fast and inexpensive method, such as estimation of urine β -galactosidase activity, could be used in evaluating the therapeutic efficacy of steroids or non-steroidal anti-inflammatory agents in patients with acute myocardial infarction. Experimental studies, designed to assess the effectiveness of steroids in stabilizing the lysosomal membrane, have shown significant inhibition of lysosomal redistribution.^{19),20)} Clinical studies have also demonstrated encouraging results.^{18),21)}

ACKNOWLEDGMENTS

We gratefully acknowledge the help and cooperation provided by the CCU Nursing Staff of Mercy Hospital, Urbana, Dr. Ben Williams, and the Medical Staff of Christie Clinic, Champaign, and the technical assistance provided by Ms. Cindy Gibson. We greatly appreciated the expert secretarial assistance of Florence Holguin.

REFERENCES

1. Williamson JR, Schaffer SW, Ford C, Safer B: Contribution of tissue acidosis to ischemic injury in perfused rat heart. *Circulation* **53** (Suppl 1): 3, 1976
2. Tsien RW: Possible effect of hydrogen ions in ischemic myocardium. *Circulation* **53** (Suppl 1): 14, 1976
3. Katz AM, Hecht HH: The early pump failure of the ischemic heart. *Am J Med* **47**: 497, 1969
4. Hirschhorn R: Lysosomes in inflammation. *in* The Inflammatory Process, Vol 1, ed by Zweifach BW, Grant L, McCluskey R, Academic Press, p 259, 285, 1974
5. Hoffstein S, Gennaro D, Weissmann G, Hirsch J, Streuli F, Fox AC: Cytochemical localization of lysosomal enzyme activity in normal and ischemic dog myocardium. *Am J Path* **79**: 193, 1975
6. Paigen K, Peterson J: Coordinary of lysosomal enzyme excretion in human urine. *J Clin Invest* **61**: 751, 1978
7. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol method. *J Biologic Chem* **193**: 267, 1951
8. De Duve C, Beaufay H: Tissue fractionation studies 10. Influence of ischemia on the state of some bound enzymes in rat liver. *Biochem J* **73**: 610, 1959
9. Hoffstein S, Weissmann G, Fox AC: Lysosomes in myocardial infarction: studies by means of cytochemistry and subcellular fractionation, with observations on effect of methylprednisolone. *Circulation* **53** (Suppl 1): 34, 1976
10. Lancker JLV, Holtzer RL: The release of acid phosphatase and beta glucuronidase from cytoplasmic granules in the early course of autolysis. *Am J Path* **35**: 563, 1958
11. Brunk UT, Ericsson JLE: Cytochemical evidence for the leakage of acid phosphatase through ultrastructurally intact lysosomal membranes. *Histochem J* **4**: 479, 1972

12. Ricciutti MA: Myocardial lysosome stability in the early stages of acute ischemic injury. *Am J Cardiol* **30**: 492, 1972
13. Ricciutti MA: Lysosomes and myocardial cellular injury. *Am J Cardiol* **30**: 498, 1972
14. Jennings RB, Reimer KA: The fate of the ischemic myocardial cell. *in* Myocardial Infarction. New Perspectives in Diagnosis and Management, ed by Cordy E, Swan HJC, Williams & Wilkins Co, Baltimore, p 13-25, 1973
15. Trump BF, Laiho KA, Mergner WJ, Arstila AU: Studies on subcellular pathophysiology of acute lethal cell injury. *Beit Path Bd* **152**: 243, 1974
16. Brachfeld N, Gemba T: Mechanisms of cell death. Release of lysosomal hydrolases after ischemia. *Clin Res* **13**: 524, 1965
17. Shnitka TK, Nachlas MM: Histochemical alterations in ischemic heart muscle and early myocardial infarctions. *Am J Path* **42**: 507, 1963
18. Welman E, Selwyn AP, Fox KM: Lysosomal and cytosolic enzyme release in acute myocardial infarction: effect of methylprednisolone. *Circulation* **59**: 730, 1979
19. Weissmann G: Effects of corticosteroids on the stability and fusion of biomembranes. *in* Asthma, ed by Lichtenstein L, Austen KF, Academic Press, NY and London, p 221-233, 1973
20. Weissmann G, Hofstein S, Kaplan H, Gennaro D, Hirsch J, Fox AC: Early lysosomal disruption in myocardial infarction and protection by methylprednisolone. *Clin Res* **23**: 383A, 1975
21. Libby P, Maroko PR, Bloor CM, Sobel BE, Braunwald E: Reduction of experimental myocardial infarction size by corticosteroid administration. *J Clin Invest* **52**: 599, 1973