SHOCK, Vol. 12, No. 2, pp. 102–104, 1999

PLASMA GELSOLIN IS REDUCED IN TRAUMA PATIENTS

Benny Dahl,* Frank Vinholt Schiødt,† Peter Ott,† Robert Gvozdenovic,‡ Helen L. Yin,§ and William M. Lee¶

*Departments of *General Surgery, Division of Burn/Trauma/Critical Care, §Physiology, and ¶Internal Medicine, Liver Division, The University of Texas Southwestern Medical Center at Dallas; Departments of †Hepatology and ‡Orthopaedic Surgery, Rigshospitalet, Copenhagen, Denmark*

Received 26 Jan 1999; first review completed 21 Feb 1999; accepted in final form 15 Mar 1999

ABSTRACT—Tissue injury results in the release of the intracellular protein actin which is cleared from the circulation by the plasma proteins gelsolin and Gc-globulin, constituting the Extracellular Actin Scavenger System (EASS). Experimental studies have shown that excessive amounts of actin in the circulation can lead to a condition resembling multiple organ dysfunction syndrome (MODS), and we have previously demonstrated that the level of Gc-globulin is decreased after severe trauma. The purpose of the present study was to determine whether the plasma levels of gelsolin were altered in the early phase after trauma. Twenty-three consecutive trauma patients were studied. Plasma samples were assayed for gelsolin by immunonephelometry with polyclonal rabbit antihuman gelsolin prepared in our own laboratory. The median time from injury until the time the first blood sample was taken was 52 min (range 20–110) and the median Injury Severity Score (ISS) was 20 (range 4–50). The gelsolin level on admission was reduced significantly in the trauma patients compared with normal controls. The median level was 51 mg/L (7–967) vs. 207 mg/L (151–621), $P < 0.0001$. There was no correlation between admission levels of gelsolin and ISS or survival. This study illustrates that the plasma concentration of gelsolin is significantly diminished immediately after traumatic injury. Further studies are necessary to establish a role for gelsolin or EASS in the development of MODS in trauma patients. The level of serum or plasma gelsolin can be determined rapidly and accurately using a nephelometric assay.

KEYWORDS—gelsolin, multiple trauma, MODS, actin, ISS, outcome, immunonephelometry

INTRODUCTION

While multiple organ dysfunction syndrome (MODS) commonly develops after severe trauma, a full understanding of the pathophysiologic events which occur after trauma would help in the development of effective treatments. In animals, intravenous injection of large amounts of actin results in rapid death due to the formation of actin-containing microthrombi eliciting endothelial injury resembling the pathological findings in adult respiratory distress syndrome (ARDS), a condition invariably linked to MODS (1).

Actin is a ubiquitous structural protein in muscle and non-muscle cells. It exists in an equilibrium between monomers (G-actin) and polymers (F-actin) (2, 3). During normal cell turnover and especially during tissue injury, cellular actin is released into the circulation where the ionic composition of the extracellular fluid favors polymerization of actin molecules into F-actin filaments (3). The plasma proteins gelsolin and Gc-globulin appear to remove F-actin from the circulation and constitute the Extracellular Actin Scavenger System (EASS), a homeostatic mechanism. Gelsolin is an actin-severing protein that promotes F-actin depolymerization, so that G-actin monomers can bind to Gc-globulin and be cleared from the circulation (4). Complexes of G-actin:Gc-globulin are cleared primarily in the liver and in the spleen (5, 6). Gelsolin exists in a cytoplasmic and a plasma isoform encoded by the same gene (7). Plasma gelsolin, however, contains an additional 25 amino acids compared to the cytoplasmic form, and is synthesized primarily by muscle (8, 9).

Clinical studies suggest that in conditions of massive cell death, release of large amounts of cellular actin may overwhelm the EASS. Lind et al. (3) suggested that F-actin filaments, if not removed rapidly, may increase the viscosity of the extracellular fluids including plasma, leading to impaired perfusion of organs. Plasma levels of Gc-globulin are decreased in patients with acute liver failure and septic shock and correlate with the severity of the disease and with survival. Patients with the most severely decreased Gc levels (< 10% of normal values) have a poor outcome (10–12).

Since major trauma often is accompanied by tissue or cell death, it is likely that the EASS is affected in such conditions. We have recently shown that the serum level of Gc-globulin, measured by rocket immunoelectrophoresis (RIE), is reduced in patients with severe trauma (13). The purpose of the present study was to determine whether the plasma levels of gelsolin, measured by immunonephelometry, were also altered in the early phase after trauma.

MATERIAL AND METHODS

Study population

The study population consisted of 23 trauma patients, 10 women and 13 men, with a median age of 40 years (range 19–86). All patients fulfilled one or more of the criteria for severe injury described by the American College of Surgeons (14):

1. Injury to more than 1 organ system (CNS, respiration, circulation, abdomen, extremities).
2. More than 1 fracture of the long bones or the axial skeleton (pelvis/spine).
3. Fracture of the axial skeleton combined with another fracture.

