Hydroxyethyl starch 130/0.4 decreases inflammation, neutrophil recruitment, and neutrophil extracellular trap formation

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Editor’s key points

• The use of HES 130/0.4 is not recommended in sepsis but may be anti-inflammatory.
• The effect of HES 130/0.4 on aspects of leucocyte activation in a mouse model of sepsis was studied.
• HES 130/0.4 reduced neutrophil activation under inflammatory conditions.
• HES 130/0.4 has anti-inflammatory actions via effects on neutrophils.

Background. During systemic inflammation, leucocytes are activated and extravasate into damaged tissue. Activation and recruitment are influenced by different mechanisms, including the interaction of leucocytes with platelets and neutrophil extracellular traps (NET) formation. Here, we investigated the molecular mechanism by which hydroxyethyl starch (HES 130/0.4) dampens systemic inflammation in vivo.

Methods. Systemic inflammation was induced in C57Bl/6 wild-type mice by caecal ligation and puncture and cytokine concentrations in the blood, neutrophil recruitment, platelet–neutrophil aggregates, and NET formation were investigated in vivo. Intravascular adherent and transmigrated neutrophils were analysed by intravital microscopy (IVM) of the cremaster muscle and the kidneys. Flow chamber assays were used to investigate the different steps of the leucocyte recruitment cascade.

Results. By using flow cytometry, we demonstrated that HES 130/0.4 reduces neutrophil recruitment into the lung, liver, and kidneys during systemic inflammation (n=8 mice per group). IVM revealed a reduced number of adherent and transmigrated neutrophils in the cremaster and kidney after HES 130/0.4 administration (n=8 mice per group). Flow chamber experiments showed that HES 130/0.4 significantly reduced chemokine-induced neutrophil arrest (n=4 mice per group). Furthermore, HES 130/0.4 significantly reduced the formation of platelet–neutrophil aggregates, and NET formation during systemic inflammation (n=8 mice per group).

Conclusions. Our findings suggest that HES 130/0.4 significantly reduces neutrophil–platelet aggregates, NET formation, chemokine-induced arrest, and transmigration of neutrophils under inflammatory conditions.

Keywords: HES 130/0.4; inflammation; leucocyte; neutrophil extracellular traps; platelet

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During systemic inflammation, leucocytes are activated, interact with other cells, and are recruited to sites of inflammation.1 However, uncontrolled or overshooting activation and recruitment during systemic inflammatory processes may also be harmful and cause severe tissue damage leading to organ failure.2,3 The initial contact between leucocytes and endothelial cells is mediated by selectins which are expressed on inflamed endothelial cells and interact with their counter-receptor P-selectin glycoprotein ligand (PSGL)-1 on leucocytes.5 After the initial contact, leucocytes roll along the endothelium and collect several activating signals.2 Chemokines, presented on inflamed endothelial cells, bind to G-protein-coupled receptors on leucocytes and trigger an intracellular signalling cascade leading to integrin activation and leucocyte arrest.6 Leucocyte integrins then bind to their counter-receptors on endothelial cells, signal into the cell, and may fully activate the leucocyte inducing leucocyte extravasation.7 In addition, leucocytes also interact with platelets during systemic inflammation and this interaction may activate leucocytes and participate in their recruitment.6

Platelets are traditionally well recognized for their role in primary haemostasis, yet current research has shown a prominent role for platelets in leucocyte recruitment and inflammation.6 Platelets may bind to inflamed vascular endothelium and boost neutrophil recruitment by a process known as ‘secondary capturing’, in which neutrophils roll on adherent platelets expressing P-selectin,8 which binds to PSGL-1 on neutrophils.9 The interaction of platelets and neutrophils is further mediated...
by glycoproteins on activated platelets binding directly or indirectly to integrins on neutrophils.\textsuperscript{6} The formation of platelet–neutrophil aggregates is required for neutrophil recruitment into organs during systemic inflammation.\textsuperscript{10} Recently, it has been demonstrated that intimate platelet–neutrophil interactions induce the formation of neutrophil extracellular traps (NET) under inflammatory conditions.\textsuperscript{11, 12} During NETosis, neutrophils decondensate and release their nuclear DNA in long chromatin filaments that form extracellular, web-like structures.\textsuperscript{13} NETs contribute to the pathology of several inflammatory diseases.\textsuperscript{14–16}

