A hypothetical approach to handle SARS-CoV-2: Breaking or bending the viral spikes

Alaa Shaheen

Kafr El-Sharakwa Medical Center, Aga, Dakahlia, Egypt.

ORCID iD: https://orcid.org/0000-0001-7692-3336

Correspondence:

email: alaa-shaheen@outlook.com

Abstract

A new epidemic caused by SARS-CoV-2 started in Wuhan, China in 2019 and affected more than two million people around the world with high rate of mortality.

In this hypothetical article, a new prophylactic and therapeutic measure is suggested, based on observations in previous studies during electron microscopic examination of several members of the orthocoronavirinae subfamily, including the new virus SARS-CoV-2. However, this measure needs to be confirmed experimentally before consideration. Also, a preconditioning step against the cytokine storm of COVID-19 is proposed. A new line of research is also proposed to find a broad spectrum antivirus against several members of of this subfamily.

Keywords

COVID-19, Cytokine storm, fasting, formaldehyde, glutaraldehyde, ketosis, ketogenic diet, preconditioning, SARS-CoV-2, schiff base, virus spikes.

Introduction

Coronaviridae is a family of single-stranded RNA viruses that have an envelope and surface projections called spikes [1]. These viruses use the spikes to attach to host cells during infection. Orthocoronavirinae and letovirinae are subfamilies of the coronaviridae family [2]. Orthocoronavirinae is further divided into four genera named Alpha, Beta, Delta and Gammacoronaviruses.

Glutaraldehyde is a dialdehyde commonly used in laboratory research as a homobifunctional fixative [3]. It has the chemical formula $C_5H_8O_2$ with two carbonyl groups (one at each end). Formaldehyde is another aldehyde with only one carbonyl group and is also used in chemical fixation and virus inactivation [4]. It has the chemical formula CH_2O and it can polymerize to form paraformaldehyde.

Observations

In one study, during electron microscopic examination of Vero E6 cells infected with SARS-CoV (genus Betacoronavirus), Snijder et al. [5] reported that the viral spikes of secreted extracellular virions were only visible in cryofixed samples but were rarely seen in chemically fixed samples (with 1.5% glutaraldehyde). This effect of chemical fixation was also observed in the extracellular virions of the transmissible gastroenteritis coronavirus (genus Alphacoronavirus) [6] which affects newborn piglets (fixation with a mixture of 2% glutaraldehyde and 2% tannic acid). For the new virus SARS-CoV-2, virus particles in the supernatant of infected cell cultures that were inactivated with 2% paraformaldehyde also showed a degree of loss of viral spikes [7].

Interpretation

Since chemical fixation leads to the cessation of all biological processes inside cells (including the secretion of new virions), this effect of chemical fixation most probably results from direct chemical interaction between the fixative and the viral proteins (most probably the spike protein). The fixatives appear to interact with the spike protein in such a way that leads to its bending or separation from the virion. Also, since this effect is

evident in several members of the orthocoronavirinae subfamily, these fixatives appear to interact with a conserved sequence of the spike protein in all those members.

The next questions to ask are, what is the nature of this interaction? and how can we benefit from it? Several studies [8,9,10] reported that glutaraldehyde can interact to any significant extent only with lysine amino acids on the surface of proteins. The carbonyl group of glutaraldehyde reacts with the -amino group of lysine side chain to form schiff bases. Formaldehyde was also shown to form schiff bases during interaction with proteins [11]. Schiff base reaction involves the formation of a carbon-nitrogen double bond when the carbonyl group of an aldehyde or ketone reacts with a primary amine [12]. Since both fixatives form schiff bases during interaction with proteins, these bases are most likely responsible for the loss of viral spikes in the mentioned studies [5,6,7]. The formation of these bases may alter the conformation of the spike protein in a way that leads to its separation from the virion or alternatively, leads to its bending. Supporting this view, glutaraldehyde was shown to cause conformational changes in several soluble and membrane proteins [10,13], through interaction with lysine residues via schiff base reaction. Similarly, formaldehyde was also shown to induce protein conformational changes [14,15]. Based on this, other short aldehydes and ketones that have carbonyl groups capable of forming schiff bases with the spike protein could be speculated to have the same effect. One such compound could be the ketone bodies. Similar to glutaraldehyde and formaldehyde, ketone bodies can also alter the conformation of proteins they react with, most probably via schiff base reaction [16].

