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The effect of heparin administration in animal models of sepsis: A prospective study in *Escherichia coli*-challenged mice and a systematic review and metaregression analysis of published studies*

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Abstract

Introduction—If thrombosis contributes to sepsis, heparin titrated using activated partial thromboplastin times may be efficacious. We investigated heparin in preclinical models.

Methods and Main Results—In unchallenged mice ($n = 107$), heparin at 100, 500, or 2500 units/kg produced activated partial thromboplastin time levels less than, within, or greater than a prespecified therapeutic range (1.5–2.5 times control), respectively. In animals ($n = 142$) administered intratracheal *Escherichia coli* challenge, compared to placebo treatment, heparin at 100, 500, or 2500 units/kg were associated with dose dependent increases in the hazard ratios of death (hazard ratio [95% confidence interval]: 1.08 [0.66, 1.76]; 1.34 [0.80, 2.24]; 3.02 [1.49, 6.10], respectively) ($p = .001$ for the dose effect). Compared to normal saline challenge, *E. coli* without heparin (i.e., with placebo) increased the activated partial thromboplastin time ($p = .002$) close to the therapeutic range. While heparin at 100 and 500 units/kg with *E. coli* further increased activated partial thrombo-plastin time ($p < .0001$ vs. placebo) within or above the therapeutic range, respectively, these did not decrease inflammatory cytokines or lung injury. In metaregression analysis of published preclinical studies, heparin improved survival with lipopolysac-charide ($n = 23$, $p < .0001$) or surgically induced infection ($n = 14$, $p < .0001$) but not monobacterial ($n = 7$, $p = .29$) challenges.

Conclusion—Coagulopathy with sepsis or other variables, such as type of infectious source, may influence the efficacy of heparin therapy for sepsis.

*See also p. 1225.

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Keywords

sepsis; infection; pneumonia; heparin; treatment; metaregression analysis; animal models

Although not consistently documented by necropsy, excessive microvascular thrombosis stimulated by infection is speculated to contribute to the pathogenesis of sepsis (1). Despite this association, of three antithrombotic agents (antithrombin, tissue factor pathway inhibitor, and recombinant human activated protein C) tested clinically, recombinant human activated protein C alone decreased mortality significantly but only in one of several studies (2). However, all three agents increased bleeding risk (3). In this regard, heparin therapy, which clinicians conventionally titrate with activated partial thromboplastin time (aPTT), may have less bleeding risk. Low-dose heparin, while not beneficial in a sepsis randomized controlled trial, did not increase bleeding since therapy was stopped for aPTT elevation (4). Also, in a retrospective study in septic patients, therapeutic heparin for concurrent conditions (e.g., myocardial ischemia) increased sepsis survival but not bleeding (5).

Further defining heparin's usefulness for sepsis appears warranted. Pneumonia commonly causes sepsis clinically (6). We therefore investigated heparin in a mouse *Escherichia coli* pneumonia model. We hypothesized that heparin doses increasing aPTT levels into a range considered therapeutic would improve outcome. Initial experiments measured the effect of three heparin doses on aPTT levels in nonchallenged animals. Subsequent experiments tested these doses in animals challenged with intratracheal *E. coli*. We also performed a metaregression analysis and examined the effect of heparin in published preclinical sepsis models.

METHODS

Animal Care

All studies were approved by the Animal Care and Use Committee of the Clinical Center of the National Institutes of Health.

Prospective Studies

See the online supplement (Supplemental Digital Content 1, <http://links.lww.com/CCM/A224>) for more detailed methods for both prospective studies and metaregression analysis.

Design

Study 1. Effect of Heparin in Nonchal-lenged Animals—Mice (C57BL/6J) were administered intraperitoneal heparin (sterile and nonpyrogenic; Abraxis Pharmaceutical Products, Schaumburg, IL) at 100 (n = 29), 500 (n = 30), or 2500 (n = 11) units/kg or diluent (placebo, n = 32). From 0.5 to 4 hrs after treatment, randomly selected animals were anesthetized with isoflurane and had blood drawn for aPTT, prothrombin time (PT), and fibrinogen measures (Fig. 1A). An untreated group was also tested (baseline animals) (n = 5).

