

Review Article

The role of heparin and allied compounds in the treatment of sepsis

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Summary

The crosstalk between coagulation and inflammation and the propensity for microthromboembolic disease during sepsis calls for anticoagulant measures to prevent tissue hypoxxygenation and to attenuate organ damage and dysfunction. Only one anti-coagulant, recombinant human activated protein C (aPC, drotrecogin- α) has a proven survival benefit when used as an adjunctive therapy for human sepsis, partly because of its anti-inflammatory effect. However, heparin (-like compounds) may exert similar beneficial anti-inflammatory actions as aPC, in spite of the relatively narrow therapeutic window for anticoagulation.

Keywords

Heparin, sepsis, disseminated intravascular coagulation, cytokines, host defence, aPC, survival, inflammation

This narrative review is based on a Medline search of relevant basic and clinical studies published in English and discusses the potential role of heparin in modulating inflammatory responses in the treatment of animal models and human sepsis and its harmful sequelae. In any case, the results of a metaanalysis based on animal data suggest a potentially life-saving effect of heparin (-like compounds) in the treatment of sepsis. Therefore, a prospective randomized clinical trial is called upon to study effects in human sepsis.

Thromb Haemost 2007; 98: 579–586

Introduction

Sepsis, the host response to severe microbial infection, is an important health problem, being the leading cause of death in the intensive care unit. The inflammatory response to infection and tissue injury is characterized by a local and systemic release of pro- and anti-inflammatory factors and changes in the microcirculation, impairing vascular barrier function resulting in extravasation of plasma proteins and leukocytes. An essential early step in the inflammatory reaction is the migration of neutrophils to the site of infection followed by adherence to the vascular endothelium in order to permeate the vessel wall and penetrate the tissues. In this neutrophil-endothelium interface, activation of coagulation may play an important role.

Activation of an inflammatory response during infection and sepsis may result in a pro-coagulant state (1–4). Lipopolysaccharide (LPS) or endotoxin decreases anticoagulant heparan sulphate and other glycosaminoglycans (GAGs) in endothelium and increases tissue factor (TF) production and release by macrophages (4, 5). The resultant activation of coagulation, independently followed by inhibition of fibrinolysis, may result in disseminated intravascular coagulation (DIC), microvascular obstruction, and thereby organ dysfunction and damage, which is

characteristic for sepsis. The latter is accompanied by consumption and depletion of natural anticoagulants such as antithrombin (AT), protein C (PC) and tissue factor pathway inhibitor (TFPI), and release of plasminogen activator inhibitor (PAI-1) inhibiting fibrinolysis (1, 4, 6–9). Hence, repletion of anticoagulants could reverse the imbalance of coagulation to fibrinolysis in sepsis (4). Conversely, activation of coagulation has pro-inflammatory effects via the TF/factor VII complex, thrombin and fibrin, resulting in release of cytokines by leukocytes and endothelium, among others, while factor X activation may have less pro-inflammatory effects (4, 10–13). Clearly, the crosstalk between coagulation and inflammation opens new ways for treatment of sepsis (4, 12).

Recently, three major, phase III trials on the three naturally occurring anticoagulants, i.e. activated protein C (aPC) or drotrecogin- α (recombinant human aPC) (PROWESS-trial) (14), antithrombin (KyberSept-trial) (15–17) and tissue factor pathway inhibitor (OPTIMIST-trial) (18) have been performed. The PROWESS-trial was the only trial so far to demonstrate a statistically significant reduction of the (absolute) mortality rate by 6.1%. However, in all three trials, low-dose prophylactic heparin has been used as co-treatment, and study results suggested that the drug intervention was most effective and safe, with less

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Received January 4, 2007
Accepted May 17, 2007

Prepublished online August 18, 2007
doi:10.1160/TH07-01-0006

bleeding complications, in the group not receiving heparin (14, 15, 17–19).