The plasma expander hydroxyethyl starch (HES) is frequently used for fluid resuscitation in hypovolaemic patients and has been shown to have anti-inflammatory effects. Previously, HES was demonstrated to reduce neutrophil chemotaxis through monolayers of human umbilical vein endothelial cells (HUVEC),\textsuperscript{17} but it did not directly abrogate responses of neutrophils to activating agents.\textsuperscript{18, 19} Adhesion of flowing neutrophils to HUVEC that had been stimulated with tumour necrosis factor (TNF) \(\alpha\) or interleukin-1 (IL-1) was inhibited in the presence of HES, but HES had no effect on the responses of the HUVEC to these cytokines, in terms of adhesion molecule expression.\textsuperscript{19–21} Furthermore, in vitro experiments showed that HES does not inhibit adhesion per se, but modified the recruitment by endothelial cells in a differential manner, depending on TNF-\(\alpha\) dose, attributable to changes in selectin or chemokine expression at low or high dose, respectively. However, the effect of HES (130/0.4) on leucocyte recruitment and inflammation has not been investigated in vivo.

The aim of the present study was to investigate the molecular mechanism by which HES 130/0.4 influences neutrophil recruitment and inflammation during systemic inflammation.

**Methods**

**Animals**

We used 8- to 12-week-old male C57BL/6 mice. The mice were kept in a barrier facility under specific pathogen-free conditions. All animal experiments were approved by local government authorities, followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and relevant sections of the ARRIVE guidelines.

**Reagents**

Unlike otherwise stated, all reagents were obtained from Sigma–Aldrich (Taufkirchen, Germany).

**Murine model of systemic inflammation**

Systemic inflammation was induced by caecal ligation and puncture (CLP). Mice were anaesthetized by intraperitoneal injection of ketamine (125 \(\mu\)g \(\text{g}^{-1}\) body weight; Pfizer, New York, NY, USA) and xylazine (12.5 \(\mu\)g \(\text{g}^{-1}\) body weight; Bayer, Leverkusen, Germany). Sufficient anaesthesia for the analgesic withdrawal response to a nociceptive stimulus. Animals were killed by exsanguination through transcervical cardiac puncture under anaesthesia after the experiment. CLP was performed as described before.\textsuperscript{22} Briefly, a midline laparotomy incision was made after skin disinfection. The caecum was ligated 14 mm distal to the ileocecal valve so that continuity was preserved and then punctured twice with a 20 G needle. It was returned to the peritoneal cavity, and the wound was closed in two layers. Mice were then allowed to recover and had free access to food and water. Immediate postoperative analgesia was provided by subcutaneous injection of tramadol (15 mg \(\text{kg}^{-1}\)). During the observation period, analgesia was achieved using tramadol (2.5 mg \(100\text{ mL}^{-1}\)) in drinking water. Animals that failed to drink in the later stages of the observation period (>12 h) received subcutaneous injections of tramadol every 3 h. Animals in the sham group underwent the identical procedure without CLP. One hour after the procedure, the animals received 20 ml \(\text{kg}^{-1}\) HES 130/0.4 (Volulyte; Fresenius Kabi, Bad Homburg, Germany) or a control solution (balanced electrolyte solution: Isolyte; Fresenius Kabi) by i.v. injection into the tail vein through an intravascular catheter. Volulyte and Isolyte share the same electrolyte constituency (\(\text{Na}^+\) 137 mmol litre\(^{-1}\), \(\text{K}^+\) 4 mmol litre\(^{-1}\), \(\text{Mg}^2+\) 1.5 mmol litre\(^{-1}\), \(\text{Cl}^-\) 110 mmol litre\(^{-1}\), \(\text{CH}_3\text{COO}^-\) 34 mmol litre\(^{-1}\), osmolality 286.5 mmol litre\(^{-1}\)). Animals were randomized to treatment groups and the investigators were blinded to these groups. An animal group size of eight mice was analysed for each condition. Colony forming units (CFUs) in the lung, blood, and spleen 24 h after CLP were analysed as described previously.\textsuperscript{23}

