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MAJOR ARTICLE

Recombinant Human Plasma Gelsolin Improves Survival and Attenuates Lung Injury in a Murine Model of Multidrug-Resistant *Pseudomonas aeruginosa* Pneumonia

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Background. Plasma gelsolin (pGSN) is an abundant circulating protein quickly consumed by extensive tissue damage. Marked depletion is associated with later poor outcomes in diverse clinical circumstances. Repletion with recombinant human (rhu)–pGSN in animal models of inflammation lessens mortality and morbidity.

Methods. Neutropenic mice were treated with different meropenem doses ±12 mg of rhu-pGSN commencing 1 day before an intratracheal challenge with multidrug-resistant *Pseudomonas aeruginosa*. Survival, bacterial counts, and pulmonary pathology were compared between corresponding meropenem groups with and without rhu-pGSN.

Results. Overall survival was 35/64 (55%) and 46/64 (72%) in mice given meropenem without and with rhu-pGSN, respectively (Δ = 17%; 95% CI, 1–34). In control mice receiving meropenem 1250 mg/kg/d where the majority died, the addition of rhu-pGSN increased survival from 5/16 (31%) to 12/16 (75%) (Δ = 44%; 95% CI, 13–75). Survival with minor lung injury was found in 26/64 (41%) mice receiving only meropenem, vs 38/64 (59%) in mice given meropenem plus rhu-pGSN (Δ = 19%; 95% CI, 2–36).

Conclusions. In a series of dose-ranging experiments, both mortality and lung injury were reduced by the addition of rhu-pGSN to meropenem against carbapenem-resistant *P. aeruginosa*. Rhu-pGSN offers a novel candidate therapy for antibiotic-resistant pneumonia.

Keywords. acute lung injury; bacterial pneumonia; carbapenem resistance; plasma gelsolin.

Plasma gelsolin (pGSN) is an abundant protein in the blood of healthy individuals that acts to regulate inflammatory homeostasis [1–3]. pGSN is structurally similar to the intracellular isoform (cytoplasmic GSN) but serves unique biological functions [4–6]. Both forms are encoded by a single gene on human chromosome 9 under the control of different promoters. Alternative mRNA splicing programs the biosynthesis of the cytoplasmic or plasma variants. pGSN has a processed signal sequence that directs its secretion and a small N-terminal extension. There are no post-translational modifications.

As a nodal point in a coordinated system modulating host inflammatory reactions, pGSN acts through pleiotropic mechanisms to scavenge leaked intracellular contents, localize inflammatory signals, and enhance immune clearance of microbial and host-derived toxins [7–17]. By severing actin filaments, pGSN digests debris exposed by ruptured cells that impedes host defenses. It also augments macrophage microbial uptake and killing [18, 19]. Once leaked cytoskeletal actin and nuclear DNA are cleared, free pGSN can scavenge proinflammatory lipid and peptide mediators and promote resolution of local inflammatory injury. Consequently, pGSN boosts the ability of the innate immune response to clear pathogens at the infected site while tempering the injurious consequences of unbridled inflammation. Innocent bystander organs are protected from an overly exuberant systemic inflammatory response. As part of innate immunity, pGSN appears to be indifferent to the pathogen type.

Circulating pGSN concentrations decrease precipitously in a variety of serious infectious and noninfectious life-threatening conditions such as bacterial sepsis, major trauma and burns, prolonged hyperoxia, malaria, and liver injury [20–30]. The associations interconnecting the severity of the inciting injury, the magnitude of pGSN decline, and the subsequent likelihood of mortality or devastating complications (like ARDS) have been reproducibly observed in correlative studies of patients following a diverse spectrum of common insults. A recombinant human product developed for clinical use appeared generally safe and well tolerated in a recently completed dose-finding study in patients hospitalized for community-acquired pneumonia (CAP; ClinicalTrials.gov NCT03466073).

Received 18 March 2020; editorial decision 8 June 2020; accepted 11 June 2020.

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DOI: 10.1093/ofid/ofaa236

Patients with pneumonia are at potential risk for complications from the overzealous inflammatory responses that underlie acute lung injury and sepsis. Despite improved critical care, mortality rates still hover around 15%–20% in the subset of CAP patients requiring admission to an intensive care unit, and survivors can suffer long-term health consequences [31–33]. According to the World Health Organization, pneumonia affects ~450 million people globally each year, with ~4 million deaths. In the United States, 1.1 million patients with pneumonia are hospitalized each year, leading to >52 000 deaths. Critical care costs in the United States in 2010 were $108 billion (increased from $81.7 billion in 2005), accounting for 13.2% of hospital costs, 4.1% of national health expenditures, and 0.7% of the gross national product. Patients admitted with CAP who have the lowest pGSN levels at presentation have the worst outcomes [30].

