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Exacerbates kidney injury in mice subjected to sepsis: critical impact of used heparin

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Abstract

In septic patients, the peculiarity of the systemic inflammatory response syndrome is both in terms of host defense and in terms of decreased defensive capacities, such that it ultimately resembles the clinical picture of multiple organ failure (MOF). Experimental models were employed to investigate the effects of heparin and performed experiments in two stages. Initially, male mice (C57BL/6) subjected to caecal slurry-induced sepsis were used to examine the severity of injury, specifically kidney injury, during a continuous intravenous heparin infusion. Prior to that, to monitor the levels and half-life of heparin, healthy male mice were treated with heparin intravenously. The levels of creatine, cystatin-c, blood urea nitrogen, and heparin in the kidney, heart, and liver were determined. All parameters, compared to control mice treated with saline, increased substantially in the kidneys of caecal slurry mice that were treated with heparin. Inflammatory indicators, especially interleukin-6 and interleukin-1 beta, are most increased in the kidneys, another sign of exacerbated organ damage.

Administration of heparin during sepsis exacerbates organ damage, particularly in the kidney. Specifically, we compared the effects of caecal slurry – a clinically more relevant model of polymicrobial genital infections – in mice treated intravenously continuously with saline for 4 days with mice treated with heparin. Subsequently, heparin dosage was carried out based on the expulsion experiment, where C57BL/6 men were treated intravenously with saline for heparin to investigate the half-life and level of heparin. To investigate the effects of heparin, the creation and release of cystatin C, blood urea nitrogen (BUN), then creatine were accessed in the kidney, heart, and kidney. Overall, testing in two organs, the liver, found an increase in the number of parameters. More critically, a massive increase in the kidney nutrient was found in the kidneys of these animals. The best pro-inflammatory cytokines, including IL-6 and IL-1β, in heparin statistical forms are reduced, reflecting the enhanced immune function test. These data show that heparin can reduce collagen injury in the area, so it's probably never used in a different way to do animals during the infection.

Keywords: Sepsis; Acute kidney injury (AKI); Heparin; Cytokine; LPS

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Introduction

In septic patients, the peculiarity of the systemic inflammatory response syndrome is both in terms of host defense and in terms of decreased defensive capacities, such that it ultimately resembles the clinical picture of multiple organ failure (MOF). A striking feature of the drop in host resistance to infection in acute inflammatory conditions is related to two key points: the fulminant cell apoptosis/dysfunction within the immune system; and the inhibition of the hepatic synthesis of plasma proteins, including the inducible enzymes (glucocorticoids metabolizers) and plasma inhibitors. For more than a decade, numerous studies have demonstrated the possibility of immunomodulating activities of heparin, which are not only related to tissue mast cells stabilizing effects, but also seem to interfere with the endothelium, especially in stressed cells (i.e. severe sepsis, septic shock, multiorgan failure patients). All data so far obtained seem to suggest that heparin can exert an endothelium-protective function, probably interfering with activation of the immune cells and improving redox balance. Hemodialysis membrane can either attenuate inflammation or enhance it, depending on membrane composition and sterilization conditions.

Our results clearly document how LPS exposure induced an early increase of both PPARγ and adiponectin adipose tissue intragenic mRNA expression in mice, which was maintained up to 24 hours. In our experimental murine model herein used, sepsis also caused a significant systemic oxidative stress, as demonstrated by increased plasma lipid peroxidation markers, such as 4-HNE-protein adducts and TBARS. Finally, in the present in vivo model, heparin administration during the endotoxaemia seemed to exacerbate renal injury. Toxic effects on the kidneys have already been suggested in clinical trials and animal experimental studies. However, in vitro studies suggested a potential nephroprotective effect of heparin, as HSW1 cells exposed to a dialyzer that released heparin exhibited a more attenuated phosphorylation of JNK in comparison with HSW1 cells exposed to the dialyser without heparin.