Study 2. Effect of Heparin in *E. coli*-Challenged Animals—To study survival, animals were briefly anesthetized, challenged with intratracheal *E. coli* (15×10^9 CFU/kg), and then randomized to receive heparin at 100 (n = 42), 500 (n = 40), or 2500 (n = 18) units/kg or placebo (n = 42) (intraperitoneal, every 12 hrs [q12 hrs] for 48 hrs, and then q24 hrs for 48 hrs) (Fig. 1B). All animals received ceftriaxone (Roche Laboratories, Nutley, NJ) q24

hrs for 96 hrs and fluids (one dose of normal saline, 0.5 mL, subcutaneous) beginning 4 hrs after challenge. Animals were observed for up to 168 hrs.

For other measures, *E. coli*-challenged animals were randomized to heparin at 100 (n = 59) or 500 (n = 63) units/kg or placebo (n = 46) and supported as above. Randomly selected animals at 24 hrs and all those remaining at 48 hrs had blood collected for aPTT, PT, fibrinogen, thrombin-antithrombin complex (TATc), complete blood count, and 12 cytokines. Then, lungs were either lavaged for cell, protein, TATc, and quantitative bacterial measures or divided for histology and wet to dry weight ratio determinations. As noninfected controls, additional animals were challenged with intratracheal normal saline (NS), treated with placebo, and sampled at 24 or 48 hrs (n = 8 per time point). For survival and other measures, sequential weekly experiments were performed including up to 24 animals each randomized between groups.

Coagulation Measures

aPTT, PT, and fibrinogen levels were measured with a biphasic transmittance method (Trilogy, Drew Scientific, Dallas, TX). TATc levels were measured by enzyme-linked immunosorbent assay (Enzygnost TAT micro, Dade Behring, Newark, DE).

Other Blood and Lung Lavage

Complete blood count, plasma cytokines (interleukin-1 α [IL-1 α], IL-1 β , IL-2, IL-4, IL-6, IL-10, tumor necrosis factor alpha, gamma interferon, granulocyte macrophage colony-stimulating factor, migratory inhibitory protein-1 α , monocyte chemoattractant protein-1, and RANTES [regulated on activation, normal T-cell expressed, and secreted]) (Mouse Lincoplex Kit, Millipore, St. Charles, MO) and lung lavage cell and protein were measured (7). Individual lung histology injury parameters (interstitial capillary congestion and edema; alveolar edema, hemorrhage, and fibrin; and pneumocyte hyperplasia), an overall lung pathology score, alveolar neutrophil infiltration, and lung wet to dry weight ratios were measured or calculated (see the online supplement [Supplemental Digital Content 1, <http://links.lww.com/CCM/A224>]).

Statistics

Prospective Preclinical Studies—The effects of heparin on aPTT and PT levels were analyzed with a two-group *t* test with unequal variance (8). Survival (hazard ratio of death) was analyzed with a Cox proportional-hazard model accounting for heparin treatment and dose category in each experiment (9). Actual dose was used in a separate Cox proportional-hazard model to assess the dose-response relationship. All other data were analyzed with a linear mixed model (10). Data were transformed (log10) as indicated to meet the model of assumption of normality and constant variance. A total pathology score was calculated by summing the scores (from 0 to 4) for the six individual measures. For survival and aPTT levels (primary end points), a *p* value of .05 was considered significant. For all other parameters, a *p* value of .01 was considered significant to informally control for multiple comparisons. Statistical analyses were conducted using SAS version 9.1. Animal numbers were based on power analysis and prior laboratory experience (see the online supplement [Supplemental Digital Content 1, <http://links.lww.com/CCM/A224>]).

Metaregression Analysis of Published Pre-clinical Studies—An English-language search using MEDLINE and Embase was conducted of animal studies published from 1960 to the present with the following terms: sepsis, septic shock, heparin, anticoagulant, antithrombotic, and treatment. Included studies had to compare the effects of heparin treatment to placebo on mortality in sepsis models employing either a bacterial or bacterial toxin challenge in the absence of other anti-inflammatory agents (e.g., anticytokine or

antiendotoxin). Other abstracted data included species, route (intravenous, intraperitoneal, or other), and type (monobacterial, lipopolysaccharide [LPS], or surgically induced infection with intestinal perforation or ischemia [termed surgical poly-microbial infection]) of septic challenge; route, dose, and timing of heparin treatment; presence or absence of concurrent antibiotic or fluid support; and duration of observation. Two or more experiments from the same paper were treated as separate studies.

