

## Recombinant plasma gelsolin infusion attenuates burn-induced pulmonary microvascular dysfunction

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**Rothenbach, Patricia A., Benny Dahl, Jason J. Schwartz, Grant E. O'Keefe, Masaya Yamamoto, William M. Lee, Jureta W. Horton, Helen L. Yin, and Richard H. Turnage.** Recombinant plasma gelsolin infusion attenuates burn-induced pulmonary microvascular dysfunction. *J Appl Physiol* 96: 25–31, 2004. First published May 2, 2003; 10.1152/jappphysiol.01074.2002.—Reduced plasma concentrations of the extracellular actin-binding proteins gelsolin and Gc-globulin correlate with pulmonary failure and death in humans after injury. The purpose of this study was to investigate the role of plasma gelsolin in the pathophysiology of inflammation-induced lung injury. We postulated that plasma gelsolin levels decrease at an early time point after burn injury and that the intravenous infusion of gelsolin prevents burn-induced pulmonary microvascular dysfunction. Adult Sprague-Dawley rats were randomized to undergo a 40% body surface area thermal injury (Burn) or manipulation without burn (Sham). Plasma gelsolin and Gc-globulin concentrations were determined at various times during the first 6 days of injury by Western blotting. Other animals were randomized to receive either recombinant human gelsolin (0.078, 0.78, or 7.8 mg) or albumin (7.8 mg) before and 8 h after Burn or Sham. Twenty-four hours later, pulmonary microvascular permeability was assessed by measuring the capillary filtration by use of an isolated, perfused lung model. We found that plasma gelsolin levels of burn-injured rats decreased to 10% of normal levels within 12 h and remained below normal levels for up to 6 days postinjury. Gc-globulin values also fall, but to a lesser extent and only transiently. Treatment of burned animals with intravenous infusions of recombinant human gelsolin prevented the increase in pulmonary microvascular permeability that accompanies this injury. Our findings are consistent with the hypothesis that plasma gelsolin depletion contributes to the pathophysiology of pulmonary microvascular dysfunction during inflammation.

Gc-globulin; microvascular permeability; capillary filtration coefficient; isolated perfused lung model

PLASMA CONTAINS TWO SECRETED proteins, Gc-globulin and plasma gelsolin, that coordinately depolymerize actin filaments. Gelsolin severs actin filaments to promote their rapid depolymerization (33), whereas Gc-globulin binds to actin monomers to shift the actin monomer-polymer equilibrium toward depolymerization and to prevent repolymerization (12). The existence of these so-called actin-scavenging proteins engenders the hypothesis that they serve to clear actin, the abundant but normally intracellular protein, that is exposed to

extracellular spaces or released into the circulation after tissue injury (18). The spillage of large amounts of actin may overwhelm the capacity of the circulating actin-scavenging proteins, resulting in their depletion and the persistence of actin within the microvasculature. The latter is postulated to contribute to the pathogenesis of organ injury at sites removed from the primary insult (3, 18, 23). The principal evidence for this hypothesis has been 1) a precipitous drop in circulating levels of Gc-globulin and gelsolin after severe injury or illness (3, 18, 23); 2) the demonstration of actin and actin-gelsolin complexes in the plasma of critically ill patients, including those with acute respiratory distress syndrome (ARDS) (20, 23); 3) a relationship between reduced plasma levels of gelsolin and Gc-globulin and poor clinical outcome in a wide spectrum of diseases (4, 20, 23, 27, 28, 31); 4) the observation that actin-containing plasma from ARDS patients is toxic to endothelial cells in culture (9); and 5) one observation that the intravenous infusion of actin into rats in excess of the capacity of the actin-scavenging system caused pulmonary microvascular injury (14).

If plasma gelsolin prevents local and remote microvascular injury by ameliorating the toxic effects of actin released into the microcirculation, the early depletion of circulating gelsolin levels documented clinically after injury may contribute to the progression of the injury state. A decrease in gelsolin level may exacerbate microvascular dysfunction both at the site of injury and at remote vascular beds, particularly the lungs. We therefore postulate that plasma gelsolin levels should decrease at an early time point in inflammation-induced lung injury and that the intravenous infusion of gelsolin may prevent microvascular dysfunction at sites remote from the primary injury.

