**Role of PMN Elastase on Ischemic Myocardial Injury in Evolving
Myocardial Infarction:
Correlation with Clinical Parameters and Intervention
by Protease Inhibitor Ulinastatin**

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The purposes of this study were to investigate the sequential changes of PMN elastase during evolving myocardial infarction, and also to ascertain whether or not ulinastatin (UL), a clinically useful protease inhibitor, would affect the extent of ischemic myocardial injury.

The levels of plasma PMN elastase (as α_1 -proteinase inhibitor-elastase complex) were measured once in 13 normal controls, and at intervals in 30 acute myocardial infarction (AMI) patients given UL and 30 AMI controls on conventional therapy, and compared between the groups.

The levels in control group on conventional therapy were significantly higher from 6 to 72 hours after the onset than those in normal controls. Maximum PMN elastase levels in non-survivors ($n = 7$) were significantly higher than in survivors ($n = 23$) at the 6-month follow-up (288.7 ± 75.8 vs. $188.1 \pm 56.9 \mu\text{g/l}$, $p < 0.01$).

The maximum level of PMN elastase in patients given UL was significantly lower than that in the control group (162.2 ± 96.2 vs $207.3 \pm 70.1 \mu\text{g/l}$, $p < 0.05$), and the peak CK-MB in patients given UL was significantly lower than that in controls (252.3 ± 150.9 vs $360.1 \pm 239.6 \text{ IU/l}$, $p < 0.05$).

Early mortality (seen at 6-month follow-up) in patients administered UL was significantly lower than that of the treated controls (3.3% vs 23.3%, $p < 0.05$).

Analysis of changes in PMN elastase levels suggested that UL would be clinically beneficial for reduction of ischemic myocardial injury.

THE leukocytes have been shown to play an important role in the promotion of ischemic myocardial damage in cases of acute myocardial infarction^{1,2}. It is considered that the lysosomal enzymes, as well as the production of superoxide and the activation of the lip-oxygenase system of metabolism of arachidonic acid, contribute to this destructive action of the

leukocytes.

Granulocyte (PMN) elastase, which is believed to be intimately involved with leukokinetics, has been suspected to be the substance that causes adult respiratory distress syndrome (ARDS) and multiple organ failure (MOF)³, and it has been reported that the cause of these in pulmonary emphysema may be a disproportion in the relative levels of granulocyte elastase and α_1 -proteinase inhibitor (α_1 -PI)⁴. Moreover, in septicemia, granulocyte elastase acts as an augmenting factor whose level reflects the severity of the case, and aids in its prognosis⁵.

Key words:

PMN elastase
Acute myocardial infarction
Ulinastatin
Myocardial salvage
Early mortality

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Fig.1. PMN Elastase-Proteinase Inhibitor-Complex: Assay⁶

In the present study, we examined the role of granulocyte elastase in evolving acute myocardial infarction (AMI); and we also investigated the efficacy of the protease inhibitor ulinastatin in protecting ischemic myocardium.

MATERIALS AND METHODS

Sixty patients who had suffered acute myocardial infarction within the previous 24 hours were used as subjects. The protease inhibitor ulinastatin was administered to 30 patients of them, and the other 30 acted as the control group. In addition, 13 hospital staff volunteers

and blood donors for surgical operations were employed as healthy controls.

For administration, 200,000 units of ulinastatin were dissolved in 20 ml of physiological saline solution; and every 8 hours after the end of the blood sampling carried out on admission, this was injected as a bolus injection until a total of 3,000,000 units had been administered. The first dose was given 6.2 ± 4.1 hours after the onset of the infarction.

The granulocyte elastase levels were determined by the solid-phase enzyme-linked immunosorbent assay (ELISA⁶), whose principle is described in Fig. 1, both at several points within

Fig.2. Serial changes of PMN elastase- α_1 PI complex in AMITABLE I CORRELATION OF PMN ELASTASE- α_1 PI COMPLEX MAX WITH CARDIAC HEMODYNAMICS AND LABORATORY DATA

				<i>r</i> value	<i>P</i> value
PMN elastase- α_1 PI complex Max	vs	WBC	Max	0.60	$p < 0.01$
"	"	CK	"	0.35	NS
"	"	CK-MB	"	0.23	NS
"	"	GOT	"	0.32	NS
"	"	LDH	"	0.29	NS
"	"	PCWP at the time of admission	"	0.39	$p < 0.05$
"	"	CVP	"	0.12	NS
"	"	CI	"	-0.01	NS
"	"	SVI	"	-0.01	NS

24 hours and at 24-hour intervals after onset until 144 hours later, at five points (for hours 24-48, 48-72, 72-96, 96-120, and 120-144 hours).