The effect of heparin treatment on the odds ratio of death was analyzed for each study. Heterogeneity among studies was assessed using the Q statistic and I^2 value (11). A random-effects metaregression analysis investigated the influence of recorded variables on the effects of heparin across studies (12). Due to significantly different treatment effects across challenge type (see Results), we conducted random-effects meta-analysis to estimate the overall impact of heparin treatment for each challenge type (13). Heterogeneity among species within each challenge type was assessed using random-effects metaregression. Based on the mouse pneumonia study, the effect of heparin dose was assessed using a weighted Spearman test. Dose potentially influenced heparin's effect in published monobacterial challenge studies only (see below). Sensitivity analysis determined whether individual studies contributed disproportionately to significant results. Potential publication bias and its influence on observed heparin effects were also evaluated (14, 15). Summary odds ratios are reported when heterogeneity was low (I^2 30% or less) or represented quantitative differences in otherwise similarly directed effects among studies. Meta-analyses and multivariate random-effects metaregression were done using Comprehensive Meta-analysis version 2 (<http://www.meta-analysis.com/>) and SAS Proc Mixed, respectively (12).

RESULTS

Prospective Preclinical Studies

Study 1: Effect of Heparin in Nonchallenged Animals—Comparing untreated animals (baseline) and those receiving placebo, aPTT (Fig. 2A), PT, and fibrinogen levels (data not shown) did not differ significantly. Compared to placebo, while heparin at 100 units/kg did not, 500 units/kg did increase aPTT levels similarly at all time points in an overall pattern that was significant ($p = .001$ averaged over time). Heparin at 2500 units/kg produced aPTT levels above the upper detection limit throughout (Fig. 2). Compared to placebo, heparin at 2500 units/kg, but not at lower doses, increased PT levels ($p = .0002$ averaged over time, data not shown). Finally, no heparin dose altered fibrinogen levels significantly (data not shown). The mean ($\pm$ SEM) value for aPTT when averaged over untreated and placebo animals was 47.7 ± 3.0 secs. Using this value as a control and based on what is recommended clinically, a therapeutic aPTT range for this model (i.e., 1.5 to 2.5 times the control) might be estimated as 72 to 120 secs (Fig. 2A) (16). Notably, heparin at 500 units/kg maintained aPTT levels within or close to this range throughout. Heparin at 100 or 2500 units/kg produced aPTT levels that were below or above this range, respectively.

Study 2: Effect of Heparin in *E. coli*-Challenged Animals—In *E. coli*-challenged animals, compared to placebo, treatment with heparin doses of 100, 500, or 2500 units/kg increased the hazard ratios of death (hazard ratio [95% confidence interval] 1.08 [0.66, 1.76]; 1.34 [0.80, 2.24]; 3.02 [1.49, 6.10] respectively) in a dose-dependent pattern ($p = .0013$ for the effect of dose) (Fig. 3). Heparin at 2500 units/kg increased the hazard ratio significantly ($p = .002$). This dose was higher than ones used clinically and was not investigated further.

Noninfected animals challenged with intratracheal NS had aPTT levels that did not differ significantly at 24 and 48 hrs and were similar to levels in nonchallenged animals from study 1 (Fig. 2). Compared to NS challenge, *E. coli* in placebo-treated animals (i.e., in the

absence of heparin) increased aPTT at 24 and 48 hrs ($p = .002$) (Fig. 2B). In *E. coli*-challenged animals, compared to placebo treatment, heparin at 100 and 500 units/kg increased aPTT levels at 24 and 48 hrs ($p < .0001$ for either dose averaged over time) but these increases were greater with the higher dose ($p = .001$ comparing the two doses). Different from what was seen in study 1 in noninfected animals, heparin at 100 units/kg with infection increased aPTT into the therapeutic range and heparin at 500 units/kg produced levels well above this range.