**PMN recruitment into different organs**

Four, 8, 12, and 24 h after CLP, mice were killed and perfused with 20 ml phosphate-buffered saline (PBS) via the right and left ventricle. The lung, liver, and kidneys were removed, followed by mechanical tissue homogenization and digestion with collagenase and hyaluronidase at 37°C for 60 min. Neutrophils were analysed by flow cytometry (BD FacsCanto; Becton Dickinson, Heidelberg, Germany) based on the expression of CD45 (clone 30-F11, BD Biosciences, Franklin Lakes, NJ, USA), GR-1 (clone RB6-8C, 5 purified from hybridoma supernatant), and Ly6B.2 (clone 7/4, AbD Serotec, Düsseldorf, Germany). FACs data were processed using FlowJo (version 7.5.5; Tree Star, Inc., Ashland, OR, USA).

**Intravital microscopy of the cremaster and kidney**

To investigate the influence of HES 130/0.4 administration on leucocyte recruitment during systemic inflammation in vivo, we performed intravital microscopy (IVM) of the cremaster muscle and kidney as described previously.\textsuperscript{24–27}

**PMN capturing and chemokine-induced neutrophil arrest in vitro**

To investigate neutrophil capturing and chemokine-induced arrest, we used a whole blood-perfused murine microflow chamber system as described previously.\textsuperscript{24, 26} Murine whole blood was withdrawn from the left ventricle into a heparinized syringe from mice which had received an i.v. injection of 20 ml \(\text{kg}^{-1}\) HES 130/0.4 or control solution 30 min before. For neutrophil capturing, glass capillaries were coated with P-selectin.
HES 130/0.4 reduces inflammation

(20 μg ml⁻¹, R&D Systems, Minneapolis, MN, USA) for 2 h and then blocked for 1 h using casein (Thermo Fisher Scientific, Bonn, Germany). The number of rolling cells was analysed after 2 min of perfusion with whole blood at a shear stress of 5–6 dyn cm⁻². To investigate the chemokine-induced firm arrest of rolling neutrophils, a separate set of flow chambers was used. This flow chambers were coated for 2 h with P-selectin (20 μg ml⁻¹; R&D Systems), ICAM-1 (15 μg ml⁻¹; R&D Systems), and CXCL-1 (25 μg ml⁻¹; R&D Systems) or P-selectin and ICAM-1 as a control. After this treatment, the capillaries were blocked with casein for 1 h. Rolling and adhering cells per field of view were counted after 2 min of perfusion with whole blood at a shear stress of 5–6 dyn cm⁻². Videos were recorded using an upright microscope equipped with an SW40/0.75 objective and a digital camera (Sensicam QE, Cooke Corporation, Germany) and analysed using ImageJ (National Institutes of Health, USA). The ratio of adherent to rolling cells was calculated.

Quantification of platelet–neutrophil aggregates

The amount of platelet–neutrophil aggregates in murine blood in vivo was determined as described previously. For

Fig 1 HES 130/0.4 reduces leucocyte adhesion and transmigration during systemic inflammation in vivo. IVM of the cremaster muscle or the kidney after inducing systemic inflammation or sham operation. (A) Rolling flux fraction, (B) rolling velocity, and (C) number of intravascular adherent leucocytes in post-capillary venules of the cremaster muscle (n=8). (D) Number of leucocytes that transmigrated from the vessel into the surrounding tissue (n=8). (E) Number of intravascular adherent leucocytes in the cortical venules of the kidney after 4 h (n=8).
the quantification of platelet–neutrophil aggregates in vitro, isolated washed platelets were stimulated with thrombin (0.1 units ml⁻¹) at 37°C for 5 min and mixed with bone marrow-derived leucocytes obtained from wild-type mice. The reaction was stopped with the addition of 400 μl ice-cold PBS and the samples were incubated with CD45-PerCP antibody (clone 30-F11), CD41-PE antibody (clone MWReg30), Ly-6B.2-FITC antibody (clone 7/4), and Gr1-antibody (clone RB6-8C5) conjugated with Alexa633 for 10 min. Samples were analysed on a FACSCanto flow cytometer (BD Biosciences). FACS data were processed using FlowJo (version 7.5.5; Tree Star, Inc.).

