Hypothesis

Hypothesis of a potential antiviral drug

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DOI: 10.22034/HBB.2022.19

Received: August 7, 2022; **Accepted:** August 22, 2022

ABSTRACT

The approval of mRNA vaccine technique against COVID-19 opens a door to research and the creation of new drugs against different infectious pathologies or even cancer, since for several diseases the therapeutic options are limited, and different viral diseases are treated only symptomatically. For these reasons, this study proposed a hypothesis supported by biological studies, that it provides a theoretical basis for the possible development of a drug that used the mRNA technique and the ribonucleolytic action of a ribonuclease for a possible antiviral therapy, and analyzed a future perspective of this technique in order to provide a bibliographic basis on this hypothesis and motivate researchers to carry out biological studies on this topic.

***Keywords*:** Ribonuclease, antiviral drug, antiviral agent

INTRODUCTION

Viruses are intracellular parasites that throughout the history of human civilization have generated various diseases such as AIDS, poliomyelitis, viral hepatitis, Ebola, influenza, COVID-19, among a few other pathologies that have depleted the world population and some have significantly reduced the quality of life of people who suffer from them [1]. Some of the examples that can be mentioned is the 1918 pandemic due to the influenza virus, which caused the death of approximately

30 million people worldwide [2]. HIV/AIDS, which at the end of the 20th century was estimated to have 34.3 million people suffering from it, [1] and which continues to be one of the biggest health problems worldwide, since it is estimated that 7.7 million deaths in the last 10 years are related to HIV [3].

One of the viruses that has recently paralyzed the world is SARS-CoV-2, the cause of the COVID-19 disease. This virus was detected in December 2019, and shortly after, on March 11, 2020, the World Health Organization (WHO) declared it a pandemic [1]. By June 2022, the WHO estimated that there were 6,305,358 deaths attributable to COVID-19 [4]. For the reasons stated above, it is necessary to develop new drugs and antiviral treatments that provide new therapeutic options that can cover diseases that are currently treated symptomatically.

With the recent approval of mRNA (messenger ribonucleic acid) vaccines against SARS-CoV-2, a door is opened to new treatments that could use this technology for the production of new drugs. The objective of this study is to provide the theoretical basis and literature review of a potential antiviral drug never before developed that employs this well-studied technology. As well as encouraging researchers to carry out studies on this topic in order to better understand the medical application of ribonucleases.

Viral structure and replication

The structure of a virus is generally based on a nucleic acid molecule covered by a protein capsule, [5] whose subunits are generally organized as icosahedral or helical [2]. In addition, some viruses have a phospholipid envelope also composed of proteins and glycoproteins, which is external to the capsule [5]. Finally, the virus may contain enzymes or other proteins that allow initial replication in a cell [6].

In this case, viruses that contain positive and negative chain RNA will be taken into account, the same that is internalized in the cell through the recognition and union of viral adhesion proteins, which are found in the capsid or in the envelope of the virus, with the receptors of the target cell [6]. Viral adhesion proteins in enveloped viruses are glycoproteins, as in the case of SARS-CoV-2, whose envelope contains Spike (S) glycoproteins that adhere to ACE2 receptors on the host cell and mediate virus internalization [7].

The penetration of the virus into the interior or cytosol of the host cell depends on the structure of the virus, since non-enveloped viruses enter the cell by endocytosis or viropexy, while enveloped viruses fuse their envelope with the plasma membrane of the host cell and in this way the nucleic acid molecule (RNA) is introduced directly or with the capsid (nucleocapsid) [6]. Once in the cell cytoplasm, the nucleocapsid or envelope is removed by the action of an acidic environment or by proteases found in endosomes.

