The Anti-inflammatory Effects of Ulinastatin
in Traumatic Patients with a Hemorrhagic Shock

Kyung Hye Park

The Graduate School
Yonsei University
Department of Medicine
The Anti-inflammatory Effects of Ulinastatin
in Traumatic Patients with a Hemorrhagic Shock

A Master's Thesis
Submitted to the Department of Medicine
and the Graduate School of Yonsei University
in partial fulfillment of the
requirement for the degree of
Master of Medical Science

Kyung Hye Park

January 2007
This certifies that the master’s thesis of

Kyung Hye Park is approved

Thesis Supervisor : Kang Hyun Lee M.D.

Thesis Committee Member : Sung Oh Hwang M.D.

Thesis Committee Member : Jae Won Choi M.D.

The Graduate School

Yonsei University

January 2007
 감사의 글

이 논문이 나오기까지 바쁘신 와중에도 아김없는 지도를 해 주신 이강현 교수님께 깊이 감사드립니다. 또한 느 정원해 주시고 지도해 주신 황성오 교수님, 귀중한 시간을 내어 조언해 주신 최재원 교수님께 감사드립니다. 그리고 관심을 가져주시고 조언해 주신 김현 교수님, 김선휴 선생님, 장용수 선생님께도 감사드립니다.

제 연구를 적극적으로 도와 준 응급의학과 의료원들과 응급실 간호사분들에게도 고마움을 전하고 싶습니다. 그리고 진단검사의학과 권오건 선생님과 봉근 후에도 시간을 내어 혈청 검사를 해 주신 임상병리사 진혜경 선생님에게도 감사드립니다.

석사 논문을 완성시킨 기쁨을 누리게 해 주신 위의 모든 분들에게 감사드립니다.

마지막으로 낳아주시고 길리주신, 물심양면으로 큰 발을 응원해주신 아버지, 어머니께도 고개 숙여 감사드립니다. 교사와 공무원으로 바쁘게 생활하면서도 석사학위를 따신 두 분의 노력이 제게 큰 힘이 되었습니다.

논문 쓰며 혼들어하는 나를 마음으로 위로해주신 동생 경화, 경석이에게도 고마움을 전합니다.

이 논문을 내 사랑하는 가족들에게 바칩니다.
Index

Figure index ................................................................. iii
Table index ................................................................. iv
Abstract ................................................................. v
I. Introduction ........................................................... 1
II. Subjects and Methods .............................................. 2
   1. Subjects ............................................................. 2
   2. Methods ............................................................ 2
      2.1. Patients ......................................................... 2
      2.2. Administration of ulinastatin .............................. 3
      2.3. Measurements of serum TNF-α, IL-6 and PMNE ......... 3
      2.4. Analysis of results ........................................... 4
   3. Statistical analysis ............................................... 6
III. Results ............................................................... 7
   1. Demographic data for the patients ............................. 7
   2. Comparison of laboratory data between the control group and the ulinastatin group ......................................................... 8
   3. Comparison of serum TNF-α, IL-6 and PMNE levels between the control group and the ulinastatin group ......................................................... 10
      3.1. Serum TNF-α levels .......................................... 10
      3.2. Serum IL-6 levels ............................................ 11
      3.3. Serum PMNE levels .......................................... 13
   4. Treatment and the final results ................................ 14
      4.1. Transfusion, treatment and mortality ....................... 14
      4.2. Comparison of SIRS score, MODS score and APACHE III between
the control group and the ulinastatin group

IV. Discussion

V. Conclusion

References

Abstract in Korean
Figure Index

Fig. 1. The study design ................................................................. 5
Fig. 2. A. Effects of ulinastatin on serum TNF-α levels .................. 10
Fig. 2. B. Changes of serum TNF-α levels after admission ................ 11
Fig. 3. A. Effects of ulinastatin on serum IL-6 levels ..................... 12
Fig. 3. B. Changes of serum IL-6 levels after admission .................. 12
Fig. 4. A. Effects of ulinastatin on serum PMNE levels .................. 13
Fig. 4. B. Changes of serum PMNE levels after admission ............... 14
Table Index

Table 1. Demographic data of the enrolled patients .......................... 7
Table 2. Comparison of laboratory data between the control group and the ulinastatin group .......................................................... 8
Table 3. Transfusion, treatment modality and the final results between the control group and the ulinastatin group ............................. 15
Table 4. SIRS score, MODS score and APACHE III 48 hours after admission .................................................................................. 17
Table 5. Comparison of SIRS score, MODS score and APACHE III between the control group and the ulinastatin group ..................... 18
Abstract

The Anti-inflammatory Effects of Ulinastatin in Traumatic Patients with a Hemorrhagic Shock

Kyung Hye Park
Dept. of Medicine
The Graduate School
Yonsei University

**Background:** Ulinastatin, a glycoprotein from human urine, inhibits the proteolytic action and has an anti-inflammatory effect on tissues. Ulinastatin reduces the renal dysfunction associated with the ischemia-reperfusion of the kidney as well as the blood transfusion-induced Polymorphonuclear Leukocyte Elastase (PMNE) which may injure a variety of tissues and organs. However, the effect of ulinastatin on traumatic hemorrhagic shock has rarely been reported.

**Purpose:** The aim of this study was to investigate the use of ulinastatin in association with the suppression of plasma proinflammatory cytokine and PMNE and the good prognosis in the patients with traumatic hemorrhagic shock.

