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Dexmedetomidine does not directly inhibit neutrophil extracellular trap production

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Editor—Dexmedetomidine is a highly selective α2-adrenergic receptor agonist widely used in clinical anaesthesia as a sedative and analgesic agent. It has also been associated with numerous anti-inflammatory effects in preclinical models, including protection against leucocyte-mediated acute lung injury (ALI) after caecal ligation puncture, reducing pulmonary oedema in lipopolysaccharide-induced ALI, and attenuating cell injury in experimental severe acute pancreatitis via the cholinergic anti-inflammatory pathway. In a recent case report, clinical improvement upon dexmedetomidine treatment was suggested to have spared a patient with COVID-19 with worsening hypoxaemia from mechanical ventilation. Indeed, there are ongoing clinical trials registered to examine dexmedetomidine in palliative sedation for severe COVID-19 and to evaluate its immunomodulatory profile in patients recovering from COVID-19-related acute respiratory distress syndrome (ARDS).

Neutrophil extracellular trap formation (NETosis) is a specialised cell death process in which release of chromatin components such as DNA and histones provides a framework for trapping and killing invading microbes. However, when dysregulated, NETosis can also aggravate harmful inflammatory responses, including those driving the pathogenesis and thrombosis of severe COVID-19 in lungs and other major organs.

In 2020, Jain and colleagues hypothesised that ’given the anti-inflammatory effects of dexmedetomidine, it too may inhibit NETosis and be beneficial in COVID-19 patients’. They went on to provide a detailed schematic illustration of the many feedforward mechanisms potentiating NETosis during COVID-19 and the molecular pathways through which they predicted dexmedetomidine could act to inhibit NET activation.

Our research group has a longstanding interest in the biology and pathobiology of NETs in animal models of infectious diseases such as necrotising fasciitis and bacterial...
pneumonia,11 and recently we studied NET phenotypes in critically ill patients with COVID-19.12 In parallel, we have examined how NETosis is modulated by common medications including statins,13 tamoxifen,14 desferoxamine,15 and propofol.16 With this background, we tested the hypothesis that dexmedetomidine inhibits human NETosis.

Blood was collected from healthy adults under a protocol approved by the University of California San Diego Institutional Review Board (IRB), and neutrophils were isolated using the PolyMorphPrep™ Kit (Fresenius Kabi, Oslo, Norway) per the manufacturer’s instructions. The effective sedative concentration of dexmedetomidine in plasma has been estimated to be 0.2–3.2 ng ml⁻¹ (~1–16 nM).17 We stimulated neutrophils to produce NETs by exposure to live methicillin-resistant Staphylococcus aureus (MRSA) or to the classical NET inducer phorbol myristate acetate (PMA) at 25 nM, in the presence or absence of dexmedetomidine at final concentrations of 0.5, 5, 50, and 500 ng ml⁻¹. For all dexmedetomidine exposures, no

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**Fig 1.** (a) Fold change in NETosis triggered by either methicillin-resistant Staphylococcus aureus (MRSA; stationary phase, MOI 10, n=4) or phorbol myristate acetate (PMA; 25 nM, n=5) in the presence of increasing concentrations of dexmedetomidine. NET production (extracellular DNA) was quantified using PicoGreen dye. (b) Immunocytochemical analysis of NETosis in response to PMA (25 nM) in the presence or absence of several concentrations of dexmedetomidine (assessed using Transwell inserts with a 3 µm pore size as described previously, n=5). (c) Time course of reactive oxygen species (ROS) production by human neutrophils (measured at indicated time points using H₂DCFDA, n=3) in the presence or absence of dexmedetomidine either alone or with PMA. (d) Chemotaxis of human neutrophils in response to 100 nM N-formylmethionyl-leucyl phenylalanine (fMLP) in the presence or absence of several concentrations of dexmedetomidine (assessed using Transwell chemotaxis (405 nm) fluorescence). (e) Phagocytosis time course of S. aureus bioparticles in the presence and absence of several concentrations of dexmedetomidine, n=4. (f) MRSA killing by human neutrophils (MOI 10) in the presence of dexmedetomidine at 5 ng/ml (25 nM) and dexmedetomidine and yohimbine at indicated concentration as compared with the respective control without cells expressed as % colony-forming units (CFU) ml⁻¹, n=8. (g) MRSA killing by human neutrophils (MOI 10) in the presence of several concentrations of yohimbine as compared with the respective control without cells expressed as % CFU ml⁻¹, n=3. (h) amount of MRSA per g of either liver or kidney tissue after an 24 h ivu intraperitoneal (i.p.) challenge of CD-1 mice that received either dexmedetomidine or phosphate-buffered saline (PBS) i.p. at time of infection and 1 h after infection, n=24, 12 in each group. One-way analysis of variance with post hoc analysis was used to assess significance for data shown here, with the exception of the MRSA i.p. challenge, where unpaired Student’s t-test was used. *P<0.05; **P<0.01. CTL, control; DEX, dexmedetomidine; H₂DCFDA, 2',7'-dichlorodihydrofluorescein diacetate; NETOsis, neutrophil extracellular trap formation; NETs, neutrophil extracellular traps; n.s., not significant; YOH, yohimbine.
inhibition of NET production by MRSA- or PMA-stimulated neutrophils was seen via PICO green quantification of extracellular DNA release (Fig. 1a) or immunocytochemistry using antibodies against myeloperoxidase (Fig. 1b).