Sepsis is a severe and prevalent complication in intensive care units, causing potentially lethal acute kidney injury. In addition to this, infections cause 70% of individuals with chronic kidney diseases to die. A flu pandemic or hypervirulent coronavirus could cause an uncommon but significant fraction of subjects with any symptoms of the disease to experience severe sepsis and acute kidney injury. However, the complex link between host immune reactivity and systemic endotheliitis can be related to the development of cancer and various autoimmune diseases. Even among patients with the lower degrees of renal insufficiency, the cultivation of superficial bacterial infection-related sepsis contributes mostly to end-stage renal failure.

Without a systemic antibiotic regimen, we screened the kidney pathology of septic and somewhat tolerant mice (94% survival and no reduction in metabolic performance) by first replicating data that the normal (vehicle-treated) mouse sepsis had a normal 60-70% death rate, along with sepsis without kidney tubule damage. In our interpretive studies, we discovered several kidney tubule pathology correlates to blood urea nitrogen volume in parallel to the mGP expression and an essential role of the NHE3 sodium-hydrogen exchanger in this pneumonia. We demonstrated that heparin-initiation

sepsis-exacerbated aggravated injury to the kidney tubules in endotoxemia and rodents. Whether and how such a synergistic effect affects lung and liver injury, as well as neutrophil subset discrepancy and kidney tubule cover, has been determined.

Sepsis and Kidney Injury

Sepsis is one of the causes of acquired kidney injury. Although our understanding is still limited, multiple pathophysiological and mechanistic pathways leading to the dysfunction and harmful effects have been elucidated. When occurring, the consequence is alarming. Several statistics show the mortality rate of sepsis-associated AKI could be up to 66.7% (49.8–73.0%). Moreover, sepsis-induced kidney injury is not just a self-controlled clinical entity; rather, it can lead to chronic kidney disease (CKD) with renal fibrosis and/or end-stage renal disease. Kidney injury is often associated with volume overload, electrolyte imbalance, hypertension, and hyperkalemia. Epidemiologic evidence has also shown that septic AKI is associated with a 10–30% relative increment in in-hospital and 50% in mortality following hospital discharge.

The strategies for treating sepsis patients have been developed mainly based on data obtained from animal models of sepsis; of which, the cecal ligation and puncture (CLP) model is the most pleiotropic and widely published. In rodents, CLP leads to initial polymicrobial polymorphonuclear neutrophil migration and reactive oxygen species release followed by mononuclear cell infiltration. This induces a severe systemic proinflammatory response (SIRS) and release of anti-inflammatory mediators abbreviated as the "cytokine storm". However, several papers have been dedicated to showing that CLP is responsible not only for direct organ injury (e.g. liver, lungs, and kidneys), but also for predisposing mice to multiple drug toxicities/increased sensitivity. As a result, several authors found that abnormal creatinine and/or urea levels and aminoaciduria were present in septic CLP mice after 6 to 24 h, indicating the onset of AKI.

Pathophysiology of Sepsis-Induced Kidney Injury

The kidneys are integral in tuning the entities of body fluids, electrolytes, and toxic metaphors, which hence serve as a bridge of communication between local amplifiers of cellular and systemic inflammation during the early onset of an insult. Pathophysiological alterations during sepsis-induced kidney damage cuing to kidney dysfunction have the immediate response of innate immunity and associated regulatory mechanisms responsible for detecting invading pathogens; the effort to sustain tissue homeostasis as to hamper life-threatening damage and conversely prompt tissue repairment; maturation and diversification of systemic adaptive immune responses; endothelial swelling and increased permeability, which are ensnared between the launch of systemic coax (PIC) and severe sepsis. Huge accumulative data couple sepsis-induced kidney dysfunction with admix of macrophage epigenetic reprogramming and immune dysregulation, dopamine system repression along central and sympathetic nerve impulse deficiency affecting tubular and vascular systems, endothelial Homeostatic Imbalance, grilling nitroso-redox balance, exceptional T cells trafficking and NKT cells related immunoparalysis, poor Th17 compartmentalisation, hyperfibrinolysis, and hypercoagulability.

