Inhaled and systemic heparin as a repurposed direct antiviral drug for prevention and treatment of COVID-19

Authors: Carina Conzelmann, Janis A Müller, Lukas Per khofer, Konstantin MJ Sparrer, Alexander N Zelikin, Jan Mü nch, and Alexander Kleger

Here, we advocate a wholly evolutive opportunity for the treatment of COVID-19 disease by repurposing a long-serving medical agent with an excellent history of clinical use, namely heparin. Heparin is best known as an anticoagulant, but it also exhibits direct antiviral activity against many enveloped viruses and has anti-inflammatory activity. The high incidence of thromboembolic events in COVID-19 patients suggests that coagulopathy plays an important role in the SARS-CoV-2 pathogenesis. This already makes heparin a unique, potentially curative agent that can be used immediately to help resolve the ongoing crisis associated with SARS-CoV-2 infection and COVID-19 disease. We demonstrate here in vitro that heparin does indeed inhibit SARS-CoV-2 infection. The three concurrent modes of activity of heparin (antiviral, anticoagulant and anti-inflammatory) against SARS-CoV-2/COVID-19 form a unique therapeutic combination. Thus, repurposing of heparin to fight SARS-CoV-2 and COVID-19 appears to be a powerful, readily available measure to address the current pandemic.

KEYWORDS: COVID-19, heparin, SARS-CoV-2, Inhalation, pulmonary coagulation

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Introduction

COVID-19 is an emerging infectious disease with a high case fatality rate. It is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is transmitted between humans by respiratory droplets, aerosols or rarely via contaminated surfaces. SARS-CoV-2 is a beta coronavirus which was described in Wuhan, China in December 2019 for the first time. It is genetically 79.5% identical to SARS-CoV which caused the SARS outbreak in 2002 and similar to the MERS-CoV (Middle East respiratory syndrome-related coronavirus) discovered in 2012.

The virus primarily infects the respiratory tract, causing no or mild symptoms in the majority of cases, while a smaller proportion develops severe disease with pneumonia and, upon spread to other body compartments, multi-organ failure. Currently, patients with COVID-19 are mostly treated with supportive care. Accordingly, literature survey reveals that the highest success registered to date lies not in the realm of antiviral drugs to address the infectivity of SARS-CoV-2, but is instead due to this symptomatic and supportive treatment of COVID-19 itself. Widely available vaccination strategies are not expected before the year 2021. As the development of novel drugs usually takes years, the fastest route to establish a new treatment option is to repurpose an existing drug. Indeed, a number of drug preparations developed in another context are currently being evaluated in clinical trials for COVID-19. Of these, the direct antiviral agent remdesivir has recently received an emergency use authorisation from the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). However, according to the most recent data, remdesivir only shortens hospitalisation and weakens disease course without reducing overall mortality. The first repurposed drug was dexamethasone, which reduced 28-day mortality among patients receiving invasive mechanical ventilation, as recently communicated by the RECOVERY consortium and published as a preprint manuscript. Conversely, several promising repurposed formulations have failed. Active viral replication and shedding seems to take place in the upper respiratory tract during the early phase of COVID-19, followed by either immune-mediated viral clearance or transmission to the lower respiratory tract. In its later stages, COVID-19 presents with progressive respiratory failure, possibly caused by viral replication in the lungs and cytokine-induced hyperinflammation. Hence, one strategy to prevent clinical deterioration and respiratory failure might be to impede locoregional viral replication in the early disease phase, eg by inhaled drug formulations.
Thromboembolic events during COVID-19

Besides obvious respiratory symptoms, the broad tropism of SARS-CoV-2 appears responsible for a variety of atypical manifestations of COVID-19.15 Accordingly, a series of manuscripts reported the importance of anticoagulation to prevent and/or treat thromboembolic complications in COVID-19 patients.16–19 Along these lines, a recent autopsy study, among others, confirmed thromboembolic events as a major cause of death in critically ill COVID-19 patients, all pointing toward an obviously relevant prothrombotic condition caused by SARS-CoV-2.17,19 Thus, systemic anticoagulation becomes one of the major columns in lowering death rates in critically ill COVID-19 patients. A recent study conducted a propensity score matching to illuminate the importance of anticoagulation to prevent and/or treat thromboembolic complications in COVID-19 patients.20–23 Specifically, inhaled dosages of up to 150,000 IU/d heparin marked a threshold dose to cause systemic effects detected by activated partial thromboplastin time (aPTT) prolongation while causing no relevant side effects in patients with distinct chronic lung disease.11,20 Accordingly, in 2006 an orphan designation to improve mucosal clearance in cystic fibrosis patients with inhaled heparin has been provided by the EMA. Further to being the most prominent approved anticoagulant, heparin is also known to have antiviral activity against diverse viruses, including SARS-CoV-2 (Fig 1).24–27 Thus, the overall effect of heparin to treat SARS-CoV-2/COVID-19 is unique, and, unlike directly acting antivirals, covers both the pathogen and the ensuing disease. Specifically, it has three modes of action: direct antiviral,25,26,28 anti-inflammatory,29 and anticoagulant,30 as outlined below.