Compared to NS challenge, *E. coli* in placebo-treated animals increased PT and fibrinogen levels at 48 hrs, and overall these effects were significant ($p = .01$) (Fig. 4A, B). *E. coli* did not alter plasma TATc levels significantly but did increase lung lavage TATc levels at both times ($p < .0001$) (Fig. 4C, D). In *E. coli*-challenged animals, compared to placebo, neither heparin dose altered PT or fibrinogen measures or plasma or lung lavage TATc levels significantly (Fig. 4A–D).

Consistent with prior studies in this mouse model, compared to NS challenge, *E. coli* in placebo-treated animals decreased circulating neutrophils, lymphocytes, and platelets and increased hemoglobin, plasma IL-1 β , IL-6, IL-10, granulocyte macrophage colony-stimulating factor, migratory inhibitory protein-1 α , monocyte chemoattractant protein-1, and RANTES along with lung neutrophils, histopathology injury score, lavage protein and wet to dry weight ratios significantly at either 24 or 48 hrs or both ($p = .01$) (data not shown) (17). Lung lavage in infected animals demonstrated *E. coli* at both time points. In *E. coli*-challenged animals, compared to placebo treatment, heparin at 100 and 500 units/kg did not alter any parameter significantly, except at 24 hrs, when the higher dose increased lung lavage neutrophil ($\log_{10}$ [cells/mL]) (5.35 ± 0.04 vs. 5.68 ± 0.07) and protein ($\log_{10}$ [mg/dL]) (4.30 ± 0.05 vs. 4.46 ± 0.05) concentrations significantly or close to it ($p = .0006$ and .02, respectively).

Metaregression Analysis of Published Preclinical Studies

The literature search produced 29 published reports, including 45 studies (experiments) (18–46) (Fig. 5). These employed differing species, routes, and types of septic challenge and regimens of heparin treatment (Table 1). Some included antibiotics or fluid and observation periods varied (24 to 720 hrs, data not shown). There was considerable heterogeneity in the effects of heparin across the studies (Q statistic 102.7, $I^2 = 57.2\%$, $p < .001$). Based on univariate metaregression of the variables recorded, five did not significantly influence the effect of heparin on survival ($p = .37$), while type of septic challenge (monobacterial, LPS, or surgical polymicrobial infection) ($p = .0003$), species ($p = .003$), antibiotics ($p = .01$), and route of infection ($p = .08$) did. However, after accounting for challenge type, the association between the remaining three variables and the effects of heparin approached significance for species ($p = .06$) or were no longer significant ($p = .73$). Studies for each challenge type were separately analyzed using a random-effects model.

Heparin did not improve survival significantly in any of the seven studies employing a monobacterial challenge (Fig. 6). However, there was evidence of moderate heterogeneity across the seven studies (i.e., heparin had little effect in five and was clearly on the side of harm in two) (Q statistic 10.1, $I^2 = 40.9\%$, $p = .12$). Variability in the effect of heparin was potentially related to species ($p = .06$) and dose ($p = .13$). Combining the seven studies, heparin increased the odds ratio of death (odds ratio = 1.65) although not significantly (95% confidence interval, 0.66, 4.14; $p = .29$). Additional sensitivity analysis did not change this effect meaningfully. There was no apparent publication bias across the seven studies (Egger's regression, $p = .64$).

Across the 23 LPS challenge studies, heterogeneity was modest (Q statistic 30.4, $I^2 = 27.7\%$, $p = .11$) and heparin decreased the odds ratio of death (odds ratio [confidence interval] 0.15 [0.09, 0.25], $p < .0001$) (Fig. 6). Species did not alter the effect of heparin significantly ($p = .16$). There was evidence of publication bias for LPS studies (Egger's regression, $p = .002$). However, neither correcting for this bias nor performing sensitivity analysis negated heparin's significant benefit.

For the 15 studies using surgical polymicrobial infection models, there was moderate to high heterogeneity (Q statistic 30.4, $I^2 = 54.0\%$, $p = .007$) (Fig. 6). This heterogeneity was due partially to one outlier, a single mouse study employing the highest heparin dose, in which treatment was significantly harmful (24). With this outlier excluded, heterogeneity was reduced (Q statistic 20.1, $I^2 = 35.4\%$, $p = .09$) and overall heparin showed benefit (0.23 [0.13, 0.41] $p < .0001$). This effect of heparin did not vary significantly among species (excluding the outlier) ($p = .58$). Although there was evidence of publication bias among the 14 studies (Egger's regression, $p = .02$), neither correcting for it nor performing sensitivity analysis negated heparin's benefit.

