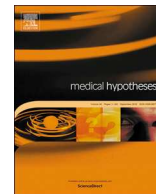




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Letter to Editors

Prospective vaccination of COVID-19 using shRNA-plasmid-LDH nanoconjugate



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ABSTRACT

COVID-19 is the pandemic outbreak that is caused by SARS-CoV-2 virus from December, 2019. Human race do not know the curative measure of this devastating disease. In today's era of nanotechnology, it may use its knowledge to develop molecular vaccine to combat this disease. In this article we are intended to propose a hypothesis on the development of a vaccine that is molecular in nature to work against COVID-19. The nanoconjugate may comprise with the inorganic nanoparticle layered double hydroxide intercalated with shRNA-plasmid that have a sequence targeting towards the viral genome or viral mRNA. This nanoconjugate may be used as a nasal spray to deliver the shRNA-plasmid to the target site. The nanoconjugate will have several advantages such as they are biocompatible, they forms as stable knockdown to the target cells and they are stable in the nasal mucosa.

Background

COVID-19 is caused by an RNA virus that produces Severe Acute Respiratory Syndrome (SARS). Coronavirus 2 (SARS-CoV-2) is the causative organism of this pandemic disease from 2019 [1]. This virus like similar other viruses live and multiply in living cells. The viral replication occurs in 7 stages i.e., Adsorption, Entry, Uncoating, Transcription, Synthesis of virus components, Virion assembly and Release Stage [2]. In the first stage of adsorption the virus attaches itself with the cell membrane of the host cell and injects its genetic material (in this case the RNA) into the host initiating the infection process. In the second entry stage by the method of endocytosis the cell membrane of the host cell encloses the vacuole. In the third stage of uncoating the virus genome is released by stripping of the virus coat protein. After this stage the 1st step of protection may take place by the use of shRNAs that may be incorporated within the host cell far before the infection.

shRNAs are nothing but the short hairpin RNA molecules that have special power of gene silencing with target sequence [3]. shRNAs may be delivered to the target cells by the help of nanoparticles. Nanoparticles are small particles with size smaller than 100 nm. They may be of different types such as inorganic, organic or polymeric nanoparticles. Layered double hydroxide (LDH) is a type of inorganic nanoparticle that is highly biocompatible, and has the ability to protect the shRNA from enzymatic degradation [4]. LDH have layers in their nano structure and they intercalate the shRNA-plasmid within those layers. Due to their cationic nature they are easily internalized by the cell as the cell membrane have anionic property [5]. After the uptake of this nanoconjugate the shRNA-plasmid is incorporated within the genome of the cell and start transcribing the pre-shRNA. This pre-shRNA is transported to the cytoplasm from the nucleus by Exportin 5. In the cytoplasm they are processed by Dicer and loaded with RISC (RNA-induced silencing complex). Here the sense strand is degraded and the antisense strand directs RISC to the RNA molecules that have the complementary sequence with the shRNA [6]. Here, in the 1st line of protection, after the uncoating stage when the single stranded RNA genome is released, they may have a complementary sequence with the shRNA that may help the

genome to be degraded by the RISC.

The fourth stage of viral replication is transcription. In this stage, the RNA genome starts to transcribe mRNAs for the production of protein molecules. In the 2nd line of protection the shRNAs with complementary sequence of the important viral mRNA molecules may help to degrade the viral mRNAs and suppress the translation of important viral proteins. However, this vaccine is a novel method of combating SARS-CoV-2, as this types of vaccines are new in literature so they may be termed as 'molecular vaccine'.

In this article we are intended to give a hypothesis on the production of molecular vaccine that may protect our body from COVID-19.

Hypothesis

COVID-19 is a disease condition that is incurable in today's world. Invention of a vaccine is the need of the hour. In this era of nanotechnology we may use nanoparticles to develop a molecular vaccine for this disease condition. We may use layered double hydroxide as a nanoparticle in conjugation with shRNAs with specific sequence that may target the viral RNA to degrade them as soon as they enter within the cell. The conjugate may be used as a nasal spray to the probable effectible location as a prophylaxis.

Probable methodology

LDH nanoparticles synthesis

The synthesis method of LDH nanoparticle is by co-precipitation method with Mg:Al molar ratio of 2:1. The precursor solution should be prepared by dissolving magnesium nitrate hexahydrate, $Mg(NO_3)_2 \cdot 6H_2O$ and aluminium nitrate nonahydrate, $Al(NO_3)_3 \cdot 9H_2O$ in decarbonated water that should achieve final concentrations of 0.32 (M) and 0.16 (M), respectively. Within the solution above 0.5 (M) sodium hydroxide, NaOH should be titrated in a sealed three necked flask under inert atmosphere with constant stirring at 1100 RPM using a magnetic stirrer that should be under continuous monitoring of the pH

till it arrives at 11.5. The aging should be performed by stirring for further 24 h and the precipitate thus formed should be collected and washed repeatedly in decarbonated water by centrifugation at $5200 \times g$ for 5 min. Finally freeze drying should be performed at -82°C and 20 Pa pressure to get free flowing nanocrystalline LDH powder.

