

Heparin: the cheap alternative for immunomodulation in sepsis?

Megan S Robertson

Severe sepsis, involving a systemic inflammatory response caused by infection and acute organ dysfunction, is a common reason for intensive care unit admission. It is also one of the most common non-cardiac causes of death in ICU patients, resulting in about 150 000 deaths per annum in Europe, over 200 000 deaths per annum in the United States, and an estimated 1400 deaths daily worldwide.¹ Furthermore, the incidence of sepsis resulting in hospital admission is reportedly increasing, at a rate of up to 9% per year in the US.²

Recent Australian studies have documented the annual incidence of severe sepsis in adults treated in Australian and New Zealand ICUs at about 0.70 per 1000 population.^{3,4} The binational study showed that 11.8% of all ICU admissions involved severe sepsis, either on admission or during the ICU stay, and that 32.4% of patients with severe sepsis died within 28 days of diagnosis, while 37.5% died during their hospital stay. Thus, it is estimated that severe sepsis is responsible for over 5000 deaths in hospital annually across Australia and New Zealand.³

Management of severe sepsis

The mainstay of treatment for severe sepsis in Australia is aggressive antibiotic therapy combined with supportive care for associated organ dysfunction. Over the past two decades, many studies have examined the ability of agents that modulate the inflammatory response associated with severe sepsis to reduce the incidence of organ dysfunction, but none have proved successful until the PROWESS (Recombinant human protein C Worldwide Evaluation in Severe Sepsis) study.⁵

In March 2001, Bernard et al, for the PROWESS study group, published their landmark study demonstrating the efficacy of recombinant human activated protein C (rh-APC) in reducing mortality in patients with severe sepsis.⁵ In a randomised, blinded, placebo-controlled, multicentre international study that included 1690 patients with severe sepsis, the investigators showed a 6.1% absolute reduction in 28-day mortality with rh-APC (mortality, 24.7% [rh-APC] versus 30.8% [placebo]; $P=0.005$), representing a reduction in the relative risk of death of 19.4%.⁵

Activated protein C is an endogenous human protein involved in the coagulation cascade, and treatment with

ABSTRACT

Severe sepsis, involving a systemic inflammatory response caused by infection and acute organ dysfunction, is one of the most common non-cardiac causes of death in intensive care unit patients worldwide. The mainstay of treatment for severe sepsis is aggressive antibiotic therapy combined with supportive care for associated organ dysfunction. In 2001, recombinant human activated protein C was shown to improve survival in patients with severe sepsis, but two further studies of the immunomodulatory effects of endogenous anticoagulant agents did not show any survival benefit.

Importantly, all three studies showed potential confounding by patients receiving low-dose heparin during the study period, raising the possibility that heparin may also have important immunomodulatory actions. This has increased the focus on the non-anticoagulatory actions of heparin, and its potential therapeutic immunomodulatory effects during severe sepsis. This review summarises the known clinical effects of heparin and explores the significant implications of its widespread therapeutic use.

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rh-APC is associated with systemic anticoagulation.⁶ Improved understanding of human inflammatory and coagulation processes has shown significant overlap and interaction between the inflammatory process and the coagulation cascade,^{7,8} and rh-APC is also postulated to have anti-inflammatory effects at multiple sites.^{5,6}

The PROWESS study was the first to demonstrate a survival benefit in ICU patients with severe sepsis treated with an agent that aimed to modulate the host inflammatory response to sepsis. Two more studies were published soon after which investigated the potential benefits of endogenous anticoagulant agents in severe sepsis. The KyberSept trial enrolled 2314 patients with severe sepsis in a blinded, placebo-controlled, randomised study of antithrombin III, and showed no difference in mortality at 28 days.⁹ Similarly, OPTIMIST (Optimized Phase 3 Tifacogin In Multicenter International Sepsis Trial) included 1754 patients with severe sepsis in a blinded, placebo-controlled, randomised study of recombinant

tissue factor pathway inhibitor, and showed no mortality difference at 28 days.¹⁰

All three studies excluded patients receiving full-dose anticoagulation with either unfractionated heparin (UFH) or coumarin agents, but allowed use of low-dose UFH prophylaxis against deep vein thrombosis or use of UFH in line flushes. Importantly, all three studies reported an interaction between the study drug and low-dose UFH administration during the 96-hour study dosing period, stating that each study drug was less effective in reducing mortality from severe sepsis when low-dose UFH was administered concomitantly.^{5,9-11} Data review shows that administration of low-dose UFH was associated with a lower mortality rate than no low-dose UFH, in the placebo group, but that the addition of UFH to the study drug did not reduce mortality in the treatment groups (Table).