To test this hypothesis, we utilized a standardized rat burn model to induce a defined pulmonary injury (19, 24, 37). We found that burn injury induced a rapid decrease in plasma gelsolin levels within 2 h, and the decrease persisted for at least 6 days after burn. In contrast, Gc-globulin values decreased initially but became much higher than control levels subsequently. We used an ex vivo lung perfusion model to accurately quantitate pulmonary microvascular permeability and pulmonary hemodynamics. Rats sustaining a 40% total body surface area burn characteristically develop increased pulmonary microvascular permeability, a parameter that can be quantitated by determining the capillary filtration coefficient

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( $K_f$ ). We found that intravenous infusion of recombinant human gelsolin completely prevented the increase in pulmonary microvascular permeability in burn-injured rats. This study provides important new evidence to support the hypothesis that circulating gelsolin has an important protective function in the pathophysiology of systemic inflammatory states.

## METHODS

### *Animal Model of Acute Burn Injury*

Sprague-Dawley rats (300 g; Charles River Laboratories, Wilmington, MA) underwent cutaneous burn injury as previously described (19, 24, 37). Briefly, the animals were anesthetized with isoflurane and secured in a template that exposed the skin of the back. This area was immersed in 100°C water for 12 s, causing a full-thickness burn of 40% of the body surface area (designated as Burn) or room temperature water (designated as Sham). The burn-injured animals received 4 ml of lactated Ringer solution per kilogram body weight per percent body surface area burned administered intraperitoneally immediately after injury. A prior report from our laboratory demonstrated that this resuscitation protocol ameliorated the mortality rate of burn-injured animals given lesser volumes of fluid resuscitation (15). Burn-injured animals resuscitated in this manner return to normal activity including food and water consumption within 45 min of injury and appear to make normal quantities of urine (unpublished observations). The animal treatment protocols were approved by the Institutional Animal Care and Research Advisory Committee of the University of Texas Southwestern Medical Center.

### *Determination of Plasma Gelsolin and Gc-globulin Concentrations*

At 2, 6, 12, 24, 72, and 144 h after Burn or Sham, the animals were anesthetized, and blood obtained by cardiac puncture was placed in a heparinized tube ( $n = 4-6$  samples per experimental group per time point). The blood was centrifuged and the plasma decanted for protein electrophoresis. The plasma protein concentration was determined in duplicates with the Bio-Rad protein assay kit. Plasma samples were diluted sixfold with distilled water, and 2  $\mu$ l were analyzed by SDS-PAGE after boiling in SDS gel sample buffer. Western blotting was performed with the use of antibodies specific for Gc-globulin (rabbit anti-human Gc-globulin) or gelsolin (rabbit anti-mouse anti-serum; Ref. 32). These antibodies react specifically with the respective rat proteins. The immunoreactive bands were visualized with the ECL detection system (Amersham, Arlington Heights, IL). The intensity of the stained bands was determined by densitometry and the values expressed as optical density per square millimeter of the stained band. Serial dilutions were analyzed, and samples within the linear range were used for quantitation. Burn and Sham groups were compared by use of the unpaired, two-tailed Student's *t*-test (Sigma-Stat, version 2.0; SPSS, Chicago, IL).

### *Effect of Burn Injury on Plasma Protein Concentration and Hematocrit*

Because burn injury may cause the extravasation of plasma proteins into the site of injury or tissues remote from the injury (5), we measured the total protein concentration in the plasma of animals 2, 4, 6, 12, 24, 72, and 144 h after Burn or Sham. The animals were anesthetized, and blood was drawn into a heparinized syringe by cardiac puncture. The plasma protein concentration was measured by using a Bio-Rad protein assay kit and was expressed as grams per deciliter. Samples were obtained from four to six animals in each experimental group at each time point.

A decrease in plasma protein concentration may be related to the loss of proteins from the intravascular space or the addition of crystalloid solutions into the intravascular space, i.e., hemodilution. In

contrast to plasma proteins, red blood cells do not move across the microvasculature during acute inflammation. By measuring the hematocrit of animals 2, 4, and 24 h after Burn or Sham, we used red blood cells as nonpermeable markers of changes in blood volume and cellular concentration. The hematocrit was determined by placing heparinized blood into capillary tubes, which were then centrifuged for 60 s at 6,500 rpm. The hematocrit measurements are expressed as a percentage of whole blood that is red blood cells. Samples were obtained from four to six animals per experimental group at each time point assessed.