Recombinant human pGSN (rhu-pGSN) reduces the mortality of penicillin-sensitive and penicillin-resistant pneumococcal pneumonia, even with delayed administration when therapy is initiated after the mice are visibly ill [34]. Although it also enhances the uptake and killing of gram-negative (as well as gram-positive) bacteria in vitro [19], the efficacy of rhu-pGSN in gram-negative pneumonia has not been previously studied in vivo. In acute lung injury induced with hyperoxia or infection, rhu-pGSN limits the influx of neutrophils into bronchoalveolar fluid [34, 35]. We therefore exploited an established model of highly lethal, multidrug-resistant *Pseudomonas aeruginosa* pneumonia in neutropenic mice to determine whether the addition of rhu-pGSN to inadequate carbapenem therapy would increase survival and/or diminish acute lung injury [36].

METHODS

Production of rhu-pGSN

Recombinant human plasma gelsolin (rhu-pGSN), provided by BioAegis Therapeutics, was produced in *E. coli* and subsequently lyophilized for reconstitution. Human and murine pGSN are highly (~93%) homologous (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). Vehicle controls containing formulation components were used for the comparator mice.

Bacteria Strain and Growth Conditions

P. aeruginosa UNC-D is a sputum isolate from a patient with cystic fibrosis kindly provided by Dr. Peter Gilligan at the University of North Carolina [36]. Bacteria were cultured on trypticase soy agar (TSA) plates and in Lennox broth at 37°C with shaking of broth cultures. Minimum inhibitory concentrations for the UNC-D strain are ceftazidime (32 μg/mL), meropenem (8 μg/mL), imipenem (16 μg/mL), tobramycin (32 μg/mL), piperacillin (16 μg/mL), aztreonam (4 μg/mL), colistin (1 μg/mL), and fosfomycin (256 μg/mL). Bacteria were prepared for animal challenge studies by culturing bacteria in Lennox broth overnight and washing the bacteria into 1X PBS before diluting to a final concentration based on OD_{600}-based estimates and a final 50-μL delivery dose. Bacterial concentrations were confirmed by serial dilution and colony enumeration on TSA plates.

Murine Respiratory Infection Model

The BALB/c infection model of *P. aeruginosa* UNC-D strain [36] was specifically designed to test for adjunctive therapies that might result in improved efficacy of failing meropenem monotherapy against a multidrug-resistant (MDR) *P. aeruginosa* UNC-D strain resistant to several clinically important antibiotics including meropenem. Previous experience has demonstrated that this model is most informative when examining novel compounds using meropenem doses that provide ~50% mortality with meropenem treatment alone [36]. Mice were housed and treated in accordance with standard animal experimentation guidelines at the University of Louisville. Briefly, female BALB/c mice were rendered neutropenic using cyclophosphamide injections (150 mg/kg) on days –5 and –3 before infection, typically resulting in a ~90% drop in neutrophil counts. Approximately $10^{5.5}$ CFU of UNC-D was directly instilled into the lungs by intubation-mediated intratracheal instillation. Meropenem (Hospira, Lake Forest, IL, USA) was administered by subcutaneous injection beginning at 3 hours postinfection and q8h for 5 days. To determine if rhu-pGSN adjunctive therapy improves the efficacy of meropenem, 12 mg/d of rhu-pGSN was administered by intraperitoneal injection of 0.3 mL at on days –1, 0, 1, 2, 3, 4, and 5 postinfection. Mice were monitored for development of illness every 8 hours after infection for 7 days, including temperatures measured via transponders implanted subcutaneously before the initiation of the studies (BioMedic Data Systems, Seaford, DE, USA). Moribund mice were humanely killed and scored as succumbing to the infection at the next time point. Tissues samples were harvested for bacterial counts and pathology as previously described [36]. Mice surviving to 7 days were scored as surviving infection and killed; tissues were similarly processed. Lung histopathology was scored in a blinded fashion by a board-certified veterinary pathologist. A 4-point, 4-criteria system (inflammation, infiltrate, necrosis, and other including hemorrhage) with a maximum score of 16 points was used to evaluate lung pathology. Points for each criterion were assigned as follows: no (0), minimal (1), mild (2), moderate (3), and severe (4) pathologic findings. Despite the overall reproducibility of the model, a sharp inflection point with respect to the survival benefit and meropenem dose can sometimes obscure the benefits of adjunctive interventions.