**Subjects and Methods:** Nineteen patients who were admitted to the emergency department for trauma with hemorrhagic shock from June 2006 to October 2006 were
enrolled. Eleven patients received ulinastatin at random. Ulinastatin 100,000 IU was intravenously administered every 8 hours for a total of 300,000 IU. Measurements of serum PMNE, Tumor Necrosis Factor Alpha (TNF-α) and Interleukin 6 (IL-6) were taken before ulinastatin treatment, at 24 hours, 2 days, 3 days and 7 days after admission. We compared the Systemic Inflammatory Response Syndrome (SIRS) score, the Multiple Organ Dysfunction Syndrome (MODS) score and the Acute Physiology, Age, Chronic Health Evaluation (APACHE) III between the control group and the ulinastatin group.

**Results:** There were no significant baseline differences between the control group and the ulinastatin group. Furthermore, there were no significant differences in laboratory data, treatment and mortality between the control group and the ulinastatin group. The serum PMNE levels of the ulinastatin group were lower than the control at the second hospitalized day (11.58±5.57 vs 4.33±1.21, p=0.19). Serum TNF-α and IL-6 levels of the ulinastatin group decreased 24 hours after admission and were lower than the control, however, there were no significant differences.

**Conclusion:** Ulinastatin 300,000 IU leads to decrease the serum PMNE in traumatic patients with a hemorrhagic shock on the second day of hospitalization.

**Key Words:** Ulinastatin, Hemorrhagic shock, Polymorphonuclear Leukocyte Elastase, Tumor Necrosis Factor Alpha, Interleukin 6
I. Introduction

Shock resulting from life-threatening hemorrhage induces the ischemic injuries of the all organs as well as tissues. Furthermore, this kind of damage occurs during the resuscitation period. In both hemorrhagic shock and systemic inflammations (i.e. burn, acute pancreatitis, sepsis), neutrophils become active, inflammatory cytokine increases, whereby these systemic metabolic change can lead to an acute respiratory distress syndrome and microischemia of the liver, and can impair the function of the kidney, heart and the brain. As a result, the multiple organ failure is the leading cause of mortality\textsuperscript{1-3}.  

Interleukin-6 (IL-6), Tumor Necrosis Factor Alpha (TNF-\textalpha) and Polymorphonuclear Leukocyte Elastase (PMNE) begin to increase in the early inflammation stage, stimulating various tissues and organs, and lead to a systemic inflammatory response. In order to suppress this kind of cytokine, various studies regarding an antibody against cytokine or a protease inhibitor have been attempted\textsuperscript{4}.  

Ulinastatin, a glycoprotein with a molecular weight of 67,000 daltons, derived from human urine, has an anti-inflammatory activity to suppress PMNE, TNF-\textalpha, IL-6, IL-8\textsuperscript{5-8}. In animal studies, the effects of ulinastatin on the artificially induced hemorrhagic shock have been reported\textsuperscript{8,9}, whereas the studies about the inflammatory cytokine or PMNE and clinical trials to hemorrhagic shock have not been reported. The purpose of this study was to investigate the anti-inflammatory effects of ulinastatin on the proinflammatory cytokines and PMNE in traumatic patients with a hemorrhagic shock.
II. Subjects and Methods

1. Subjects

This was a randomized controlled trial using nineteen adult patients who were admitted to the emergency department for trauma from June to October 2006 were enrolled. The enrolled patients with a traumatic hemorrhagic shock had arrived within six hours after an accident and were 18 years or older.

Patients with any documented preexisting heart failure, chronic renal failure, liver cirrhosis or chronic obstructive pulmonary disease were excluded. Other exclusion criteria included cardiopulmonary resuscitation-performed patients, or patients with severe brain injury which was the main cause of death or morbidity.

2. Methods

1) Patients

The enrolled patients were divided into two groups randomly. Patients who were admitted on the even days composed the control group. Furthermore, those admitted on the odd days made up the ulinastatin group to whom ulinastatin was administrated.
2) Administration of ulinastatin

Ulinastatin was administrated to the enrolled patients receiving blood transfusion and fluid immediately after a hemorrhagic shock was diagnosed. Ulinastatin is commercially known as Ulistin® (Ulinastatin 100,000 IU/2ml, Han Lim Pharm. Co., Ltd, Seoul, Korea). 100,000 IU ulinastatin with 100 ml normal saline for duration of 30 minutes at a time, every 8 hours, for a total of three times.

3) Measurements of serum TNF-α, IL-6 and PMNE

In the ulinastatin group, measurements were taken of the plasma concentrations of PMNE, IL-6 and TNF-α before injecting ulinastatin, 24 hours, 2, 3, and 7 days after injection. In the control group, the plasma concentrations of PMNE, IL-6 and TNF-α were measured upon admission to the Emergency Room (ER), 24 hours, 2, 3 and 7 days after admission. After centrifuging the blood samples of the patients (MF 600, Hanil Science Industrial, Seoul, Korea), the collected serum was kept in a freezer and then dissolved to examine by use of Enzyme-Linked Immunosorbent Assay (ELISA; PhD™ System, BIO RAD, USA).

Serum PMNE was measured in terms of the concentration of PMNE-α1-antitrypsin complex using PMNE/α1-proteinase inhibitor complex ELISA kit (Calbiochem®, EMD Biosciences, Inc., Darmstadt, Germany). Furthermore, serum IL-6 was measured using Human IL-6 immunoassay kit (Quantikine®, R&D Systems, Inc., Minnesota, USA).
Lastly, serum TNF-α was measured using Human TNF-α /TNFSF1A immunoassay kit (Quantikine®, R&D Systems, Inc., Minnesota, USA).