Therefore, we reviewed the effects of heparin (-like compounds) beyond coagulation, during sepsis and shock. For this narrative review, English papers have been included, as found in a Medline search. We will address the properties of heparin as a chemical compound, anticoagulant and anti-inflammatory drugs, both *in vitro* as well as in animal experiments and human studies, during sepsis and shock, to enforce the idea that a randomized trial of low/prophylactic or even therapeutic doses of heparin is needed, in the treatment of sepsis and shock in man.

Heparin as an anti-coagulant and immune-modulating agent

Heparin is a naturally occurring GAG, produced by mast cells in intestines or lung, basophils in blood, and endothelial cells (20–22). Heparin consists of alternating chains of uronic acid and glucosamine, sulphated to a varying degree. The pharmaceutical drug is extracted from porcine or bovine mucosa; unfractionated heparin weighing 13,000 – 15,000 Dalton (Da) and fractionated or low-molecular-weight heparin 3,000 – 5,000 Da (23, 24). Heparin exerts anti-coagulant properties by binding to AT and heparin cofactor II (21, 24). The bond to AT, which requires a unique pentasaccharide sequence, results in a conformational change, potentiating the ability of AT to inactivate the coagulation factors IXa, Xa, XIa and XIIa (25). Following inactivation of factor Xa, prothrombin cannot be converted to thrombin, thereby inhibiting conversion of fibrinogen to fibrin and clotting of blood. Moreover, heparin directly inhibits throm-

bin, but since this requires a minimum chain length of 18 saccharides to simultaneously bind thrombin and AT, it is exclusive to unfractionated heparin. Fractionated heparins and pentasaccharides exert more specific anti-factor X activity than unfractionated heparin (21, 26). Inhibition of thrombin-induced feedback activation of coagulation factors V and VIII contributes to the anticoagulant activity of heparin (27). Endothelial release of TFPI and reduced expression of TF and PAI-1 may also play a role (28). Heparin reverses the pro-coagulant properties of stimulated endothelial cells (29).

The anticoagulant properties of heparin (-like compounds) during endotoxaemia and sepsis may even surpass drotrecogin- α 's (7–9, 13, 30, 31). Heparin as treatment for activated coagulation during sepsis, however, might impair interaction of AT with GAGs in endothelium (17, 32–35), and might deplete the endothelium and blood of TFPI in spite of increased synthesis (28, 35). These phenomena may partly explain the lack of survival benefit of AT/TFPI in the heparin-cotreated patients in large multicenter trials (15, 17, 18). Thrombin pre-treatment of LPS-induced shock in animals protected against coagulation activation and mortality in animals, potentially because of enhancement of aPC, since thrombin binds to thrombomodulin thereby activating protein C (36). Heparin (-like compounds) synergize with the favourable effects of aPC on neutrophil-endothelial interactions (37), but may antagonize aPC by promoting the aPC inhibitor (38) or augment aPC in anti-coagulation by activating aPC-induced inhibition of factor V, thus aggravating the risk of bleeding (39). This may explain the seemingly less favourable effects of drotrecogin- α in heparin-cotreated sepsis patients in the PROWESS trial (14).