Statistical Analyses

In total, 3 comparable experiments were independently performed using this model. Titration experiments were done

when a new batch of meropenem was to be used to estimate the effective dose $(ED)_{50}$ for each lot of antibiotic before the formal experiments. We tallied overall survival and survival with minimal lung injury (defined post hoc as histopathology scores ≤2) for the experiments overall and for experimental conditions where the meropenem-only control groups protected ≤50% of the mice. The 95% confidence intervals and *P* values for differences in the proportions of surviving mice between treatment arms with and without rhu-pGSN were computed via normal approximation to the binomial distribution. For the individual experimental conditions where the mortality rate in the control meropenem group approximated 50% or more, survival curves were analyzed by the log rank test, temperature data were analyzed by 2-way analysis of variance (ANOVA), and bacterial burden and pathology scores were analyzed by 1-way ANOVA with Tukey post-test multiplicity adjustment. The prespecified primary end point was survival 7 days post–infectious challenge. During analysis of these data, we thought that a "survival-plus" end point to examine survival with healthy lungs (histopathology score ≤2) was a clinically meaningful extension of a good outcome. Bacterial burden and temperature response were not included in this 2-pronged composite because they were not direct measures of clinical improvement.

RESULTS

Rhu-pGSN Improved Survival of Mice Infected With *P. aeruginosa*

To determine whether rhu-pGSN could improve the efficacy of meropenem against pulmonary infection, female BALB/c mice were made neutropenic with cyclophosphamide (n = 8), infected with MDR *P.* aeruginosa, and treated with varying doses of meropenem to determine the dose at which meropenem therapy begins to fail in this model (ie, approached the ED_{50} for meropenem). Mice were treated with the selected doses of meropenem with or without rhu-pGSN for 5 days postinfection and monitored for the development of moribund disease for 7 days postinfection (Table 1). In both experiments 1 and 2, treatment with 1250 mg/kg/d of meropenem resulted in ≤50% survival, indicating failure of meropenem treatment and allowing us to ascertain whether adjunctive therapy with rhu-pGSN could improve efficacy. Focusing on animals receiving this dose, the addition of rhu-pGSN numerically increased the number of animals that survived to the end of each study (Figure 1A/B). Combining the 2 sequential studies, 31% of the mice receiving meropenem alone survived for 7 days compared with 75% survival when the mice were given meropenem with rhu-pGSN (Δ = 44%; 95% CI, 13–75; *P* = .0238) (Figure 1C). A third experiment using a different lot of meropenem that demonstrated a higher than predicted meropenem efficacy (75% survival in the meropenem-only group) did not show a difference in survival rates between the treatment groups (Table 1).

To ascertain if the increased survival with rhu-pGSN therapy was associated with decreased bacterial burden in the lungs, colony counts were determined from the lungs of mice receiving 1250 mg/kg/d at the time of death (Figure 2). We observed a general trend suggesting that rhu-pGSN improved control of bacterial burden in the lungs of infected mice compared with meropenem alone, but a statistically significant difference in bacterial counts was only observed in the second study (*P* = .0273).

Overall survival for all the dosing groups in the 3 experiments combined was 35/64 (55%) and 46/64 (72%) in mice treated with meropenem without or with rhu-pGSN, respectively (Δ = 17%; 95% CI, 1–34). Although treatment with adjunctive rhu-pGSN increased the efficacy of meropenem against pulmonary infection with *P. aeruginosa*, inhibition of bacterial proliferation in the lungs may only partially explain the observed benefit. Interestingly, we observed that meropenem alone controlled spread from lung to spleen in both studies, but that pGSN allowed splenic colonization in some animals. Although this observation was not significant in any study alone, combining data demonstrated a significant increase in splenic counts in pGSN-treated mice. In conjunction with improved survival, these observations are consistent with rhu-pGSN exerting an opsonic effect that enhanced splenic uptake.