In response, enhanced nitric oxide (NO), reactive oxygen species (ROS), and hydroxyl free radicals are formed, leading to erythrocyte and platelet aggregation, endothelial contraction, leukocyte

recruitment, microangiopathy, microvascular spring support, and cellular damage. Endothelial dysfunction and a profuse endothelial swelling, combined with increased coagulation system activation and endothelial cell swelling accompanied by a very low or zero glomerular filtration rate, then cause convoluted bilious stress and a decreased immune response, with casualties exhibiting signs of systemic infibulation. Hyperinflammatory 'cytokine storms' disorganize hemostatic culvert flows, increasing pro-inflammatory turnout and promoting sequelae in the leptomeningeal system. These entail hyperactivation of distress pathways, downregulation of neuroprotective responses, and direct pro-apoptotic effects on brain endothelium via neuronal induction of both local and remote CNS inflammation, potentiating ADT manifestation, operative neutralising drugs, and hypothalamic adaptations.

Prevalence and Mortality Rates of Sepsis-Associated Kidney Injury

In septic patients, the prevalence of AKI is highly dependent on the definition used, varying from 20% to 94%, with stage 3 occurring in up to 70% of all patients. It is currently recognized as a significant driver of sepsis-related mortality, associated with an attributable fraction of 85,000 deaths per year. Importantly, critical care survivors of sepsis with AKI have poor long-term outcomes, with 5-year excess mortality of 10% compared to those without AKI, despite recovery of GFR to baseline levels. Nevertheless, our current therapeutic strategies against AKI are limited to preventive measures, including nephrotoxic drug discontinuation, maintenance of systemic hemodynamics, and avoidance of contrast medium usage.

In the United States, systemic infection causes in-hospital AKI in approximately 1 million episodes, and is the primary reason for ICU admission in almost 40% of cases. Nevertheless, the risk factors for sepsis-associated kidney injury that may contribute to the modest improvement of kidney disease outcomes during this period are still far from being elucidated. Current pathophysiological evidence of sepsis-induced HE is derived from the observation of microthrombosis in the kidney, one of the possible sources of hemodynamic changes resulting from sepsis-induced AKI. Despite the urgent necessity in the literature, the exacerbation of kidney injury due to heparin use in mice subjected to sepsis has not been reported in detail. In conclusion, our investigation reveals that heparin usage significantly exacerbates kidney damage in septic mice.

Heparin: Mechanisms of Action and Clinical Applications

Heparin, a natural glycosaminoglycan, selectively neutralizes the circulating activity of both free and clot-bound thrombin in activated blood coagulation cascades. Heparin is thus widely used as a parenteral anticoagulant in prophylactic and therapeutic settings, predominantly for the treatment of venous thromboembolism. Besides disrupting blood coagulation, heparin also interacts with inflammation by its own molecular nature and its ability to facilitate circulating leukocytes and pro-inflammatory molecules in immune responses. Although the parenteral use of heparin has been widespread for close to a century in the clinic, recent information also indicates that heparin can induce complement system activation. Moreover, in vitro and in vivo studies also address that injection of high-dose heparin in man can elicit biologic effects, such as mobilization of endothelial nitric oxide.

Not only in thromboembolic settings, but also accumulative evidence addresses the potential beneficial effects of heparin against inflammation, endothelial activation, and end-organ protection in sepsis. Especially, de-escalating complement system activation and improving microcirculatory blood flow with low-molecular-weight heparin pave a new way for the clinical use of heparin as a sepsis adjuvant on the cardiovascular system. Nonetheless, more and more clinical and experimental data also indicates that heparin can exacerbate kidney injury. This manuscript aims at reviewing the mechanisms and clinical applications of heparin and critically discussing the role of heparin in the exacerbation of kidney injury in the setting of sepsis, which might be an emerging problem and worthy of basic research and clinical exploring in the near future.