### *Intravenous Gelsolin Infusions*

In another experiment, Burn and Sham animals received 0.078, 0.78, or 7.8 mg of recombinant human gelsolin (molecular weight 82,000; a gift of Susan Goelz, PhD, Biogen, Cambridge, MA;  $n = 4-6$  in each experimental group) or an equivalent amount of bovine serum albumin (molecular weight 66,000; Fisher Scientific, Fair Lawn, NJ;  $n = 6$  and 4 for Burn and Sham, respectively) in 500  $\mu$ l of sterile 0.9% NaCl solution. These agents were administered intravenously immediately before and again 8 h after Burn or Sham. Twenty-four hours later, pulmonary microvascular permeability was measured by calculating  $K_f$  by use of a gravimetric technique. The measurement of  $K_f$  at this time point for the experiments involving gelsolin was based on a time curve in which pulmonary  $K_f$  was measured 2, 4, 8, and 24 h after Burn.

### *Measurements in Isolated, Perfused Lungs*

*Ex vivo lung perfusions.* The methods for perfusing rat lungs have been described previously (36). Briefly, animals were anesthetized after Burn or Sham, and the pulmonary artery, left atrium and trachea were cannulated. The lungs and heart were excised en bloc and suspended from a force transducer (TSD125C, BioPac Systems, Santa Barbara, CA) for continuous measurement of lung weight. The lungs were perfused with Krebs-Henseleit buffer (Sigma Chemical, St. Louis, MO) containing 3% bovine serum albumin at 8 ml/min and ventilated with room air at 60 cycles/min (Harvard rodent ventilator, model 683, Millis, Massachusetts). Pulmonary arterial (Ppa) and left atrial pressure (Ppv) were continuously measured with pressure transducers (TSD 104A, BioPac Systems) with zero reference at the level of the apex of the lung.

*Quantitation of pulmonary microvascular functions.* CAPILLARY FILTRATION COEFFICIENT. Pulmonary microvascular permeability was quantitated by determining the  $K_f$  as previously described (1, 35, 36). This methodology was chosen because it is one of the most sensitive and specific measures of microvascular permeability, much more sensitive than wet-to-dry weight ratios and other commonly used assays of tissue microvascular permeability. Furthermore, this methodology quantitates microvascular permeability independently of hydrostatic pressure changes (7).

After 30 min of ex vivo perfusion, capillary pressure ( $P_c$ ) was measured by using the double-occlusion technique (35, 36). Ppv was then elevated to 10 mmHg, and 5 min later  $P_c$  was again measured. The  $K_f$  value, expressed as grams per minute per mmHg per 100 g of body wt, was calculated as shown in Eq. 1

$$K_f = \frac{\Delta W / \Delta T}{\Delta P} \quad (1)$$

where  $\Delta W$  is the change in lung weight between minutes 2 and 5 of increased outflow pressure,  $\Delta T$  is the length of time of elevated outflow pressure, and  $\Delta P$  is the difference between  $P_c$  after and before increase of Ppv to 10 mmHg.

PULMONARY VASCULAR RESISTANCE. Pulmonary vascular resistance ( $R_t$ ) was determined as previously described (1, 35, 36). After 30 min of ex vivo perfusion, Ppa and Ppv were recorded.  $R_t$  was calculated as shown in Eq. 2

$$R_t = \frac{P_{pa} - P_{pv}}{\dot{Q}} \quad (2)$$

where  $P_{pa}$  and  $P_{pv}$  represent the pulmonary artery and venous pressures, respectively, and  $\dot{Q}$  is the flow rate through the lung. The pulmonary circulation can be represented as a simple linear model in which  $P_{pa}$  is separated from  $P_c$  by a precapillary resistance ( $R_a$ ) and  $P_c$  is separated from  $P_{pv}$  by a postcapillary resistance ( $R_v$ ) (1, 35, 36). Therefore,  $R_a$  and  $R_v$  can be calculated as follows

$$R_a = \frac{P_{pa} - P_c}{\dot{Q}} \quad (3)$$

$$R_v = \frac{P_c - P_{pv}}{\dot{Q}} \quad (4)$$

All resistance calculations ( $R_t$ ,  $R_a$ , and  $R_v$ ) were normalized for body weight and expressed as mmHg per milliliter per minute.

#### Statistical Analysis

All data are expressed as means  $\pm$  SD. The sample size in each group was four to nine animals. The data were compared by a Kruskal-Wallis one-way analysis of variance on ranks with a Dunn's post hoc comparison or an unpaired, two-tailed Student's *t*-test. Statistical significance was considered for a type 1 error of  $<5\%$ .