4) Analysis of results

Upon admission and 48 hours after admission to the ER, the Systemic Inflammatory Response Syndrome (SIRS) score, the Multiple Organ Dysfunction Syndrome (MODS) score, the Acute Physiology, Age, Chronic Health Evaluation (APACHE) III, transfusion amounts, cause of death and the duration of ICU admission of the control group and the ulinastatin group were compared (Fig. 1).
Fig. 1. The study design

* IRB: institutional review board

b GCS: Glasgow coma scale
c CPR: cardiopulmonary resuscitation
d COPD: chronic obstructive pulmonary disease
e TNF: tumor necrosis factor
f IL: interleukin
g PMNE: polymorphonuclear leukocyte elastase
h SIRS: systemic inflammatory response syndrome
i MODS: multiple organ dysfunction syndrome
j APACHE: acute physiology, age, chronic health evaluation
3. Statistical analysis

Data were summarized and coded into a software (SPSS 13.0 for windows, SPSS Inc., Chicago, IL). Statistical analysis were performed with the Mann-Whitney U-test, Pearson chi-square test for demographic data, comparing parameters of the two groups, and the Wilcoxon test for comparison between the SIRS score, the MODS score and the APACHE III within the same group. A $p$ value $<$0.05 was considered statistically significant.
III. Results

1. Demographic data for the patients

The control group consisted of eleven patients, and the ulinastatin group consisted of eight. The mean age of each group were 48.0±17.1, 48.7±11.1 respectively, and of each group, five and eight patients were males respectively. There was no significant difference in the two groups (p>0.05). Regarding the injury mechanism, 8 were traffic accidents, 3 motorcycle accidents, 5 falls, 1 stab injury, 1 cultivator accident and 1 collision by rock. Injury Severity Score (ISS) was 27.1±22.5, 20.6±11.6 respectively (p=0.901), Revised Trauma Score (RTS) was 10.4±1.9, 10.3±1.8 respectively (p=0.858). However, there was no significant difference (Table 1).

Table 1. Demographic data of the enrolled patients

| Characteristics        | Control group (n=8) | Ulinastatin group (n=11) | P value |
|------------------------|--------------------|--------------------------|---------|
| Age, years             | 48.0±17.1          | 48.7±11.1                | 1.000²  |
| Sex, male/female, n    | 5/3                | 8/3                      | 0.636⁴  |
| Injury mechanism, n    |                    |                          | 0.040⁴  |
| Pedestrian             | 2                  | 1                        |         |
| Driver                 | 0                  | 1                        |         |
| Passenger              | 1                  | 3                        |         |
| Motorcycle             | 3                  | 0                        |         |
| Fall                   | 0                  | 5                        |         |
| Stab injury            | 0                  | 1                        |         |
| etc                    | 2                  | 0                        |         |
ISS\textsuperscript{a}  
27.1±22.5 \quad 20.6±11.6 \quad 0.901\textsuperscript{c}

RTS\textsuperscript{b}  
10.4±1.9 \quad 10.3±1.8 \quad 0.858\textsuperscript{c}

\textsuperscript{a} Mean±SD.

\textsuperscript{a} ISS: injury severity score

\textsuperscript{b} RTS: revised trauma score

\textsuperscript{c} Mann-Whitney U-test

\textsuperscript{d} Pearson Chi-square test

2. Comparison of laboratory data between the control group and the ulinastatin group

White blood cell counts, neutrophil counts, hemoglobin, pH, base excess and lactate were not significantly different between the control group and the ulinastatin group (p>0.05) (Table 2).

Table 2. Comparison of laboratory data between the control group and the ulinastatin group

