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Intravenous administration of ulinastatin (human urinary trypsin inhibitor) in severe sepsis: a multicenter randomized controlled study

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Take-home message: Ulinastatin, a protease inhibitor, inhibits several pro-inflammatory proteases and decreases inflammatory cytokine levels and mortality in experimental sepsis. In this pilot study, intravenous administration of ulinastatin (200,000 IU 12 hourly for 5 days) reduced mortality, new onset of organ dysfunction, duration of mechanical ventilation and hospital stay in patients with severe sepsis when started within 48 h of onset of failure of one or more organs.

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Abstract *Purpose:* Ulinastatin, a serine protease inhibitor, inhibits several pro-inflammatory proteases and decreases inflammatory cytokine levels and mortality in experimental sepsis. We studied the effect of ulinastatin on 28-day all-cause mortality in a double-blind trial in patients with severe sepsis in seven Indian hospitals. *Methods:* Patients with sepsis were randomized within 48 h of onset of one or more organ failures to receive intravenous administration of ulinastatin (200,000 IU) or placebo 12 hourly for 5 days. *Results:* Of 122 randomized subjects, 114 completed the study (55 receiving ulinastatin, 59 receiving placebo). At baseline, the mean APACHE II score was 13.4 (SD = 4.4), 48 (42 %) patients were receiving mechanical ventilation, 58 (51 %) were on vasopressors, and 35 % had multiple organ failure. In the modified intention-to-treat analysis (patients receiving six or more doses of study drugs), 28-day all-cause mortality was 7.3 % with ulinastatin (4 deaths) versus 20.3 % (12 deaths) with placebo ($p = 0.045$). On multivariate analysis too, treatment with ulinastatin (odds ratio 0.26, 95 % CI 0.07–0.95; $p = 0.042$) independently decreased 28-day all-cause mortality. However, the mortality difference did not reach statistical significance in the intention-to-treat analysis [10.2 % (6/59 deaths) with ulinastatin versus 20.6 % (13/63 deaths) in the placebo group; $p = 0.11$]. The ulinastatin group had lower incidence of new-onset organ failure (10 vs. 26 patients, $p = 0.003$), more ventilator-free days (mean ± SD 19.4 ± 10.6 days vs. 10.2 ± 12.5 days, $p = 0.019$), and shorter hospital stay (11.8 ± 7.1 days vs. 24.2 ± 7.2 days, $p < 0.001$). *Conclusions:* In this pilot study, intravenous administration of ulinastatin reduced mortality in patients with severe sepsis in the modified intention-to-treat analysis, but not in the intention-to-treat analysis.

Keywords Serine protease inhibitor · Inflammatory mediators · Bacterial infection · Septic shock · Pneumonia · Abdominal sepsis

Introduction

Sepsis is a common cause of morbidity and mortality in critically ill patients, and its incidence is increasing worldwide annually [1, 2]. The pathogenesis of sepsis is complex and is believed to be initiated by the interaction between pathogen-associated molecular patterns and pattern recognition receptors on host immune cells [3, 4]. This sets off a series of pro-inflammatory mechanisms including synthesis and release of cytokines and complement, chemotaxis and activation of neutrophils, and initiation of coagulation [3–5]. These, in turn, have widespread effects on other cells including inflammatory cells, immune response, endocrine and autonomic nervous systems, and vascular endothelium, mainly aimed at limiting spread or eliminating the infecting pathogen [3–6]. Current opinion suggests that the systemic inflammatory response syndrome (SIRS) that characterizes severe sepsis results from an excessive activation of pro-inflammatory mediators, which have pleiotropic effects that overwhelm the body's anti-inflammatory mechanisms, leading to widespread vascular, endothelial, and organ dysfunction that is often fatal [3–6].