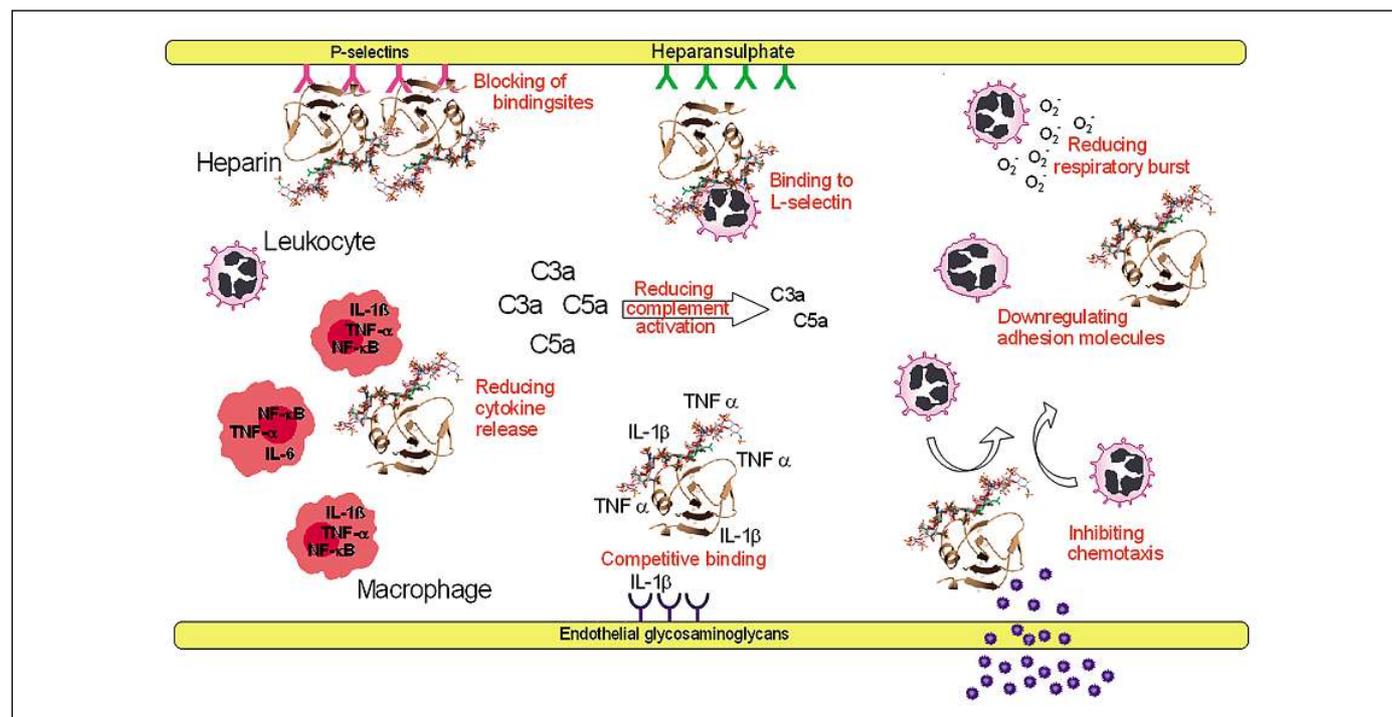


Figure 1: Schematic presentation of potential mechanisms of endogenous heparin sulphate and exogenous heparin (-like compounds) in the blood and interaction with neutrophils and endothelium.

Anti-inflammatory actions

Heparin may play a role in inhibiting the production and release of pro-inflammatory factors (Fig. 1) (20). Thrombin has pro-inflammatory properties and increases endothelial permeability and endothelin production (11). AT and aPC inhibit nuclear factor (NF)κB, a transcription factor responsible for gene expression of pro-inflammatory factors in monocytes and endothelium (33–35, 40). Heparin might thus modulate anti-inflammatory effects of AT or aPC (35, 40). The anti-inflammatory action of heparin may relate to inhibition of thrombin formation, even though chemically modified heparin-like compounds without anticoagulant action may have preserved anti-inflammatory properties (11, 24, 41–44). Both (un)fractionated and modified, non-anticoagulant heparin inhibit the activation of NFκB, the pro-inflammatory responses after ischaemia/reperfusion, and attenuate endothelial dysfunction by enhancing nitric oxide (NO) and prostacyclin (44–47). Similarly, (non-anticoagulant) heparin improves endothelial function and vascular reactivity during hyperdynamic sepsis (41). Although not demonstrable in whole blood or during human endotoxaemia, (un)fractionated heparin (sulphate) inhibited gene expression, production and release of pro-inflammatory cytokines by LPS-stimulated monocytes and other cells, mediated by thrombin and NFκB activation *in vitro* (9, 11, 48–52).