**Statistical analysis**

The power analysis on the required group size, which was performed before initiating the experiments, was performed with G*Power software (G*Power 3.1.7 by Axel Buchner, University of Duesseldorf, Germany). Statistical analysis was performed with SPSS (version 21.0) using the Wilcoxon test or t-test as appropriate. More than two groups were compared using one-way analysis of variance followed by the Bonferroni testing. Data distribution was assessed using the Kolmogorov–Smirnov test or Shapiro–Wilks test. All data are represented as mean and SD. A P-value of <0.05 was considered as statistically significant. See Supplementary material for further information.

**Results**

HES 130/0.4 reduces leucocyte adhesion and transmigration during systemic inflammation after CLP in vivo.

The administration of HES130/0.4 did not change the number of neutrophils interacting with the endothelial cells (“rolling flux fraction”) and the rolling velocity under non-inflammatory and inflammatory condition (Fig. 1A and B). Animals treated with HES 130/0.4 or control solution had the same number of intravascular adherent leucocytes (Fig. 1C) and transmigrated leucocytes (Fig. 1D) in post-capillary venules of the cremaster muscle after sham operation. However, IVM of the cremaster muscle revealed a significant increase in the number of adherent (Fig. 1C) and transmigrated leucocytes (Fig. 1D) in mice with systemic inflammation compared with control mice. The administration of HES 130/0.4 under systemic inflammatory conditions significantly decreased the number of adherent (Fig. 1C) and transmigrated leucocytes (Fig. 1D) compared with mice which received the control solution.
Similar to the IVM of the cremaster muscle, IVM of the kidney revealed a significant increase in the number of intra-vascular adherent leucocytes in the cortical venules of the kidney during systemic inflammation (Fig. 1E). The administration of HES 130/0.4 significantly decreased the number of adherent leucocytes compared with mice which received the control solution (Fig. 1E).

To exclude the possibility that the systemic inflammation significantly reduces arterial blood pressure, which might alter perfusion of the microcirculation and subsequently reduces neutrophil recruitment, we measured the systolic arterial blood pressure in control mice and mice suffering from systemic inflammation. The arterial blood pressure in these groups was not different (Supplementary Fig. S1). To exclude the possibility that the application of HES 130/0.4 at doses of 20 ml kg$^{-1}$ over a period of 1 h, a dose similar to that used in patients, causes adverse haemodynamic effects in mice or increases vascular permeability, we measured the systolic arterial blood pressure and lung wet/dry ratio in control mice after administration of HES 130/0.4 over 1 or 2 h. There was no difference in systolic arterial blood pressure or lung wet/dry ration between the groups, indicating that HES 130/0.4 administration at 20 ml kg$^{-1}$ over 1 h does not alter the systolic arterial blood pressure or vascular permeability (Supplementary Fig. S1).

**HES 130/0.4 does not affect neutrophil capturing, but reduces chemokine-induced neutrophil arrest in vitro**

In order to analyse whether HES 130/0.4 affects neutrophil capturing, we performed flow chamber assays. Neutrophils in whole blood from animals treated with HES 130/0.4 and control solution showed the same number of rolling neutrophils on P-selectin (Fig. 2A). To investigate neutrophil rolling, we coated the flow chambers with either P-selectin and