Many of the intermediaries in the systemic inflammatory processes are serine proteases. These include trypsin, thrombin, chymotrypsin, kallikrein, plasmin, neutrophil elastase, cathepsin, neutrophil protease-3, and coagulation factors IXa, Xa, XIa, and XIIa [7, 8]. It is now being recognized that besides their proteolytic activity, these proteases have an important role in regulation of inflammation through inter- and intracellular signaling pathways [8, 9]. To counter-regulate the effect of these proteases, several protease inhibitors are produced by the liver in the presence of inflammation; these include acute phase reactants such as α_1-antitrypsin and proteins of the inter-α-inhibitor family [9]. Urinary trypsin inhibitor is one such important protease inhibitor found in human blood and urine; it has been also referred to in the literature as ulinastatin or bikunin [10, 11]. It is an acidic glycoprotein (molecular weight 30 kDa) and Kunitz-type serine protease inhibitor composed of 143 amino acid residues and includes two Kunitz-type domains [7, 10]. It is cleaved from the larger inter-α-trypsin inhibitor molecule by neutrophil elastase in the presence of inflammation, and is believed to play an important anti-inflammatory role [8–12].

Studies in patients have shown that there is a decrease in serum levels of ulinastatin in sepsis, with the lowest levels being found in patients with severe sepsis and septic shock [12]. As compared to wild-type mice, mortality due to experimental sepsis is higher in genetically modified knockout mice that lack the genes for synthesis of urinary trypsin inhibitor [11, 13]. Several preclinical studies have shown a reduction in the systemic inflammatory response and organ dysfunction due to sepsis in animals treated with ulinastatin [13–17]. On the basis of a few small clinical studies that have shown a trend towards reduced mortality and duration of hospitalization with ulinastatin in severe sepsis [18, 19], some authors have suggested that ulinastatin may have a role as a novel therapy in severe sepsis [20, 21]. We therefore conducted this pilot study to evaluate the efficacy of ulinastatin in Indian patients with severe sepsis.

Methods

This randomized, double-blind, placebo-controlled trial was conducted in the intensive care units (ICUs) of seven tertiary care hospitals in India; the study protocol was approved by the institutional review boards at each center. The study protocol is available from the Indian clinical trials registry at http://www.ctri.nic.in (clinical trial number CTRI/2009/091/000650).

Adults, aged 18–60 years (both inclusive), with severe sepsis, admitted to the ICU between September 2009 and June 2010 were eligible for enrollment into the study. Sepsis was defined as evidence of infection (defined as presence of white blood cells in a normally sterile body fluid, perforated abdominal viscus, evidence of pneumonia, or presence of a condition associated with a high risk of bacterial infection, e.g., ascending cholangitis), and the presence of at least three of the four SIRS criteria [22]. Severe sepsis was defined as the presence of sepsis as defined above, along with dysfunction of at least one organ or system. Cardiovascular system failure was defined as systolic blood pressure $\leq$90 mmHg or mean arterial pressure $\leq$70 mmHg for at least 1 h despite adequate fluid resuscitation, or the use of vasopressors to maintain arterial pressure above these levels or unexplained metabolic acidosis (pH $\leq$ 7.30 or base deficit $\geq$5.0 mmol/L) with plasma lactate greater than 1.5 times the upper limit of normal. Renal failure was defined as urine output <0.5 mL/kg/h for 1 h, or serum creatinine levels greater than 2.5 times the upper limit; respiratory failure as PaO_2/FiO_2 $\leq$250 in the presence of other dysfunctional organs or $\leq$200 if only lung; hematologic dysfunction by platelet count <80,000/mm^3 or 50 % drop in preceding 3 days [22]. Only patients with organ dysfunction of $\leq$48 h duration were eligible for inclusion.

Pregnant or breastfeeding women, patients with platelet count <30,000/mm^3, history of organ transplantation, poorly controlled neoplasm, end-stage chronic kidney or liver disease, and patients weighing >135 kg were excluded. Patients in whom limitation of care was planned or who were expected to die within the next 24 h were also excluded.

Block randomization (block size of 4) using a computer-generated sequence was used to ensure balance

between treatments; blinding of the sequence was maintained in sealed, opaque envelopes. Blinding of treatment allocation in participating centers was done using serially numbered packages of the study medication; package numbered 1 was to be used for the first patient, and so on. After obtaining written informed consent, patients were randomized in a 1:1 ratio to receive ulinastatin or placebo in addition to standard care. Randomized patients received an intravenous infusion of either 200,000 IU ulinastatin (U-tryp, Bharat Serum and Vaccines Ltd, India) or identical placebo dissolved in 250 mL of 0.9 % saline given intravenously over 1 h every 12 h for 5 days in a double-blind evaluation. For patients with fluid restriction, 100 mL of 0.9 % saline could be used. Infusion could be interrupted for 1 day, if there was greater than three times increase in liver enzymes over baseline levels.