All patients but one patient sustained blunt trauma. Mechanisms of trauma are shown in Table 1.

The time of injury was established from police and ambulance records, and only patients primarily admitted within 2 h after the injury were included after

Address reprint requests to Benny Dahl MD PhD, Department of General Surgery, Division of Burn/Trauma/Critical Care, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235-9031.

TABLE 1. **Mechanisms of injury**

Mechanism of injury	Number of patients
Pedestrian/bicyclist hit by motor vehicle	11
Fall from height	8
Motor vehicle collision	3
Explosion	1
Stabbing wound through spine	1

informed consent. All 23 patients had a blood sample taken on arrival to the emergency department. The median time from injury until the first blood sample was taken was 52 min (range 20–110).

To describe the time course of gelsolin after injury, 11 patients had blood samples taken the first 6 days of hospitalization.

At discharge or death, all patients were scored according to the Injury Severity Score (ISS) (15, 16). The median ISS was 20 (range 4–50). Trauma-related death was defined as death within 30 days after the trauma. Seven patients died and 16 survived. The study was approved by the local ethics committee, and patients were included after informed consent from the patient or the next of kin.

Control group

The population of normal individuals consisted of 25 healthy blood donors, who had an extra blood sample taken immediately after donation of 450 mL blood. The population consisted of 10 women and 15 men with a median age of 32 years (range 18–58).

Analyses or patients and methods

Gelsolin concentration was quantified in sera from 23 trauma patients, and in 25 age- and sex-matched healthy individuals.

Immunonephelometry was performed using a Behring Nephelometer (Behring Diagnostics Inc, Westwood, MA), according to the method described by Wians et al. (17). The principle of immunonephelometry is based on a positive linear relationship between the scattering of a light source by antigen-antibody complexes and the concentration of antigen (i.e., gelsolin). Briefly, a working gelsolin standard solution (400 μg/mL) was prepared by manually diluting a 1 mg/mL solution of purified gelsolin 1:2.5 with N-Diluent (Behring Diagnostics Inc). The working standard solution and patient's plasma samples were assayed in duplicate using an immunoglobulin fraction of rabbit serum prepared by immunization of New Zealand White rabbits (18).

Statistics

Data were tested for normal distribution using the Kolmogoroff-Smirnov test. Due to the non-normal distribution of data, comparison between groups was done using Mann-Whitney rank sum test. Likewise, all results are expressed as median and range, and the correlation between gelsolin values and ISS was calculated using Spearman's rank test. $P < 0.05$ was considered significant.

RESULTS

The gelsolin levels measured by this assay were reduced significantly in the trauma patients compared with normal controls: 51 mg/L (7–967) vs. 207 mg/L (151–621), $P < 0.0001$ (Figure 1). There was no correlation between admission levels of gelsolin and ISS ($P = 0.7$). Neither was there any significant difference in admission levels of gelsolin in survivors compared to non-survivors ($P = 0.86$).

Using a cut-off level of 150 mg/L, no normal controls had plasma gelsolin concentrations below that level, and only 5 of the 23 trauma patients had gelsolin concentrations above that level.

In the patients with serial blood samples, the lowest levels of gelsolin were found on day 1 after the injury. Thereafter, the gelsolin level gradually increased to reach the normal range on day 6. Again, no correlation between survival or ISS was found.

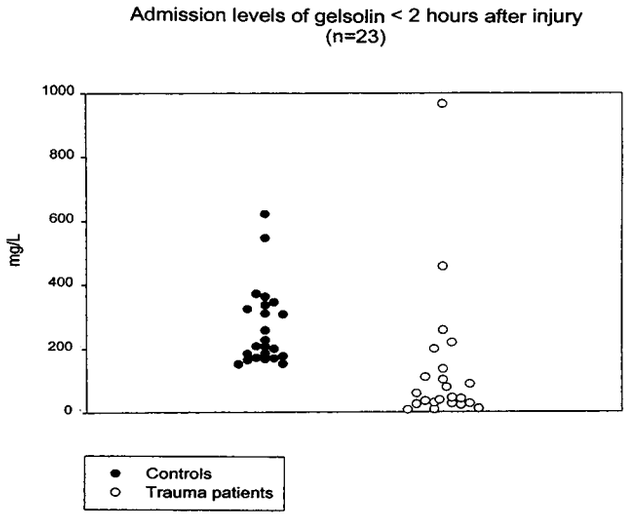

FIG. 1.