**Fig 3**

HES 130/0.4 influences the release of inflammatory mediators during systemic inflammation. Plasma levels of CXCL1 (a), CXCL2 (a), IL-6 (c), and HMGB-1 (a) 4, 8, 12, and 24 h after sham or CLP procedure (n=8).
Fig 4. HES 130/0.4 influences neutrophil recruitment during systemic inflammation after inducing systemic inflammation. Neutrophil recruitment into the lung (A), liver (B), and kidney (C) and histological lung injury score (D) 4, 8, 12, or 24 h after induction of systemic inflammation or sham procedure (n = 8). Serum creatinine (E) and GPT levels (F) 24 h after induction of systemic inflammation or sham procedure (n = 6).
ICAM-1 or P-selectin alone. Neutrophils from mice treated with HES 130/0.4 or the control solution had the same rolling velocity on P-selectin alone (Fig. 2a). HES 130/0.4 did not influence the rolling velocity on P-selectin and ICAM-1 compared with the control solution (Fig. 2a). During capturing and rolling on inflamed endothelial cells, neutrophils are exposed to chemokines which cause firm arrest. To investigate a possible effect of HES 130/0.4 on chemokine-induced arrest, flow chambers were coated with P-selectin/ICAM-1/CXCL-1 or P-selectin/ICAM-1 as a control. In the absence of CXCL-1, only a low number of neutrophils adhered in the flow chambers perfused with whole blood from HES 130/0.4 or control-treated animals (Fig. 2c). However, neutrophil adhesion to P-selectin/ICAM-1/CXCL-1 was significantly reduced in mice with HES 130/0.4 compared with mice treated with the control solution (Fig. 2c).

**HES 130/0.4 influences the release of inflammatory mediators during systemic inflammation**

The plasma levels of the pro-inflammatory mediators CXCL1, CXCL2, IL-6, and HMGB-1 significantly increased during the course of the systemic inflammation (Fig. 3a–d). The application of HES 130/0.4 significantly reduced the plasma levels of the pro-inflammatory mediators CXCL1, CXCL2, IL-6, and HMGB-1 (Fig. 3a–d).

**HES 130/0.4 influences neutrophil recruitment during systemic inflammation**

The induction of systemic inflammation caused the recruitment of neutrophils into the lung and liver as early as 8 h and into the kidney 12 h after inducing systemic inflammation (Fig. 4a–c). After inducing systemic inflammation, the administration of HES 130/0.4 significantly reduced the number of recruited neutrophils in the lungs after 8 h (Fig. 4a) and in the liver and kidney after 12 and 24 h (Fig. 4b and c). The administration of HES 130/0.4 after induction of systemic inflammation significantly reduced histological lung injury score (Fig. 4a) and serum creatinine levels (Fig. 4c). However, it did not affect hepatic (Fig. 4f) tissue damage.

In order to address the haemodynamic effects of HES and the crystalloid control solution, we analysed heart rate, systolic arterial blood pressure, haematocrit, and serum lactate 24 h after the induction of systemic inflammation. Neither the haematocrit nor global haemodynamic variables (heart rate, systolic arterial blood pressure) were significantly different between the groups (Supplementary Fig. S2). In order to investigate the effect of HES on pathogen clearance, animals were killed 24 h after induction of systemic inflammation and we analysed the number of CFUs in the blood, lung, and spleen. The administration of HES 130/0.4 significantly increases the number of CFUs in the spleen and lung (Fig. 5a–c).

![Graphs showing bacterial clearance in blood, lung, and spleen](https://academic.oup.com/bja/article-abstract/114/3/509/2919995)

**Fig 5** Bacterial clearance. CFUs in the blood (a), lung (b), and spleen (c) from mice receiving HES or a control solution 24 h after induction of systemic inflammation or sham procedure (n=6).
A B

Sham Systemic inflammation

Control HES Control HES Control HES Control HES Control HES Control HES

4 h 8 h 12 h 24 h 4 h 8 h 12 h 24 h

PMNs PLTs PMNs PLTs PMNs PLTs PMNs PLTs

Unstimulated Thrombin

P=0.000 P=0.034

P=0.017

P=0.018

P=0.039

P=0.018

C D

Control HES

PMNs PLTs PMNs PLTs

Unstimulated Thrombin

Lung Liver Kidney

HES pretreatment

PMNs PLTs PMNs PLTs

Unstimulated Thrombin

E

HES pretreatment

P=0.001

P=0.045

P=0.019

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HES 130/0.4 reduces the formation of circulating platelet–neutrophil aggregates and NET formation

As formation of platelet–neutrophil aggregates is known to be involved in systemic and pulmonary inflammation, we investigated whether the administration of HES 130/04 after inducing systemic inflammation modulates this cell–cell interaction. During systemic inflammation, the number of platelet–neutrophil aggregates significantly increased (Fig. 6A). The administration of HES 130/0.4 reduced the number of circulating platelet–neutrophil aggregates at 4, 8, 12, and 24 h after inducing systemic inflammation compared with mice treated with the control solution (Fig. 6A). Similar to these findings, the pretreatment of platelets, but not neutrophils, with HES 130/0.4 also significantly reduced the formation of platelet–neutrophil aggregates in vitro (Fig. 6B).