Table 1. Survival: All Treatment Groups in the 3 Experiments

	Meropenem 1750 mg/kg/d		Meropenem 1500 mg/kg/d		Meropenem 1250 mg/kg/d		Meropenem 1000 mg/kg/d		Meropenem all Doses	
Experiment No.	- rhu-pGSN	+ rhu-pGSN	- rhu-pGSN	+ rhu-pGSN	- rhu-pGSN	+ rhu-pGSN	- rhu-pGSN	+ rhu-pGSN	- rhu-pGSN	+ rhu-pGSN
1	6/8	8/8	6/8	6/8	4/8	7/8	—	—	16/24	21/24
2	—	—	6/8	7/8	1/8	5/8	0/8	1/8	7/24	13/24
3	—	—	—	—	12/16	12/16	—	—	12/16	12/16
Totals, No.	6/8	8/8	12/16	13/16	17/32	24/32	0/8	1/8	35/64	46/64
% (95% CI)	75	100	75	81	53	75	0	13	54.7 (42.5–66.9)	71.9 (60.9–82.9)
Between-group difference (95% CI), %									17.2 (0.8–33.6)	
Nominal *P* value (2-sided)									.044	

n/N = number of surviving mice/number of treated mice. Meropenem doses were administered subcutaneously beginning at 3 hours postinfection and q8h thereafter for 5 days. Rhu-pGSN was administered as 12 mg via intraperitoneal injection on days –1, 0, 1, 2, 3, 4, and 5.

Abbreviation: rhu-pGSN, recombinant human plasma gelsolin.

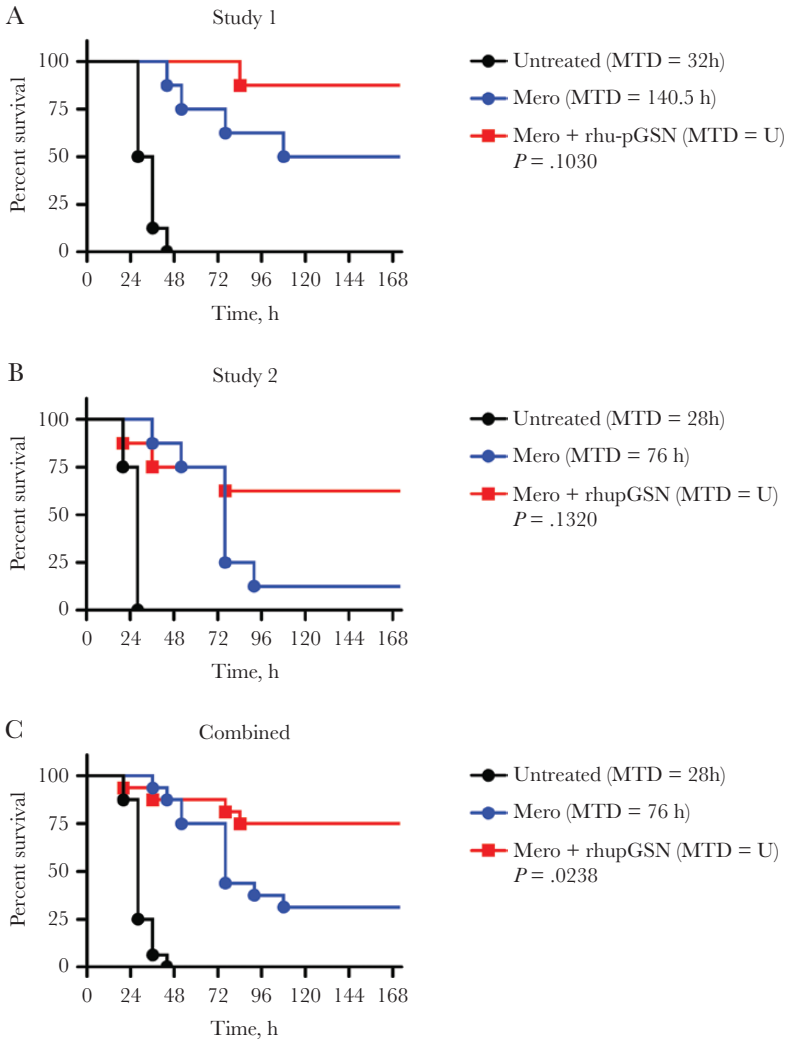

Figure 1. Survival benefit is achieved by combining rhu-pGSN with meropenem. BALB/c- mice made neutropenic with cyclophosphamide (BALB/c-Cy mice) were infected with the UNC-D strain of *P. aeruginosa* and treated with meropenem either alone (1250 mg/kg/d subcutaneously q8h for 5 days beginning 3 hours postinfection) or in combination with pGSN (12 mg/d intraperitoneally daily for days –1 to +5). Mice were killed upon reaching end point criteria or at the study conclusion on day 7. Survival analysis was conducted by log-rank test using the first 2 studies of n = 8 group size (A and B), where the control mortality rate at day 7 was ≥50% with the same 1250-mg meropenem dose. The results were then analyzed by combining these 2 separate studies (C). The *P* values refer to the survival advantage of combination therapy over meropenem alone. Abbreviations: Mero, meropenem; MTD, mean time to death; rhu-pGSN, recombinant human plasma gelsolin.