In addition to the study medications, patients also received antibiotics, intravenous fluids, enteral or parenteral nutrition, transfusion of blood and blood products, and supportive care for organ dysfunction including mechanical or non-invasive ventilation, vasopressors (noradrenaline, adrenaline, dopamine, or vasopressin), or dialysis as per the standard treatment protocols followed in each ICU. Investigators were encouraged to follow the Surviving Sepsis Campaign guidelines. No concomitant medications were prohibited in the study.

Baseline characteristics including demographics, pre-existing conditions, organ dysfunction, infection, and hematologic and other laboratory tests were assessed within the 24 h prior to infusion of the first dose of study medication. Blood samples were also obtained on the day of discharge. Patients were followed up till 28 days after the start of treatment. The primary end-point for this study was 28-day all-cause mortality. Secondary end-points included onset of new organ failure, duration of vasopressor use, ventilator-free days till day 28, and length of hospital stay. New-onset organ failure was defined as occurrence of organ failure after randomization. The definitions used were the same as those used in inclusion criteria; liver dysfunction was defined as serum bilirubin $\geq$2.0 mg/dL when bilirubin was $<$2.0 mg/dL at baseline and central nervous system failure was defined as Glasgow coma scale (GCS) $\leq$10 after randomization in patients who had baseline GCS $>$10.

Statistical analysis

Data analysis was carried out using the Graph Pad Instat and SPSS version 14 software package. Forward stepwise multiple logistic regression analysis was used for assessment of the primary end-point. Other categorical data were compared between the treatment groups by Pearson's Chi-squared test or Fisher's exact test, as appropriate. The unpaired *t* test was used for continuous variables and the Mann–Whitney test for ordinal data like the APACHE II score.

Sample size calculation was performed assuming a 28-day all-cause mortality of 30 % in the control group and 10 % in the study group. A sample size of 59 completed patients in each group was required to attain a power of 80 % at a significance level of 5 %. The primary efficacy end-point of 28-day all-cause mortality was assessed in the modified intention-to-treat population which was defined a priori. This primary analysis population included all randomized patients who had received at least six doses of the study medication. Safety was assessed in the safety population, defined as all subjects who received even a single dose of the study medication. Mortality difference in the intention-to-treat population that included all randomized subjects was analyzed as a secondary end-point.

Results

During the 10-month study period, 224 patients met the inclusion criteria. Of these, 102 patients were excluded; 99 because of presence of one or more exclusion criteria, or refusal of consent to participate in the study and three patients were not enrolled as they were expected to die within 24 h (Fig. 1). Of the 122 randomized subjects, 114 completed the study (55 subjects in the ulinastatin group and 59 subjects in the control group). Five subjects discontinued from the study (three subjects withdrew consent and two due to protocol violation). Three patients who died within 48 h of enrollment were not included for analysis in the modified intention-to-treat population. Of the 114 patients who completed the study, one patient in the ulinastatin group received six doses and one patient each in the placebo group received six and seven doses; all others received the 10 scheduled doses of study medications.

Baseline characteristics

At baseline, the two treatment groups were similar with respect to demographic characteristics, cause of sepsis, number of organs affected, pattern of organ dysfunction, and need for vasopressors or mechanical ventilation (Table 1). Infection was microbiologically confirmed in 26 patients (23 %). Approximately 35 % of patients had multiple organ failure at baseline, 51 % required vasopressors, and 42 % received mechanical ventilation. For details of organisms causing sepsis and antibiotics used, see Electronic Supplementary Material.