While the confounding effect of low-dose UFH treatment in these studies suggests that this treatment may be beneficial in reducing mortality associated with severe sepsis, it is difficult to interpret post-randomisation events in clinical studies.¹² It is important to acknowledge that patients were not randomised to receive UFH or no UFH, and it is not known whether baseline characteristics were comparable between the groups.¹² There are currently no prospective randomised data to support the finding, but given the potential benefit that would be gained if low-dose UFH was shown to reduce mortality in severe sepsis — an estimated 300 lives per annum in Australia and over 14 000 lives internationally — a randomised blinded study is urgently required to provide definitive evidence to support or refute these findings.

Heparin in severe sepsis

The findings on UFH use in patients with severe sepsis generated much discussion in the medical literature.¹³⁻¹⁶ Although there are currently no prospective randomised data to support the findings, many studies, both in animal models and humans, have suggested that UFH has significant immunomodulatory effects, in addition to its well established anticoagulant effects.

Heparin as an anticoagulant

Heparin is an endogenous glycosaminoglycan which is found exclusively in mast cell granules and appears in the circulation as a free carbohydrate after degranulation. Endogenous biosynthesis of heparin results in heterogeneously sized molecules with a molecular weight of 3000–30000 Da, similar to those found in exogenous UFH preparations.

These variable-sized UFH molecules exhibit broad biological activity and bind to a wide spectrum of proteins, including cytokines, growth factors, extracellular matrix proteins and leukocyte proteases.¹⁷ Interestingly, only a third of molecules bind to antithrombin, and this binding occurs through a specific pentasaccharide sequence. It is likely that many other protein–UFH interactions also occur via specific carbohydrate moieties not yet described, but some may be via non-specific highly acidic negatively charged sulfated regions.

The anticoagulatory effects of UFH are well described:¹⁸

- Heparin binds to antithrombin via a unique pentasaccharide sequence, resulting in a conformational change in antithrombin which accelerates about 1000-fold the ability of antithrombin to inactivate coagulation factors IXa and Xa.
- Heparin and antithrombin inactivate thrombin by forming a ternary complex, which requires that heparin bind simultaneously to thrombin and antithrombin. This action is only possible with heparin fractions composed of at least 18 saccharide units, and therefore can occur with UFH but not with low-molecular weight heparin (LMWH).¹⁹
- Heparin binds to heparin cofactor II and catalyses thrombin inactivation. This action occurs only with heparin fractions with at least 24 saccharide units, and does not occur with LMWH.
- Inactivation of thrombin prevents fibrin formation and also thrombin-induced activation of factors V and VIII.
- Heparin promotes the release of tissue factor pathway inhibitor from vascular endothelial sites, thus reducing the procoagulant activity of tissue factor–VIIa complex. This action is more marked with UFH than with LMWH.²⁰

Mortality of patients with severe sepsis treated with an immunomodulatory drug or placebo, by concomitant therapy with low-dose unfractionated heparin (UFH)^{5,9-11}

Study	Mortality in placebo group				Mortality in study-drug group			
	With UFH		No UFH		With UFH		No UFH	
PROWESS	179/637	28.0%	80/203	39.0%	158/634	25.0%	52/216	24.0%
KyberSept	na	36.6%	na	43.6%	na	39.4%	na	37.8%
OPTIMIST	179/600	29.8%	117/274	42.7%	204/600	34.0%	97/280	34.6%

na = not available.



- Heparin increases both the rate and affinity of the reaction between tissue factor pathway inhibitor and factor Xa, which rapidly inactivates tissue factor–factor VIIa.²⁰

Heparin as an immunomodulator

Heparin also has many immunomodulatory actions that may affect the systemic response to sepsis, thus suggesting its potential to be therapeutically useful in this setting.