Heparin exerts direct antiviral activity

Heparin is a broadly active antiviral agent that inhibits different enveloped viruses including coronaviruses.25–27 Applying heparin per inhalationem could imply that concentrations of heparin would be highest in tissues that are most affected by SARS-CoV-2, namely the upper and lower respiratory tract. Of note, inhaled heparin has a proven broad distribution in the respiratory tract including the alveolar space.30,31 Specifically, the antiviral activity of heparin is based on affinity of viral glycoproteins to negatively charged glycosaminoglycans such as sulfated heparin, which are ubiquitously expressed on the surface of mammalian cells. It has recently been demonstrated in a published and a preprint article that soluble heparin interacts with the SARS-CoV-2 spike protein28,31 and inhibits SARS-CoV-2 spike pseudovirus entry.31 In line with these findings, another preprint article showed that porcine heparin prevents wildtype SARS-CoV-2 infection of Vero E6 cells.29 Thus, we here set out to determine the antiviral activity of heparin against SARS-CoV-2. We conducted a viral plaque reduction assay in Vero E6 cells inoculated with a Dutch SARS-CoV-2 isolate in the absence or presence of various concentrations of heparin. Heparin not only resulted in a decrease in the number of plaques (Fig 1a,b)
but in particular also resulted in a decrease in the size of the plaques (Fig 1a). In order to quantify the plaque sizes, we calculated the total virus-induced cytopathic effect per well using a custom ImageJ macro as the percentage of non-stained area within one well (Fig 1c). This evaluation shows that in the presence of 500–1,000 µg/ml heparin, viral replication is almost completely inhibited, and at 125–250 µg/ml suppressed by more than 60%. Thus, heparin prevents SARS-CoV-2 infection and subsequent replication with a half maximal inhibitory concentration (IC_{50}) below 125 µg/ml.

### Anti-inflammatory and anticoagulant effects of heparin in the lung

Apart from these direct antiviral properties, heparin is known to have anti-inflammatory activity via, among other mechanisms, its ability to neutralise a variety of cationic immune mediators including IL-6 and IL-8 and to inhibit chemotaxis.\(^3\)\(^5\)\(^6\) In mammals, heparin is found in mast cells, suggesting its physiological usefulness in mucosal and connective tissue. Inhaled heparin has been used in patients with cystic fibrosis for its proposed mucolytic activity and a reduction of connective tissue. Inhaled heparin is being used in patients with mast cells, suggesting its physiological usefulness in mucosal and connective tissue. Inhaled heparin is being used in patients with

