Medical Hypotheses 146 (2021) 110470

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Polycations and polyanions in SARS-CoV-2 infection

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ARTICLE INFO

Keywords:
COVID-19
SARS-CoV-2
Therapy
Neutrophils
Polycations
Polyanions
Heparin

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 pandemia [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-mediate 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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https://doi.org/10.1016/j.mehy.2020.110470
Received 24 October 2020; Accepted 17 December 2020
Available online 4 January 2021

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 lysophopspahyatides [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-infloammatory 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.

Acknowledgement

Supported by an endowment fund by the late Dr. SM Robbins, Cleveland, Ohio, USA.

References

[1] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
[2] Ni W, Yang X, Yang D, et al. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Crit Care 2020;24:422.
[3] Tang Y, Liu J, Zhang D, Xu Z, Ji J, Wen C. Cytokine storm in COVID-19: the current evidence and treatment strategies. Front Immunol 2020;11:1708.
[4] Wang J, Li Q, Yin Y, et al. Excessive neutrophils and neutrophil extracellular traps in COVID-19. Front Immunol 2020;11:2063.
[5] Fuchs TA, Abed U, Goosmann C, et al. Novel cell death program leads to neutrophil extracellular traps. J Cell Biol 2007;176:231–41.
[6] Schonrich G, Raftery MJ. Neutrophil extracellular traps go viral. Front Immunol 2016;7:366.
[7] Fuchs TA, Brill A, Duerschmied D, et al. Extracellular DNA traps promote thrombosis. Proc Natl Acad Sci USA 2010;107:15880–5.
[8] Bonaventura A, Liberale L, Carbone F, et al. The pathophysiological role of neutrophil extracellular traps in inflammatory diseases. Thromb Haemost 2018;118:6–27.
[9] Middleton EA, He XY, Denorme F, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood 2020;136:1169–79.
[10] Ginsburg I, Kohen R. Synergistic effects among oxidants, membrane-damaging agents, fatty acids, proteinases, and xenobiotics: killing of epithelial cells and release of arachidonic acid. Inflammation 1995;19:101–18.
[11] Ginsburg I. Cationic polyelectrolytes: a new look at their possible roles as opsonins, as stimulators of respiratory burst in leukocytes, in bacteriolysis, and as modulators of immune-complex diseases (a review hypothesis). Inflammation 1987;11:489–515.
[12] Lipman KM, Dodelson R, Hays RM. The surface charge of isolated toad bladder epithelial cells. Mobility, effect of pH and divalent ions. J Gen Physiol 1966;49:501–16.
[13] Ma Y, Poole K, Goyette J, Gaus K. Introducing membrane charge and membrane potential to T cell signaling. Front Immunol 2017;8:1513.
[14] Verma S, Bednar V, Blount A, Hogue BG. Identification of functionally important negatively charged residues in the carboxy end of mouse hepatitis coronavirus A59 nucleocapsid protein. J Virol 2006;80:4344–55.
[15] Ginsburg I. Cationic polyelectrolytes: potent opsonic agents which activate the respiratory burst in leukocytes. Free Radic Res Commun 1989;8:11–26.
[16] Ginsburg I. Multi-drug strategies are necessary to inhibit the synergistic mechanism causing tissue damage and organ failure in post infectious sequelae. Inflammopharmacology 1999;7:207–17.
[17] Weiss RJ, Esko JD, Tor Y. Targeting heparin and heparan sulfate protein interactions. Org Biomol Chem 2017;15:5656–68.
[18] Ginsburg I, Sela MN, Morag A, et al. Role of leukocyte factors and cationic polyelectrolytes in phagocytosis of group A streptococci and Candida albicans by neutrophils, macrophages, fibroblasts and epithelial cells: modulation by anionic polyelectrolytes in relation to pathogenesis of chronic inflammation. Inflammation 1981;5:289–312.
[19] Wildhagen KC, Garcia de Frutos P, Reutelingsperger CP, et al. Nonanticoagulant heparin prevents histone-mediated cytotoxicity in vitro and improves survival in sepsis. Blood 2014;123:1098–101.
[20] Tang N, Bai H, Chen X, Gong J, Li D, Sun Z, et al. Anticoagulant treatment is associated with decreased mortality in severe coro-navirus disease 2019 patients with coagulopathy. J Thromb Haemost 2020;18:1094–9.
[21] Gozzo L, Viale P, Longo L, Vitale DC, Drago F. The potential role of heparin in patients with COVID-19: beyond the anticoagulant effect. A review. Front Pharmacol 2020;11:1307.
[22] Lang J, Yang N, Deng J, et al. Inhibition of SARS pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans. PLoS ONE 2011;6:e23710.

[23] Milewska A, Nowak P, Owczarek K, et al. Entry of human coronavirus NL63 into the cell. J Virol 2018;92.
[24] Kim SY, Jin W, Sood A, et al. Glycosaminoglycan binding motif at S1/S2 proteolytic cleavage site on spike glycoprotein may facilitate novel coronavirus (SARS-CoV-2) host cell entry. bioRxiv 2020 (Preprint).
[25] Hippensteel JA, LaRiviere WB, Colbert JF, Langouet-Astrie CJ, Schmidt EP. Heparin as a therapy for COVID-19: current evidence and future possibilities. Am J Physiol Lung Cell Mol Physiol 2020;319:L211–7.