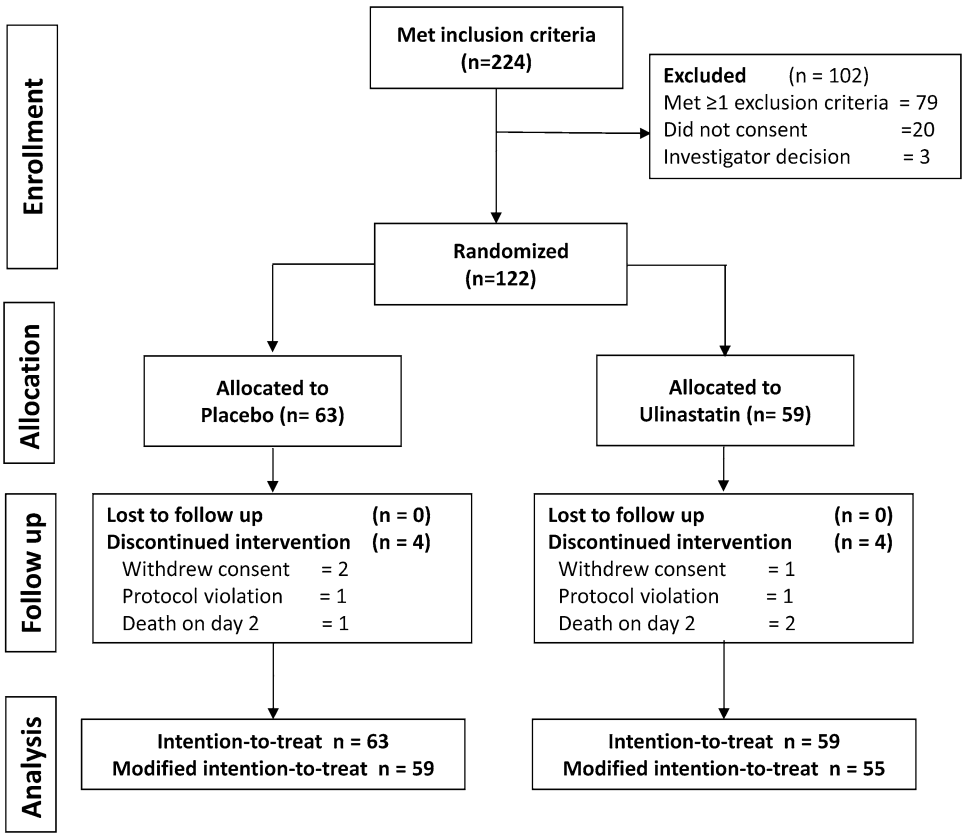

Fig. 1 CONSORT diagram of the study

Outcomes

The 28-day mortality, the primary end-point of this study, showed a significant difference (Table 2) in the two treatment groups; there were four deaths (7.3 %) in the ulinastatin group versus 12 deaths (20.3 %) in the placebo group. On stepwise multiple logistic regression, treatment with ulinastatin was found to produce a statistically significant decrease in risk of death (odds ratio 0.26, 95 % confidence limits 0.07–0.95; $p = 0.042$). Other variables independently associated with mortality were acute renal failure and mechanical ventilation at baseline (Table 3). Of the four deaths in the ulinastatin group one patient each died as a result of ARDS, acute renal failure, refractory shock, and multiple organ failure. Of the 12 deaths in the placebo group five patients died as a result of ARDS, five as a result of refractory shock, and one each as a result of intracranial hemorrhage and multiple organ failure. All deaths were attributed by the investigators to progression of underlying sepsis. The Kaplan–Meier plot for survival in the two groups is shown in Fig. 2.

Among the secondary end-points, there were 6 deaths (10.2 %) in the ulinastatin group ($n = 59$) in the intention-to-treat population which included all randomized subjects versus 13 deaths (20.6 %) in the control group ($n = 63$; $p = 0.11$). New onset of organ dysfunction was seen in 10 subjects in the ulinastatin group and 26 subjects in the placebo group ($p = 0.003$). Number of ventilator-free days up to day 28 (mean ± SD) was significantly more (19.4 ± 10.6 days) in the ulinastatin group and 10.2 ± 12.5 days in the placebo group ($p = 0.019$). Mean hospital stay too was less in the ulinastatin group by an average of 12.4 days ($p = 0.001$).

Complications

Intracranial hemorrhage was the only unexpected serious adverse event in one patient; this patient was from the placebo group. There were no infusion-related adverse events associated with treatment with ulinastatin.