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**References**

1. Wadman M, Cousin-Frankel J, Kaiser J, Matacic C. A rampage through the body. Science 2020; 368:356–60.
2. Meselson M. Droplets and aerosols in the transmission of SARS-CoV-2. N Engl J Med 2020; 382:2063.
3. Beigel JH, Tomashek KM, Dod LE et al. Remdesivir for the treatment of COVID-19 - Preliminary Report. N Engl J Med 2020, in press (DOI: 10.1056/NEJMoa2007764).
4. Pushpakom S, Jiao F, Eyers PA et al. Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discovery 2019;18:41–58.
5. Li G, De Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). Nat Rev Drug Discov 2020;19:149–50.
6. Graham BS. Rapid COVID-19 vaccine development. Science 2020;368:945–6.
7. Dixon B, Santamaria JD, Campbell DJ. A phase 1 trial of nebulised heparin in acute lung injury. Curr Care 2008;12:R64.
8. Horby P, Lim WS, Emberson J et al. Effect of dexamethasone in hospitalized patients with COVID-19: preliminary report. medRxiv 2020; 2020.2006.2022.20137273.
9. Geleris J, Sun Y, Platt J et al. Observational study of hydroxychloroquine in hospitalized patients with Covid-19. N Engl J Med 2020;382:2x01–8.
10. Cao B, Wang Y, Wen D et al. A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 2020;382:1787–99.
11. Wölfel R, Corman VM, Guggemos W et al. Virological assessment of hospitalized patients with COVID-19. Nature 2020;581:645–9.
12. Dixon B, Schultz MJ, Smith R et al. Nebulized heparin is associated with fewer days of mechanical ventilation in critically ill patients: a randomized controlled trial. Curr Care 2010;14:R180.
13. Smoot P. Nebulized heparin for the treatment of COVID-19 induced lung injury. https://clinicaltrials.gov/ct2/show/NCT04397510.
14. Gilead Sciences Inc. Working to supply remdesivir for COVID-19. www.gilead.com/purpose/advancing-global-health/covid-19/working-to-supply-remdesivir-for-covid-19.
15. Puelles VG, Lutgehetmann M, Lindenmeyer MT et al. Multiorgan and renal tropism of SARS-CoV-2. N Engl J Med 2020;383:590–2.
16. Helms J, Tacquard C, Severac F et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive Care Med 2020, in press (DOI: 10.1007/s00134-020-06062-x).
17. Bompard F, Monnier H, Saab I et al. Pulmonary embolism in patients with Covid-19 pneumonia. Eur Respir J 2020;56:2001365.
18. Stoneham SM, Milne KM, Nutall E et al. Thrombotic risk in COVID-19: a case series and case-control study. Clin Med 2020;20:e76–e81.
19. Wichmann D, Sperhake JP, Lutgehetmann M et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. Ann Intern Med 2020, in press (DOI: 10.7326/M20-2003).
20. Yang Y, Tang H. aberrant coagulation causes a hyper-inflammatory response in severe influenza pneumonia. Cell Mol Immunol 2016;13:432–42.
21. Choudhury R, Barrett CD, Moore HB et al. Salvage use of tissue plasminogen activator (tPA) in the setting of acute respiratory distress syndrome (ARDS) due to COVID-19 in the USA: a Markov
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decision analysis. World J Emerg Surg 2020;15:29.

22 Oduah EI, Linhardt RJ, Sharfstein ST. Heparin: past, present, and future. Pharmaceuticals (Basel) 2016;9:38.

23 Laterre PF, Wittebole X, Dhainaut JF. Anticoagulant therapy in acute lung injury. Crit Care Med 2003;31:5329–36.

24 Yip LY, Lim YF, Chan VN. Safety and potential anticoagulant effects of nebulised heparin in burns patients with inhalational injury at Singapore General Hospital Burns Centre. Burns 2011;37: 1154–60.

25 Lang J, Yang N, Deng J et al. Inhibition of SARS pseudovirus cell entry by lactoferin binding to heparan sulfate proteoglycans. PLOS ONE 2011;6:e23710.

26 Mycroft-West C, Su D, Ellis S et al. The 2019 coronavirus (SARS-CoV-2) surface protein (Spike) S1 receptor binding domain undergoes conformational change upon heparin binding. bioRxiv 2020; 2020.02.2029.971093.

27 Vicenzi E, Canducci F, Pinna D et al. Coronaviridae and SARS-associated coronavirus strain HSR1. Emerg Infect Dis 2004;10:413–8.

28 Mycroft-West CJ, Su D, Pagani I et al. Heparin inhibits cellular invasion by SARS-CoV-2: structural dependence of the interaction of the surface protein (spike) S1 receptor binding domain with heparin. bioRxiv 2020; 2020.04.28.066761.

29 Young E. The anti-inflammatory effects of heparin and related compounds. Thrombosis Research 2008;122:743–52.

30 Bendstrup KE, Jensen JI. Inhaled heparin is effective in exacerbations of asthma. Respir Med 2000;94:174–5.

31 Markart P, Nass R, Ruppert C et al. Safety and tolerability of inhaled heparin in idiopathic pulmonary fibrosis. J Aerosol Med Pulm Drug Deliv 2010;23:161–72.

32 Monagle K, Ryan A, Hepponstall M et al. Inhalational use of antithrombotics in humans: Review of the literature. Thromb Res 2015;136:1059–66.

33 Kim SY, Jin W, Sood A et al. Characterization of heparin and severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) spike glycoprotein binding interactions. Antiviral Res 2020;181:104873.

34 Tandon R, Sharp JS, Zhang F et al. Effective inhibition of SARS-CoV-2 entry by heparin and enoxaparin derivatives. bioRxiv 2020; 2020.06.08.140236.