Review

The effectiveness of using ulinastatin as a therapeutic agent in the treatment of sepsis

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Abstract

Clinical trials have shown that ulinastatin (UTI), an anti-inflammatory substance, can improve outcomes for sepsis patients.[1-3] The treatment, usually administered intravenously, has been shown to reduce mortality rates[1] and attenuate sepsis-induced inflammation[4] and organ damage.[5] Newer research has attempted to explain the mechanism behind its ability to reduce inflammation by measuring various inflammatory markers.[6,7] Ulinastatin has a role in reducing TNF-α, IL-1 and IL-68 as well as (CRP),[6] TLR-4 and MyD88.[7] These are all released into the bloodstream during inflammation.

This review will explore research from peer-reviewed scientific journals to investigate the effectiveness of using ulinastatin in reducing mortality, inflammation and organ damage in sepsis. It will also explore the change in effectiveness of ulinastatin when combined with other treatments such as glutamine[9,10] or administered differently (e.g. intraintestinally).[9,11]

Abbreviations

ALT – Alanine aminotransferase
ALP – Alkaline phosphatase
CLP – Caecal ligation and puncture
CRP – C-reactive protein
IL – Interleukin
LDH – Lactate dehydrogenase
MAPK – Mitogen-activated protein kinases
MyD88 – Myeloid differentiation primary response 88
PCT – Procalcitonin
TLR – Toll-like receptor
TNF – Tumour necrosis factor
UTI – Ulinastatin
WBC – White blood cell

Introduction

Sepsis is currently defined as a "life-threatening organ dysfunction caused by a dysregulated host response to infection".[12] It is an extremely prevalent and deadly condition, with 33.1 million cases reported worldwide in 2017, with an estimated 11 million deaths.[13]

In sepsis, both the pro-inflammatory and anti-inflammatory pathways are upregulated, leading to tissue damage and organ dysfunction.[14] Inflammatory imbalance is a major component in the pathogenesis of sepsis[15] so inhibiting inflammatory response has become a key target in its treatment.[16]

Ulinastatin is a serine protease inhibitor extracted from human urine and blood.[8,17] Ulinastatin can inhibit proteases such as trypsin, thrombin and kallikrein, which have roles in inflammation and coagulation, both critical in sepsis pathophysiology.[8] Ulinastatin can prevent neutrophil infiltration and possibly supress the MAPK (mitogen-activated protein kinases) pathway as it has been shown to inhibit the production of TNF-α, IL-1 and IL-68 making it a useful therapeutic agent. Many studies have been carried out to test the efficacy of ulinastatin administration as a treatment option for sepsis.[8,16]

Methods

Primary research papers were selected from the database Medline. Studies were included in the search if they included the key terms "septi*" (which searched for "sepsis", "septic" etc.) and "ulinastatin", "urinary trypsin inhibitor" which were published in the last 5 years. Only peer-reviewed primary research papers were selected. It was ensured that authors had published additional research in the field of sepsis to ensure competency and that, where possible, they utilised randomisation in their study to reduce bias. Since it is currently a small area of research, the search was widened to articles published

in the last 10 years and some additional papers were selected to improve the scope of the review.

Results

1. Reducing Mortality

Karnad et al suggested that ulinastatin administration could reduce mortality in patients with severe sepsis.[1] This was a randomised, double-blind, placebo-controlled trial of 114 sepsis patients. Participants either received 200,000 IU ulinastatin or a placebo intravenously every 12 hours for 5 days in addition to their ongoing treatment.[1] The 28-day mortality was significantly lower in the ulinastatin group, compared to the placebo group ($p=0.042$) and so was "new onset of organ dysfunction" (10 versus 26 patients; $p=0.003$) and "mean hospital stay" (11.8 versus 24.2 days; $p=0.001$). An earlier study using mice utilised caecal ligation and puncture (CLP) to create a sepsis model and 30 mice were split into three groups: a sham group, CLP group and CLP + ulinastatin group.[2] Their results showed that 7-day survival was significantly higher in the group treated with ulinastatin (70% versus 40%; $p=0.0187$). It was found that inflammatory cytokines, specifically TNF-α, IL-6 and IL-10, were significantly reduced following administration of ulinastatin ($p<0.05$). A recent retrospective study split 130 participants into two equal groups.[3] Both groups received the conventional treatment for sepsis, but one group received ulinastatin for 7 days in addition. Conventional treatment was dependent on symptoms but included antibiotics and ventilation. The intervention group had significantly lower APACHE II scores (mean of 6.4 compared to 11.7; $p<0.01$), which accurately predict hospital mortality.[18]