The interaction of platelets with neutrophils is known to induce NET formation in vivo. To investigate a possible effect of HES 130/0.4 on NET formation, we analysed soluble NET components in the blood 8 h after inducing systemic inflammation or sham operation. Systemic inflammation led to a significant increase in soluble NET components in the blood compared with control mice (Fig. 6C). HES 130/0.4 significantly reduced soluble NET components in the blood after inducing systemic inflammation compared with the control solution (Fig. 6C). In addition, NET immunostaining in tissue sections from the lung, liver, and kidney was decreased in HES 130/0.4-treated animals after inducing systemic inflammation compared with mice treated with the control solution (Fig. 6D). Similar to platelet–neutrophil aggregate formation, the pretreatment of platelets, but not neutrophils, with HES 130/0.4 significantly reduced NET formation in vitro (Fig. 6E).

Discussion

This study demonstrates that HES 130/0.4 reduces inflammation by decreasing the number of platelet–neutrophil aggregates, NET formation, and leucocyte recruitment into different organs during systemic inflammation induced by CLP in mice. These results suggest that HES 130/0.4 has anti-inflammatory effects caused by different mechanisms.

Leucocyte recruitment into inflamed tissue proceeds in a cascade-like fashion. Disturbing one step of this adhesion cascade reduces leucocyte recruitment, which can be beneficial in the context of ischaemia/reperfusion injury and abacterial inflammation or harmful during bacterial infection. In vitro studies demonstrated that the administration of HES reduces neutrophil adhesion to endothelial cells in flow chamber assays. However, which step of the leucocyte adhesion cascade is influenced by the administration of HES was not investigated. Furthermore, the studies have not tested whether the reduced adhesion caused by HES results in a reduced leucocyte extravasation. Our in vivo study demonstrates that HES 130/0.4 (waxy maize) does not influence either the initial interaction with the endothelium or the rolling velocity of leucocytes. It has been shown that HES reduces selectin expression on HUVEC in vitro and reducing selectin expression on inflamed endothelial cells diminishes leucocyte–endothelial interactions. However, our in vivo experiments demonstrated that the administration of HES 130/0.4 did not change the number of interacting leucocytes with the endothelium, suggesting that this mechanism does not exist in vivo or that the reduction in selectin expression is so small that it does not influence leucocyte capturing. Furthermore, the administration of HES 130/0.4 during non-inflammatory and inflammatory conditions did not change the expression of adhesion molecules and chemokine receptors (PSGL-1, L-selectin, LFA-1, Mac-1, and CXCR2) on the cell surface of leucocytes (Supplementary Fig. S3), ruling out that the initial steps of the leucocyte recruitment cascade are influenced by the administration of HES 130/0.4. These findings are in line with previous reports investigating the effect of HES on neutrophil function in vitro. However, we demonstrated that HES 130/0.4 reduces neutrophil adhesion in vivo and in vitro by reducing chemokine-induced arrest. The reduced chemokine-induced arrest seen after the administration of HES 130/0.4 might be caused by a reduced function of cell surface receptors, since HES binds to cell surface receptors.

Flow chamber experiments, which exclude endothelial cells, suggest that the function of cell receptors on leucocytes is influenced by HES 130/0.4. As the administration of HES 130/0.4 does not completely abolish neutrophil recruitment, it could be that bacterial elimination is not affected by HES 130/0.4, but that the overwhelming leucocyte recruitment and inflammation during systemic inflammation are reduced and consequently tissue damage is diminished. However, this possible positive effect has to be investigated in future studies. In addition, HES has been demonstrated to increase cardiac index and improve microcirculation, in part via increased oxygen delivery due to increased plasma colloid oncotic pressure and viscosity during haemorrhagic shock, which might limit endothelial inflammation. This may have also contributed to the decreased neutrophil activation in our study.