## RESULTS

### Effect of Burn Injury on Plasma Gelsolin and Gc-Globulin Concentration

Burn injury significantly altered the plasma concentration of gelsolin (Fig. 1, A and B). As early as 2 h after burn, plasma gelsolin decreased by  $\sim 40\%$  compared with Sham animals ( $P = 0.007$ ,  $n = 5$ ) (Fig. 1, A and C). By 6 h, the gelsolin level in Burn animals decreased by 70% ( $P = 0.004$ ) (Fig. 1, B and C). By 12 h postinjury, the plasma concentration of gelsolin was  $\sim 10\%$  of time-matched controls ( $P = 0.0008$ ). At 24, 72, and 144 h postinjury, plasma gelsolin levels gradually increased but remained significantly less than those of controls ( $P \leq 0.03$ ). Normalization of plasma gelsolin values to the amount of total plasma proteins detected no statistically significant difference between the Burn and Sham groups at 2 h ( $P = 0.07$ ); however, the differences at 6 ( $P < 0.001$ ), 12 ( $P < 0.001$ ), 72 ( $P = 0.01$ ) and 144 h ( $P = 0.04$ ) were significant.

The plasma concentration of Gc-globulin was also significantly changed by acute burn injury (Fig. 1, B and C), but in a different direction than gelsolin after 2 h. The Gc-globulin level decreased to 50% of control within 2 h ( $P = 0.01$ ), then rose progressively such that the concentration was doubled by 72 h ( $P = 0.003$ ). It returned to control levels by 144 h after injury (Fig. 1C). When the Gc-globulin levels were standardized to the amount of plasma protein, the difference between Burn and Sham groups was statistically significant at 2, 12, and 72 h postinjury ( $P = 0.02$ , 0.002, and 0.02, respectively).

### Effect of Burn Injury on Plasma Protein Concentration and Hematocrit

To determine whether the burn-induced reduction in plasma gelsolin concentration was related to a nonspecific decrease in all circulating plasma proteins, the plasma total protein concentration was determined in Burn and Sham animals at various times after injury. The plasma protein concentration of

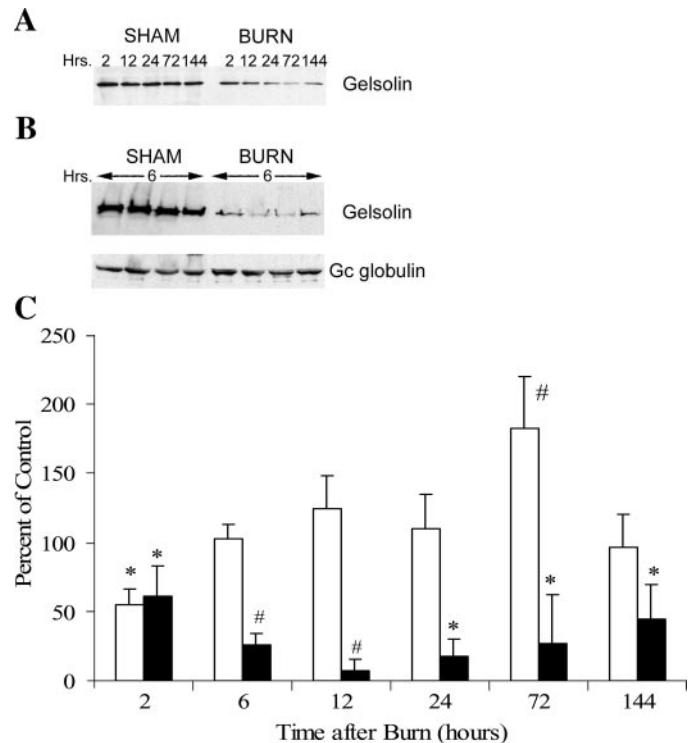


Fig. 1. Effect of burn injury on plasma gelsolin and Gc-globulin levels. The amount of gelsolin and Gc-globulin present in the plasma of animals sustaining a 40% total body surface area full-thickness burn (Burn) was compared with that of manipulated but uninjured controls (Sham). A: Western blot of gelsolin in the plasma of Burn and Sham animals at 2, 12, 24, 72, and 144 h. B: Western blot of gelsolin and Gc-globulin in the plasma of Burn and Sham animals at 6 h. Four different animals were analyzed per group. C: amount of Gc-globulin (open bars) and gelsolin (solid bars) in the plasma of burn-injured animals was expressed as a percent of the amount of Gc-globulin and gelsolin in the plasma of the Sham group. Plasma from 4–6 animals for each group at each time point was analyzed on the same gel. Values are means  $\pm$  SD. \* $P < 0.01$  vs. Sham at this particular time point; # $P < 0.001$  vs. Sham at this particular time point.

burn-injured animals was reduced by 15–30% at nearly every time assessed compared with the Sham group (Fig. 2A).