Once inside, the viral RNA encodes RNA-dependent RNA polymerases. In the case of positive-strand viral genomes such as coronaviruses or picornaviruses, where the viral RNA binds to ribosomes and acts as an mRNA (Messenger Ribonucleic Acid), so it can be infectious on its own, but in the case negative-strand RNA genomes, such as paramyxoviruses or rhabdoviruses, require a polymerase in the capsid that is internalized in the cytoplasm along with the RNA [6]. In the case of retroviruses, in addition to the positive-stranded RNA genome, an RNA-dependent DNA polymerase or reverse transcriptase is internalized that transcribes it into complementary circular DNA [5].

Finally, the structural and accessory proteins synthesized by the translation of the viral RNA, surround the replicated RNA and form nucleocapsids that can subsequently go outside the cell through cell lysis, if it is a virus with a naked capsid, or by budding if the virus has an envelope, as in the case of SARS-CoV-2, where the structural and accessory proteins are inserted into the intermediate endoplasmic reticulum-golgi complex for the assembly of the virion in which the positive-strand RNA is subsequently incorporated and leaves the cell by fusion of the vesicle, which contains the virion, with the cytoplasmic membrane (budding) [8].

Ribonuclease 1 and its antiviral action

RNase 1 or human pancreatic RNase belongs to the RNase, a family that consists of eight members [9]. This enzyme is a glycoprotein with a molecular weight of 15 kDa without glycosylation, consisting of 128 amino acids and glycosylated at Asn 34 (asparagine) [10]. It exhibits ribonucleolytic activity, both on single-stranded and double-stranded RNA, [11] which catalyzes the cleavage of phosphodiester bonds on various substrates, showing a greater substrate preference at the cytokine binding site over uridine. [12]

It has been observed that extracellular RNA, that is, the one that is released by vascular injury, ischemia or microbial infection, has harmful activity influencing inflammatory processes, but this can be effectively counteracted by the administration of exogenous RNase 1, since in several animal studies it had been observed that the exogenous administration of RNase 1 has significantly improved inflammatory processes in rat models of strokes and myocardial infarctions [9,13].

In addition, there are several studies that corroborated the antiviral activity of RNases, such as the study carried out by Shah and Ilinskaya, where it was shown that the extracellular ribonuclease of bacilli, when internalized in epithelial cells infected with the Influenza A virus (H1N1), had an antiviral activity, since when the virus eliminated its capsid and the viral RNA was released from the endosome to the cytosol, it was hydrolyzed by RNase [14]. Similarly, in the research carried out by De Yang *et al.* in 2003, where a conjugate of RNase 1 and albumin was inoculated in mice infected with the Influenza A and Influenza B viruses, the result of which was a high antiviral activity [10]. Likewise, it has been observed that recombinant pancreatic RNase inhibits the viral replication of the HIV-1 virus in cultures of activated T lymphocytes [15,16].

In another study, vesicles were made from the plasma membrane of HEp-2 cells, which were loaded with RNAse A. These vesicles were incorporated into HEp-2 cell culture in the presence of Newcastle Disease Virus Virions (NDV), the fusion of these vesicles and the internalization of RNase A in HEp-2 cells were observed, as well as an inhibition of viral replication in these cells together with the acceleration of cell death in cells infected by the virus, while in uninfected cells no cytotoxic effect of ribonuclease was detected, since it was rapidly inactivated by ribonuclease inhibitor protein [17].

Drug development

The development of this potential antiviral drug is based on mRNA technology, and the antiviral action of ribonuclease 1, so a possible development of the drug will be briefly reviewed below.

The RNAse1 gene that codes for ribonuclease A, member of family 1, located in 38.p14 and whose sequence is found in GenBank, [18] should be considered. Once the *in silico* sequence is obtained, it is synthesized and cloned into a

DNA template plasmid is subsequently transcribed in vitro to mRNA, [19] and the nucleosides are modified with the incorporation of N1-methyl-pseudouridine instead of uridine, to improve the half-life of the RNA and its translation [20]. In addition, the signal peptide must be modified or substituted so that the final location of RNase 1 is in the cytosol [21,22].