DISCUSSION

Heparin treatment sufficient to increase aPTT levels in the mouse pneumonia model did not increase survival or reduce lung injury. With higher doses, it increased the hazard ratio of death. Heparin treatment in published preclinical studies, although beneficial in LPS and surgical infection models, was not protective in ones employing monobacterial challenge.

Heparin may have lacked benefit in the present pneumonia model for several reasons. First, infection alone had antithrombotic effects (i.e., increased aPTT), which may have negated potential benefit with heparin. Second, despite the proposed anti-inflammatory effects of antithrombotic agents in sepsis, no heparin dose limited cytokine production or lung injury (47, 48). Third, the dose, duration, or timing of heparin may have been insufficient to produce benefit. However, this regimen did correspond with the period of greatest mortality in the model. Furthermore, in published monobacterial models, differing heparin regimens also did not improve outcome. While it is possible that prolonging treatment beyond 96 hrs might be beneficial, the large animal number necessary to investigate potential change in the limited mortality occurring at this late time (roughly 10% of the total mortality) would be prohibitive. Fourth, the model's high mortality rate may have precluded benefit with heparin. However, hydrocortisone treatment was beneficial in this same model with a similar high mortality rate (7). Fifth, although heparin increased aPTT levels during infection, it did not alter TATc levels significantly, and anticoagulation may have been insufficient to show benefit. Finally, an intact thrombotic response may be necessary for microbial clearance (49, 50). However, heparin did not alter lung lavage bacterial counts.

The highest heparin dose studied (2500 units/kg) worsened survival, possibly because it caused excessive bleeding. It was not reasonable, however, to expend additional animals investigating this possibility, since this heparin dose was higher than ones used clinically. The trend observed over all the doses however raises the possibility that even heparin at 500 units/kg adversely effected survival. Such a trend is consistent with increased lung lavage neutrophil and protein concentrations in this group.

Together, our pneumonia and published preclinical studies suggest that heparin efficacy in animal models may depend on the type of septic challenge. While nonbeneficial in monobacterial models, heparin was beneficial in LPS and almost all surgical polymicrobial infection models. Perhaps these three challenges have differing effects on coagulation. Similar to our model, in the only published study providing aPTT data after monobacterial

challenge, aPTT increased >2 fold from baseline (28). Conversely, in the one study reporting such data after LPS challenge, aPTT was close to normal in placebo-treated animals but increased to 120 secs with heparin (39). It is also possible that the effects of thrombosis with LPS or surgical infection differ from those of monobacterial challenge. LPS administration causes rapid widespread activation of endothelial coagulant activity in animal models, and heparin may produce benefit with little risk (51, 52). During surgical infection, tissue disruption may release tissue factor and augment microbial abscess formation (30). Heparin here could limit abscess formation and facilitate microbial clearance (30).

Infection in our pneumonia model also increased plasma PT and fibrinogen levels and TATc in lung lavage. While PT increases may have been due to mechanisms similar to those increasing aPTT, fibrinogen increases appear more consistent with the stimulation of acute-phase reactants by sepsis. Increased TATc levels in lung lavage but not plasma raises the possibility that excessive thrombosis and fibrinolysis was compartmentalized at the site of infection. These findings support investigations of nebulized antithrombotic therapy for pneumonia (53).

A prior review of heparin therapy for sepsis included a meta-analysis of nine preclinical studies (48). Eight are included in the present investigation, while one also testing LPS antiserum was excluded. This published review reported that heparin was beneficial across the nine studies. However, six studies employed either LPS (n = 5) or surgical infection (n = 1) challenges. Two monobacterial models (without LPS antiserum) did not show benefit. Factors potentially influencing heparin's efficacy were not examined. While our systematic review and metaregression analysis did not include all the elements of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA), critical ones (e.g., explicit search terms, multiple databases examined, predefined inclusion and exclusion criteria, prospective plan of analysis, flow diagram of the search results, stated and reported measure of effect, assessment of heterogeneity, etc.) were (54). The results of our analysis suggest that heterogeneity among preclinical models testing heparin is indeed an important variable which requires exploration.