| Parameters                  | Control group (n=8) | Ulinastatin group (n=11) | \( P \) value\textsuperscript{a} |
|-----------------------------|---------------------|--------------------------|---------------------------------|
| At admission                |                     |                          |                                 |
| pH                          | 7.42±0.72           | 7.35±0.15                | 0.265                           |
| Base excess (mmol/L)         | -6.1±3.4            | -8.2±5.2                 | 0.364                           |
| Lactate (mmol/L)             | 4.1±2.5             | 5.4±3.1                  | 0.600                           |
| WBC\textsuperscript{b} (10^9/L) | 22.4±5.8           | 22.4±8.1                 | 0.934                           |
| Neutrophil (10^9/L)          | 18.3±5.9            | 17.9±7.8                 | 0.741                           |
|                      | Value 1  | Value 2  | p-value |
|----------------------|----------|----------|---------|
| Hemoglobin (g/dL)    | 10.6±2.4 | 10.7±3.2 | 0.934   |
| **At 24 hours after admission** |          |          |         |
| pH                   | 7.40±0.06 | 7.29±0.35 | 0.733   |
| Base excess (mmol/L) | -2.1±2.7  | -4.7±14.5 | 0.435   |
| Lactate (mmol/L)     | 2.6±2.6   | 3.7±3.3   | 0.425   |
| WBC (10⁹/L)          | 11.6±4.1  | 8.8±2.1   | 0.241   |
| Neutrophil (10⁹/L)   | 9.7±3.7   | 6.6±3.7   | 0.257   |
| Hemoglobin (g/dL)    | 10.9±1.5  | 9.5±2.4   | 0.434   |
| **At 2 days after admission** |          |          |         |
| pH                   | 7.42±0.03 | 7.44±0.06 | 0.699   |
| Base excess (mmol/L) | 0.1±1.5   | 0.9±3.4   | 0.606   |
| Lactate (mmol/L)     | 0.7±0.2   | 1.4±0.7   | 0.134   |
| WBC (10⁹/L)          | 10.4±3.5  | 9.2±2.7   | 0.562   |
| Neutrophil (10⁹/L)   | 8.5±3.8   | 7.5±1.2   | 1.000   |
| Hemoglobin (g/dL)    | 9.3±0.9   | 9.3±0.9   | 0.816   |
| **At 3 days after admission** |          |          |         |
| pH                   | 7.42±0.03 | 7.41±0.04 | 0.698   |
| Base excess (mmol/L) | 0.2±0.8   | -0.63±3.19| 0.794   |
| Lactate (mmol/L)     | 0.8±0.3   | 1.0±0.9   | 0.724   |
| WBC (10⁹/L)          | 8.2±2.9   | 8.3±1.6   | 0.563   |
| Neutrophil (10⁹/L)   | 6.5±3.1   | 6.8±1.5   | 0.465   |
| Hemoglobin (g/dL)    | 9.4±1.1   | 9.1±0.9   | 0.353   |
| **At 7 days after admission** |          |          |         |
| pH                   | 7.45±0.05 | 7.46±0.03 | 0.721   |
| Base excess (mmol/L) | 2.4±2.9   | 1.4±2.2   | 0.471   |
| Lactate (mmol/L)     | 0.9±0.5   | 1.1±0.7   | 1.000   |
| WBC (10⁹/L)          | 10.2±3.5  | 9.8±5.4   | 0.655   |
| Neutrophil (10⁹/L)   | 7.4±3.6   | 7.7±3.1   | 0.732   |
| Hemoglobin (g/dL)    | 10.5±1.1  | 10.3±1.4  | 0.654   |

* Mean±SD.

a Mann-Whitney U-Test

b WBC: white blood cell
3. Comparison of serum TNF-α, IL-6, and PMNE levels between the control group and the ulinastatin group

1) Serum TNF-α levels

The serum TNF-α concentration increase up to the third day of hospitalization in the control group, however, it was a lower concentration than the initial on the 7th day. On the other hand, TNF-α levels decreased continuously within the ulinastatin group over the period of 7 days. However, there were no significant difference between the mean of serum TNF-α of the control group and the ulinastatin group (Fig. 2).
Fig. 2. A. Effects of ulinastatin on serum TNF-α levels. B. Changes of serum TNF-α levels after admission. HD0 means before injection of ulinastatin. (HD: hospitalized day)

2) Serum IL-6 levels

In the ulinastatin group, the serum concentration of IL-6 was 141.77±113.59 pg/ml after 1 day and then decreased to 52.82±29.68 pg/ml after the next day. But the control group showed an increase of serum IL-6 from 78.31±52.95 pg/ml to 100.70±42.57 pg/ml by the next day, however, decreased thereafter. The means of the control group and the ulinastatin group were not different (Fig. 3).
Fig. 3. A. Effects of ulinastatin on serum IL-6 levels. B. Changes of serum IL-6 levels after admission. HD0 means before infusion of ulinastatin. (HD : hospitalized day)
3) Serum PMNE levels

The concentration of serum PMNE in the control group increased continuously and was 11.58±5.57 ng/ml on the second day of hospitalization. In the ulinastatin group, serum PMNE kept up with the increased trend showing a small difference and lower average. The plasma concentration of PMNE of the ulinastatin group on the second hospital day was 4.33±1.21 ng/ml and was lower than that of the control group significantly (p=0.019). The change between the second hospitalized day and the admitted day was statistically significant (p=0.045) (Fig. 4).
Fig. 4. **A.** Effects of ulinastatin on serum PMNE levels. **B.** Changes of serum PMNE levels after admission. HD0 means before infusion of ulinastatin. (HD : hospitalized day)

4. Treatment and the final results

1) Transfusion, treatment and mortality
Within the 24 hours after admission to the ER, the total transfusion amount of packed red blood cells, fresh frozen plasma, and platelet concentration were not different between the two groups (p>0.05).

Within the control group, six of the eight patients underwent an operation, one had a conservative treatment, and one moribund discharged. In the ulinastatin group, there were six conservations and five operations. The treatment modalities in both groups were not significantly different (p=0.117).

One patient died as a result of a hemorrhagic shock in the control group. In the ulinastatin group, two patients died: one a hemorrhagic shock and the other of multiple organ dysfunction syndrome (Table 3).

Table 3. Transfusion, treatment modality and the final results between the control group and the ulinastatin group

| Parameters | Control group (n=8) | Ulinastatin group (n=11) | P value |
|------------|---------------------|--------------------------|---------|
| In 24 hours transfusion | | | |
| pRBC\textsuperscript{a} | 5.0±5.1 | 9.3±9.8 | 0.376\textsuperscript{f} |
| FFP\textsuperscript{b} | 0.8±1.5 | 2.9±3.3 | 0.089\textsuperscript{f} |
| PC\textsuperscript{c} | 0 | 2.2±3.7 | 0.117\textsuperscript{f} |
| Total transfusion | | | |
| pRBC | 7.9±8.3 | 11.6±11.2 | 0.430\textsuperscript{f} |
| FFP | 3.8±9.1 | 3.4±3.4 | 0.137\textsuperscript{f} |
| PC | 0 | 2.9±4.0 | 0.062\textsuperscript{f} |
| Treatment | | | |
| Conservation | 1 | 6 | 0.117\textsuperscript{g} |
Operation 6 5
Moribound discharge 1 0
ICU\textsuperscript{d} admission 12.0±19.2 5.8±6.3 0.787\textsuperscript{f}
Result 0.644\textsuperscript{g}
Discharge alive 6 6
Transfer 1 3
Death 1 2
Cause of death 0.672\textsuperscript{g}
Hypovolemia 1 1
MOF\textsuperscript{e} 0 1