A reduction in plasma protein concentration in the burn-injured animals may have resulted from hemodilution due to the absorption of lactated Ringer administered immediately after burn injury. We therefore measured the hematocrit of animals sustaining Burn or Sham at 2, 4, and 24 h postinjury, times at which plasma gelsolin levels were reduced by 40–80%. The hematocrit of burn-injured animals was not different than that of the Sham group at 2 and 24 h postinjury but was 23% greater than that of the Sham group 4 h postburn injury ( $P < 0.001$ ) (Fig. 2B).

*Effect of gelsolin on burn-induced increases in pulmonary  $K_f$ .* Burn-induced changes in  $K_f$  were maximal at 24 h when compared with earlier time points, and hence this time point was chosen for those experiments examining the effect of gelsolin on burn-induced changes in pulmonary microvascular permeability. The  $K_f$  of lungs measured 24 h after Burn was significantly greater than that of measurements taken 2 ( $P = 0.001$ ) and 4 h ( $P = 0.005$ ) after acute burn injury as well as that of the Sham group ( $P < 0.001$ ) (Fig. 3A).

The intravenous infusion of recombinant human gelsolin (7.8 mg immediately before and 7.8 mg again 8 h after injury) totally prevented the increase in  $K_f$  associated with burn injury



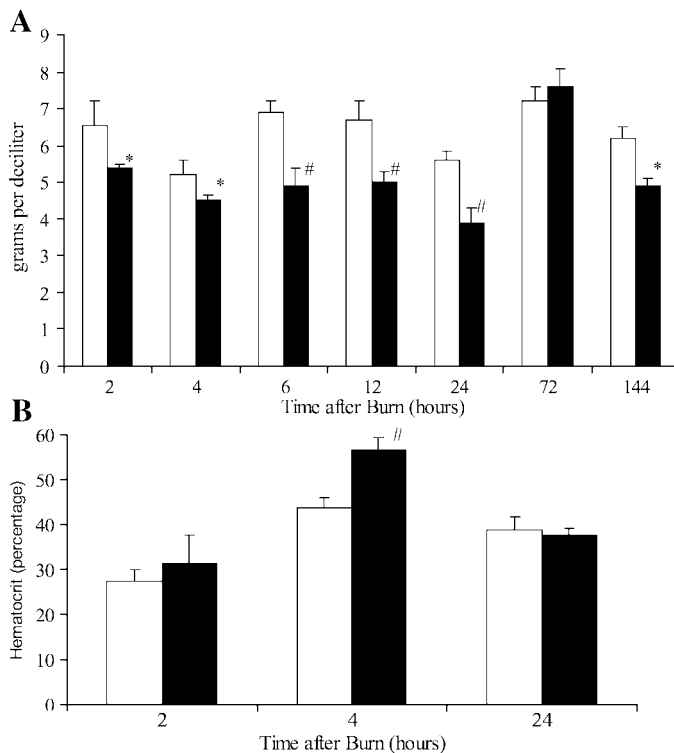


Fig. 2. Effect of burn injury on plasma total protein concentration and hematocrit. Total protein concentration and hematocrit in the blood of Burn animals was compared with Sham controls. *A*: total protein concentration in the plasma of Burn (solid bars) and Sham animals (open bars) at various times postinjury. Samples were obtained from 4–6 animals at each time point in each group. *B*: hematocrit of Burn (solid bars) and Sham animals (open bars) at various times postinjury. Samples were obtained from 4 or 5 animals at each time point in each group. Values are means  $\pm$  SD. \* $P \approx 0.01$ ; # $P \approx 0.001$ .