Rhu-pGSN Limits Acute Lung Injury

The lack of an unambiguous relationship between reduced bacterial loads in the lungs and increased survival in mice that received rhu-pGSN raised the possibility that rhu-pGSN protection might be mediated by alternative or additional mechanisms. Because pGSN modulates inflammation, we investigated whether adjunctive rhu-pGSN therapy diminished lung injury in *P. aeruginosa*–infected animals receiving 1250 mg/kg/d. Representative sections of lung tissue harvested from animals were blindly scored for pathology by a board-certified veterinary pathologist. Addition of rhu-pGSN to meropenem reduced host lung damage ($P = .0035$ and $P = .1514$) (Figure 3A, B). Combining the data from these 2 independent studies, the mean pathology score for mice receiving meropenem alone was 6.86, whereas the mean pathology score for mice that received both meropenem and rhu-pGSN was 2.53 ($P = .0049$) (Figure 3C).

Based on these observations that rhu-pGSN protected against lung damage, we expanded our analysis to include mice receiving doses of meropenem above and below 1250 mg/kg/d. The overall survival rates of mice receiving different doses of meropenem for 3 individual experiments are shown in Table 1. Animals surviving infection for 7 days were grouped as either demonstrating near normal lung histology (pathology scores ≤2) or signs of lung pathology (pathology scores >2). Retrospectively using this criterion, overall survival with minor lung injury was found in 26/64 (41%) mice receiving only meropenem vs 38/64 (59%) mice given meropenem plus

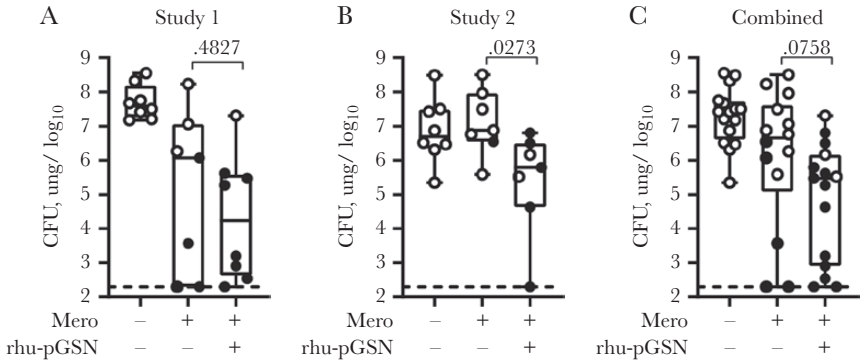

Figure 2. Rhu-pGSN reduces bacterial counts in the lungs. BALB/c-Cy mice were infected with the UNC-D strain of *P. aeruginosa* and treated with meropenem either alone (1250 mg/kg/d subcutaneously q8h for 5 days beginning 3 hours postinfection) or in combination with pGSN (12 mg/d intraperitoneally daily for days –1 to +5). Mice were killed upon reaching end point criteria (open circle) or at the study conclusion on day 7 (closed circle). Bacteria were enumerated from homogenized lung by plate count. Individual and combined data were analyzed for the first 2 studies with pairwise analysis of meropenem therapy alone (Mero) vs in combination with pGSN. The *P* values refer to unpaired Student *t* test comparisons of combination therapy vs meropenem alone. The lines at the bottom of the graph indicate the limit of detection. Abbreviations: CFU, colony-forming units; rhu-pGSN, recombinant human plasma gelsolin.

rhu-pGSN (Δ = 19%; 95% CI, 2–36) (Table 2). We then imposed arbitrary but clinically reasonable exclusion limits of ≥75% and ≤25% for the control survival rate to eliminate the noise generated by highly effective and ineffective meropenem doses. In this middle ground of responsiveness to meropenem alone, another exploratory post hoc analysis yielded favorable outcomes (survival with near-normal lungs) in 12/32 (37.5%) with only meropenem and in 27/32 (84.4%) with the combination of meropenem and rhu-pGSN (Δ = 47%; 95% CI, 26–68). Using surviving mice as the denominator, near-normal lung histopathology was found in 26/35 (74.3%) and 38/46 (82.6%), respectively, with meropenem treatment alone vs meropenem and rhu-pGSN combined therapy. These data together indicate that the addition of rhu-pGSN may decrease lung injury caused by *P. aeruginosa* infection treated only with antibacterial agents.