- Heparin binds to L- and P-selectin, which have important roles in leukocyte recruitment in inflammation. P-selectin is expressed on activated endothelium, and L-selectin on leukocytes; both are involved in leukocyte attachment. Heparin has been shown to decrease neutrophil influx in a murine model of peritonitis.²¹
- Heparin has been reported to attenuate the rise in serum vascular cell adhesion molecule-1 (s-VCAM-1), when given in high doses (1000 U/h) and early in the course of acute ischaemic stroke.²² VCAM-1 is an adhesion molecule which is expressed by activated endothelium and mediates adhesion of monocytes and lymphocytes, but not neutrophils. Reduced levels of s-VCAM-1 were also associated with better clinical outcome in these patients.²²
- Heparin enhances lipopolysaccharide-stimulated release of tissue necrosis factor- α and interleukin-8 from human monocytes *in vitro*.^{23,24} Animal models of sepsis with endotoxaemia have suggested that UFH may improve clinical outcome.^{25,26}
- Heparin inhibits the respiratory burst of neutrophils. UFH also binds to superoxide dismutase and can therefore neutralise superoxide radicals and other similar molecules.¹⁷
- Studies examining the effect of heparin on platelet activation have yielded conflicting results. Platelet activation by thrombin is mediated via platelet receptor glycoprotein 1b, which binds with high affinity to thrombin via its heparin binding site. Thus heparin can interfere with thrombin–glycoprotein 1b interaction and inhibit thrombin-induced platelet activation.¹⁹ Interestingly, recent studies in patients with unstable angina pectoris and non-ST elevated myocardial infarction suggest that LMWH is more effective in inhibiting platelet activation than UFH.²⁷
- Heparin-binding proteins include a wide array of biologically active proteins, such as chemokines, growth factors, adhesion molecules, cytotoxic peptides, and tissue destructive enzymes. Heparin is proposed to limit inflammation by binding to, and modulating the activity of, these proteins, thus limiting cellular activation.^{17,19}
- Heparin reduces eosinophil recruitment after nasal allergen challenge in patients with atopic rhinitis.²⁸ Heparin has also been reported to attenuate antigen-induced skin and bronchial responses^{29,30} and to prevent exercise-induced asthma when inhaled before exercise.^{31,32}
- Heparin limits mast cell degranulation and inhibits mast cell-mediated reactions.³³
- Heparin demonstrates antiproliferative effects and limits angiogenesis and neointimal hyperplasia, possibly by reducing monocyte infiltration into areas of vascular injury.³⁴
- Heparin is used extensively in affinity chromatography techniques because of its high binding affinity for growth factors, proinflammatory chemokines, extracellular matrix proteins and leukocyte proteases.^{17,19}

The increasing understanding of the immunomodulatory effects of heparin has led to studies of their clinical implications. Currently, the beneficial immunoactive actions of heparin have been investigated in the treatment of inflammatory bowel disease,³⁵ asthma,³¹ acute ischaemic stroke²² and acute myocardial infarction.²⁷ Heparin has also been shown to improve survival and reduce metastases in patients with cancer treated with heparin for prophylaxis against venous thromboembolism.³⁶

Conclusions

Over the past decade, there has been renewed interest in the non-anticoagulatory effects of heparin and its ability to modulate cellular activity and inflammation. Based on this interest and data from the three studies of endogenous anticoagulants in sepsis, it is clearly important to study the potential clinical effect of heparin therapy in severe sepsis.

In addition to the potential to reduce mortality from sepsis, the use of heparin rather than rh-APC in sepsis has international humanitarian significance. Currently, a standard 96-hour infusion of rh-APC in a 70 kg man costs about A\$13 858, while UFH 5000 U 8-hourly for 96 hours costs about A\$8.76. Thus, if UFH was found to have equal effectiveness to rh-APC in reducing mortality in sepsis, it would result in substantial cost savings and be a novel treatment with potentially wide application in less developed nations.

Author details

Megan S Robertson, Senior Specialist
Intensive Care Unit, Royal Melbourne Hospital, Melbourne, VIC.
Correspondence: megan.robertson@mh.org.au

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