Proposed measure: ketosis

There are three types of ketone bodies in the human blood [17]. These include beta-hydroxybutyrate, acetoacetate and the least abundant one, acetone. They are synthesized in the liver from fatty acids and released in the blood to act as a source of energy when there is a shortage of carbohydrate supply to the body. Thus, the level of ketone bodies increases in the plasma during periods of fasting and starvation. In fact, there are observations strongly suggesting that acetoacetate can form schiff bases when reacting with proteins and can alter their secondary structure [16], similar to the effect of the mentioned fixatives. Hence, ketone bodies are expected to effectively react with the viral spikes and break or bend them similar to the fixatives, however this needs to be tested experimentally before consideration. If this could be confirmed, then the induction of ketosis would have a therapeutics as well as a prophylactic potential. In infected patients, the loss of viral spikes after virions secretion from infected cells can prevent the spread of infection to other cells and tissues in the body. Also, virus particles in body secretions will not be able to infect other persons, thus rendering the infected patients noninfectious. In the uninfected persons, the presence of high titre of ketone bodies in the blood can make them immune against infection. Overall, this may slowdown the rapid spread of infection all over the world.

There are several ways to induce ketosis in the body, such as fasting, ketogenic diet and ketone supplements. Fasting would represent the most economic solution. However, only controlled and regulated intermittent fasting should be considered, which is long enough to induce ketosis but not enough to weaken the immune system and the entire body. Ketogenic diet is a diet that has a high-fat and low-carbohydrate content and has been

used regularly to treat children with refractory epilepsy and some congenital metabolic disorders [18,19]. Medium-chain triacylglycerol (MCT) diet is an alternative diet that is more ketogenic. However, both diets should only be consumed under the guidance of physicians since such diets are not suitable for some people [18]. There are also some ketone supplements to raise the level of ketone bodies in the blood such as ketone esters and salts [20]. These should also be used under the guidance of physicians. Ketone body infusion (especially acetoacetate) could also be considered in severe or terminal cases. An important point to consider is that the ketone body titre in the blood should not rise to the level of ketoacidosis. Aside from the harmful effects of ketoacidosis, the formation of schiff bases with proteins decreases in acidic medium [11], which may in turn inhibit viral spike loss and decrease the efficacy of the procedure.

Noteworthy, studies showed that fasting can have anti-inflammatory effects. For example, several studies [21,22,23] reported that the levels of pro-inflammatory cytokines such as interleukin 1β (IL-1β), IL-6, IL-8, IL-10, IL-16, IL-18, monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor α (TNFα) significantly decreased in the plasma during fasting, many of which are involved in the cytokine storm associated with COVID-19 [24]. Another study [25] reported the down-regulation of genes of several cytokines in peripheral blood mononuclear cells during fasting. Thus, fasting could be used also as a preconditioning procedure to lower the levels of cytokines in the body and thus decrease the severity of the cytokine storm that may occur during possible subsequent SARS-CoV-2 infection, and to decrease the rate of mortality associated with this condition.

The consumption of ketogenic diets may also have some anti-inflammatory effects. For instance, ketogenic diet was shown to inhibit the increase in the levels of IL-1β and TNFα in the plasma of rats during inflammation and also the increase in lymphocyte counts [26]. Interestingly, ketogenic diet was shown also to increase a specific type of T cells in the lungs of mice and to make them highly resistant to Influenza A virus infection and disease [27]. There is also evidence of anti-inflammatory effects of ketogenic diet in humans [28] (several pro-inflammatory cytokines are affected such as TNF-α,IL-6, IL-8 and MCP-1). Hence, ketogenic diet consumption may also be considered as a preconditioning procedure against the cytokine storm of SARS-CoV-2 infection. A mixture of both, fasting and ketogenic diet, may also be considered for preconditioning.