Plasma Gelsolin Speeds Resolution of the Host Systemic Response

As part of monitoring disease progression, we followed host temperature over the course of infection. For this model, all mice tend to exhibit a steady decrease in body temperature within the first 24 hours of infection. For mice that receive efficacious treatments, their temperatures eventually return to normal, while the temperature of mice that receive subefficacious treatments will continue to decline [36]. The time course of temperature normalization allows assessment of differences in recovery rates between different treatments. Focusing on the dosing regimens approaching the targeted ED_{50} for meropenem alone in these experiments, we investigated whether pGSN sped the restoration of temperature homeostasis in mice surviving infection. In the 2 studies achieving a survival advantage, mice typically experienced ~10°F decrease in body temperature within the first 24 hours after infection (Figure 4). Mice treated

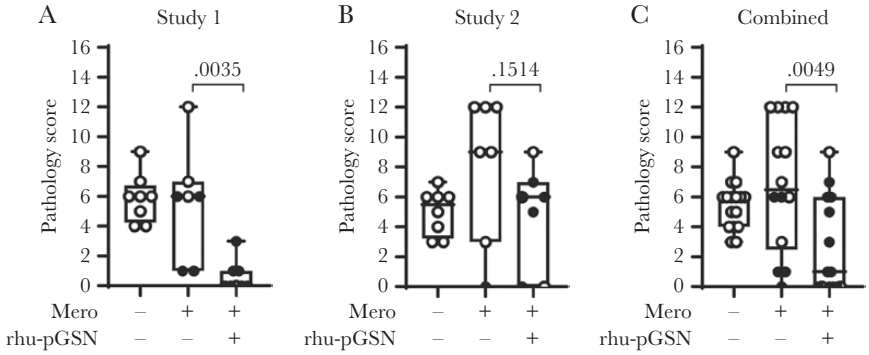

Figure 3. Rhu-pGSN limits infection-induced lung injury. BALB/c-Cy mice were infected with the UNC-D strain of *P. aeruginosa* and treated with meropenem either alone (1250 mg/kg/d subcutaneously q8h for 5 days beginning 3 hours postinfection) or in combination with pGSN (12 mg/d intraperitoneally daily for days –1 to +5). Mice were killed upon reaching end point criteria (open circle) or at the study conclusion on day 7 (closed circle). A representative section of lung was excised from the lung and processed for hematoxylin and eosin staining and scoring. Data were analyzed for the first 2 studies with pairwise analysis of meropenem therapy alone (Mero) or meropenem in combination with pGSN. The *P* values refer to unpaired Student *t* test comparisons of combination therapy vs meropenem alone. Abbreviation: rhu-pGSN, recombinant human plasma gelsolin.

Table 2. Survival With Near-Normal Lung Histopathology: All Treatment Groups in the 3 Experiments

	Meropenem 1750 mg/kg/d		Meropenem 1500 mg/kg/d		Meropenem 1250 mg/kg/d		Meropenem 1000 mg/kg/d		Meropenem all Doses	
Experiment No.	- rhu-pGSN	+ rhu-pGSN	- rhu-pGSN	+ rhu-pGSN	- rhu-pGSN	- rhu-pGSN	- rhu-pGSN	+ rhu-pGSN	- rhu-pGSN	+ rhu-pGSN
1	6/8	6/8	6/8	6/8	2/8	6/8	—	—	14/24	18/24
2	—	—	2/8	7/8[a]	1/8	1/8[a]	0/8	1/8[a]	3/24	9/24
3	—	—	—	—	9/16	11/16	—	—	9/16	11/16
Totals, No.	6/8	6/8	8/16	13/16	12/32	18/32	0/8	1/8	26/64	38/64
% (95% CI)	75	75	50	81	38	56	0	13	40.6 (28.6–52.7)	59.3 (47.3–71.4)
Between-group difference (95% CI), %									18.8 (1.7–35.8)	
Nominal *P* value (2-sided)									.039	

n/N = number of surviving mice with composite Lung Injury Scores ≤2/number of treated mice. Meropenem doses as indicated were administered subcutaneously beginning at 3 hours postinfection and q8h thereafter for 5 days. Rhu-pGSN was administered as 12 mg via intraperitoneal injection on days –1, 0, 1, 2, 3, 4, and 5.