Discussion

This prospective, double-blind, randomized, placebo-controlled trial of ulinastatin in patients with severe sepsis showed that intravenous administration of ulinastatin in a dose of 200,000 units twice daily for 5 days was associated with a reduction in 28-day all-cause mortality (the primary end-point) to 7.3 versus 20.3 % in the placebo control group. A few small studies, published in Chinese-language journals, have shown lower mortality in patients

Table 1 Baseline characteristics in patients with severe sepsis randomized to placebo and ulinastatin treatment groups

Characteristics	Placebo group ($n = 59$)	Ulinastatin group ($n = 55$)	p value
Age (years)[b]	36.7 ± 12.5	37.5 ± 12.9	0.70
Gender			
Males	50 (85 %)	38 (69 %)	0.05
Females	9 (15 %)	17 (31 %)	
Serum creatinine (mg/dL)[c]	1.0 (0.81, 1.39)	1.0 (0.8, 1.57)	0.97
Platelet count ($\times 10^3/\mu L$)[c]	163 (101, 264)	191 (115, 242)	0.44
Total leucocyte count ($\times 10^3/\mu L$)[c]	12.8 (8.1, 21.0)	13.6 (7.4, 18.3)	0.55
Serum C reactive protein (mg/L)	132 ± 116	104 ± 112	0.41
Systemic inflammatory response			
3 of 4 SIRS criteria	33 (56 %)	27 (49 %)	0.46
4 of 4 SIRS criteria	26 (44 %)	28 (51 %)	
Organ dysfunction[a]			
Cardiovascular	31 (52.5 %)	28 (50.9 %)	0.86
Renal	9 (15.3 %)	8 (14.5 %)	0.92
Respiratory	27 (45.8 %)	29 (52.7 %)	0.46
Hematological	10 (16.9 %)	8 (14.5)	0.73
Metabolic acidosis	8 (13.6 %)	4 (7.3 %)	0.27
Central nervous system	7 (11.9 %)	8 (14.6 %)	0.67
Number of organs affected			
One organ	38 (64.4 %)	36 (65.5 %)	0.91
Two organs	17 (28.8 %)	16 (29.1 %)	
Three organs	3 (5.1 %)	3 (5.5 %)	
Four organs	1 (1.7 %)	0	
Infection causing sepsis			
Respiratory	25	22	0.79
Abdominal	22	18	
Urinary tract	4	4	
Central nervous system	0	1	
Skin and soft tissue	4	3	
Bloodstream infection	4	7	
Type of infection			
Community-acquired	44 (75 %)	42 (76 %)	0.82
Nosocomial	15 (25 %)	13 (24 %)	
Organisms isolated			
Gram negative organisms	11	7	0.66
Gram positive organisms	3	2	
Candida spp.	1	2	
APACHE II score[b]	13.5 ± 6.5	13.2 ± 5.9	0.81
Vasopressors use	30 (50.8 %)	28 (50.9 %)	0.99
Mechanical ventilation	24 (41 %)	24 (44 %)	0.75
Non-invasive	2	4	
Invasive	22	20	
Chronic comorbid conditions[a]			
Diabetes mellitus	6	9	0.96
COPD	4	5	
Chronic liver disease	3	2	
Tuberculosis	4	4	
Congestive heart failure	4	2	
HIV with AIDS	1	1	
Chronic kidney disease	1	1	
Stroke	0	1	
Autoimmune vasculitis	1	1	
No comorbid conditions	37	38	

[a] Some patients had more than one organ dysfunction or comorbid condition
[b] Values are mean ± standard deviation
[c] Values are median (interquartile range)

treated with ulinastatin [19, 23, 24]. Small randomized controlled trials have also been conducted by a single group of investigators comparing standard treatment with intravenous administration of ulinastatin in combination with alpha thymosin and found a reduction of mortality in patients with severe sepsis [25–27]. Since these studies have all used a combination of two novel agents, it is unclear whether the survival benefit seen was due to either one of these agents, or the combination. A small Korean study showed that mortality was lower in patients

Table 2 Outcomes in patients with severe sepsis in the placebo and ulinastatin groups