In vascular adherence, migration and activation of leukocytes, heparin sulphate may play a modulating role. Heparin inhibits the chemotaxis of normal human neutrophils (53, 54). The effects seemed to relate primarily to inhibition of C5a, a positively charged peptide that may be neutralized by the negatively charged heparin. *In vivo* and *in vitro*, (non-anticoagulant) heparin derivatives prevented in part complement activation and subsequent neutrophil activation (55, 56). Furthermore, heparin is the most potent enhancer of all naturally occurring GAGs, of the inhibitory capacity of C1-inhibitor (57, 58). Heparan sulphate may bind and present cytokines/chemokines, enhancing IL-8-induced chemotaxis (59). By competing with heparin sulphate in binding these cytokines/chemokines, intravascular heparin could partly antagonize these events at the neutrophil-endothelial interface (46, 59–61). Neutrophil-derived heparin binding protein may interact with endothelial GAGs, altering the cytoskeleton and increasing permeability and neutrophil transmigration (59, 62). GAGs and heparins promote endothelial barrier function (63). Heparan sulphate in the endothelium also synergizes with adhesion molecules in neutrophil adherence, chemotaxis and endothelial interactions (60, 61, 64, 65). Conversely, heparin in the blood is able to bind to leukocytes by L-selectin, and to endothelium by glycosylation-dependent cell ad-

Table 1: Animal models of sepsis and shock and evaluation of effects of treatment with heparin or allied compounds.

Species	Model and design	Effect parameter	Effect of heparin or allied compound	Reference	Year
Rat	Intravenous LPS injection. Heparin administration at different intervals after injection.	24 hour mortality	Significant mortality reduction when heparin was administered up to 2 hours after injection	Filkins et al.	1968
Rabbit	Intraperitoneal injection with <i>Pasteurella multocida</i> . One group receiving heparin (1,000 IU/4 h), other group receiving saline.	24 hour mortality	No difference in mortality	Corrigan et al.	1975
Rabbit	Intraperitoneal inoculation with type B meningococci in mucin	Mortality Survival time Schwartzman phenomenon	No improvement of mortality or survival time Amelioration of Schwartzman phenomenon	Gaskins et al.	1976
Guinea pig	IV infusion of live <i>E.coli</i> O111:B4. One group pre-medicated with heparin (n=21), controls received saline 0.9% (n=18)	48 hour survival	Heparin 94% (20/21) Saline 38.8% (7/18) p<0.005	Dunn et al.	1983
Pig	Fecal <i>E. coli</i> peritonitis. One group (n=5) receiving continuous heparin infusion, control group (n=5) receiving lactated Ringer's solution.	Mean survival time	Heparin: 18.8 ± 2.2 hr Ringer's: 11.9 ± 2.8 hr (p<0.05)	Griffin et al.	1990
Rat	Abdominal sepsis by puncturing intestinal wall. One group (n=50) receiving sc heparin 1 day before surgery until 5 th day after. Control (n=50) sc distilled water. **	Early death (<24 h) Death (< 7 days) Average survival (h)	<i>Heparin vs. Control</i> 6% vs. 28% (p=0.0078) 70% vs. 82% (p=0.096 (n.s.)) * 103.2 hours vs. 68.8 hours (p<0.05)	Sun et al.	1997
Sheep	Continuous infusion with <i>E. coli</i> endotoxin (10 ng/kg/min) for 72 hours. One group receiving heparin (40 IE/ kg/ hr)(n=7). Second receiving hirudin (500 units/ kg/h)(n=7). Third receiving saline (n=8).	72 hour mortality Cardiac output Oxygenation Hyaline membranes	<i>Heparin vs. Control</i> 43% vs. 100% No decrease in heparin group No decrease in heparin group No formation in heparin group	Schiffer et al.	2002
Mouse	Two consecutive injections with <i>S. Marcerens</i> . One group (n=14) receiving 2 x 5 IU of unfractionated heparin (iv and sc). Controls (n=12) receiving saline.	30 hour mortality Mult. organ failure Cytokine levels	<i>Heparin vs Control</i> 50% vs. 75% (p=0.06) Sign. decrease heparin group No differences	Slofstra et al.	2005

*n.s. = not significant; *data derived from figures and graphs in the original article; **sc = subcutaneous.