Abbreviation: rhu-pGSN, recombinant human plasma gelsolin.

[a]A total of 3 mice (all in experiment #2) were killed at 20 hours postchallenge but had no lung injury; there was 1 mouse in each of the 3 meropenem + rhu-pGSN Rx groups. Excluding these 3 mice from the rhu-pGSN tallies yields a final count of 38/61 (62.3%).

with meropenem alone who were to survive to day 7 began to restore their body temperatures toward 95°F within 3–5 days postinfection. In contrast, the restoration of host body temperature was much more rapid in mice treated with rhu-pGSN and meropenem, where survivor body temperatures returned to 95°F by day 2. Thus, adjunctive rhu-pGSN not only improved survival and lung pathology, but also accelerated systemic recovery of the host as measured by temperature curves. In the third experiment, where a survival advantage with rhu-pGSN was not seen, no difference in the temperature course was observed between treatment arms.

DISCUSSION

Rhu-pGSN improved survival when added to meropenem in an established murine model of severe multidrug-resistant *P. aeruginosa* pneumonia. Normalization of temperature in

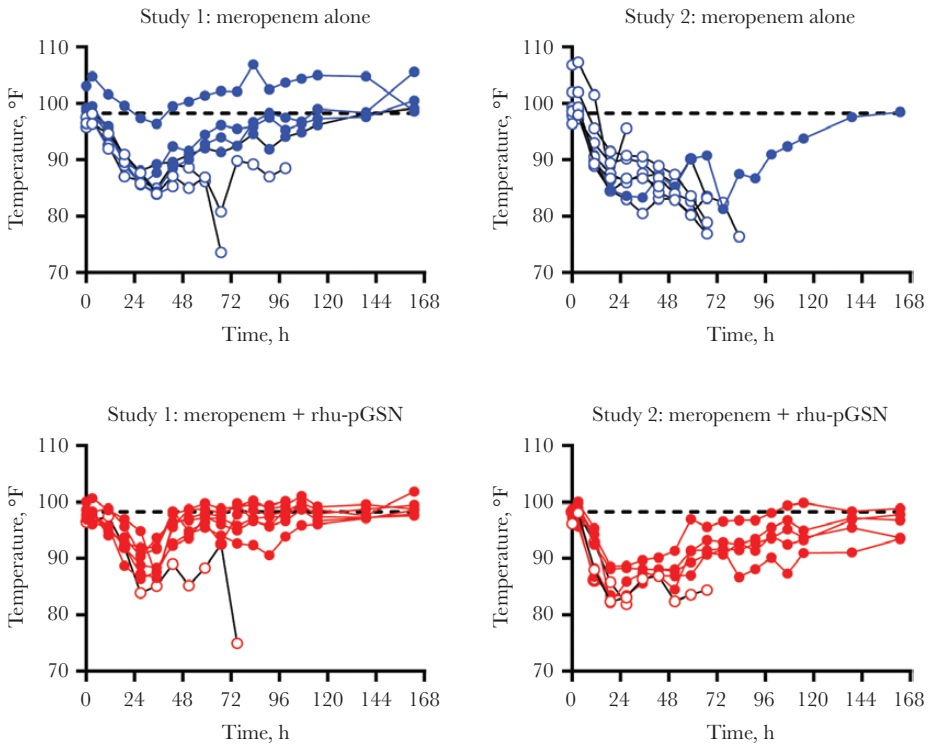

Figure 4. Restoration of baseline temperature in meropenem- and meropenem-plus-rhu-pGSN-treated mice. BALB/c-Cy mice were infected with the UNC-D strain of *P. aeruginosa* and treated with meropenem either alone (1250 mg/kg/d subcutaneously q8h for 5 days beginning 3 hours postinfection) or in combination with pGSN (12 mg/d intraperitoneally daily for days –1 to +5). Animal temperatures were monitored every 8 hours postinfection until the end of the study. Mice were sacrificed upon reaching end point criteria (open circles) or at the study conclusion on day 7 (closed circles). Abbreviation: rhu-pGSN, recombinant human plasma gelsolin.

surviving mice generally occurred more rapidly with adjunctive rhu-pGSN therapy than with meropenem alone. Lungs from rhu-pGSN recipients generally had fewer viable bacteria. Furthermore, rhu-pGSN reduced the degree of acute lung injury in surviving animals, which potentially represents a clinically important advance in the treatment of serious bacterial pneumonia. Taken together, our findings suggest that the survival advantage afforded by the addition of rhu-pGSN to meropenem treatment was likely due in large part to a rhu-pGSN-mediated reduction in the bacterial load and severity of lung injury during the course of infection.