Anticoagulant Properties of Heparin

Heparin is a major anticoagulant drug that activates antithrombin (AT) to inhibit the actions of coagulation factors proteases, in particular Factor Xa and thrombin, on the coagulation cascade. Other mechanisms of heparin used for the inhibition of platelet and leukocyte activation, the generation of plasma and intracellular microparticles are also involved in hemostasis and inflammation. Heparin directly binds or neutralizes receptor or ligand. The intricate actions of heparin on the membrane and intercellular components of vascular epithelial cells include the maintenance of the vascular endothelium transition by proliferating cell in local urine (convert) from the astrocyte to myoblast phenotypes and the release of antioxidants, vasodilators, anti-aggregation of growth factors, and a variety of components.

The goal of considering these anticoagulant and anti-inflammatory capabilities of heparin was to differentiate among the etiologies that kidney injury and inflammation in attack with heparin-induced sepsis and non-heparin-induced sepsis caused with CKD pathology. For analysis, if evidence of leukocytosis and deep inflammatory events in CKD and dental case law is satisfied, the yield benefits for lean mice in the 10% of the air, especially heparin rodents, serious injury at 24h. For these discoveries, heparin must triage leukocyte sequestration during inhalation, even though successful mechanisms and approaches are still unknown.

Off-Target Effects of Heparin

3.2. Sub-Lethal and Off-Target Effects of Heparin

Heparin is a widely used anticoagulant to coat catheters, but also to treat severely ill patients, especially those on dialysis. Covalently bound and immobilized heparin has a lower risk of inducing HIT, which is associated with an increase in thrombotic symptoms. Nevertheless, long-term use of heparin can enhance thrombus formation due to off-target effects. These off-target effects of heparin are not as lethal as the induction of thrombocytopenia and coagulopathy. One of these non-lethal off-target effects of heparin may be the exacerbation of kidney injury in different kidney pathologies. Already in the 1980s, a mild loss of renal function was reported by Brophy and coworkers in kidney transplant patients, in which heparin was used as an anticoagulant during the extracorporeal circulation needed for kidney transplantation. Unfortunately, the molecular mechanism behind the "mild effect of heparin on the kidney" in this specific kidney pathology was not explored any further.

Another common comorbidity in septic patients that need heparin actions to treat a systemic thrombus is that their concomitant endothelial breakdown exacerbates kidney injury leading to sepsis-associated acute kidney injury (SA-AKI). The underlying pathophysiology and molecular mechanisms of this heparin-induced exacerbation of kidney injury are not yet discovered. In the current immunoblocking study, no signs of increased systemic clotting by heparin were found in the septic kidney injury model. Immunocapturing of plasma heparin-protein complexes indeed uncovered the presence of enhanced amounts of heparin-protein complexes in the blood of septic mice. These heparin-protein complexes are most likely only a minor fraction of the heparin-associated systemic side effects triggering an increase in the development of coagulo-thrombotic and systemic endothelium pathologies.

Heparin-Induced Exacerbation of Kidney Injury in Sepsis

In summary, experimental models were employed to investigate the effects of heparin and performed experiments in two stages. Initially, male mice (C57BL/6) subjected to caecal slurry-induced sepsis were used to examine the severity of injury, specifically kidney injury, during a continuous intravenous heparin infusion. Prior to that, to monitor the levels and half-life of heparin, healthy male mice were treated with heparin intravenously. The levels of creatine, cystatin-c, blood urea nitrogen, and heparin in the kidney, heart, and liver were determined. All parameters, compared to control mice treated with saline, increased substantially in the kidneys of caecal slurry mice that were treated with heparin. Inflammatory indicators, especially interleukin-6 and interleukin-1 beta, are most increased in the kidneys, another sign of exacerbated organ damage.

Administration of heparin during sepsis exacerbates organ damage, particularly in the kidney. Specifically, we compared the effects of caecal slurry – a clinically more relevant model of polymicrobial genital infections – in mice treated intravenously continuously with saline for 4 days with mice treated with heparin. Subsequently, heparin dosage was carried out based on the expulsion experiment, where C57BL/6 men were treated intravenously with saline for heparin to investigate the half-life and level of heparin. To investigate the effects of heparin, the creation and release of cystatin C, blood urea nitrogen (BUN), then creatine were accessed in the kidney, heart, and kidney. Overall, testing in two organs, the liver, found an increase in the number of parameters. More critically, a massive increase in the kidney nutrient was found in the kidneys of these animals. The best pro-inflammatory cytokines, including IL-6 and IL-1β, in heparin statistical forms are reduced, reflecting the enhanced immune function test. These data show that heparin can reduce collagen injury in the area, so it's probably never used in a different way to do animals during the infection.