\textsuperscript{a} Mean±SD.
\textsuperscript{b} pRBC: packed red blood cells
\textsuperscript{c} FFP: fresh frozen plasma
\textsuperscript{d} PC: platelet concentrate
\textsuperscript{e} ICU: intensive care unit
\textsuperscript{f} MOF: multiple organ failure
\textsuperscript{g} Mann-Whitney U-test
\textsuperscript{h} Pearson Chi-square test

2) Comparison of SIRS score, MODS score and APACHE III between the control group and the ulinastatin group

In the ulinastatin group, SIRS score and APACHE III decreased significantly (2.3±0.9 vs 0.8±0.9, p=0.03; 42.7±28.6 vs 24.9±23.8, p=0.02). MODS score decreased after 48
hours admission, but there was no difference in the two groups (4.0±3.7 vs 2.3±3.2, p=0.10). In the control group, SIRS score and APACHE III decreased also (2.8±1.0 vs 0.6±0.8, p=0.03; 45.0±28.2 vs 16.9±13.1, p=0.02). However, the MODS score was not different (2.3±1.5 vs 2.5±2.3, p=0.59) (Table 4).

The means of SIRS score, MODS score and APACHE III of two groups were not different significantly (Table 5).

Table 4. SIRS score, MODS score and APACHE III 48 hours after admission

| Parameters      | At study entry | 48 hours after admission | P value<sup>a</sup> |
|-----------------|----------------|--------------------------|---------------------|
| **Control group (n=8)** |                |                          |                     |
| SIRS<sup>b</sup> score | 2.8±1.0        | 0.6±0.8                  | 0.026               |
| MODS<sup>c</sup> score  | 2.3±1.5        | 2.5±2.3                  | 0.596               |
| APACHE<sup>d</sup> III  | 45.0±28.2      | 16.9±13.1                | 0.018               |
| **Ulinastatin group (n=11)** |                |                          |                     |
| SIRS score      | 2.3±0.9        | 0.8±0.9                  | 0.026               |
| MODS score      | 4.0±3.7        | 2.3±3.2                  | 0.102               |
| APACHE III      | 42.7±28.6      | 24.9±23.8                | 0.017               |

<sup>a</sup> Mean±SD.

<sup>b</sup> Wilcoxon test.

<sup>c</sup> SIRS: systemic inflammatory response syndrome

<sup>d</sup> MODS: multiple organ dysfunction syndrome

<sup>d</sup> APACHE: Acute physiology, Age, Chronic health evaluation
Table 5. Comparison of SIRS score, MODS score and APACHE III between the control group and the ulinastatin group

| Parameters          | Control group (n=8) | Ulinastatin group (n=11) | P value<sup>a</sup> |
|---------------------|---------------------|--------------------------|---------------------|
| At admission        |                     |                          |                     |
| SIRS<sup>b</sup> score | 2.8±1.0             | 2.3±0.9                  | 0.224               |
| MODS<sup>c</sup> score | 2.3±1.5             | 4.0±3.7                  | 0.530               |
| APACHE<sup>d</sup> III | 45.0±28.2           | 42.7±28.6                | 0.563               |
| At 2 days after admission |                       |                          |                     |
| SIRS score          | 0.6±0.8             | 0.8±0.9                  | 0.702               |
| MODS score          | 2.5±2.3             | 2.3±3.2                  | 0.610               |
| APACHE III          | 16.9±13.1           | 24.9±23.8                | 0.451               |
| At 3 days after admission |                       |                          |                     |
| SIRS score          | 0.3±0.5             | 0.3±0.5                  | 1.000               |
| MODS score          | 1.8±1.3             | 1.9±1.6                  | 1.000               |
| APACHE III          | 18.0±17.9           | 15.3±9.1                 | 0.886               |
| At 7 days after admission |                       |                          |                     |
| SIRS score          | 0.4±0.8             | 1.0±1.0                  | 0.250               |
| MODS score          | 1.0±1.1             | 1.7±1.7                  | 0.455               |
| APACHE III          | 19.5±16.3           | 17.4±14.5                | 0.886               |

<sup>a</sup> Mean±SD.

<sup>a</sup> Mann-Whitney U-test.

<sup>b</sup> SIRS: systemic inflammatory response syndrome

<sup>c</sup> MODS: multiple organ dysfunction syndrome

<sup>d</sup> APACHE: Acute physiology, Age, Chronic health evaluation
IV. Discussion

One-hundred years ago, it was reported that human urine had the capacity to inhibit trypsin\textsuperscript{10}. In 1955, the protein with antitryptic activity in urine was isolated\textsuperscript{10}. One main function of bikunin, a urinary trypsin inhibitor, is to inhibit serine protease, especially elastase and to suppress neutrophils, lymphocytes and macrophages increased by infection and inflammation\textsuperscript{11}. Now commonly known as ulinastatin, this urinary trypsin inhibitor inhibits cell apoptosis by free radicals and lipid peroxidation in renal ischemia-reperfusion injuries and has a suppressive effect against mitochondrial injury\textsuperscript{8}.