2. Reducing inflammation

Multiple studies have investigated the potential anti-inflammatory mechanism of ulinastatin in sepsis. One study investigated 263 sepsis patients with 179 receiving ulinastatin and 84 in the control group.[6] On day 3, CRPs in the ulinastatin group were not shown to be significantly lower than the control group (124 mg/l versus 83.8 mg/l; $p=0.061$). A 2017 study focusing on sepsis-induced spinal cord inflammation investigated the effect of ulinastatin on the TLR4/MyD88/NF-κB pathway, an inflammatory pathway in sepsis.[7] 120 rats were used and 110 of those underwent CLP to induce sepsis. 50 in the CLP subgroup received ulinastatin intrathecally of varying concentrations. The remaining 10 control rats underwent a sham operation, a surgical procedure where there is no therapeutic intervention. The higher doses significantly reduced TLR4 expression ($p<0.05$) and MyD88 expression ($p<0.05$) compared to untreated rats, leading to greater production of anti-inflammatory cytokines.[4]

3. Reducing organ and tissue injury

A 2019 study used 50 mice divided into five equal groups: sham, ulinastatin alone, CLP alone, and two groups received different strengths of intraperitoneal ulinastatin following CLP.5 Ulinastatin was found to significantly increase survival time (43.5 hours versus 35.2 hours; $p<0.05$) compared to untreated mice with sepsis.[5] Another main finding was that ulinastatin alleviates liver injury as in treated mice, there was significantly reduced "increase in serum levels of ALT (alanine aminotransferase), ALP (alkaline phosphatase) and LDH (lactate dehydrogenase)" ($p<0.05$) compared to CLP-only mice.[5] Histological examination reinforces this point as treated mice have a significantly lower area of liver tissue degeneration ($p<0.01$). Another study used 60 rats to model sepsis using CLP.[19] Microscopy showed that septic intestinal tissue was severely damaged, characterised by features such as "partially detached villi".

4. Ulinastatin as a combined treatment

Many studies have investigated the effectiveness of ulinastatin administration in combination with other treatments. A recent study used 60 rats, split into four equal groups.[9] All received CLP, followed by either saline, ulinastatin, glutamine or ulinastatin and glutamine combined. Sepsis symptoms were improved in all treatment groups, particularly with the combined treatment, where symptoms were mildest. Inflammation was also significantly reduced, with procalcitonin (PCT), IL-6, TNF-α and IL-1β levels decreasing the most upon administration of the combined treatment. A human study investigated a similar concept, this time with rhubarb instead of glutamine.[10] Similar methods were used, with 75 patients split into four groups; control (receiving "basic treatment"), ulinastatin, rhubarb, and ulinastatin and rhubarb combined. The level of inflammation was measured using three indicators, CRP, PCT and white blood cells (WBC) and all were significantly decreased ($p<0.05$; **Figure 1**) in treatment groups compared to the control group. However, there was no significant difference between the combined group and the other treatment groups. The same pattern was seen when comparing APACHE II score, tissue perfusion and liver/kidney function.