Fig 6 HES 130/0.4 reduces the formation of circulating platelet–neutrophil aggregates and NET formation. (a) Platelet–neutrophil interactions in vivo in whole blood samples (n=8). (b) Thrombin-induced formation of platelet–neutrophil aggregates in vitro after incubation of neutrophils (PMNs), platelets (PLTs), or both with HES 130/0.4 or control solution (n=4). (c) Circulating NET components in the blood of control mice or mice receiving HES 130/0.4 or control solution after inducing systemic inflammation in vivo (n=8). (d) Representative images of immunofluorescence staining against NETs (red), neutrophils (green), platelets (grey), and nuclear DNA (blue) of the lung, liver, and kidney tissue sections from mice after either control solution or HES 130/0.4 h after inducing systemic inflammation. (e) Thrombin-induced NET formation of isolated neutrophils co-incubated with thrombin-activated platelets in vitro after incubation of neutrophils (PMNs), platelets (PLTs), or both with HES 130/0.4 or control solution (n=4).
Platelets play an important role in many inflammatory conditions, and it has been shown that the interaction of platelets with neutrophils is important for neutrophil recruitment into the lung and kidney and for NET formation. Eliminating platelets or reducing the interaction of platelets with neutrophils diminishes inflammation and neutrophil recruitment. During systemic inflammation, the administration of HES 130/0.4 reduced the number of platelet–neutrophil aggregates and NET formation. Platelet–neutrophil aggregate formation requires the interaction of distinct surface adhesion molecules on both platelets and neutrophils. Binding of HES to cell surface receptors may reduce the functionality of these receptors and subsequently reduce the formation of the aggregates. Our in vitro data indicate that HES primarily reduces aggregate formation by affecting the platelets. Furthermore, NET formation, which relies on the interaction between platelets and neutrophils under different inflammatory conditions, was also reduced as a consequence of the reduced formation of platelet–neutrophil aggregates caused by HES 130/0.4. NETs may activate endothelial cells and subsequently contribute to the regulation of vascular permeability and leucocyte recruitment. However, no research on the effect of HES on NET formation has been published to date. Thus, this is the first study to demonstrate that the administration of HES 130/0.4 reduces NET formation in vivo. Importantly, we also demonstrated that while neutrophil recruitment and NET formation were decreased after HES administration, the number of CFUs in the blood, lung, and spleen was increased in these animals. These data suggest that HES administration leads to increased bacterial growth and dissemination, probably through modulating systemic inflammation and host defence.

Another important finding of this in vivo study is that the administration of HES 130/0.4 after inducing systemic inflammation by CLP significantly reduced the blood concentrations of different pro-inflammatory mediators (CXCL1, CXCL2, IL-6, and HMGB-1). These results are in line with studies showing that the administration of HES reduced the production of pro-inflammatory mediators in an in vivo model of endotoxaemia mediated by preventing LPS-induced NF-κB activation. Accordingly, blocking neutrophil recruitment and the formation of platelet–neutrophil aggregates also reduces the concentration of pro-inflammatory cytokines in the blood.

This study has several limitations. The use of HES in critically ill patients with sepsis and septic shock has recently been shown to be associated with an increased risk of renal dysfunction and renal replacement therapy. The European Medicines Agency does not recommend the use of HES as a resuscitation fluid in patients with sepsis. Thus, it remains a matter of current debate to gauge the impact of the effects of HES on leucocyte recruitment and organ injury during systemic inflammation.

In summary, the data from this study show that the administration of HES 130/0.4 during systemic inflammation modulates inflammation by reducing the number of platelet–neutrophil aggregates, NET formation, and leucocyte recruitment into different organs.

Supplementary material
Supplementary material is available at British Journal of Anaesthesia online.

Authors’ contributions
J.R. and C.B.: performed the experiments, analysed the data, and wrote the manuscript. H.V.A.: revised the manuscript. F.K. and N.G.: performed experiments. A.Z.: conceived of the study, analysed the data, and wrote the manuscript. J.R. and C.B. contributed equally to this study and share first authorship.

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Declaration of interest
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References
4 Ley K. Arrest chemokines. Microcirculation 2003; 10: 289–95

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