( $P \leq 0.05$ ; Fig. 3*B*). Gelsolin had no effect on the  $K_f$  of the lungs of uninjured animals. By assuming a blood volume of 24 ml per 300 g rat (J. Horton, personal communication), each bolus of 7.8 mg gelsolin administered should provide an intravascular concentration of  $\sim 327 \mu\text{M}$  before equilibration with extravascular fluids. This level is similar to the plasma concentration of endogenous gelsolin in normal, uninjured humans (211  $\mu\text{g}/\text{ml}$ ) and rabbits (368  $\mu\text{g}/\text{ml}$ ) (21). Gelsolin infusions at 1/10 or 1/100 of the calculated replacement dose (0.78 and 0.078 mg) did not prevent burn-induced increases in pulmonary microvascular permeability (Fig. 3*B*).

**Effect of gelsolin on burn-induced changes in pulmonary hemodynamics.** Besides increasing microvascular membrane permeability, inflammatory states may be associated with vasoconstriction and increased intravascular hydrostatic pressure. The latter may exacerbate the movement of fluid and proteins from the microvascular space into the interstitium. We therefore measured the resistance within various segments of the pulmonary vascular bed to determine whether burn injury alters pulmonary hydrostatic pressure. In contrast to the dramatic effects burn injury had on pulmonary microvascular permeability, it had no effect on vascular resistance or capillary pressure (Table 1). Similarly, there was no difference in these measurements in the lungs of animals treated with gelsolin when compared with controls. Lower concentrations of gelsolin (0.078 and 0.78 mg) also had no demonstrable effect on these parameters (data not shown).

## DISCUSSION

The purpose of this study was to determine the effect of cutaneous burn injury on plasma gelsolin and Gc-globulin levels and to investigate whether intravenous administration of gelsolin could protect animals from burn-induced changes in pulmonary microvascular dysfunction. We found that a full-thickness burn produced a rapid and profound reduction in the concentration of circulating gelsolin that persisted for as long as 6 days postinjury. In contrast, Gc-globulin levels decreased initially but subsequently rose to levels higher than control. Recombinant human gelsolin, when administered immediately before and 8 h after burn injury at concentrations that restored normal plasma gelsolin levels, completely prevented the increase in pulmonary microvascular permeability characteristic of this model. These data suggest that gelsolin may preserve endothelial barrier function during systemic inflammatory injuries.

Enhanced pulmonary microvascular permeability is a fundamental component of the systemic inflammation that accompanies acute injury such as severe burns. In contrast to experimental models in which exposure of the lung to toxic substances causes marked pulmonary edema, the alterations in pulmonary microvascular function associated with more clin-