It is known that the effect of ulinastatin is dose-dependant\textsuperscript{6, 12, 13}. In this study, 300,000 IU, three times per day was used, for this is the commonly recommended dosage for acute circulatory failure. This is comparison to the 50,000 IU/kg selected in canine experiments\textsuperscript{9, 14}, and 1,500,000 IU for a period of five days selected in clinical studies\textsuperscript{7, 15}. In Japan, Ulinastatin 6,000 IU/kg was permitted as the maximum for safety\textsuperscript{16}. Although side effects of ulinastatin are thought to include nausea, vomiting and hypersensitivity reaction, etc, these side effects were not seen in this study, nor have they been seen in others. Furthermore, in most animal studies, ulinastatin was administrated before the induction of a hemorrhagic shock or a septic shock, and in clinical trials before a laparotomy or blood transfusion\textsuperscript{5, 9, 14}. However, ulinastatin was prescribed after diagnosing a traumatic hemorrhagic shock in the ER in this study.

Serum TNF-\(\alpha\), IL-6, PMNE were chosen as the inflammatory mediators associated with a hemorrhagic shock. Specifically, serum TNF-\(\alpha\) is thought to be an important factor
among the three because it is secreted by the activated macrophage, stimulating other inflammatory cytokines and bringing inflammatory cells to tissues\textsuperscript{17}. Furthermore, IL-6 is secreted from the cells by early inflammatory reaction. In the rat model, trauma-induced hemorrhagic shock increased plasma levels of the liver enzyme alanine aminotransferase (ALT), a marker of liver injury, showing significant correlation with IL-6\textsuperscript{18}. Witthaut \textit{et al.}\textsuperscript{19} reported that serum IL-6 values in a septic shock were significantly higher 150 times than those of the controls, therefore IL-6 was the main cytokine of infection and inflammation. Finally, neutrophils secrete PMNE when inflammation occurs, which can injure every tissue and organ\textsuperscript{20, 21}.

Protease such as elastase is typically seen to be increased in case of inflammation and/or infection, and any substance which can inhibit this protease results in an anti-inflammatory effect\textsuperscript{22}. $\alpha_1$-protease inhibitor ($\alpha_1$-PI) and ulinastatin are the intrinsic physiologic protease inhibitor which can suppress PMNE activity. However, in inflammatory tissues, $\alpha_1$-PI loses its ability to function in the acidic conditions, but ulinastatin can continue to inhibit PMNE\textsuperscript{23}. In addition, ulinastatin protects the endothelial cell against neutrophil-mediated injury not only by inactivating the extracellular elastase secreted by neutrophils, but also by acting directly on neutrophils and suppressing the production and secretion of the activated elastase from them\textsuperscript{13}.

There are the animal studies about the antibodies against rat IL-6 and TNF-$\alpha$. Toth \textit{et al.}\textsuperscript{18} reported that ALT was suppressed by two thirds after injecting anti-IL-6 in a hepatic injury of the resuscitated rat from a trauma-induced hemorrhagic shock. Furthermore, Vallejo \textit{et al.}\textsuperscript{24} found that treatment with TNF-$\alpha$ receptor antagonist abrogated
inflammatory mediators and left ventricular dysfunction before a hemorrhagic shock or at the time of resuscitation.

Ulinastatin has effectiveness in animal studies with a septic shock and a hemorrhagic shock induced experimentally\(^9,14\). Specifically, in the septic shock canine model study, ulinastatin improved blood pressure and lactic acid levels. Interestingly, although ulinastatin does not have anti-microbial activity, the ulinastatin-treated group was found to have a bacterial count that was significantly decreased, and a high survival rate\(^14\). It is thought that ulinastatin might activate the reticuloendothelial system and the phagocytosis\(^14\). Furthermore, in hemorrhagic shock, the protective effect of ulinastatin might be associated with the up-regulation of Bcl-2, a kind of inhibitor of the cell apoptosis\(^8\).

Based on the literature, we hypothesized that ulinastatin would inhibit the inflammatory cytokines such as TNF-\(\alpha\), IL-6 and PMNE. But in actuality, there were no difference found among the averages of TNF-\(\alpha\), IL-6 and PMNE concentrations except for PMNE on the second day of hospitalization.

Aosasa et al.\(^6\) reported that ulinastatin decreased the TNF-\(\alpha\) production of lipopolysaccharide (LPS)-stimulated monocytes, but there was no significant difference. Serum TNF-\(\alpha\) concentration was low when ulinastatin was used before LPS stimulation and the serum concentration of TNF-\(\alpha\) was in inverse proportion to the amount of ulinastatin. In this study, after ulinastatin was injected to the ulinastatin group, serum TNF-\(\alpha\) level was lower than the initial serum TNF-\(\alpha\) levels. Nonetheless, serum TNF-\(\alpha\) concentrations of the control group increased until the third hospitalized day.
Serum IL-6 concentrations of the ulinastatin group decreased by half after one day of ulinastatin injection and from the second day of hospitalization. Serum IL-6 concentration was lower than initial IL-6 concentration in the ER within the two groups. Nishiyama et al.\textsuperscript{5)} proposed that ulinastatin might be useful to inhibit blood transfusion-induced increase of serum PMNE concentrations but not IL-6 after a laparotomy. In their study, serum PMNE levels increased to a lesser extent than the control group by 50 percent. Tani et al.\textsuperscript{15)} used a total of 1,500,000 IU of ulinastatin for 5 days after laparotomy. Within the control group and the urinary trypsin inhibitor (UTI) group, PMNE concentrations were not different statistically but moved within a narrow range in the UTI group. In another study, laparotomy was taken and ulinastatin was administered to the patients at the same time. In this instance, although serum PMNE levels did not decrease significantly, coagulation and fibrinolysis was inhibited significantly\textsuperscript{16}).