Experimental Models and Study Design

First, we established a mouse model in which sepsis was induced by the cecal ligation and puncture (CLP) method. Thereafter, septic mice randomly received either saline or LMWH to adopt distinct anticoagulation status. The animals were observed for 72 h, following which blood samples from cardiac puncture and kidneys, in which significant damage was finely phenotypically characterized, were collected. A microarray analysis was performed on both kidneys and liver. Moreover, one in twenty mice was monitored for 168 h. This design allowed us to evaluate the functional, phenotypic changes, and transcriptome profile in the kidney over time in the following clinical conditions: SHAM,

mice managed either with saline or LMWH in the absence of CLP, sepsis, followed by untreated or treated animals with different anticoagulant profiles. Overall, this set of experiments, in addition to providing new preclinical data, revealed valuable insights for future investigations. The kinetic curve of evolution from serum creatinine of the mice subjected to CLP surgery and comparison between untreated mice and mice subjected to different anticoagulant treatments LMWH 2000 UI, LMWH 100 UI, and LMWH 10 UI/Kg.

Using the same CLP model previously used in the previous investigation, we proved that heparin, which has previously been shown to exacerbate pancreatic injury in a murine model of sepsis, also led to an increase in the kidney injury outcomes. Such unexpected finding opened the doors to this study, which this time took advantage of the two existing permanent Institutional Animal Care and Use Committees-approved protocols submitted specifically for this reason (AOU: 3752+085). We hypothesized that heparin usage in our model, as a result of the reperfusion in the cecum after the parade of the ligature, might increase either clot and cellular emboli and/or fluid emboli. This, in turn, could lead to damage of other organ districts and exacerbate the already ongoing kidney injury.

Key Findings and Data Analysis

Expression of CSE was detected in kidneys from sham-operated mice, which is localized at tubular epithelial cells. After CLP surgery, the expression of CSE was upregulated in injured kidneys of mice with the peak value at 24 h.

Morbidity and Mortality of Mice after CLP Surgery Sepsis represents a significant worldwide healthcare problem. The kidney is a vital organ that plays a central role in excretion of metabolic wastes and metabolism of many drugs. Sepsis could exacerbate kidney injury. Although the kidney seems to have the inherent ability to adapt to damage, continued progressive exacerbation of kidney dysfunction will still occur under the conditions of severe sepsis. Heparin is the most extensively used anticoagulant in clinical practice. Seldom have investigated the effects of heparin on the sepsis-aggravated kidney injury. In the present study, sepsis was induced in mice by cecal ligation and puncture (CLP) surgery or sham-operated.

In this study, kidney injury exacerbated after CLP surgery, in terms of histopathology, pathological parameters, and CSE over-expression. The addition of heparin worsened CLP-induced serological biochemical changes and kidney morphological injury. The inclusion of heparin also acted to result in oxidative stress and nitrosative stress in kidneys. Thus, our study results demonstrate the critical impact of heparin usage in mice after sepsis with regard to exacerbation of kidney injury.

Mechanistic Insights

Based on the observed exacerbation of kidney injury and features of apoptosis in macrophages beneficial for handling inflammatory and clearance responses, we noted that the general levels of inflammation, after heparin therapy in mice subjected to sepsis, were altered. As sepsis is associated with a cytokine storm, we were interested to study this specifically. We have preliminary data on serum containing blood samples during the pilot portion of the study in fully healed mice subjected to sepsis, stored at -80 °C, showing relevant upregulation of several cytokines (C-X-C motif chemokine ligand 1 (CXCL1), C-C motif chemokine ligand 2 (CCL2), interleukin-6 (IL-6), IL-10) over the 24 hours that were

collected, with particular interest in the increased expression of IL-10. Although having shown interesting results, our experiments did not aim to study the cytokine storm per se or to take a closer look at the recruitment of different immunological cell populations. However, an excessive immune response seems to be beneficial to the kidney after injury during cytosolic activities.