Employing the Merck-1-Test kit, which is based on a modification of the method of Oliver, for the measurement of creatine kinase (CK) and CK-MB, we made determinations every 2 hours, for the first 24 hours after onset; for the second

24 hours, every 4 hours, between hours 48 and 72, every 6 hours; and thereafter every 12 hours.

For the examination of the hemodynamics within the heart, the Swan-Ganz catheter method was applied in all subjects at the time of admission.

Statistically analyses were done using Student's t-test or Welch's t-test.

Fig.3. Comparison of PMN elastase α_1 -PI complex between survival and dead groups in AMI

TABLE II COMPARISON OF LABORATORY DATA BETWEEN THE SURVIVAL AND THE DEAD GROUPS

	Survival N = 23	Dead N = 7	t	p value
PMN elastase α_1 -PI complex Max ($\mu\text{g/l}$)	188.1 ± 56.9	288.7 ± 75.8	3.64	$p < 0.01$
WBC Max ($/\text{mm}^3$)	11922 ± 3046	13614 ± 2314	1.31	NS
CK Max (IU/l)	3392.5 ± 2684.2	4163.4 ± 2757.9	0.64	NS
CK-MB Max (IU/l)	359.9 ± 247.8	360.9 ± 210.6	0.0088	NS
LDH Max (IU/l)	1194.2 ± 636.3	1544.3 ± 756.8	1.18	NS
GOT Max (IU/l)	298.3 ± 194.0	429.0 ± 246.9	1.41	NS

RESULTS

- Relationship between changes with time in granulocyte elastase level and severity (results from 30 patients not administered ulinastatin).

(1) Changes in granulocyte elastase level in acute myocardial infarction

The variations in the granulocyte elastase level taking place after acute myocardial infarction in the 30 patients who did not receive ulinastatin are shown in Fig. 2. In comparison with the healthy controls, these subjects showed signifi-

cant increases from 6 to 72 hours after onset.

(2) Relationships between maximum granulocyte elastase levels in acute myocardial infarction and (i) maximum values in laboratory data, and (ii) cardiac hemodynamics at admission time

Table I presents the correlations of the maximum levels of granulocyte elastase with the maximum values in laboratory data and the cardiac hemodynamics at the time of admission in AMI. Significant correlations were seen with the maximum leukocyte count and with pulmonary capillary wedge pressure (PCWP).

(3) Comparison of changes with time in

TABLE III-A COMPARISON OF BACKGROUNDS (AGE, SEX, SEVERITY)

	Age	Sex (Cases)**		Attacks (Cases)**				Killip classification (Cases)**			
		Male	Female	1st	2nd	3rd	4th	I	II	III	IV
Ulinastatin group N = 30	60.9 ± 10.1	21	9	23	5	1	1	21	3	4	2
Control group N = 30	64.3 ± 9.9	23	7	26	4	0	0	22	5	3	0

PCWP (mmHg)	CVP (cmH ₂ O)	CI (l/min/ m ²)	SVI (ml/beat/ m ²)	WBC· MAX (/mm ³)
16.5 ± 6.9	9.8 ± 4.1	2.42 ± 0.51	30.1 ± 8.5	13267 ± 295
17.1 ± 7.8	9.3 ± 3.7	2.54 ± 0.73	34.6 ± 11.8	12316 ± 297

*There are no significant differences between two groups

**(χ^2 test)

TABLE III-B COMPARISON OF BACKGROUNDS (Treatment within a Week)

	Ca antagonists	β -blockades	Nitrates	Catecholamines		Lidocaine	Diuretics
				DA	DB		
Ulinastatin group N = 30	13	0	22	7	4	17	17
Control group N = 30	14	0	15	7	4	15	17

Thrombolytic therapy UK IC·IV, t-pA IV, pro UK IV	Anti coagulant or anti platelet therapy heparin, warfarin aspirin, ticlopidine
13	11
17	4

*There are no significant differences between two groups
(χ^2 test)

granulocyte elastase levels in the survivor group and in the early-death group after acute myocardial infarction

In Fig. 3 are shown the changes with time in the levels of granulocyte elastase in the group of subjects who survived and in those who died within 6 months of their attacks. From 24 hours

after the onset of AMI the early-death group showed significantly lower levels of PMN elastase compared to those in the survivors.