5. Comparing methods of administration

Some studies compare the effectiveness of different ulinastatin administration methods. The study by Yang et al in 2017.[19] involved the administration of ulinastatin either intraintestinally or intraperitoneally and the two methods were compared. It was shown that intraintestinal treatment resulted in a higher average survival rate than intraperitoneal treatment but these results were not significant.[19] A similar study divided 90 rats into five groups: sham (n=10), sepsis without ulinastatin (n=20), intraintestinal ulinastatin (n=20), intravenous ulinastatin (n=20) and two-route intraintestinal and intravenous ulinastatin (n=20).[11] The 5-day survival was noticeably higher in the two-route group, but again not significantly compared to one-route treatments ($p>0.05$).

Discussion

1. Reducing Mortality

Karnad et al provides evidence which suggests that the administration of ulinastatin in sepsis may improve patient outcomes, but does not explore the mechanism behind this,[1] unlike more recent studies such as Xu et al.[6] Karnad et al was a randomised, double-blind trial which reduces bias.[1] However, since it is a human trial, it is difficult to standardise the environment as participants received ulinastatin in addition to standard, location-dependent care. The research by Xu et al was conducted retrospectively which presents limitations as the dosage of ulinastatin and patient population being inconsistent.[6] An earlier study using mice came to similar conclusions.[2] This was a randomised study with standardised conditions which allows for reduced bias, giving us a more accurate view of the treatment effect. We can therefore infer that ulinastatin administration may contribute to reducing inflammation. This explains the improved survival rate as organ failure can be a result of the inflammatory response[20] in one model of sepsis.[15]

2. Reducing inflammation

Xu et al suggests that ulinastatin is not effective at reducing inflammation caused by CRP.[6] This demonstrates that there are limitations to ulinastatin's anti-inflammatory potential. However, Xie et al demonstrated with statistical significance that ulinastatin is effective in reducing TLR4 and MyD88 expression, suggesting that the TLR4/MyD88/NF-κB pathway may be attenuated by ulinastatin.[7] The study was random and used an adequate sample size calculated using a desired power of 0.8 which ensures the results are useful and accurate. This suggests that UTI can reduce sepsis-induced inflammation but further research into cost effectiveness is required to assess whether the impact and effectiveness of this makes it a worthwhile treatment option.

3. Reducing organ and tissue injury

Ulinastatin administration has a prominent role in reducing sepsis-

induced organ and tissue injury in murine models.[5,21] Song et al suggests with statistical significance that ulinastatin can reduce organ and tissue injury caused by sepsis.[5] The study utilised randomisation to assign mice to each group to avoid bias, which is crucial due to the smaller sample size. All mice were accustomed to a standard condition before the study which makes the of the study location less relevant and therefore the research is more applicable to a global population as location related bias is reduced. Yang et al uses microscopy to show that intestinal tissue damage was attenuated upon administration of ulinastatin.[19] Both studies demonstrate the effectiveness of ulinastatin in reducing organ injury in sepsis patients. They also share many aspects of their experimental design, including the use of murine models and using randomisation to assign treatment groups which makes the studies both reliable and comparable.[5,19]

4. Ulinastatin as a combined treatment

Wang et al demonstrates that ulinastatin has a greater therapeutic potential when used as a combined treatment with glutamine, as opposed to using ultinastatin alone.[9] However, Meng et al suggests that combining ulinastatin with rhubarb has no significantly better outcomes than using ulinastatin alone.[10] It does, however, reiterate that ulinastatin is effective at improving sepsis patient outcomes. Both studies use animal models which while useful, cannot be assumed to be comparable to a human popultation. The two studies together show that there is potential for ulinastatin effectiveness to increase when combined with another drug but more research is needed to determine which combination works best.

5. Comparing methods of administration

Liao et al shows that intestinal injury was reduced (as villus height and villus height to crypt depth ratio was the highest compared to the other treatment options) in the two-route treatment group, where ulinastatin is administered both intraintestinally and intravenously[11]. However, the 5-day survival was not significantly different when compared to intravenous administration alone. Additionally, Yang et al failed to prove any difference in outcomes when comparing intravenous and intraintestinal administration.[19] The statistical insignificance in both studies may be due to the small sample size and lack of power. If this is the case, it may provide an avenue for further research which will enable clinicians to optimise treatment based on cost, ease of use and drug efficacy whilst minimising risk of complications.