DISCUSSION

This study demonstrates that the plasma concentration of gelsolin measured within 2 h after blunt trauma is reduced to an average of 25% of normal values. This result corresponds to our previous findings regarding EASS in trauma patients, where Gc-globulin levels were markedly reduced on admission (13), although the reduction in gelsolin levels is even more profound than that observed for Gc. The very low gelsolin levels are presumed to result from consumption of scavenging proteins due to massive actin release and the process of clearance of filamentous actin. However, since gelsolin is primarily synthesized in muscle (4), it would be released from muscle in trauma settings and may partially ameliorate the gelsolin depletion observed. Thus, gelsolin depletion in the circulation might be even more impressive if not for the simultaneous release of actin and gelsolin from damaged muscle tissue.

Gelsolin depletion was observed in our previous study of muscle diseases; all patients in that study had elevated creatinine kinase levels due to either ischemic injury or polymyositis, and none were due to blunt trauma (18). Using quantitative immunoblotting, the gelsolin levels were reduced to approximately 50% of normal levels in that study vs. the more profound changes in the present one. However, the samples analyzed in the earlier study were collected over days rather than hours, and the insults were less acute than those observed in the trauma patients.

There are likely to be several other determinants of gelsolin levels after trauma besides tissue release and consumption. Clearly, an inflammatory response is part of traumatic injury and gelsolin levels may be affected by cytokine secretion. Yin et al. (19) and Johnston et al. (20) have shown that macrophages are capable of secreting gelsolin, and the mobilization of macrophages to sites of injury may also play a role in determining gelsolin plasma levels.

The gelsolin level dropped dramatically within 2 h of injury.

There are other examples of early response to trauma. Increased plasma levels of the cytokine interleukin-6 have been demonstrated within 1 to 2 h after traumatic injury (21, 22). Additionally, the plasma concentration of tumor necrosis factor (TNF) is increased within hours after combined trauma and hemorrhage (23, 24). A recent study by Hecke et al. (25) found that the concentration of complement C3a at the time of the injury was correlated to survival. Of interest, patients who subsequently developed sepsis had a higher level of C3a at the time of injury compared to patients who did not develop complications. Although this difference did not reach significance, it suggests that late complications after trauma may depend on pathophysiological processes which take place early after the injury.

Based on our previous studies of other tissue injury settings (severe hepatic, pancreatic or myocardial necrosis) in which the level of EASS proteins (Gc or gelsolin) correlated with the extent of injury, we were surprised that no correlation between gelsolin levels and either ISS or survival was found. Although virtually all patients had sustained extensive blunt trauma, and 10 had major muscle lacerations (crush injuries), *post hoc* analysis of the results omitting patients with crush injuries from the group did not change the lack of correlation between admission levels of gelsolin and survival or injury severity. All but 5 patients had subnormal levels of gelsolin on arrival. These 5 patients did not differ from the rest of the patients regarding survival, ISS, time to blood sample, age, sex, or crush injury vs. non-crush injury. We are aware that the present study holds limited statistical power due to the relatively low number of patients included. We do find, however, that it provides a clear answer to the question of whether or not gelsolin levels are altered in the early phase after trauma. Further studies on larger patient populations are needed to determine whether any subgroups of patients have affected their EASS in a special way. Larger studies will also provide a more detailed understanding of the change in plasma gelsolin over time after injury. The fact that both components of the EASS are affected in the early phase after injury indicates a possible role of gelsolin and Gc-globulin in the systemic inflammatory response syndrome (SIRS) after trauma. Since uncontrolled SIRS leads to MODS, this makes it attractive to speculate that modulation of EASS might prevent organ complications after injury.

In conclusion, using a new and accurate quantitative assay, we have determined that gelsolin, one of the essential proteins of the actin-scavenging system, is markedly and rapidly depleted in most patients after trauma. Nephelometric measurement of gelsolin levels provides a rapid and accurate method of determining gelsolin levels in plasma or serum. While this technique is readily applied to evaluation of clinical samples, a larger study will be necessary to determine whether there is a correlation between gelsolin levels and type or extent of trauma.

ACKNOWLEDGMENT

B. Dahl is supported by The Danish Research Council (Grant # 9602006), Danish Foundation for the Advancement of Medical Science, Jeppe Juhl and Ovita Juhls Foundation, Bloddonorernes Sikrings- og Forskningsfond, and NOVO Nordisk Foundation. H.L. Yin is supported by the Robert A. Welch Foundation.