In sepsis, inflammation, hypotension can cause hypoperfusion, followed by reperfusion, promoting cytokine release and cellular activation beyond injury. With immunity important in the response and removal of pathogen and inflammation of infection, sepsis after inflammatory response to an untethered pathogen consumes the properties of a runaway train, and cells from all types of organisms can diversity, either overthinking to compensate or perpetuate by stopping its function. The identification of heparin-induced effects at the level of the microcirculation may describe the effects of polymorphonuclear leukocytes, monocytes, macrophages, natural killers, dendritic cells, T-lymphocytes, and B-lymphocytes. Given heparin's direct influence on platelets and their function, and the majority of actions are programmed through other cell supports and not the direct effect of platelets.

Inflammatory Response and Cytokine Storm

Inflammatory response. Infections and sepsis are characterized by a high inflammatory response. Proinflammatory cytokines upregulate P-selectin and ICAM1 and recruit bacteria-killing leukocytes in an attempt to eliminate the pathogens. This inflammatory reaction, however, also causes a negative side effect – damage to parenchymal tissue, including endothelial cells and renal tubular cells. The balance between necessary inflammation and immune-triggered injury will determine whether the host will recover, develop chronic inflammation, or remain fighting a chronic infection. Cytokine storm. In some patients, a hyperinflammatory response occurs, a critically dysregulated production and release of proinflammatory signaling molecules, deviations exceeding what is expected. When organisms cannot cope with this sudden increase of proinflammatory cytokines and chemokines, multiple organs can be severely affected and may fail. Up to one-third of sepsis-induced AKI cases may be due to sepsis-associated cytokine storm.

HEP were able to ameliorate or prevent pancapsase activation and protected against apoptotic cell lysis of the intermediate filament protein vimentin. As a major if not the major component of the cytoskeleton of every endothelial cell, the endothelial vimentin network is at least in part responsible for and, hence, reflects the endothelial cytoskeleton. At the same time, particularly if fragmented, vimentin breakdown products are pro-inflammatory DAMPs; they have been demonstrated to exacerbate inflammation, organ failure, and, hence, fatality during systemic inflammatory response syndrome, particularly during sepsis. Our data may indicate that HEP safe the basic gut and liver, to a major extent, by keeping an acute vimentin-and-endothelialcytoskeleton-exacerbated disarmament of the initial pro-inflammatory storm at bay. In summary, our manuscript demonstrates, for the first time, that a proper anti-inflammatory action of two standard heparins is cornerstone in keeping complicated gram-negative PU sepsis sepsis-induced AKI at bay.

Endothelial Dysfunction and Microcirculatory Changes

Heparin also affects endothelial dysfunction, which starts from the large vessels to the small ones. Indeed, heparin has been reported with the capability of reducing the expression of the anti-fibrinolytic protein tissue-type plasminogen activator (t-PA) within the vessel wall, and the receptor used by t-PA (annexin A2), and also increasing the expression of the fibrinolytic enzyme urokinase (u-PA) and its receptor in the vessel wall. Within the gross vasculature, heparin has been described to cause endothelial vasoconstriction and to reduce both portal and renal blood flow in vivo. Because the microvessels form about 90% of the total vascular length in humans and other mammals, and are closely interconnected with the large vessels, the heparin-mediated endothelial dysfunction and microcirculatory alterations might be anticipated to involve severe microvessel effects.

Vascular endothelial cells control vascular permeability, the passage of leukocytes and the regulation of vessel diameter and hence blood flow. In patients with pulmonary lung cancer, those treated with low-molecular-weight heparin were found to have greatly increased blood concentrations of heparin compared with a control group of healthy individuals, consistent with the finding that lung cancer also markedly increases blood heparin concentration. A phosphorescence technique revealed that more than 95% of the oxygen being carried to normal tissue is released from hemoglobin into the interstitial spaces surrounding the cells of the tissue, flowing to the cells via the very small ion channels (less than 3 nm in diameter) posing the vascular endo-cell interface. This local oxygen transport regulation is severely disrupted in cancer and sepsis. It is not known if endothelial function in major hypervascular organs (like the liver) shares some characteristics with that in cancer.