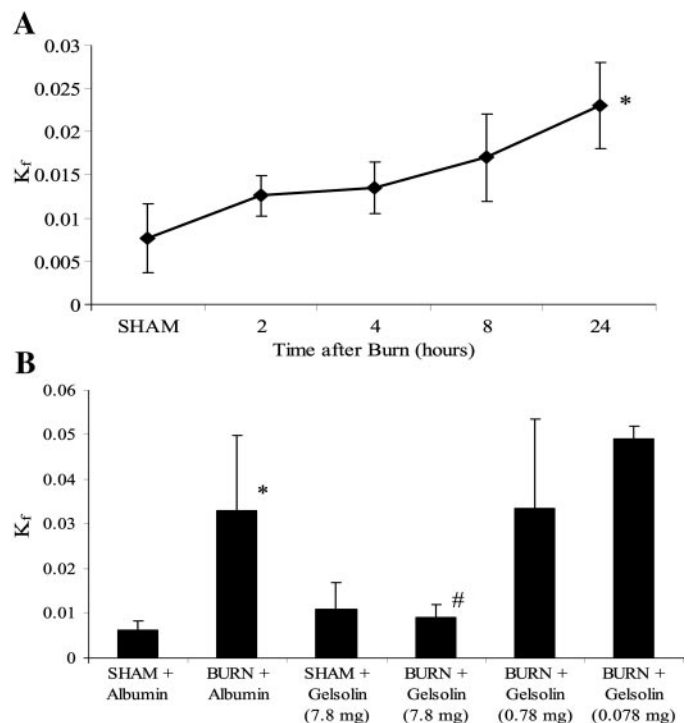


Fig. 3. *A*: time-course for burn-induced increases in pulmonary capillary filtration coefficient ( $K_f$ ).  $K_f$  was measured by using a gravimetric technique in ex vivo buffered perfused rat lungs 2, 4, 8, and 24 h after Burn injury. Sample size was 4–10 animals in each group. \* $P \leq 0.001$  vs. Sham, 2 and 4 h groups. *B*: effect of human recombinant gelsolin on burn-induced increase in the pulmonary  $K_f$ . The animals received 7.8, 0.78, or 0.078 mg of human recombinant gelsolin or 7.8 mg of bovine serum albumin in 500  $\mu\text{l}$  of sterile 0.9% NaCl solution immediately before and again at 8 h after injury.  $K_f$  was measured 24 h after Burn by using a gravimetric technique. Sample size was 4–6 animals in each group injected with 7.8 or 0.78 mg of protein and 2 per group injected with 0.078 mg of protein.  $K_f$  is expressed in  $\text{g}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}\cdot 100 \text{ g body wt}^{-1}$ . \* $P < 0.05$  vs. Sham + Albumin; # $P < 0.05$  vs. Burn + Albumin.

Table 1. *Effect of gelsolin and burn injury on pulmonary hemodynamics*

	Capillary Pressure, mmHg	Total Vascular Resistance, mmHg · ml <sup>-1</sup> · min	Precapillary Resistance, mmHg · ml <sup>-1</sup> · min	Postcapillary Resistance, mmHg · ml <sup>-1</sup> · min
Sham	11.16 ± 0.56	3.01 ± 0.57	2.01 ± 0.55	0.99 ± 0.03
Burn	10.91 ± 1.3	3.73 ± 1.10	2.76 ± 1.08	0.97 ± 0.18
Sham + gelsolin	10.25 ± 1.45	2.74 ± 1.10	1.91 ± 1.08	0.89 ± 0.17
Burn + gelsolin	8.23 ± 3.72	4.00 ± 0.81	3.28 ± 0.5	0.71 ± 0.58

Values are means ± SD. Burn, animals immersed in 100°C water for 12 s, causing a full-thickness burn of 40% of the body surface area; sham, animals immersed in room-temperature water.

ically relevant animal models such as acute burn injury are much more modest. Light microscopy of the lungs of burn-injured animals demonstrated focal neutrophil recruitment without gross interstitial or intra-alveolar edema (data not shown), findings consistent with those reported by other investigators (6, 34). This experimental observation is consistent with clinical experience in which acute burn injury, in the absence of smoke inhalation, is rarely associated with florid pulmonary edema. In the present study, pulmonary microvascular permeability changes were quantitated by measuring the  $K_f$ , which allows detection of very small changes in membrane permeability, independent of hydrostatic pressure (7).

Changes in pulmonary microvascular function during systemic inflammatory states are due to a complex interplay of pro- and anti-inflammatory mediators including neutrophils, complement, and various cytokines. Our study supports the hypothesis that circulating gelsolin has an essential beneficial role in protecting the pulmonary microvasculature from systemic inflammatory injuries. Until now, the principal evidence supporting this notion has been the occurrence of a large and early reduction in the plasma concentration of gelsolin after severe injury or illness and correlation between reduced plasma gelsolin concentrations and poor clinical outcome in many, but not all, cases (4, 18, 20, 23, 31). For example, Mounzer et al. (23) reported that the plasma gelsolin level in severely injured trauma patients was about half that of uninjured volunteers and that 77% of the patients who developed significant pulmonary complications had plasma gelsolin concentrations more than two standard deviations below the mean of the control group. Others have reported reduced plasma gelsolin concentrations in patients suffering acute hepatic failure, myocardial infarction, septic shock, myonecrosis, and acute lung injury and have related this to increased risk of morbidity and mortality (16, 20, 31).

Our study shows a significant reduction in circulating gelsolin levels within the first 2 h of injury, with the decrease persisting for at least 6 days postburn. The very early reduction in circulating gelsolin levels is consistent with that reported clinically in severely injured patients (4, 23). The diminution in plasma gelsolin levels after acute burn injury may reflect the leakage of gelsolin, as well as other plasma proteins, across the microvasculature at the site of the burn and in other microvascular beds (5), enhanced clearance of gelsolin-actin complexes by the liver (21), and reduced plasma gelsolin synthesis by skeletal muscle. The higher rates of loss of plasma gelsolin relative to total plasma proteins at the later time points suggest that plasma gelsolin is selectively removed from the circulation, that it is not recycled once it leaked out of the vasculature, or that it is not replenished by de novo synthesis. The prolonged depression in gelsolin levels up to 6 days after burn is

consistent with reduced synthesis. Plasma gelsolin is synthesized primarily in skeletal muscle (17), and burn injury promotes skeletal muscle degradation (10). It is unlikely that the reduction in circulating gelsolin levels is due to expansion of the intravascular fluid compartment by the infusion of crystalloid resuscitation fluids. This notion is supported by the observations that plasma gelsolin concentrations remained significantly reduced even when normalized to circulating plasma protein levels, and the hematocrit of burn-injured animals was never less than that of the Sham group, as one would expect had hemodilution been present. Irrespective of how circulating gelsolin is decreased during burn injury, the absolute reduction in the amount of plasma gelsolin would limit its protective effects against inflammation for a prolonged period.