The reference range of serum PMNE levels was reported as 20–180 μg/L\textsuperscript{25}) or 21–165 μg/L\textsuperscript{5).} We did not check the normal serum concentration of PMNE, but the reference range was 0.15–3.0 ng/ml. The lowest value was 1.49 ng/ml and the highest value was 19.88 ng/ml. In the clinical study about laparotomy or blood transfusion, the peak value of serum PMNE was seen immediately after an operation or a blood transfusion. Ulinastatin administration were seen to gradually decrease serum concentrations of PMNE, but the serum concentration of PMNE were shown to increase three-fold in the control group\textsuperscript{5,16).} In this study, serum PMNE levels increased more than two-fold on the second hospitalized day within the control group, but serum PMNE concentration was suppressed from increasing in the ulinastatin group. In addition, serum
IL-6 showed its peak value upon admission to the ER, and then was found to gradually
decrease in the ulinastatin group, however in the control group, serum IL-6 levels after
one day of hospitalization was at its highest value, and then was decreased. Similarly, it
has been shown in other studies that within septic shock patients, serum IL-6
concentration was peaked on the first day of diagnosis and then decreased slowly, and
serum IL-6 concentration showed the maximum after 24 hours in the rat model
hemorrhagic shock study\textsuperscript{18, 19}.

The laboratory data, physiologic results like the SIRS score, MODS score and APACHE
III and the mortality had no difference between the two groups. First of all, this may be
due to the small number of patients and that is one limitation of this study. Secondly, it
may be because ultimately the control group underwent the same medical procedures as
the ulinastatin group, such as a blood transfusion and fluid therapy, and having been
required an operation. Therefore, a larger number of patients and a longer period for
clinical study is needed. Furthermore, studies regarding the various dosages of ulinastatin
for a traumatic hemorrhagic shock patients are needed.
V. Conclusion

Ulinastatin was shown to prevent the increase of serum PMNE levels in traumatic patients with a hemorrhagic shock.
1. Brun-Buisson C. The epidemiology of the systemic inflammatory response. Intensive Care Med 2000;26:S64-74.

2. Maitra SR, Gestring M, El-Maghrabi MR. Alternations in hepatic 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase and glucose-6-phosphatase gene expression after hemorrhagic hypotension and resuscitation. Shock 1997;8:385-8.

3. Lee CC, Marill KA, Carter WA, Crupi RS. A current concept of trauma-induced multiorgan failure. Ann Emerg Med 2001;38:170-6.

4. Riedemann NC, Neff TA, Guo RF, Bernacki KD, Laudes IJ, Sarma JV, et al. Protective effects of IL-6 blockade in sepsis are linked to reduced c5a receptor expression. J Immunol 2003;170:503-7.

5. Nishiyama T, Hanaoka K. Do the effects of a protease inhibitor, ulinastatin, on elastase release by blood transfusion depend on interleukin 6? Crit Care Med 2001;29:2106–10.

6. Aosasa S, Ono S, Mochizuki H, Tsujimoto H, Ueno C, Matsumoto A. Mechanism of the inhibitory effect of protease inhibitor on tumor necrosis factor α production of monocytes. Shock 2001;15:101–5.
7. Sato Y, Ishikawa S, Otaki A, Takahashi T, Hasegawa Y, Suzuki M, et al. Induction of acute-phase reactive substances during open-heart surgery and efficacy of ulinastatin. Inhibiting cytokines and postoperative organ injury. Jpn J Thorac Cardiovasc Surg 2000;48:428–34.

8. Chen CC, Liu ZM, Wang HH, He W, Wang Y, Wu WD. Effect of ulinastatin on renal ischemia-reperfusion injury in rats. Acta Pharmacol Sin 2004;25:1334-40.

9. Ohnishi H, Suzuki K, Niho T, Ito C, Yamaguchi K. Protective effects of urinary trypsin inhibitor in experimental shock. Jpn J Pharmacol. 1985;39:137-44.

10. Fries E, Blom AM. Bikunin-not just a plasma proteinase inhibitor. Int J Biochem Cell Biol 2000;32:125-37.

11. Pugia MJ, Lott JA. Pathophysiology and diagnostic value of urinary trypsin inhibitors. Clin Chem Lab Med 2005;43:1-16.

12. Yano T, Anraku S, Nakayama R, Ushijima K. Neuroprotective effect of urinary trypsin inhibitor against focal cerebral ischemia-reperfusion injury in rats. Anesthesiology 2003;98:465-73.
13. Nakatani K, Takeshita S, Tsujimoto H, Kawamura Y, Sekine I. Inhibitory effect of serine protease inhibitors on neutrophil-mediated endothelial cell injury. J Leukoc Biol 2001;69:241-7.

14. Tani T, Aoki H, Yoshioka T, Lin KJ, Kodama M. Treatment of septic shock with a protease inhibitor in a canine model: a prospective, randomized, controlled trial. Crit Care Med 1993;21:925-30.