REFERENCES

1. Haddad JG, Harper KD, Gouth M, Pietra GG, Sanger JW: Angiopathic consequences of saturating the plasma scavenger system for actin. *Proc Natl Acad Sci* 87:1381–5, 1990.
2. Carlier M-F: Actin: protein structure and filament dynamics. *J Biol Chem* 266:1–4, 1991.
3. Lind SE, Smith DB, Janmey PA, Stossel TP: Depression of gelsolin levels and detection of gelsolin-actin complexes in plasma of patients with acute lung injury. *Am Rev Respir Dis* 138:429–34, 1988.
4. Liu YT, Rozelle AL, Yin HL: The gelsolin family of actin-filament severing and capping proteins. In Maruta H, Kohama K (eds): *G proteins, Cytoskeleton and Cancer*. Austin, TX: R.G. Landes Co., pp 19–35, 1998.
5. Lind SE, Smith DB, Janmey PA, Stossel TP: Role of plasma gelsolin and the vitamin D-binding protein in clearing actin from the circulation. *J Clin Invest* 78:736–42, 1986.
6. Dueland S, Nenseter MS, Drevon CA: Uptake and degradation of filamentous actin and vitamin D-binding protein in the rat. *Biochem J* 274: 237–41, 1991.
7. Kwiatkowski DJ, Stossel TP, Orkin SH, Mole JE, Colten HR, Yin HL: Plasma and cytoplasmic gelsolins are encoded by a single gene and contain a duplicated actin-binding domain. *Nature* 323:455–8, 1986.
8. Kwiatkowski DJ, Mehl R, Izumo S, Nadal-Ginard B, Yin HL: Muscle is the major source of plasma gelsolin. *J Biol Chem* 263:8239–43, 1988.
9. Lee WM, Galbraith RM: The extracellular actin-scavenger system and actin toxicity. *New Eng J Med* 326:1335–41, 1992.
10. Lee WM, Reines D, Watt GN, et al.: Alterations in Gc-protein levels and complexing in septic shock. *Circ Shock* 28:249–55, 1989.
11. Schiødt FV, Bondesen S, Petersen I, Dalhoff K, Ott P, Tygstrup N: Admission levels of serum Gc-globulin: predictive value in fulminant hepatic failure. *Hepatology* 23:713–8, 1996.
12. Schiødt FV, Ott P, Bondesen S, Tygstrup N: Reduced serum Gc-globulin concentrations in patients with fulminant hepatic failure: Association with multiple organ failure. *Crit Care Med* 25:1366–70, 1997.
13. Dahl B, Schiødt FV, Ott P, Kiaer T, Bondesen S, Tygstrup N: Gc-globulin in the early course of multi-trauma. *Crit Care Med* 26:285–9, 1998.
14. American College of Surgeons, Commitee on Trauma: Hospital resources for optimal care of the injured patient. *Bull Am Coll Surg* 64:42–9, 1979.
15. American Association for Automotive Medicine: *The Abbreviated Injury Scale (AIS)*. 1990 Revision. Des Plaines, Illinois,USA: 1990.
16. Baker SP, O'Neill B, Haddon W, Long WB: The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. *J Trauma* 14:187–96, 1974.
17. Wians FH, Lin W, Brown LP, Schiødt FV, Lee WM: Immunonephelometric quantification of group-specific component protein in patients with acute liver failure. *Liver Transplant Surg* 3:28–33, 1997.
18. Suhler E, Lin W, Yin HL, Lee WM: Decreased plasma gelsolin concentrations in acute liver failure, myocardial infarction, septic shock, and myonecrosis. *Crit Care Med* 25:594–8, 1997.
19. Yin HL, Kwiatkowski DJ, Mole JE, Cole FS: Structure and biosynthesis of cytoplasmic and secreted variants of gelsolin. *J Biol Chem* 259:5271–6, 1984.
20. Johnston PA, Yu FX, Reynolds GA, Yin HL, Moomaw CR, Slaughter CA, Sudhof TC: Purification and expression of gCap39. An intracellular and secreted Ca2(+)-dependent actin-binding protein enriched in mononuclear phagocytes. *J Biol Chem* 265:17946–52, 1990.
21. Hoch RC, Rodriguez R, Manning Teal: Effects of accidential trauma on cytokine and endotoxin production. *Crit Care Med* 21:839–45, 1993.
22. Di Padova F, Pozzi C, Tondre Mjeal: Selective and early increase of IL-1 inhibitors, IL-6 and cortisol after elective surgery. *Clin Exp Immunol* 85:137–42, 1991.
23. Ayala A, Wang P, Ba ZF, Perrin MM, Ertel W, Chaudry IH: Differential alterations in plasma IL-6 and TNF levels after trauma and hemorrhage. *Am J Physiol* 260:R167–71, 1991.
24. Ayala A, Perrin MM, Meldrum DR, Ertel W, Chaudry IH: Hemorrhage induced and increase in serum TNF which is not associated with elevated levels of endotoxin. *Cytokine* 2:170–4, 1990.
25. Hecke F, Schmidt U, Kola A, Bautsch W, Klos A, Kohl J: Circulating complement proteins in multiple trauma patients - correlation with injury severity, development of sepsis, and outcome. *Crit Care Med* 25:2015–24, 1997.