The first line of host defense against infection involves a focused inflammatory response. However, excessive local and systemic inflammation can be injurious to vital organs near and far from the primary infection site. The strict localization and ultimate resolution of acute inflammation are complex processes whose regulation is critically important but only incompletely understood. pGSN serves to modulate inflammatory processes by at least 3 different actions: (i) debriding viscous content leaked from disrupted cells by scavenging actin and other danger-associated molecular patterns (DAMPs) at the site of injury; (ii) augmenting macrophage uptake and killing of microbial pathogens; and (iii) as the precipitating insult subsides, complexing proinflammatory mediators, dampening their local effects and preventing their systemic spread to uninvolved organs [7–19, 34, 35, 37, 38]. As the acute injury recedes, pGSN promotes resolution of the inflammatory process and limits the resultant damage.

We explored the possible benefits of rhu-pGSN treatment added to meropenem in highly lethal, multidrug-resistant *P. aeruginosa* pneumonia in a neutropenic mouse model. All mice died within ~24 hours of infection without immediate antimicrobial therapy. Rhu-pGSN as sole treatment slightly prolonged average survival by ~12 hours. To decide on the dose of meropenem that would yield ≥50% mortality, titration experiments were performed with each batch of antibiotic. Nonetheless, outcomes were not always predictable, leading to mortality rates ≤25% or ≥75% for the meropenem controls in some trials. Under such extreme conditions, the possible benefits of adjunctive rhu-pGSN on outcome might be masked because the mice were either too sick or not sick enough. Nonetheless, rhu-pGSN given with meropenem was more efficacious than meropenem alone under most conditions.

These preclinical data further strengthen the growing body of evidence that rhu-pGSN as an adjunct to standard-of-care modalities might be effective in enhancing survival while limiting lung injury. Moreover, in a recently completed, small, double-blinded, placebo-controlled, dose-escalation study, intravenous repletion of rhu-pGSN in patients hospitalized on general medical wards with modestly severe community-acquired pneumonia appeared safe and well tolerated (ClinicalTrials.gov NCT03466073). Even with supraphysiological levels throughout the dosing interval, neither serious nor drug-related adverse events were observed in rhu-pGSN recipients given 3 consecutive days of therapy.

Using an established model of murine gram-negative pneumonia, bacterial colony counts from alveolar lavage and histopathological lung injury scores at the time of death were higher in mice receiving meropenem alone compared with mice treated with meropenem and rhu-pGSN, although there was considerable variability observed within and between experiments. Both mortality and parenchymal injury were lessened by the addition of rhu-pGSN to meropenem, most prominently in situations where meropenem alone was relatively ineffective. The clinically meaningful composite end point measured in our hypothesis-generating analyses merits testing in different animal models of lung infection and ultimately in proof-of-concept clinical trials.

Acknowledgments

Recombinant human plasma gelsolin was provided by BioAegis Therapeutics Inc. The authors gratefully acknowledge the technical support provided by Daniel Cramer, Xiaoxian Duan, Jon Gabbard, Tia Pfeffer, William Severson, and Julie Sotsky at the University of Louisville Center for Predictive Medicine for Biodefense and Emerging Infectious Diseases. We thank James Bolognese for his instructive statistical guidance during the design and analyses of the reported experiments and Dr. Abla Tannous for her helpful feedback during the preparation of this manuscript.

Financial support. These studies were supported by National Institutes of Health/National Institute of Allergy and Infectious Diseases contracts HHSN272201000033I-HHSN27200003-Task A72 and HHSN272201000033I-HHSN27200006-Task A111 to Drs. Lawrenz and Warawa.

Potential conflicts of interest. S.L.L. is the Chief Executive Officer and M.J.D. is the Chief Medical Officer of BioAegis Therapeutics, which is developing rhu-pGSN for clinical use, and they own stock in the company. T.P.S. was the founding scientist of the company but tragically and unexpectedly died while this manuscript was actively in progress. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Author contributions. All coauthors have contributed to, seen, and approved the final submitted version of the manuscript, except for T.P.S., who suddenly died while this manuscript was in preparation.

Prior presentation. This work has not been submitted elsewhere for publication, except as a presentation at ASM Microbe 2020.

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