Future directions

There are several questions that need to be investigated. For example, what is the exact nature of interaction between the fixatives and viral proteins? Does this interaction occur outside cells only or also inside cells? What happens to the spikes? Are they lost or just bent due to structural changes after the formation of schiff bases? Is schiff base reaction solely responsible for this effect or is it a part of a sequence of reactions? Are there any other reactions in addition to the schiff base reaction that may have caused this effect?
The effect of ketone bodies should also be examined using cryofixation. Finally, research should be aimed to find or formulate compounds that can function like the fixatives against corona viruses (broad spectrum antivirus). This could be needed if there are future attacks by mutated or new members of this family.

Conclusion

- Schiff base reaction appears to break or bend the spikes of SARS-CoV-2.

- Only after experimental confirmation, ketone bodies - especially acetoacetate - could be considered to provide immunity against SARS-CoV-2 (alternative to vaccination).

- Fasting and consumption of ketogenic diet may precondition the body to decrease the severity of the cytokine storm if subsequent infection occurs.

References

1. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol. 2015;1282:1-23. https://doi.org/10.1007/978-1-4939-2438-7_1

2. King AMQ, Lefkowitz EJ, Mushegian AR. et al. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2018). Arch Virol. 2018;163(9):2601-2631. https://doi.org/10.1007/s00705-018-3847-1

3. Dey P. Fixation of Histology Samples: Principles, Methods and Types of Fixatives. In: Basic and Advanced Laboratory Techniques in Histopathology and Cytology. Springer, Singapore. 2018 pp 3-17. https://doi.org/10.1007/978-981-10-8252-8_1

4. Thavarajah R, Mudimbaimannar VK, Elizabeth J, et al. Chemical and physical basics of routine formaldehyde fixation. J Oral Maxillofac Pathol. 2012;16:400–405 https://dx.doi.org/10.4103%2F0973-029X.102496

5. Snijder EJ, van der Meer Y, Zevenhoven-Dobbe J, et al. Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus

replication complex. J Virol. 2006;80(12):5927-5940. https://doi.org/10.1128/JVI.02501-05

6. Salanueva IJ, Carrascosa JL, Risco C. Structural maturation of the transmissible gastroenteritis coronavirus. J Virol. 1999;73(10):7952-7964.

7. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-733. https://doi.org/10.1056/NEJMoa2001017

8. Bowes JH, Cater CW. The interaction of aldehydes with collagen. Biochim Biophys Acta. 1968;168(2):341-352. https://doi.org/10.1016/0005-2795(68)90156-6

9. Yonath A, Sielecki A, Moult J, et al. Crystallographic studies of protein denaturation and renaturation. 1. Effects of denaturants on volume and X-ray pattern of cross-linked triclinic lysozyme crystals. Biochemistry. 1977;16(7):1413-1417. https://doi.org/10.1021/bi00626a027

10. Chang LS, Lin SR, Yang CC. Glutaraldehyde cross-linking alters the environment around Trp(29) of cobrotoxin and the pathway for regaining its fine structure during refolding. J Pept Res. 2001;58(2):173-179. https://doi.org/10.1034/j.1399-3011.2001.00909.x

11. Metz B, Kersten GF, Hoogerhout P, et al. Identification of formaldehyde-induced modifications in proteins: reactions with model peptides. J Biol Chem. 2004;279(8):6235-6243. https://doi.org/10.1074/jbc.M310752200

12. Hameed A, Al-Rashida M, Uroos M, et al. Schiff bases in medicinal chemistry: a patent review (2010-2015). Expert Opin Ther Pat. 2017;27(1):63-79. https://doi.org/10.1080/13543776.2017.1252752