	Placebo group ($n = 59$)	Ulinastatin group ($n = 55$)	p value
28-day all-cause mortality	12 (20.3 %)	4 (7.3 %)	0.045*
New-onset organ dysfunction[a]			
Cardiovascular	9	4	0.18
Respiratory	8	4	0.27
Hematological	7	4	0.41
Hepatic	3	2	1.0
Renal	4	2	0.68
Central nervous system	3	2	1.0
Total number of patients with new-onset organ dysfunction	26	10	0.003
Duration of vasopressor use (days)	1.7 ± 2.1	1.7 ± 1.9	0.92
Duration of mechanical ventilator (days)	11.9 ± 9.6	7.5 ± 7.5	0.18
Ventilation-free days (till day 28)	10.2 ± 12.5	19.4 ± 10.6	0.019
Length of hospital stay (days)	24.2 ± 7.2	11.8 ± 7.1	<0.001

* p value is by univariate analysis. Multivariate analysis results are in Table 3

[a] New-onset organ dysfunction was defined as organ dysfunction occurring after administration of the first dose of the study medication. Criteria used for individual organ dysfunction were same as those used for inclusion in the study. In addition, liver dysfunction was defined as post-baseline serum bilirubin ≥2.0 mg/dL when bilirubin was <2.0 mg/dL at baseline and central nervous system dysfunction was defined as post-baseline GCS ≤10 when baseline GCS was >10

Table 3 Variables associated with 28-day all-cause mortality on forward stepwise multiple logistic regression analysis

Variable	Odds ratio[a] (OR)	95 % CI of OR	p value
Renal failure	6.37	1.70–23.8	0.006
Need for mechanical ventilation	3.36	1.01–11.2	0.048
Treatment with ulinastatin	0.26	0.07–0.95	0.042

Treatment with ulinastatin was independently associated with decreased mortality compared with treatment with placebo after adjusting for other baseline characteristics including age, gender, Glasgow coma scale, specific organ system failure, number of organs failed, need for vasopressors and need for mechanical ventilation

[a] OR <1 indicates reduced risk of death; OR >1 indicates increased risk of death

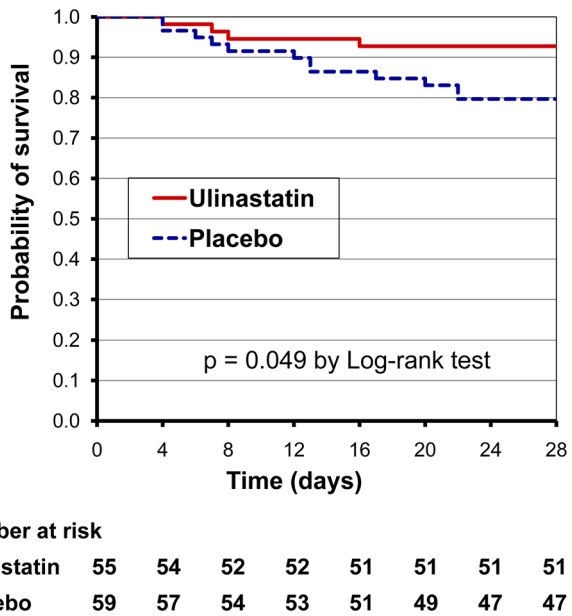

Fig. 2 Kaplan–Meier analysis of the probability of survival of patients with severe sepsis treated with ulinastatin or placebo (modified intention-to-treat cohort)

with severe sepsis treated with ulinastatin (18.6 vs. 27 % in the control group) [18]. Our results further corroborate these studies and suggest that treatment with ulinastatin may reduce mortality in severe sepsis in humans.

Highly selective serine protease inhibitors that act on only a few steps in the multipronged inflammatory response involved in the pathogenesis of sepsis like coagulation (tissue factor pathway inhibitor, activated protein C, thrombomodulin, and antithrombin III), complement cascade (C1 inhibitor), or neutrophil elastase (sivelestat) have failed to provide substantial clinical benefit in clinical trials [3–6, 21]. In contrast, ulinastatin inhibits a wide variety of pro-inflammatory serine protease enzymes including trypsin, thrombin, kallikrein, plasmin, cathepsin, neutrophil elastase, neutrophil protease-3, and coagulation factors IXa, Xa, XIa, and XIIa [7–11, 28]. Although the exact mechanism of action of ulinastatin in sepsis is not clear, it is likely that it may attenuate the inflammatory response by acting at multiple sites. In animal models of sepsis, exogenously administered ulinastatin has been shown to reduce levels of TNF-α, IL-1, IL-6, cytokine-induced neutrophil chemoattractant-1 (CINC-1), myeloperoxidase, free oxygen radicals,

