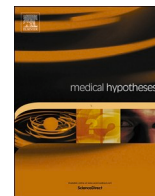




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## Polycations and polyanions in SARS-CoV-2 infection

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### ABSTRACT

We hypothesize that polycations, such as nuclear histones, released by neutrophils COVID-19 aggravate COVID-19 by multiple mechanisms: (A) Neutralization of the electrostatic repulsion between the virus particles and the cell membrane, thereby enhancing receptor-mediated entry. (B) Binding to the virus particles, thereby inducing opsonin-mediated endocytosis. (C) Adding to the cytotoxicity, in conjunction with oxidants, cytokines and other pro-inflammatory substances secreted by cells of the innate immunity system. These effects may be alleviated by the administration of negatively charged polyanions such as heparins and heparinoids.

### Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological cause of coronavirus disease 2019 (COVID-19), the respiratory illness responsible for the current world pandemic [1].

### The hypothesis

In this short communication, we propose that polycations such as histones secreted by neutrophils following viral infection may aggravate COVID-19 by several mechanisms that may be mitigated by the administration of negatively charged polyanionic drugs such as heparins and heparinoids.

### Evaluation of the hypothesis

COVID-19 is initiated by viral infection of susceptible cells of the respiratory tract mainly by receptor (ACE2)-mediated endocytosis [2], followed by viral multiplication and, consequently, activation of the innate immune system. The latter involves mobilization of inflammatory neutrophils by chemotaxis, their accumulation in infected areas such as the lungs, and secretion of toxic proinflammatory agents and various cytokines that are responsible for the severe consequences, and sometimes fatality, of the disease (the cytokine storm) [3].

COVID-19 is involved in excessive neutrophils and dysregulated neutrophil extracellular traps (NETs) [4]. The latter are three-

dimensional lattices of decondensed chromatin decorated with cationic histones and additional antimicrobial proteins that are released from neutrophils upon stimulation [5]. Pathogens, including respiratory viruses, induce NET formation that physically trap and kill them as part of the innate immune response [6]. In access, NETs can mediated tissue damage and hypercoagulability [7] and they are associated with acute and chronic inflammation [8]. In COVID-19, their dysregulated formation is thought to be associated with thrombosis [9]. In addition to NET formation, neutrophils secrete a plethora of various cytokines, reactive oxygen and nitrogen species, hypochlorous acid, lipase, and the membrane-penetrating agents phospholipase A2 and lysophosphatase which directly permeabilize lung cells [10]. They also secrete polycationic proteins such as histones, cathepsin, elastase, cationic proteinases and the Cathelicidin antimicrobial peptides, LL37, [11]. We hypothesize that these agents may have several effects that aggravate COVID-19.

(A) Both lung cells [12,13] and viruses, including viruses of the Corona family [14], carry negative-charged domains on their surfaces that may result in electrostatic repulsion that mitigates infection. Polycations such as histones may neutralize negative charges on the virus and cell surfaces, reduce their electrostatic repulsion, and thereby, enhance the adsorption and entry of viruses by receptor-mediated endocytosis, thus, facilitating the spreading of the virus.

(B) Polycations have been shown to opsonize negatively-charged surfaces of cells, bacteria and viruses and facilitate their phagocytosis not only by professional phagocytes such as neutrophils and

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macrophages, but also by fibroblasts, endothelial cells and epithelial cells [15]. A similar mechanism may facilitate opsonin-mediated endocytosis of SARS-CoV-2 to the lung epithelia in COVID-19.

(C) Many polycations are cytotoxic, adding to the deleterious effects on the lung epithelia of substances such as cytokines, reactive oxygen and nitrogen species as well as phospholipase A2 and lysophosphatidates [15]. Cell damage in COVID-19 may also be augmented by microbial co-infections: bacteriolysis caused by antibiotics or cationic lysozyme release membrane-injuring and perforating agents [16].

The effects of the polycations might be inhibited to some extent by the administration of highly negatively charged polyanions such as heparins and heparinoids. Heparin is a member of a family of long, linear, highly sulfated heparans that have the highest negative charge density of any known biomolecule [17]. This charge allows heparin to strongly interact with proteins, such as the serine protease inhibitor antithrombin-III that provides it with anticoagulant activity. However, hundreds of other biologically relevant, heparin-protein interactions with potential clinical significance have been described, including the ability to neutralize polycations released by neutrophils [18]. For example, it has been reported that heparin binds histones and prevents histone-mediated cytotoxicity independent of its anticoagulant properties [19].

Heparin has been proposed as a therapeutic modality for COVID-19 mainly as an anticoagulant [20]. Severe SARS-CoV-2 infection with acute lung injury is complicated with coagulopathy: disseminated intravascular coagulation is involved in the majority of deaths [20]. Therefore, The World Health Organization (WHO) recommended in these patients thrombo-prophylaxis with either unfractionated or low molecular weight heparin (<https://www.who.int/publications/i/item/clinical-management-of-covid-19>). However, in addition to anticoagulant, heparin possesses anti-inflammatory, anti-complement, immunomodulatory as well as anti-viral activities [21].

Anti-inflammatory effects of heparin were reported both in the vasculature and in the airway, which could beneficially affect COVID-19. They fall into two mechanisms [21]: (A) Interaction with proinflammatory proteins. (B) Prevention of the influx and adhesion of inflammatory cells to the diseased areas.

As for its anti-viral potential, we suggest that heparin may neutralize the effect of polycations on the virus adsorption and entry. In addition, surface heparan sulfate has been demonstrated to be essential for entry and infectivity of human coronaviruses [22,23], including the SARS-CoV-2 [24], by strongly binding to the spike protein thus facilitating the interaction of the virus with its receptor [23]. Heparin may bind the spike protein and function as a competitive inhibitor for viral entry, thus decreasing infectivity. Interestingly, shorter-length heparins, comparable to those used in anticoagulation therapy, did not appreciably bind the spike protein [22,24]. Clinical trials aim to demonstrate the benefit of heparin therapy should, therefore, include evaluation of the disease course and time to virus clearance, markers of direct antiviral activity, rather than solely coagulation status [25]. Despite this immense potential, no clinical data is yet available.

## Conclusions

We propose that substances, such as cationic histones secreted by neutrophils, in addition to their direct cytotoxic effects, may enhance infection of lung cells, and thereby aggravate the disease, while the administration of heparin and heparinoids might have to other therapeutic effects in addition to their anti-coagulating effect. Other agents of therapeutic value may be antioxidants such as ascorbate, N-acetyl cysteine, glutathione, and antioxidant agents present in fruits and vegetables, and also the proteinase inhibitor aprotinin, and also steroids and additional anti-inflammatory agents which may reduce the cytotoxic effect of agents secreted by cells in response to the viral infection. Finally, a co-infection with SARS-CoV-2 and influenza virus may occur

during winter times creating a toxic synergy between the two viruses. The recent development of vaccines against SARS-CoV-2 infection is a blessed promising tactic to lower the morbidity and mortality rates of COVID-19. However, the recent appearance of viral variant is a disturbing news – only time will tell if vaccination will effectively protect against these mutants.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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