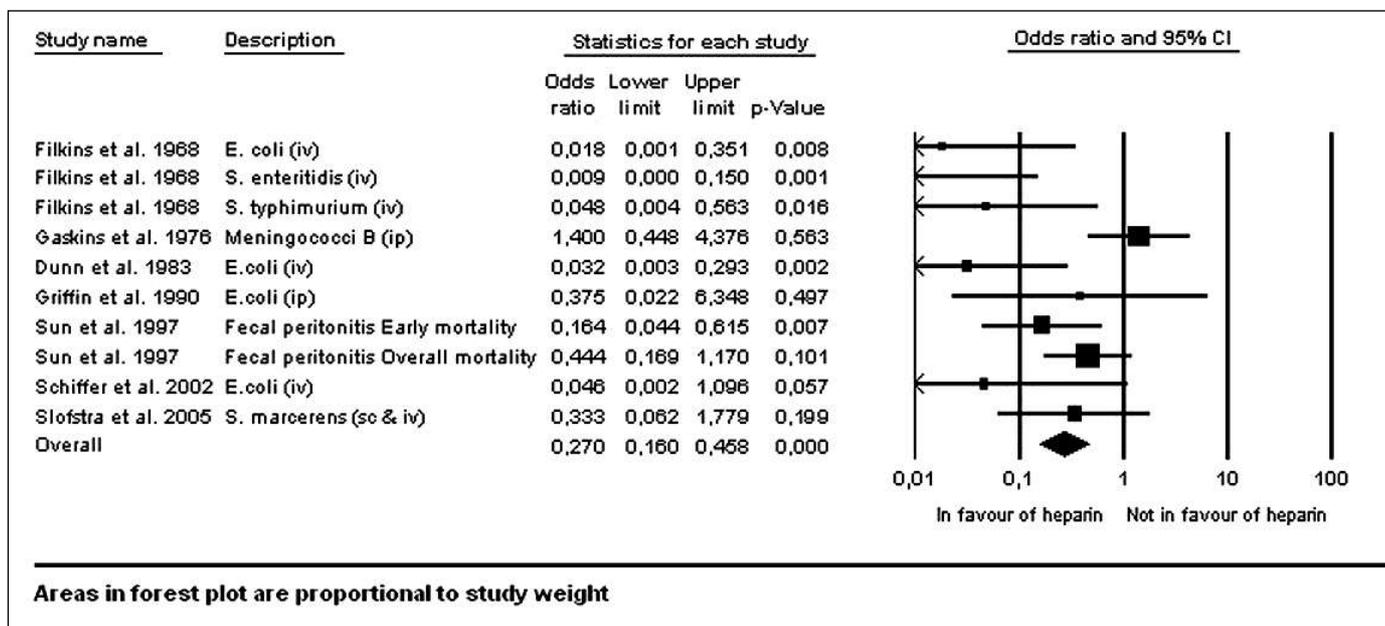


Figure 2: Results of a meta-analysis (Comprehensive Meta-Analysis version 2, www.Meta-Analysis.com, NIH USA) of all animal sepsis studies reporting survival data upon treatment with heparin. It is shown that treatment had a significant survival benefit.

hesion molecule-1 (GlyCAM-1) and P-selectin (9, 64–67). Heparin may downregulate endothelial intercellular adhesion molecule-1 (ICAM-1) and L-selectin expression more than that of E-selectin, even in a human endotoxaemia model (9, 43, 68–70). Heparin binds to the leukocyte integrin Mac-1 [CD11b/CD18], a versatile adhesion molecule interacting with ICAM-1 in promoting leukocyte adhesion to endothelial cells (71). Additional beneficial effects of heparin (-like compounds) are a decrease of neutrophil influx in murine peritonitis (65), decreased superoxide anion generation during a respiratory burst (72, 73), decreased neutrophil degranulation and inhibition of elastase and cathepsin G (59, 72–75), and decreased leukocyte phagocytosis (54).