15. Tani T, Abe H, Endo H, Hanasawa K, Kodama M. Effects of a urinary trypsin inhibitor on acute circulatory insufficiency after surgical operation. Am J Surg 1998;175:142-5.

16. Nishiyama T, Yokoyama T, Yamashita K. Effects of a protease inhibitor, ulinastatin, on coagulation and fibrinolysis in abdominal surgery. J Anesth 2006;20:179-82.

17. Cohen J. The immunopathogenesis of sepsis. Nature 2002;420:885-91.

18. Toth B, Yokoyama Y, Schwacha MG, George RL, Rue III LW, Bland KI, et al, Insights into the role of interleukin-6 in the induction of hepatic injury after trauma-hemorrhagic shock. J Appl Physiol 2004;97:2184-9.
19. Withaut R, Busch C, Fraunberger P, Walli A, Seidel D, Pilz G, et al. Plasma atrial natriuretic peptide and brain natriuretic peptide are increased in septic shock: impact of interleukin-6 and sepsis associated left ventricular dysfunction. Intensive Care Med 2003;29:1696-1702.

20. Duswald KH, Jochum M, Schramm W, Fritz H. Released granulocytic elastase: An indicator of pathobiochemical alterations in septicemia after abdominal surgery. Surgery 1985;98:892-9.

21. Pacholok SG, Davies P, Dorn C, Finke P, Hanlon WA, Mumford RA, et al. Formation of polymorphonuclear leukocyte elastase: alpha 1 proteinase inhibitor complex and A alpha (1-21) fibrinopeptide in human blood stimulated with the calcium ionophore A23187. A model to characterize inhibitors of polymorphonuclear leukocyte elastase. Biochem Pharmacol 1995;49:1513-20.

22. Mania-Pramanik J, Potdar SS, Vadigoppula A, Sawant S. Elastase: a predictive marker of inflammation and/or infection. J Clin Lab Anal 2004;18:153-8.

23. Ogawa M, Nishibe S, Mori T, Neumann S. Effect of human urinary trypsin inhibitor on granulocyte elastase activity. Res Commun Chem Pathol Pharmacol 1987;55:271-4.
24. Vallejo JG, Nemoto S, Ishiyama M, Yu B, Knuefermann P, Diwan A, et al.

Functional significance of inflammatory mediators in a murine model of resuscitated hemorrhagic shock. Am J Physiol Heart Circ Physiol 2005;288:1272-7.

25. Neumann S, Gunzer G, Hennrich N, Lang H. "PMN-elastase assay": enzyme immunoassay for human polymorphonuclear elastase complexed with alpha 1-proteinase inhibitor. J Clin Chem Clin Biochem 1984;22:693-7.
출혈성 쇼크가 동반된 외상 환자에서

Ulinastatin의 항염증 효과

연세대학교 대학원 의과학 박경혜

배경 및 목적: Ulinastatin은 사람의 소변에서 분리 정제된 단단백질로서 단백 분해 효소를 저해하고 항염증 작용이 있다. 또한 신장의 혈액 순상을 줄이고 수혈 후 주요 장기에 손상을 유발하는 Polymorphonuclear Leukocyte Elastase (PMNE)를 억제하는 효과가 있다고 알려져 있다. 그러나 외상에 의한 출혈성 쇼크에서는 ulinastatin의 효과는 잘 알려져 있지 않다. 본 연구에서는 출혈성 쇼크를 동반한 외상 환자에서 ulinastatin 투여가 환자의 예후에 좋은 영향을 주는지 알아보고자 한다.

대상 및 방법: 2006년 6월부터 10월까지 응급실에 내원한 출혈성 쇼크를 동반한 외상 환자를 대상으로 하였다. 응급실 내원 당시에 출혈성 쇼크가 진단된 환자 중 무작위로 정하여 실험군에 해당하는 환자에게 ulinastatin 을 1회에 10만
단위 석 8시간 간격으로 총 3회 투여하였다. 투여 전, 투여 후 24시간, 2일째, 3일째, 7일째에 PMNE, Tumor Necrosis Factor Alpha (TNF-α), Interleukin 6 (IL-6)을 측정하였고, 내원 당시와 내원 후의 Systemic Inflammatory Response Syndrome (SIRS) score, Multiple Organ Dysfunction Syndrome (MODS) score와 Acute Physiology, Age, Chronic Health Evaluation (APACHE) III를 비교하였다.

결과: 대상 환자는 모두 19명으로 대조군은 8명, 실험군은 11명이었으며, 두 군의 Injury Severity Scale은 차이가 없었다 (p=0.091). 그리고 두 군의 혈액학적 검사 소견, 치료, 사망률 등에도 유의한 차이가 없었다. PMNE의 농도는 내원 2일째 대조군이 11.58±5.57 ng/ml, 실험군이 4.33±1.21 ng/ml로 두 군 간의 유의한 차이를 보였다 (p=0.19). TNF-α, IL-6는 내원 1일째부터 감소하면서 대조군보다 낮은 값을 보였으나 의미 있는 차이는 없었다.

결론: Ulinastatin 30만 단위는 출혈성 쇼크를 동반한 외상 환자의 혈청 PMNE 농도를 내원 2일째 ulinastatin을 투여하지 않은 환자보다 의미 있게 낮춘다

핵심되는 말: Ulinastatin, Hemorrhagic shock, Polymorphonuclear Leukocyte Elastase, Tumor Necrosis Factor Alpha, Interleukin 6