X-ray diffraction (XRD), particle size and TG/DTA analysis may be performed for the characterization of the LDH nanoparticle.

Isolation of plasmid from *E. coli*

For the isolation of plasmid shRNA 2.5% LB broth medium should be autoclaved and ampicillin or any other antibiotic should be added upto a final concentration of 100 $\mu\text{g}/\text{ml}$ depending upon the antibiotic resistance of the *E. coli* purchased. Then the *E. coli* transfected with Lentiviral shRNA-plasmid should be added and cultured overnight at 37°C under vigorous shaking. Next morning the plasmid should be isolated using any plasmid isolation kit available in the market and should be isolated according to the manufacturer's protocol, followed by the quantification of plasmid by measuring absorption of the solution at 260 nm, and should be characterized using the gel retardation assay.

Intercalation of shRNA-plasmid in LDH nanoparticle

A suspension of the LDH nanoparticles should be prepared using DNase-free water with a final concentration of 10 $\mu\text{g}/\mu\text{l}$. The suspension should be sonicated for 10 min before making the mixture shRNA-plasmid at the mass ratios of shRNA-plasmid: LDH, 1:25, 1:50, 1:75 and 1:100, followed by incubation at different time points, such as 30 min or 45 min or 60 min at 37°C for the preparation of the final intercalate of shRNA-plasmid-LDH nanoconjugate. The intercalation should be checked using 1% agarose gel containing ethidium bromide that should be loaded with 0.1 μg of the shRNA-plasmid. The DNA loading buffer should be used for electrophoresis in TBE buffer containing ethidium bromide at 80 V for approximately 45 mins. The bare plasmid should be used as a control and the final gel images should be captured using a UV transilluminator [5,7].

Conclusion

Currently, there is no such vaccine for COVID-19 although some are in clinical trials. The vaccines available in today's world are mainly that activates the immune system but molecular vaccines have a separate principle. Molecular vaccines use the shRNA-plasmids for silencing the viral RNA before it starts to produce viral protein molecules. Molecular

vaccines have an advantage over other currently available vaccines as they may be used as a treatment module for viral infections as well. Moreover, if one can choose a target site on the viral genome that is common to other viruses while selecting the shRNA sequence then, that nanoconjugate will become the common vaccine for wide variety of viruses. Further, the use of shRNA makes a stable knockdown of the cell line so it have better efficacy than small interfering RNAs (siRNAs) [8]. These advantages of the molecular vaccine may become the most upcoming new modality of vaccination in today's world.

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.

References:

- [1] Hui DS, I Azhar E, Madani TA, Ntoumi F, Kock R, Dar O, Ippolito G, Mchugh TD, Memish ZA, Drosten C, Zumla A, Petersen E. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health — The latest 2019 novel coronavirus outbreak in Wuhan, China. *Int J Infect Diseases* 2020;91:264–6. PubMed PMID: 31953166. Epub 01/14. eng.
- [2] Peiris JSM. 57 - Coronaviruses. In: Greenwood D, Barer M, Slack R, Irving W, editors. *Medical microbiology*. Eighteenth ed. Edinburgh: Churchill Livingstone; 2012. p. 587–93.
- [3] Brummelkamp TR, Bernards R, Agami R. A system for stable expression of short interfering RNAs in mammalian cells. *Science (New York, NY)* 2002;296(5567):550–3. PubMed PMID: 11910072. Epub 2002/03/23. eng.
- [4] Acharya R. The recent progresses in shRNA-nanoparticle conjugate as a therapeutic approach. *Mater Sci Eng, C* 2019;104:109928.
- [5] Acharya R, Alsharabasy AM, Saha S, Rahaman SH, Bhattacharjee A, Halder S, et al. Intercalation of shRNA-plasmid in Mg–Al layered double hydroxide nanoparticles and its cellular internalization for possible treatment of neurodegenerative diseases. *J Drug Deliv Sci Technol* 2019;52:500–8.
- [6] Paddison PJ, Caudy AA, Bernstein E, Hannon GJ, Conklin DS. Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. *Genes Dev* 2002;16(8):948–58. PubMed PMID: 11959843. PubMed Central PMCID: PMC152352. Epub 2002/04/18. eng.
- [7] Acharya R, Chakraborty M, Chakraborty J. An in vitro comparative study of layered-double hydroxide nanoconjugate in the delivery of small interference and short-hairpin ribonucleic acid. *Bull Mater Sci* 2019;43(1):10.
- [8] Moore CB, Guthrie EH, Huang MT-H, Taxman DJ. Short hairpin RNA (shRNA): design, delivery, and assessment of gene knockdown. *Methods Mol Biol* 2010;629:141–58. PubMed PMID: 20387148. eng.

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