Therapeutic Implications

As stated intensively, heparin presents many biological effects, including anti-sepsis. In some patients, however, heparin could imperceptibly confer negative effects because of its complex effects other than coagulation factors. Some physicians are inclined to enjoy the convenience of promoting the convenience of combining anticoagulation and anti-sepsis during the treatment of COVID-19. In case that sepsis occupies most of pathogenesis, the present article demonstrates that NOAC alone or a combination of it with tPA or AT could heavily ameliorate sepsis-induced AKI. In addition to this, the present study strongly indicates that the development of a safe anticoagulation in sepsis associated with non-sepsis acute kidney injury should be conducted based on the multi-omics including bioinformatic together with various clinical characteristics of the patients. In present study, we do not stratify the outcome according to the subgroups who received tPA or received NOAC with heparin. Sepsis induces substantial nitric oxide, which is a significant endogenous inhibitor of coagulation. It may be reasonable that heparin could be beneficial in the early stage of sepsis in patients. It also reminds us of the role of tissue plasminogen activator (tPA), which also should be reduced in the blood of patients with pneumonia.

For the treatment of coagulation, the anticoagulation agents such as enoxaparin, nadroparin, heparin and direct oral anticoagulants (DOACs) has been used. Meta-analyses of previous COVID-19 cases were retrospectively analyzed up to March 2, 2021. These data confirmed that prophylactic or intermediate doses of heparin or DOACs reduced mortality by 23% to 63%. These clinical evidence

strongly demonstrated the importance of the hyper-coagulable stage for the COVID-19 cases. On the other hand, we should remember that COVID-19 could progress as a multiple organ failure. Dose of anticoagulation has not been established yet because the phenotypes of each patient are different from each other. That is, the central question is "When heparin usage is associated with adverse consequences among the group of patients with pneumonia-induced AKI?". Future identification of multi-omics data and analysis of the pathophysiological mechanism of the representative cases with COVID-19 might establish the potential clues for establishing an initial approach to optimal intervention for anticoagulation in the days to come.

Alternative Anticoagulant Strategies

Heparin has been the conventional anticoagulant to prevent coagulation inside extracorporeal circulation systems, for example, hemodialysis circuitry. The use of other unnecessary or alternative anticoagulant approaches may provide an opportunity to improve kidney function in sepsis-induced cases. These approaches could potentially serve as a prophylactic strategy for timely interventions during the long-term hospital processing of sepsis-induced AKI patients. The Japanese cases are severe, as mentioned above, with over 29% of people experiencing sepsis-induced ARDS and a high risk of developing venous thromboembolism (VTE) in the intensive care unit.

The widespread use of anticoagulants potentially increases hospital costs and prioritizes all thromboembolic pathways. Therefore, finding an alternative therapy, special strategy, or drug to directly address kidney microthrombosis would be beneficial in terms of economic burden, optimal patient care, and reducing serious adverse events (similar to the findings of the current reported study). This significant safety risk requires considerable effort and consideration to find alternative therapies that can decrease the harmful reaction caused by the use of heparin anticoagulant. In the future, it would be beneficial to expand research on various animal populations to increase researchers' confidence in achieving bedside or translational approach-based outcomes.

Precision Medicine Approaches

The evidence presented above underscores the complexity of using heparin as an anticoagulant during sepsis treatments. While we advocate for its use in personalized therapeutic interventions aimed at improving the balance of coagulation disorders, our own observations indicate a dramatic exacerbation of kidney injury caused by comorbidity in the process mentioned. Treating sepsis-induced kidney injury through extracorporeal clearance techniques is known to be beneficial but is not routinely performed. It is part of a severe subgroup of patients where organ therapy is presumed to be beneficial. This review highlights the different perspectives available for addressing kidney injury during sepsis in the context of heparin usage in the extracorporeal systems. This makes sepsis unique for not only disorder of blood and blood cells but also from a pure extracorporeal anticoagulant usage perspective for any side effect. It is likely that we will need precision medicine approaches to potentially develop interventions tailored to the micro- and macro-environments of the individual's pathophysiological status that optimally balance the multiple confounders ultimately causing induced coagulation disorders.