Subsequently, the mRNA must be encapsulated so that it is internalized in the target cell, which could be done with cationic lipid nanoparticles that can fuse with the cell plasma membrane [23]. These lipids could be, for example: ALC-0315, ALC-0159, 1,2-Distearoyl-Sn-glycero-3-Phosphocholine (DSPC) and cholesterol [24].

ALC-0315 is a cationic lipid that has an electrostatic interaction with the mRNA backbone which has a negative charge, this provides stability to the mRNA encapsulation [25]. ALC-0159 is a polyethylene glycol lipid conjugate which has a protective hydrophilic property. While DSPC and cholesterol itself provide the lipid bilayer structure and cholesterol also offers mobility to the other lipid components [24].

Pharmacokinetics

The form or route of administration may vary according to the virus to be combated, the site of infection and the target cells, such as the SARS-Cov-2 virus, which is transmitted by the respiratory route and infects type II pneumocytes, [26] in this case the drug can be administered by inhalation, since the lipid nanoparticles that encapsulate the mRNA can be administered by this route, such as MRT5005, which is a candidate mRNA therapy for the treatment of cystic fibrosis that is administered by inhalation via nebulization [27].

Once the drug is administered, the lipid nanoparticle membrane fuses with the plasma membrane of the target cell, where the mRNA is internalized [20]. Internalized mRNA is translated and ribonuclease 1 is subsequently synthesized. The location of this RNase 1 is in the cytosol and is determined by the modified signal peptide [21,22].

Metabolism and elimination

Natural lipid nanoparticles such as DSPC and cholesterol are metabolized like their endogenous counterparts, while ALC-0315 and ALC-0159 are metabolized by

hydrolysis of the ester functional group and the amine group [24].

The neutralization of the action of ribonuclease 1 is produced by the action of the Ribonuclease Inhibitor protein (RI) in the cytosol [28]. IR is a protein with a three-dimensional horseshoe-shaped structure that contains numerous Leucine-Rich Repeat (LRR) units. This gives it a large surface for protein-protein interaction, [29] so binding with ribonuclease is with femtomolar affinity, which inactivates it, [30] but there are no studies on its metabolism and elimination.

Mechanism of action

Ribonuclease 1, synthesized in the target cell, is located in the cytosol and cleaves the viral RNA by hydrolysis of the phosphodiester bonds, [31] once the viral RNA is internalized in the cytosol by the virus infection, or when it is transcribed into mRNA. Thus, it can reduce viral replication, by preventing the translation of viral RNA, and therefore the synthesis of viral proteins.

Importance and potential implications in medicine

The importance of the possible development of this drug is that it would provide a greater therapeutic option for viral infections and in several cases it could be the first antiviral treatment in infectious pathologies that are treated only symptomatically. Similarly, since it is a drug that is not specific for a definite viral pathogen, this drug can be administered for the treatment of different viral agents that use the same transmission route or the same target cell.

Therefore, the application of this drug would be focused on antiviral treatment, and could be used in infectious diseases and other branches of medicine such as pneumology or even in primary health care.

CONCLUSION

The development of drugs using mRNA technology is well documented, as is the ribonucleolytic action with an antiviral application of RNase 1. Thus, the main advantage of this possible drug is the excision of the viral genetic material to prevent its replication, but although it is true that there are studies that indicate its antiviral efficacy when incorporated into the intracellular space and that it also does not generate cytotoxicity, more studies are notably needed to corroborate its efficacy and safety when ribonuclease is incorporated into the cytosol. In this sense, biological studies are required to verify the

development of this drug, its efficacy and safety both at the cellular level and in complex systems.

It should definitely be emphasized that this possible drug has the peculiarity of acting not only against a viral agent but also against various RNA viral agents, so it could be compared analogically with the antibacterial spectrum of an antibiotic.

Finally, it should be noted that as these are topics with very broad applications in health science, an interdisciplinary team is needed and therefore the collaboration of several researchers with different backgrounds, so it is to be expected that there will be many advances in different branches of health science.

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