Extrapolating the findings from these preclinical studies to clinical ones is difficult. For example, factors differing in preclinical and clinical trials (e.g., timing of death, presence of comorbidities) confound conclusions based on the former. Also, the mechanisms underlying heparin's usefulness for sepsis are unclear. For example, it is unknown whether heparin's potential anti-inflammatory effects are dependent on its anticoagulant ones. However, the present findings do raise several clinical considerations. First, heparin had greater effects on aPTT levels in infected mice vs. noninfected ones. Thus, the effect of antithrombotic agents in septic patients may be difficult to predict based on results from noninfected subjects and may require titration with rapidly measurable end points to ensure safety. Second, preclinical studies suggest that the type of infection may influence the benefits of heparin. In the one prospective randomized controlled trial testing heparin in sepsis, pneumonia was the most common cause of sepsis and treatment was not beneficial (4). While this trial was limited by low mortality rates and because heparin was not restarted after threshold aPTT levels were reached, future trials of heparin in sepsis might consider stratifying patients based on factors such as type or source of underlying infection. It is possible that sepsis associated with particularly high LPS levels or with surgical sites of infection may be those most likely to benefit from this type of treatment. Ultimately, well-conducted clinical trials will be necessary to clearly define whether heparin is safe and beneficial for patients with sepsis and whether it may be superior compared to other antithrombotic agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

A, Time course of heparin treatment and coagulation measures in nonchallenged animals. *B*, Time course of heparin or placebo treatments, antibiotic and fluid administration, and laboratory measures in animals challenged with intratracheal (IT) *Escherichia coli* (*E. coli*) or normal saline (NS). aPTT, activated partial thromboplastin time; PT, prothrombin time; TATc, thrombin antithrombin complex; CBC, complete blood count; W/D, wet to dry lung weight ratio; q, every.

Figure 2.

A, Mean ($\pm_{SEM}$) activated partial thromboplastin time (*aPTT*) levels in unchallenged mice randomly selected either before (0 time) or 0.5, 1, 2, or 4 hrs after intraperitoneal treatment with heparin doses of 100, 500, or 2500 units/kg or diluent only. **B**, Mean ($\pm_{SEM}$) *aPTT* levels in mice 24 or 48 after they were randomly selected to receive intratracheal challenge with either *Escherichia coli* or normal saline (i.e., *E. coli* dose 0) followed immediately by initiation of treatment with heparin doses of 100 or 500 units/kg or placebo (heparin dose 0). Treatment was continued q12 hrs for 48 hrs and then q24 hrs for 48 hrs. The cross hatched areas denotes an *aPTT* range of 1.5–2.5 times a control value based on an average of measures in nonchallenged placebo-treated animals from **A**.

Figure 3.

A, Proportion of animals surviving following intratracheal *Escherichia coli* challenge and treatment with heparin doses of 100, 500, or 2500 units/kg or placebo. *B*, Effects of these three heparin doses on the hazard ratio of death. Compared to placebo, treatment with heparin doses of 100, 500, or 2500 units/kg increased the hazard ratios of death in a dose dependent pattern ($p = .0013$ for the effect of dose). Heparin at 2500 units/kg increased the hazard ratio significantly ($p = .002$).

Figure 4.

A–C, Panels A–C show mean (± SEM) plasma prothrombin time (PT) and fibrinogen and thrombin-antithrombin (TATc) levels, while panel D shows lung lavage TATc levels in mice 24 or 48 hrs after receiving intratracheal challenge with either *Escherichia coli* or normal saline (NS) followed immediately by initiation of treatment with heparin doses of 100 or 500 units/kg or placebo (heparin dose 0). Animals challenged with NS received placebo only.

Figure 5.
Study selection flow diagram.

Figure 6. Effect of heparin on the odds ratio of death (95% confidence interval) in published preclinical studies (experiments) from Table 1, stratified based on whether animals received bacteria, lipopoly-saccharide (*LPS*), or surgical infection challenge. The number of animals included in the treated and control group from each study is shown in Table 1.