The anti-inflammatory role of heparin may also apply to extracorporeal circuits. Reductions of circulating pro-inflammatory cytokines were found in patients on heparin-coated cardiopulmonary bypass (CPB), in part mediated by inhibition of complement activation (76–78); in rabbits, formation of TNF- α was completely prevented by prior heparin coating (79). Complement activation is inhibited by heparin and heparin coating of circuits during CPB, as measured by reduced levels of C5 and C3 split products and terminal complement complexes, and heparin (coated circuits) may thereby improve postoperative recovery (55, 78, 80–84). Expression of adhesion and activation molecules on neutrophils and macrophages and release of elastase were reduced when heparin-coated versus uncoated circuits were used, associated in part with reduced complement activation (80–83, 85). These effects have mostly been shown at very high doses of heparin.

Pro-inflammatory effects of heparin (-like compounds) have also been described, in part conflicting with above findings, and the discrepancy may relate to models, prior stimuli, doses and types of administration. High-dose heparin may decrease en-

dothelial NO production and promote cell adhesion and (platelet) aggregation (86). Production of TNF- α , IL-1 β and TF by isolated monocytes and whole blood, may be enhanced when (fractionated) heparin is added (49, 87, 88). Addition of heparin to whole blood challenged with LPS may result in an increased IL-8 production (89) and may promote stimulated macrophages to produce cytokines (90). GAGs may associate with extracellular superoxide dismutase (SOD) and thereby help to neutralize neutrophil-derived reactive oxygen species, and heparin in blood may displace protective SOD from endothelium (91, 92). Heparin may upregulate neutrophil CD11b and enhance IL-8 release *in vitro* (68). Other reports suggest a pro-inflammatory genetic response in leukocytes evoked by heparin coating of circuits (93). In a prospective trial on 200 patients on CPB with or without heparin-coated circuits, a rise in cytokine levels occurred in both groups, without significant differences (94).

Heparin treatment in sepsis animal models

Filkins et al. were the first to study the protective effect of heparin in LPS-induced shock; rats receiving heparin before or after injection of *Salmonella* and *E. coli* LPS had lower mortality than untreated rats (95).

In experimental fecal peritonitis, heparin ameliorated peritoneal fibrin deposition and favorably affected survival time, blood pressure, leukocyte counts and lung injury (96, 97), and improved the haematologic abnormalities compatible with DIC in a sepsis model in rats (98). In rodent LPS and sepsis models, treatment by heparin (-like compounds) was able to diminish coagulation activation, (renal) fibrin deposits, microthrombi in lung and liver and to improve hepatocellular function and survival (99–103). Inhalation of heparin attenuates acute lung injury (ALI) following smoke inhalation and bacterial pneumonia in

sheep, while intravenous heparin may be less helpful in sheep and rats although attenuating ALI in endotoxaemic pigs (104–107). In-vitro heparin may enhance surfactant protein gene expression in pneumocytes (108). In a recent, sustained and ovine LPS-induced shock model (109), unfractionated heparin, hirudin or saline was given. Sheep in the heparin group exhibited high survival rates because of less deterioration of cardiopulmonary function. Since both heparin and hirudin completely prevented the early LPS-induced decrease in plasma fibrinogen, the non-anticoagulant properties of heparin might have been responsible for the survival benefit. Furthermore, anticoagulation, for instance with heparin, may hardly enhance the clearance of LPS (100, 110, 111), so that the favorable effect of heparin in some of the above studies may not relate to an altered host defense by heparin. Non-anticoagulant heparin appeared to maintain vascular endothelial cell function during sepsis or after haemorrhage in rats (41, 42). In sheep receiving a continuous infusion of *E. coli* LPS, heparin treatment increased cardiac output and decreased pulmonary shunting and systemic vascular resistance index, as compared to placebo-treated animals (112).

In contrast, Corrigan et al. did not observe a survival benefit by heparin treatment in rabbits intraperitoneally injected with (Gram-negative) *P. multocida*, though fibrinogen consumption was diminished (113). In endotoxaemic hamsters (32), (un)fractionated heparin did not affect or attenuate the AT-mediated inhibition of leukocyte adhesion and capillary perfusion failure. In rabbit endotoxaemia, however, fractionated heparin, was only effective if combined with AT (6).