We found that Gc-globulin responded to burn injury in a different way. Although the plasma Gc-globulin level was decreased significantly at 2 h postburn, it recovered by 6 h and became much higher than control at 72 h. The statistically significant decrease in Gc-globulin per protein concentration in the first 2 h postburn suggests that Gc-globulin may be selectively cleared from the circulation as a result of burn injury, whereas the subsequent rise is likely due to increased synthesis by the liver as an acute-phase response. Previous studies in our laboratory have demonstrated that the kinetics of Gc-globulin appearance within the plasma after spinal operations mirrors that of other "positive" acute-phase proteins including haptoglobin and orosomucoid but not that of the "negative" acute-phase protein albumin (2). A similar pattern of Gc-globulin release was previously demonstrated by our laboratory in patients surviving multiple organ system trauma, but not those dying of their injuries (3). Lastly, exposure of cultured hepatocytes to the prototypic stimulators of the acute-phase response, interleukin-6 and dexamethasone, induces the synthesis and secretion of Gc-globulin (13). Because the Gc-globulin level was only reduced in the first 2 h postburn and admission levels of Gc globulin are predictive of survival in trauma patients (3), we postulate that the combined decrease in gelsolin and Gc-globulin at 2 h compromises the actin-scavenging capacity sufficiently to allow actin to inflict long-term damage on the lung endothelium. Another possibility is that although Gc-globulin has a role in actin scavenging, an increase in Gc-globulin per se is not sufficient to compensate for the decrease in gelsolin at later time points after burn.

The ability of intravenous infusion of gelsolin to prevent burn-induced increases in pulmonary microvascular permeability suggests that gelsolin has an important role in protecting the lung from inflammatory injury. Protection was observed with a dose of gelsolin that was calculated to replace the entire plasma gelsolin content, whereas infusion of much lower amounts of gelsolin, equivalent to that found in plasma within

12 h of Burn, were completely ineffective. These results support the idea that the burn-induced decrease in circulating gelsolin contributes to the pathogenesis of acute lung injury.

Because actin is one of the most abundant proteins within cells (5–20% cell protein by weight for nonmuscle cells and 25% for muscle; Ref. 30), the amount of actin filaments released into the extracellular compartment during burn injury could conceivably be quite large. Previous studies have demonstrated that the infusion of actin monomers into rats causes pulmonary endothelial and vascular dysfunction (14). The demonstration of actin and gelsolin-actin complexes within the plasma of patients with ARDS, but not normal controls, is also consistent with the notion that circulating actin microfilaments may contribute to the development of acute lung injury and that gelsolin may function to prevent this injury (9, 20). Actin filaments could injure the microvasculature by either a direct effect on the vascular endothelium (9, 14) or by activating platelets (26) with resulting platelet aggregation, microvascular thrombosis, and perhaps the release of proinflammatory mediators such as thromboxane and lysophosphatidic acid (LPA).

Gelsolin may also attenuate burn-induced lung injury by altering the bioactivity of LPA itself (11). LPA is released by activated platelets (8) and has a wide range of important proinflammatory effects, including platelet activation (29) and nuclear factor- $\kappa$ B activation to induce gene expression of proinflammatory molecules in endothelial cells (25). Gelsolin binds LPA with an affinity comparable to that of LPA for its receptors (Edg-2, -4, and -7; Ref. 22), and gelsolin binding to LPA alters its ability to stimulate cells in culture (11). Using the same *ex vivo* buffer-perfused lung model described in this study, we recently found that LPA increases pulmonary microvascular permeability and that this increase was reduced when gelsolin was included in the perfusate (unpublished observations).

In conclusion, the data presented in this study suggest that the exogenous administration of recombinant gelsolin prevents inflammation-induced pulmonary microvascular dysfunction. These data provide new evidence supporting the hypothesis that circulating gelsolin may have an important protective function during systemic inflammatory events.

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