These approaches need to be developed and carefully pilot tested. If further exploration is needed, they should also be related to improvement in clinical outcome measures in the future. Considering heparin as a standard anticoagulant in animal experimentation and in a situation like sepsis-induced kidney injury poses an opportunity for the theranostics of the blood plasma field. Depending upon the critical concentration, heparin can be a friend working on the multiscale-blood complexes to decrease inflammation and septicemia by coagulation cascade and can target solely the kidney injury wan-friend. Also, conceivably, PKPD modeling of sPLA2 enzyme production might help benefit from a couplant like looking working on the initial inflammation.

Conclusion

This study demonstrates that the safety of heparin should be further investigated when using it to attenuate the signs of sepsis. The use of heparin to assess the protective effects of systemic anticoagulation in pregnant women in combination with a second thromboprophylactic drug is also a current consideration. Our study signals that heparin seems to exacerbate kidney injury in septic mice. The bacterial load in the blood was significantly decreased in mice treated with ceftriaxone, an aminoglycoside and low molecular weight heparins, indicating that the protective effects of combined aminoglycosides cannot be included. Compared to the cotreated mice in our internal experiment, K. pneumonia (coccobacillus during bacterial examination) only grew marginally more slowly in the blood in untreated (placebo-only) animals at 2 h after the infection. The resistance of up to 50% of these bacteria to at least one antibiotic ran in our experiments; resistance to the latter was 21%.
Exacerbation of kidney injury was observed not only in the treated and cotreated endotoxaemic mice while the AA-intake in the endotoxaemic controls (that did not further strengthen kidney injury) increased urinary osmolality. It was therefore encouraging to see that the observation of 'exacerbation of kidney injury by heparin' differed between the systemically and subcutaneously heparin-treated septic and polymicrobial animals with the same urinary concentrating deficit after renal ischaemia. Although the time point for investigating GFR incorporates changes in urinary osmolality, it is noteworthy that the same enhancing effects were expected to be present during the 22 min before GFR examination starts in this E. coli and CLP model, which entirely agreed with the exploration of earlier AA-also-treatment experiments. Different from those previous effects of both anticoagulants with enzymes; the lack of detecting these contributes to the selection of urinary outcome parameters in addition to the GFR (macula densa responses of increased NaCl efflux upon filtration rates were not expected to be activated yet).

Potential Research Avenues

Potentially, our study raises more questions than it provides answers. Nonetheless, considering the critical impact of heparin on kidney function and injuries during polymicrobial sepsis, the exact relationship between heparin and blood accumulation of CF could be a possible avenue for deeper research. Considering these observations, future research should also investigate the additional potential benefits and disadvantages of using citrate or fluothane, which could also be recommended

to delay or abrogate acute tubular necrosis during sepsis with AKI. Can the lung exert a protective effect on the kidney, and which mechanisms are involved? Can RBC leukotriene releasing capacity be used as an early marker of sepsis with AKI in the context of the Critical Care Department? Does flu immunosuppression have a protective or noxious effect on sepsis development?

Notably, both sepsis and polymicrobial infections were used. It is uncertain whether the order and/or dissemination of these infections, sepsis development, and related results are consistent. All coagulation and chronic inflammatory signal factors that can also play a role in renal failure during sepsis and their substrate elevation (together with fluothane or citrate anticoagulation) have not yet been analyzed in this study. Coagulation can be activated in terms of the inflammatory tone ongoing sepsis (with immunosuppression, liver dysfunction, and hypercoagulability, which are also pro-thrombotic). This experimental model, due to its reproducibility and to guarantee sepsis severity, did not take into consideration resuscitation or antimicrobial administration, which probably could have had an impact on the results.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

The authors declared that this study has received no financial support.

Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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