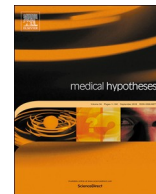




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Letter to Editors

Defensin 5 for prevention of SARS-CoV-2 invasion and Covid-19 disease



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ABSTRACT

Corona virus disease 2019 (Covid-19), a pandemic emerged recently, caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). The receptor for corona virus and influenza A is the mucosal cell membrane protein angiotensin converting enzyme 2 (ACE2), which is abundant on the membrane of alveolar cells and enterocytes. Viral spike protein 1 (S1) is the ligand, with an affinity of 14.7 nM to the receptor. The main port of entry for the virus is the upper respiratory tract, and the diagnosis is usually by PCR of the viral RNA with nasal and pharyngeal swab test. Human defensin 5 (HDEF5) is a protein encoded by the DEFA gene, secreted by Paneth cells in the small intestine and by granules of neutrophils. It has an affinity of 39.3 nM to ACE2, much higher than that of the corona S1. HDEF5 may also attach to glycosylated Corona S1 protein, make its efficiency even better. The issues to be investigated are the affinity of HDEF5 to S1 protein, the ability of recombinant HDEF5 function in attaching both ACE2 and S1, and the feasibility to perform aerosol spray of this protein. In addition, safety and efficiency should be studied in phases I, II and III clinical protocols.

Thus, an aerosol spray of HDEF5 given through the nose and throat, once to several times a day, may be a very efficient approach to prevent infection with SARS-CoV-2 as well as influenza A.

Background

Corona virus disease 2019 (Covid-19), a pandemic emerged recently, caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). On January 30, 2020 the World Health Organization (WHO) declared the outbreak as a Public Health Emergency of International Concern [1]. As of August 22, 2020, 23 million laboratory-confirmed and 800,000 deaths have been reported globally. In eight months, since the start of the pandemic, a lot of epidemiological and clinical information about the SARS-CoV-2 virus and the Covid-19 disease was assessed, sharing experience of many countries and researchers. Effort to control spread of the infection, looking for adequate tests for diagnosis, efforts for development of treatments and vaccine, and optimizing the care of critical patients, were all appreciated all over the world with a significant advance.

Similarly, to other corona viruses and influenza A, the receptor for adherence of SARS-CoV-2 is ACE2 which is abundant on respiratory and gastrointestinal mucosal cells, especially on alveolar type 2 pneumocytes [2]. The spike protein of coronavirus is divided into the S1 and S2 domains, in which S1 is the ligand for the receptor binding, and S2 is responsible for cell membrane fusion [3,4]. The affinity of S1 to ACE2 is 14.7 nM.

Some patients, especially older with background diseases or immunocompromised, progressed rapidly with Acute Respiratory Distress Syndrome (ARDS), septic shock, and multiple organ failure. 5% to 10% of patients have died [5].

Theoretically using of recombinant S1 protein, which will attach to the ACE2 receptors, may actively compete with the SARS-CoV-19 and prevent infection. A protein with higher affinity may be even better in this regard.

Hypothesis

Defensins are a family of cytotoxic peptides, involved in host defense. Human defensin 5 (HDEF5) is a 94 amino acids protein encoded by the DEFA gene on chromosome 8, secreted by Paneth cells in the small intestine and by granules of neutrophils. It has an affinity of 39.3 nM to ACE2, much higher than that of the corona S1. Wang et al looked at the efficiency of HDEF5 attachment to ACE2 and the inhibition of HDEF5 against SARS-Cov-2 in vitro [6]. They found affinity of HDEF5 binding to ACE2 of 76.2 nM, efficient blocking of SARS-CoV-2 receptor binding domain (RBD), and significant inhibition of invasion into Caco-2 cells. HDEF5 protein has also the capability to attach S1 protein through the glycosylated residue of the protein [7]. Thus, theoretically, enhances its ability to prevent the virus invasion acting dually on the ligand (S1) and the receptor (ACE2).

The next step, after preparing recombinant HDEF5, active and functioning, with high affinity to both receptor and ligand, is to make the protein available in powder, with small enough particles to be aerosolized and sprayed. Spray-drying is an effective, efficient means of producing peptide-loaded powders suitable for pulmonary delivery. If the correct formulation and spray-drying conditions can be identified, then a product can be obtained with a high yield and having a large fine-particle dose [8]. Thus, can be applied to the upper airways for prophylaxis and prevention of Covid-19 disease. The spray will be applied to high risk populations and medical teams, on a regular basis, being protective together with face masks and shields [9].

Testing hypothesis

The quality of a spray dried powder is determined by the reconstitution behavior, flowability, and morphology of the powder particles. The size, shape, weight, bulk density, and porosity of the particles

influence the flow of a powder as was demonstrated for lactose, suspensions of glass spheres, calcium carbonate crystals, plate-shaped talc, whey protein and maltodextrin [10]. Sensitive proteins should be embedded in stabilizing excipients (“carriers”) such as disaccharides or amino acids. The spray-drying of protein aqueous solution without carriers can lead to inactivation (due to unfolding or aggregation).

Several basic science and clinical steps are essential for advancing my hypothesis: preparing recombinant HDEF5 protein, protein lyophilization, preparing aerosol spray, performing clinical study of three phases for studying safety and efficiency and then randomized controlled studies of several doses and frequency of application.

Discussion

Blocking the receptor for SARS-CoV-2 virus, ACE2, on the respiratory tract epithelium, especially the alveolar type 2 pneumocytes, will probably prevent invasion of the virus, at least for a short while.

Using a spray of defensin 5 protein before potential exposure to Covid-19 active, infective patient, in addition to face mask and shield, will enhance the ability of high risk population such as the elderly, immunocompromised patients or those with background diseases, (such as chronic pulmonary diseases, diabetes mellitus, arterial hypertension, congestive heart failure, chronic renal failure, chronic liver diseases), to avoid this devastating infection.

Medical teams, especially those that take care of Covid-19 patients, may add this line of protection to their personal protective equipment (PPE) on a routine basis. This approach, together with immunization against influenza, will minimize the danger of both, corona, and influenza in the upcoming winter.

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Declaration of Competing Interest

The authors declare that they have no known competing financial

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Yaron Niv

Ministry of Health and Faculty of Medicine, Ariel University, 39 Yirmiyahu Street, Jerusalem, Israel

E-mail addresses: aron.niv@moh.gov.il, nivaron80@gmail.com.