Table 1

Summary of published studies

Author(s) (Reference no.) (yr)	Infectious Challenge			Heparin Treatment				# of Animals (Nonsurvivor/Total)		Odds Ratio of Death (95% Confidence Interval)		
	Species	Route	Type	Route	Infusion	Dose (U/kg)	Time (hrs)	Antibiotics	Fluid		Placebo	Treatment
Margaretten et al (34) (1967)	Rat	IV	LPS	IV	-	8000	0	-	-	12/12	1/12	0.01 (0.01,0.14)
Filkins et al (27) (1968)	Rat	IV	LPS	IV	-	2000	-0.25	-	-	45/62	0/12	0.02 (0.01, 0.30)
Filkins et al (27) (1968)	Rat	IV	LPS	IV	-	4000	-0.25	-	-	45/62	0/11	0.02 (0.01, 0.27)
Filkins et al (27) (1968)	Rat	IV	LPS	IV	-	4000	-0.25	-	-	12/18	0/14	0.02 (0.01, 0.35)
Filkins et al (27) (1968)	Rat	IV	LPS	IV	-	4000	-0.25	-	-	7/10	1/10	0.05 (0.01, 0.56)
Filkins et al (27) (1968)	Rat	IV	LPS	IV	-	4000	0	-	-	15/22	0/14	0.08 (0.01, 0.45)
Filkins et al (27) (1968)	Rat	IV	LPS	IV	-	4000	0.25	-	-	15/22	4/12	0.23 (0.05, 1.04)
Filkins et al (27) (1968)	Rat	IV	LPS	IV	-	4000	0.5	-	-	15/22	4/20	0.12 (0.03, 0.48)
Filkins et al (27) (1968)	Rat	IV	LPS	IV	-	4000	1	-	-	15/22	3/12	0.16 (0.03, 0.76)
Filkins et al (27) (1968)	Rat	IV	LPS	IV	-	4000	2	-	-	15/22	6/20	0.20 (0.05, 0.74)
Filkins et al (27) (1968)	Rat	IV	LPS	IV	-	4000	3	-	-	15/22	6/15	0.31 (0.08, 1.22)
Filkins et al (27) (1968)	Rat	IV	LPS	IV	-	4000	4	-	-	15/22	5/8	0.78 (0.14,4.21)
Evangelista et al (26) (1969)	Dog	IV	LPS	IV	-	150	-0.5	-	-	20/22	12/15	0.40 (0.06, 2.75)
Priano et al (37) (1971)	Dog	IV	LPS	IV	-	2100	0	-	-	9/12	9/12	1.00 (0.16, 6.35)
Herlache et al (32) (1974)	Dog	IV	LPS	IV	-	600	1	-	-	4/5	1/5	0.06 (0.01,1.39)