(4) Comparison of laboratory data in the survivor group and the early-death group

All values seen in the early-death group were elevated, but a statistically significant difference

Fig.4. Comparison of PMN elastase α_1 -PI complex between ulinastatin group and control group

Fig.5. Comparison of PMN elastase α_1 -PI complex Max between ulinastatin group and control group

was found only in the maximum granulocyte elastase level. (Table II).

2) Efficacy of ulinastatin in protection of ischemic myocardium

(1) Comparison of background factors in the ulinastatin-administered group and in the control group

A comparison of background factors in the ulinastatin-administered group and in the control group that received no ulinastatin is laid out in Table III. No significant difference could be found between the two groups in any of these factors.

(2) Comparison of changes with time in granulocyte elastase levels in the ulinastatin-administered group and in the control group

Figure 4 offers a comparison of the changes with time in the granulocyte elastase levels in the ulinastatin-treated and control groups. As the curves show, between 12 and 48 hours after onset, the granulocyte elastase levels in the ulinastatin-administered group were significantly lowered or exhibited a tendency to be lower.

(3) Comparison of maximum granulocyte elastase levels in the ulinastatin-administered group and in the control group

As Fig. 5 shows, there was a significant reduction in the maximum granulocyte elastase levels in the ulinastatin-administered group, compared with the control group.

(4) Comparison of maximum CK and CK-MB levels in the ulinastatin-treated and control groups

Figure 6 shows that the maximum CK value in the ulinastatin-administered group was 3738.5 ± 2909.4 IU/l and that in the control group was 3572.4 ± 2721.2 IU/l, so that there was no

Fig.6. Comparison of CK, CK-MB Max and interval from onset to their Max between ulinastatin group and control group

TABLE IV COMPARISON OF MORTALITY WITHIN 6 MONTHS AFTER THE ONSET OF AMI BETWEEN ULINASTATIN GROUP AND CONTROL GROUP

	Survival	Dead	Total	p value*
Ulinastatin	29 (96.7%)	1 (3.3%)	30	$p < 0.05$
Control	23 (76.7%)	7 (23.3%)	30	

*: by Fisher's exact test

statistically significant difference between the two groups; but the maximum CK-MB value in the former group, 252.3 ± 150.9 IU/l, was significantly lower than the 360.1 ± 239.6 IU/l seen in the latter group. No significant difference was observed between the times taken to reach the maximum CK and CK-MB values between the groups.

(5) Comparison of prognoses between the ulinastatin-administered and control groups

In Table IV, it is seen that the mortality in the ulinastatin-administered group was 3.3%, which was significantly lower than the 23.3% found in the control group.

DISCUSSION

In autopsies of early death after myocardial infarction, Mallory et al⁷, Lodge-Patch⁸ and Edwards⁹ reported that the invasion of the leukocyte into the myocardium beings 6 to 18 hours after onset, and is quite obvious between 2 and 4 days after. The histological changes in the acute stages of the myocardial infarction and the variations in the granulocyte elastase levels

determined in the present study show an extremely close relationship, and so we believe that our elastase measurements reflect distinctly the extent of the myocardial damage that has taken place, and therefore offer important information for the assessment of the severity of an attack. Romson et al¹⁰ found in experimental myocardial infarction that if the circulating neutrophil levels were lowered with neutrophil antiserum, the leukocyte invasion of the ischemic region was inhibited, and a significantly smaller infarction took place. The participation of lysosomal enzymes such as elastase, together with the generation of superoxide and the activation of arachidonic acid lipoxygenase metabolism, is attracting great interest in relation to this leukocyte-caused myocardial tissue damage. Thus, there is a possibility that the determination of granulocyte elastase in acute myocardial infarction can serve as an excellent means of finding out the degree of myocardial damage caused by leukocytes.

The authors investigated whether or not ulinastatin, a protease inhibitor, inhibited the granulocyte elastase level, and examined its effects on the early prognosis.

Ulinastatin is a glycoprotein with a molecular weight of 67,000. The concentration of ulinastatin that will inhibit 50% of the enzymatic action of granulocyte elastase is 5.6 U/ml¹¹ or 11 U/ml¹² the mechanism of this inhibition being antagonistic inhibition.^{11,12} The marked inhibitory effect on granulocyte elastase seen in the present study can hardly be attributed to antagonistic inhibition by ulinastatin, because, as Jönsson et al¹³ demonstrated, the affinity of ulinastatin for granulocyte elastase is far weaker

than that of α_1 -PI.