Neutrophil oxidative burst/generation of reactive oxygen species (ROS) can promote NETosis.18 We found that dexametomidine at final concentrations of 0.5, 5.0, and 50 ng ml⁻¹ did not inhibit PMA-induced neutrophil ROS production as measured by a 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA; Sigma-Aldrich, St Louis, MO, USA) fluorescence assay (Fig. 1c). While examining broader neutrophil functions, we found that similar dexametomidine concentrations did not significantly affect neutrophil chemotaxis across a Transwell membrane toward N-formylmethionyl-leucyl-phenylalanine (fMLP) (Fig. 1d), nor did it influence the efficiency of neutrophil phagocytosis of S. aureus-coated particles (pHrodo<sup>TM</sup> Red S. aureus Bioparticles; Invitrogen Corp., Carlsbad, CA, USA; Fig. 1e). In an ex vivo bactericidal assay, dexametomidine (5 ng ml⁻¹) impaired neutrophil killing of MRSA, an effect that was reversed by the α₂-adrenergic receptor antagonist yohimbine (Fig. 1f), whereas yohimbine alone did not significantly affect killing (Fig. 1g). Finally, in a murine intraperitoneal MRSA infection model approved by the University of California San Diego Institutional Animal Care and Use Committee (IACUC), treatment with 166 μg kg⁻¹ of dexametomidine i.p. at time of infection and again 1 h after bacterial challenge was associated with significantly increased recovery of bacterial colony-forming units (CFU) from kidneys 24 h later (Fig. 1h), although no change was seen in CFUs recovered from the liver.

We conclude that dexametomidine at therapeutically relevant concentrations and higher does not directly inhibit production of NETs by human neutrophils in response to commonly used NETosis inducers, nor does it significantly alter neutrophil behaviour in selected other common pheno- typic assays including ROS generation, chemotaxis, and phagocytosis. Dexametomidine slightly but significantly (1) impaired human neutrophil killing of MRSA in an α₂-adrenergic receptor-dependent manner and (2) reduced kidney bacterial burden in a murine systemic infection model, but it is premature to conclude whether these modest phenotypes are related or clinically significant for humans. Of note, dexametomidine is mainly hepatically metabolised and can reach liver concentrations much higher than plasma, after which its metabolites are primarily excreted through the kidneys.19

Our study has several limitations. First, we describe in vitro studies with purified human neutrophils and in vivo studies using mice, both relatively distant from the clinical setting. Second, the stimuli used to trigger NETosis and other neutrophil effector functions, although commonly used in the field, are not of viral origin. Follow-up ex vivo studies using COVID-19 patient blood, along with in vitro studies using activators of viral origin, will be important.

Several anaesthetic drugs are known to possess important anti-inflammatory and immunomodulatory properties, including those acting on neutrophils,27 that can influence their pharmacodynamics and clinical effectiveness. Of immediate impact, there is emerging clinical opinion that the immunomodulatory activities of dexametomidine might be harnessed to improve patient outcomes in severe COVID-19.28 Our studies, with the stated limitations, suggest that the proposed benefits do not include direct inhibition of extracellular trap formation by human neutrophils.

Authors’ contributions

Project concept: RC, VN, AM
Conduct of experiments: RC, BES, JOl, AM
Data analysis: RC, BES, AM
Data interpretation: VN, AM, RC
Editing of the manuscript: RC, AM, VC
Conduct of initial concept experiments: JOk, AM
Conduct of imaging experiment: AM, IMS
Writing of the manuscript: VN, AM

Declarations of interest

The authors declare that they have no conflicts of interest.

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High plasma dipeptidyl peptidase 3 levels are associated with mortality and organ failure in shock: results from the international, prospective and observational FROG-ICU cohort

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Editor—Shock is a common condition associated with high morbidity and mortality in the ICU. Rapid and accurate stratification of the patient in shock could improve referral to an appropriate care centre, management, and prognosis. Few biomarkers have proved their value in the stratification of shocked patients. Circulating dipeptidyl peptidase 3 (cDPP3) is a metallo-peptidase involved in the metabolism of cardiovascular peptides. Recent studies demonstrated the ability of cDPP3 to predict poor outcomes in septic or cardiogenic shock. However, the prognostic properties of cDPP3 in haemorrhagic shock remain unknown. Thus, the aim of the present study was to assess the ability of cDPP3 to predict outcome in septic, cardiogenic, and haemorrhagic shock in a substudy of the FROG-ICU study.

This study is an ancillary analysis of the FROG-ICU study (NCT01367093) which has been described. The purpose of this study was to assess the incidence of mortality in the year after ICU discharge. Patients were enrolled from August 2011 to June 2013. Haemorrhagic shock was defined as a hypovolaemic shock requiring catecholamines secondary to severe blood loss. Septic shock was defined according to the Third International Consensus definition for sepsis and septic shock. Cardiogenic shock was defined as cardiac impairment that results in reduced systolic BP (SBP) <90 mm Hg or inotrope use.