Author(s) (Reference no.) (yr)	Infectious Challenge			Heparin Treatment				# of Animals (Nonsurvivor/Total)		Odds Ratio of Death (95% Confidence Interval)		
	Species	Route	Type	Route	Infusion	Dose (U/kg)	Time (hrs)	Antibiotics	Fluid		Placebo	Treatment
Corrigan and Kiernat (21) (1975)	Rabbit	IP	<i>Pasteurella multocida</i>	IV		6000	0		10/20	17/20	5.67 (1.25, 25.61)	
Gaskins and Dalldorf (28) (1976)	Rabbit	IP	<i>Meningococ</i>	<i>ci</i> SC		10000	0		10/24	12/24	1.40 (0.45, 4.38)	
Hau and Simmons (31) (1978)	Dog	IP	MAL	SC		100	0		5/8	1/8	0.09 (0.01,1.08)	
Hau and Simmons (31) (1978)	Dog	IP	MAL	SC		1400	0		10/12	4/12	0.10(0.01,0.69)	
Coalson et al (20) (1978)	Baboon	IV	LPS	IV		3300	0		2/4	2/4	1.00 (0.06,15.99)	
O'Leary et al (35) (1979)	Rat	IP	MAL	SC	-	240	0	-	-	6/12	1/12	0.13 (0.01,1.18)
O'Leary et al (35) (1979)	Rat	IP	MAL	SC	-	960	0	-	-	6/24	1/24	0.09 (0.01, 0.94)
Davidson et al (22) (1981)	Rabbit	IP	AL	IP	-	150	0	-	-	14/33	4/11	0.78 (0.19,3.17)
Davidson et al (22) (1981)	Rabbit	IP	AL	IP		250	0		14/33	2/26	0.11 (0.02, 0.56)	
Dunn et al (24) (1983)	Guinea pig	IV	<i>Escherichia coli</i>	IV		2000	-0.5		-	9/16	18/18	29.21 (1.50,568.08)
Chalkiadakis et al (19) (1983)	Rat	IP	MAL	SC		3600	0		16/20	4/20	0.06 (0.01, 0.29)	
Gupta and Jain (30) (1985)	Dog	IP	MAL	SC	-	500	0	-	-	4/10	1/10	0.17 (0.01,1.88)
Prinz et al (38) (1986)	Rat	IP	<i>E. coli</i>	SC		1200	0		0/20	1/21	3.00 (0.12, 78.05)	
Prinz et al (38) (1986)	Rat	IP	<i>E. coli</i>	SC	-	1200	0	-	-	6/24	4/23	0.63 (0.15,2.61)
Onda et al (36) (1986)	Rat	IP	LPS	SC		100	0		5/10	1/10	0.11 (0.01,1.24)	

Author(s) (Reference no.) (yr)	Infectious Challenge			Heparin Treatment			# of Animals (Nonsurvivor/Total)			Odds Ratio of Death (95% Confidence Interval)		
	Species	Route	Type	Route	Infusion	Dose (U/kg)	Time (hrs)	Antibiotics	Fluid		Placebo	Treatment
Onda et al (36) (1986)	Rat	IP	LPS	SC		100	0			10/10	6/10	0.07 (0.01,1.50)
Hirano et al (33) (1986)	Rat	IP	CLP	SC		320	0			4/12	2/12	0.40 (0.06, 2.77)
Hirano et al (33) (1986)	Rat	IP	CLP	SC		320	0			10/12	4/12	0.10(0.01,0.69)
Schirmer et al (46) (1987)	Rat	IP	CLP	IV		600	0	-	-	10/12	4/12	0.10(0.01,0.69)
Smith et al (41) (1988)	Rat	IV	LPS	IV		1540	0	-	-	12/13	3/10	0.04 (0.01, 0.41)
Bahrani et al (18) (1989)	Rat	IP	LPS	IP		460	-1	-	-	7/10	5/10	0.01 (0.01, 0.50)
Pittet et al (45) (1989)	Sheep	IV	LPS	IV		100	0	-	-	5/5	0/5	0.43 (0.07, 2.68)
Griffin et al (29) (1990)	Piglet	IP	<i>E. coli</i>	IV		600	0			4/5	3/5	0.38 (0.02, 6.35)
Dickneite and Czech (23) (1994)	Rat	IV	<i>Klebsiella pneumoniae</i>	IV		400	1			18/20	17/20	0.63 (0.09, 4.24)
Yang and Hauptman (44) (1994)	Rat	IP	CLP	IV		3600	12			8/20	2/12	0.30 (0.05, 1.75)
Sun et al (42) (1997)	Rat	IP	ALP	SC	-	3000	-24	-	-	42/50	35/50	0.44 (0.17,1.17)
Vela et al (43) (1999)	Rat	IP	MAL	SC	-	200	0	-	-	5/30	7/30	1.52 (0.42, 5.47)
Echtenacher et al (25) (2001)	Mouse	IP	CLP	IP		5000	0			1/8	6/8	21.00 (1.50, 293.27)
Schiffer et al (39) (2002)	Sheep	IV	LPS	IV		4143	-20	-		8/8	3/7	0.05 (0.01,1.10)
Slofstra et al (40) (2005)	Mouse	IV	LPS	IV		500	-0.5			9/12	7/14	0.33 (0.06, 1.78)

IP, intraperitoneal; IV, intravenous; SC, subcutaneous; CLP, cecal ligation and puncture; ALP, appendix ligation and puncture; MAL, mesenteric artery ligation; AL, appendix ligation.