Oda et al¹⁴ incubated lysosomes isolated from rat liver in 0.17 M sucrose solution at 37°C for 60 minutes, and then, utilizing the activation of the β -glucuronidase and of the acid phosphatase in the reaction solution as indices, studied the stabilizing effect of ulinastatin on the lysosome membranes. They reported that ulinastatin significantly inhibited the liberation of these enzymes from the lysosomes. Thus, there is a strong possibility that the inhibitory action of ulinastatin on granulocyte elastase observed in the present study is an inhibition of its release resulting from stabilizing action of ulinastatin on the lysosome membrane.

Wildenthal¹⁵ ascertained that cathepsin D, one of the lysosome enzymes, was exuded from the ischemic myocardium, but no indication was given as to whether this was the cause of myocellular damage, or simply a result of it. The lysosomes of the cells of the myocardium, however, also contain large amounts of protease, and ulinastatin may contribute not only to the stability of the lysosome membranes of the granulocytes, but also to those of the myocardial cells.

Inhibitors of protease, unlike calcium antagonists and β -blockers, have not been found to have any negative inotropic action on the normal myocardium, even when administered in large amounts, and the development of a drug that can preserve the ischemic myocardium is eagerly awaited.

REFERENCES

1. ROMSON JL, HOOK BG, RIGOT VH, SCHORK MA, SWANSON DP, LUCCHESI BR: The effect of ibuprofen on accumulation of indium-111-labeled platelet and leukocytes in experimental myocardial infarction. *Circulation* 66: 1002, 1982
2. MULLANE KM, READ N, SALMON JA, MONCADA S: Role of leukocytes in acute myocardial infarction in anesthetized dogs: Relationship to myocardial salvage by antiinflammatory drugs. *J Pharmacol Exp Ther* 228: 510, 1984
3. NEUMANN S, LANG H: Recent results from clinical evaluation of the PMN elastase/ α_1 -proteinase inhibitor complex assay. In *Elastase: a new marker for inflammatory disease*, ed by FROESCHLE MC, GOETZ WA, GIT Verlag, Darmstadt, 1985, p 39
4. STOCKLEY RA: Proteolytic enzymes, their inhibitors and lung diseases. *Clin Sci* 64: 119, 1983
5. JOCHUM M, FRITZ H: Plasma level of human granulocytic elastase- α_1 -proteinase inhibitor complex (E- α_1 PI) in patients with septicemia and acute leukemia. In *Selected Topics in Clinical Enzymology*, ed by GOLDBERG D, WERNER M, Walter de Gruyter Berlin. New York 1983, p 85
6. NEUMANN S, HENNRICH N, GUNZER G, LANG H: Enzyme-linked immunoassay for human granulocyte elastase in complex with α_1 -proteinase inhibitor. In *Proteases*, HORL WH, HEIDLAND A, ed by Plenum, New York, 1984, p 379
7. MALLORY GK, WHITE PD, SALCEDO-SALGAR J: The speed of healing of myocardial infarction, a study of the pathologic anatomy in seventy-two cases. *Amer Heart J* 18: 647, 1939
8. LODGE-PATCH I: The ageing of cardiac infarcts, and its influence on cardiac rupture. *Br Heart J* 13: 37, 1951
9. EDWARDS JE: An atlas of acquired disease of the heart and great vessel 2. WB Saunders, Philadelphia and London, 1961, p 532
10. ROMSON JL, HOOK BG, KUNKEL SL, ABRAMS GD, SCHORK MA, LUCCHESI BR: Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation* 67: 1016, 1983
11. SHIBUTANI Y, KUNIHIO Y: Preventive effect of ulinastatin on tissue degradation, *Jpn Pharmacol Ther* 14: 6057, 1986
12. OGAWA M, NISHIBE S, MORI T, NEUMANN S: Effect of human urinary trypsin inhibitor on granulocyte elastase activity. *Res Commun Chem Pathol Pharmacol* 55: 271, 1987
13. JÖNSSON BM, OHLSSON K: Interactions of the complex between human urinary trypsin inhibitor and human leukocyte elastase with α_1 -proteinase inhibitor and α_2 -macroglobulin. *Hoppe-Seyler's Z Physiol Chem* 365: 1403, 1984
14. ODA T, MIYAWAKI T, SAMESHIMA T, MIYAO J: Antishock effects of urinary trypsin inhibitor, MR20. *Jpn J Anesthesiol* 33: 137, 1984
15. WILDENTHAL K: Lysosomal alterations in ischemic myocardium: Result or cause of myocellular damage? *J Mol Cell Cardiol* 10: 595, 1978