Conclusion

To conclude, most research shows statistical significance of increased survival rates of sepsis when ulinastatin is administered. The available research suggests that ulinastatin is a useful, safe, and effective therapeutic agent for sepsis and provides us with an insight into the direction of treatment options available to patients in the future. This research provides an avenue for the development of a standardised treatment to mitigate the devastating effects that sepsis can have on the human body. Although its mechanism is still not fully understood, we know ulinastatin has anti-inflammatory properties, evidenced by its reduction of inflammatory makers including CRP and TNF-α. The reduction in inflammation explains why ulinastatin significantly reduces organ injury and therefore organ failure, providing an explanation for the increased survival rate. Ulinastatin has been trailed as a combined treatment with glutamine, rhubarb, and other remedies. A variety of administration methods have also been tested. Although the results from both elements were inconclusive due to lack of statistical significance, it is possible that with further research, the optimal treatment method using ulinastatin can be determined. In further research, it will be crucial to determine many factors such as the superior administration method, timing of treatment in the disease course and safety across a variety of age groups for the best patient outcomes. This will allow for optimisation of the treatment, to ensure it remains cost effective and clinically effective.

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Hi! My name is Lucy and I'm a third-year medical student at Cardiff University. My current interests within medicine are paediatrics, dermatology and cardiology. I am also interested in research and am excited to explore this interest further. I also love dancing, spending time with family and friends and exploring new places!

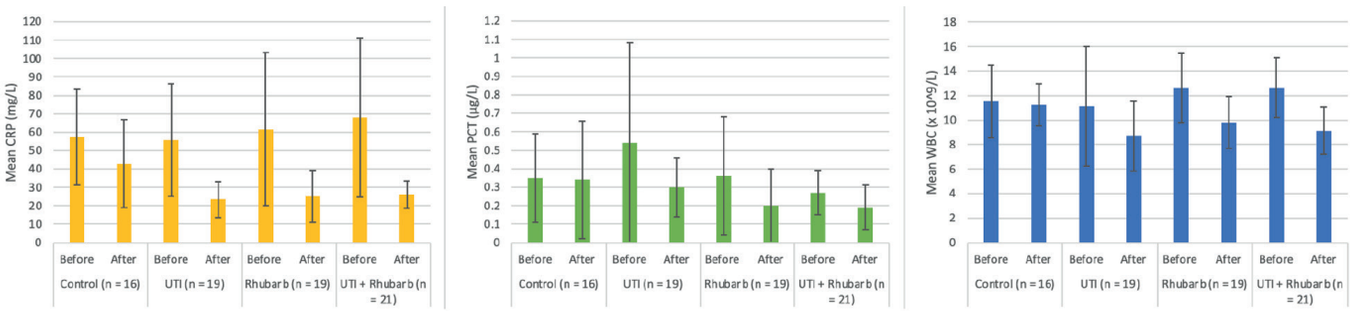

Figure 1 | Levels of inflammatory indicators before and after different treatments. 75 patients with sepsis admitted to hospital over a 19-month period participated in this single-centre, prospective, randomised trial. They were randomly split into 4 groups; control, ulinastatin (UTI), rhubarb and ulinastatin and rhubarb combined. Each group received the basic treatment, consisting of treatment for the primary disease (including antibacterial drugs), respiratory support, electrolyte and acid-base balance correction and treatment for any existing diseases. The UTI group received basic treatment alongside 200,000 units of ulinastatin three times daily. The rhubarb group received 12g of rhubarb once daily alongside basic treatment. The UTI + rhubarb group received basic treatment, ulinastatin administration and rhubarb. The level of inflammation was measured over 5 days using 3 indicators, C-reactive protein (CRP), procalcitonin (PCT) and white blood cells (WBC). The figure illustrates the starting (before treatment) and ending (after 5 days of treatment) values of these indicators with error bars representing standard deviation.
Figure created using data from Meng et al 2020.[10]