high mobility group box 1 (HMGB1), interleukin (IL)-6, interleukin-8 (IL-8), malondialdehyde, and soluble intercellular adhesion molecule-1 (sICAM-1) in serum and in organs like lung, kidney, and intestine of rats with lipopolysaccharide-induced SIRS [13–16, 28–34]. Ulinastatin also decreases phosphorylation of p38 mitogen-activated protein kinase (p38-MAPK) which in turn attenuates activation of NF-κB and downregulates expression of the TNF-α gene [33]. Studies in humans too have shown that patients with sepsis treated with intravenous administration of ulinastatin have lower serum levels of pro-inflammatory markers like TNF-α, IL-1β, IL-4, IL-6, and C-reactive protein, while levels of anti-inflammatory cytokine IL-10 was significantly higher [19, 23, 35–37]. It also reduces thrombomodulin levels and decreases endothelial dysfunction [38].

Besides reduction in 28-day all-cause mortality, ulinastatin also showed beneficial effects on some secondary end-points in the present study like new-onset organ dysfunction. Although the duration of vasopressor use was similar in the two groups, ventilator-free days were significantly higher in the ulinastatin group (19.4 vs. 10.2 days), suggesting faster recovery from severe sepsis. This also translated into a shorter mean hospital stay in the ulinastatin group.

No infusion-related adverse effects were seen in the present study. Adverse effects with ulinastatin are rare and were reported in 0.84 % of patients in a Japanese study [39]. These included increased transaminases (0.36 %), eosinophilia or leucopenia (0.16 %), rash (0.13 %), gastrointestinal symptoms (0.08 %), fever (0.02 %), and local irritation at the injection site (0.02 %). These were reported in less ill patients with pancreatitis or when the drug was used prophylactically in patients undergoing endoscopic retrograde pancreatography, for which the drug is licensed in Japan. None of these effects were seen in the present study, probably because patients were too sick to complain of these symptoms.

Our study has some limitations. Firstly, although larger than previous randomized controlled studies with ulinastatin, the number of patients is relatively small, thereby limiting the power of this study. However, power of a study is more relevant for interpreting results of clinical trials that show no difference between the two treatment arms. Secondly, patients aged >60 years were excluded. This is because unlike in western countries, the number of elderly patients seeking care in Indian ICUs is comparatively small, and limitation of care is often opted for by the family in such cases after a few days of ICU stay owing to socio-economic and cultural reasons [40, 41]. Thirdly, the baseline APACHE II scores were low although patients had significant organ dysfunction, as has also been reported in previous studies from India [42, 43]. This is because the APACHE II score allocates more points to old age than for maximum possible physiological derangement (5 points for age 65–74 years and 6 for age >75 years) [44]. Moreover, septic encephalopathy is also more common in the elderly [45], and the highest possible points for any single physiological variable in the APACHE II system are assigned to the level of consciousness (APACHE II points = 15 minus Glasgow coma score) [44]. These two factors alone could add up to 18 points per patient (6 for age and 12 for coma), accounting for high APACHE II scores in studies from western countries. These differences may limit the generalizability of our results to some extent. However, our study had some strengths too. Whereas all previous studies with ulinastatin in sepsis have been single-center studies, this was a multicenter study. Use of a double-blind design was another strength.

In conclusion, this prospective, multicenter, randomized, double-blind, placebo-controlled pilot study in patients with severe sepsis investigated a novel therapy directed against the systemic inflammatory response. We found that 5-day treatment with intravenous administration of ulinastatin in patients with severe sepsis, when started within 48 h of organ dysfunction, resulted in a reduction in 28-day all-cause mortality to 7.3 % from 20.3 % observed in the placebo group. The reduction in mortality to 10.2 % from 20.6 % in the intention-to-treat population, however, was short of statistical significance ($p = 0.11$). We also found a reduction in new onset of organ dysfunction, days of mechanical ventilation, and hospital stay. A larger randomized controlled study with ulinastatin is needed to further confirm the survival benefit seen in this pilot study and also to investigate its mechanism of action in humans.

Conflicts of interest This study was funded by Bharat Serums and Vaccines Limited, India who provided the study medications, administrative assistance, and study grants to participating centers and funded the statistical analysis by an external statistician.

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