13. Lenard J, Singer SJ. Alteration of the conformation of proteins in red blood cell membranes and in solution by fixatives used in electron microscopy. J Cell Biol. 1968;37(1):117-121. https://doi.org/10.1083/jcb.37.1.117

14. Fowler CB, Evers DL, O'Leary TJ, et al. Antigen retrieval causes protein unfolding: evidence for a linear epitope model of recovered immunoreactivity. J Histochem Cytochem. 2011;59(4):366-381. https://doi.org/10.1369/0022155411400866

15. Liu Y, Liu R, Mou Y, et al. Spectroscopic identification of interactions of formaldehyde with bovine serum albumin. J Biochem Mol Toxicol. 2011 Mar-Apr;25(2):95-100. https://doi.org/10.1002/jbt.20364

16. Bohlooli M, Ghaffari-Moghaddam M, Khajeh M, et al. The role of acetoacetate in Amadori product formation of human serum albumin. J Photochem Photobiol B. 2016;163:345-351. https://doi.org/10.1016/j.jphotobiol.2016.09.004

17. Akram M. A focused review of the role of ketone bodies in health and disease. J Med Food. 2013;16(11):965-967. https://doi.org/10.1089/jmf.2012.2592

18. Kossoff EH, Zupec-Kania BA, Amark PE, et al. Optimal clinical management of children receiving the ketogenic diet: recommendations of the International Ketogenic Diet Study Group. Epilepsia. 2009;50(2):304-317. https://doi.org/10.1111/j.1528-1167.2008.01765.x

19. Boison D. New insights into the mechanisms of the ketogenic diet. Curr Opin Neurol. 2017;30(2):187-192. https://doi.org/10.1097/WCO.0000000000000432

20. Stubbs BJ, Cox PJ, Evans RD, et al. On the Metabolism of Exogenous Ketones in Humans. Front Physiol. 2017;8:848. https://doi.org/10.3389/fphys.2017.00848

21. Aksungar FB, Topkaya AE, Akyildiz M. Interleukin-6, C-reactive protein and biochemical parameters during prolonged intermittent fasting. Ann Nutr Metab. 2007;51(1):88-95. https://doi.org/10.1159/000100954

22. Bouwman FG, de Roos B, Rubio-Aliaga I, et al. 2D-electrophoresis and multiplex immunoassay proteomic analysis of different body fluids and cellular components reveal known and novel markers for extended fasting. BMC Med Genomics. 2011;4:24. https://doi.org/10.1186/1755-8794-4-24

23. Faris MA, Kacimi S, Al-Kurd RA, et al. Intermittent fasting during Ramadan attenuates proinflammatory cytokines and immune cells in healthy subjects. Nutr Res. 2012;32(12):947-955. https://doi.org/10.1016/j.nutres.2012.06.021

24. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-1034. https://doi.org/10.1016/S0140-6736(20)30628-0

25. Elliott RM, de Roos B, Duthie SJ, et al. Transcriptome analysis of peripheral blood mononuclear cells in human subjects following a 36 h fast provides evidence of effects on genes regulating inflammation, apoptosis and energy metabolism. Genes Nutr. 2014;9(6):432. https://doi.org/10.1007/s12263-014-0432-4

26. Dupuis N, Curatolo N, Benoist JF, et al. Ketogenic diet exhibits anti-inflammatory properties. Epilepsia. 2015;56(7):e95-98. https://doi.org/10.1111/epi.13038

27. Goldberg EL, Molony RD, Kudo E, et al. Ketogenic diet activates protective γδ T cell responses against influenza virus infection. Sci Immunol. 2019;4(41). pii: eaav2026. https://doi.org/10.1126/sciimmunol.aav2026

28. Forsythe CE, Phinney SD, Fernandez ML, et al. Comparison of low fat and low carbohydrate diets on circulating fatty acid composition and markers of inflammation. Lipids. 2008;43(1):65-77. https://doi.org/10.1007/s11745-007-3132-7

Conflict of interest

The author declares that he has no conflict of interest.