Taken together, the animal experimental literature is generally in favor of a beneficial effect of heparin (-like compounds) while modes of action may depend on sepsis models, drugs, timing, dosing and routes of administration. We performed a meta-analysis (Comprehensive Meta-analysis version 2, www.meta-analysis.com, NIH USA, last accessed April 2007) of animal sepsis survival studies mentioned above and found an overall benefit of treatment by heparin (Fig. 2). Together with some preliminary human data, the analysis could underpin a future prospective randomized clinical trial.

Heparin for adjunctive treatment of human sepsis and shock

Low-molecular-weight heparins play an important role in the prophylaxis of thromboembolic disease in the critically ill patient with sepsis and shock, which are major risk factors for thromboembolic disease, although fatal pulmonary embolism may not be prevented in patients with infections (114–116).

When comparing the heparin-treated and untreated patients in the placebo arms of the PROWESS, KyberSept and OPTIMIST trials, the former seem to have a (non-significant) survival advantage (117, 118). In the KyberSept and OPTIMIST trials, patients treated with heparin alone even had a lower mortality than those treated with the study drug or placebo (119). After pooling placebo data from all three trials, the odds ratio for death of the patients who received heparin as compared to those who did not, was 0.65 ($P < 0.00001$) (120). The number-needed-to-

treat (NNT) with heparin to prevent one death, based on that analysis, would be 10, which may be similar to or even lower than the NNT for aPC (120). However, these observations are the results of post-hoc analyses, rendering it hard to draw firm conclusions because of unintended pitfalls (19, 121). Furthermore, heparin was administered on clinical grounds so that a selection bias may have occurred.

The XPRESS study (Lilly Critical Care Europe: XIGRIS[®] summary of findings in the XPRESS trial, submitted manuscript), designed to demonstrate that in adult patients with severe sepsis receiving drotrecogin- α , concomitant treatment with subcutaneous prophylactic heparin was equivalent to treatment with placebo (determined by 28-day all-cause mortality), showed that the group concomitantly treated with heparin had a trend towards a lower mortality rate, thereby suggesting a beneficial effect of heparin itself, instead of being an unsafe combination with aPC. Furthermore heparin seemed to exert protective effects in terms of thrombotic events, suggesting synergistic actions of the agents (122). The incidence of ischemic stroke was significantly higher in the group treated with drotrecogin- α alone, and venous thromboembolic events occurred more often, although not statistically significant. The number of serious bleeding events and clinically diagnosed heparin-induced thrombocytopenia (HIT) was equal in both groups (Lilly Critical Care Europe: XIGRIS[®] summary of findings in the XPRESS trial, submitted manuscript). Uncontrolled observations also suggest a beneficial effect of heparin in combination with protein C in meningococcal sepsis (123), but one uncontrolled trial of heparin in human sepsis, suggests no survival benefit in spite of amelioration of DIC (30).

Conclusion

Apart from potent anticoagulant properties heparin also has anti-inflammatory properties. Although the effects of heparin on survival in animal endotoxaemia or sepsis seem to argue in favor of heparin administration, as demonstrated by the meta-analysis in this review, these effects have, until now, not been studied in human endotoxaemia or sepsis by means of a large randomised clinical trial. Post-hoc analyses of the three major sepsis trials and the XPRESS study suggest that low-dose heparin might have favorable effects on survival. A randomised clinical trial, the (low-dose, continuously infused, unfractionated) heparin for treatment of sepsis – HETRASE – study is underway to evaluate this concept further (124). This could be supplemented by a trial of prophylactic versus therapeutic doses of low-molecular-weight heparins, but the dose and route of administration should be carefully chosen since subcutaneously administered compounds may not be absorbed well in patients on vasopressors, and bleeding risks should not be severely increased (125). Furthermore, side effects of heparin therapy, like bleeding, HIT and allergic reactions, might limit the setup of large trials. Up until now, the treatment with aPC/drotrecogin- α remains the only anticoagulant therapy with proven efficacy in human sepsis and shock (14), even though the benefit may be mainly conferred by direct anti-inflammatory rather than by anticoagulant properties (14).

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