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NEW PRINCIPLE BASED DEVELOPMENT OF NAPHTHOQUINONE AS AN UNIVERSAL PRVENTIVE DRUG(CODED AS GSPZ9) AGAINST SARS-COV-2S PROTEIN AND ITS VARIANTS

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ABSTRACT
Out of 30 trillion human cells, the only human lung cells had been endowed with furin protease attached with the receptor (ACE2)- a central point to which the dormant SARS-CoV-2 S protein, evading the human body defences including antibodies due to the defined spike protein glycosylation, accesses and attaches to furin protease without any hindrance where S protein gets cleaved into S1 and S2 protein, which causes fusion to ACE2 and entry of the virus into the human lung cell. These facts on SARS-CoV-2 S protein had been overlooked while evolving and developing vaccination, which simply elicits antibodies for spike protein engulfment, and fail to perform satisfactorily consequent to spike protein glycosylation. New principle-based an universal preventive naphthoquinone drug (GSPZ9) has been found as the best option to combat SARS-CoV-2 S proteins and its variants with no risk whatsoever The 5mg naphthoquinone as an oral dose twice a day with water or milk in order to buildup the blood volume, and $8x10^{-3}$% w/w naphthoquinone in cream base, to apply intranasally have been standardized. The intranasal application of naphthoquinone cream would neutralise the virus protein to stop its entry beyond the intranasal limitation. The blood circulating naphthoquinone, acting as an additional body defence against SARS-CoV-2 S protein neutralises it, and furin protease internally, overlying layers of naphthoquinone over the proteins of both the players to prevent their union, and S protein cleaving into S1 and S2 proteins. If by chance their union occurs, the blood circulating naphthoquinone would intervene and dehydrogenbond the weekly hydrogenbonded proteins of the two players, and then strongly hydrogenbond both the players' proteins to prevent their remarrying.

KEYWORDS: SARS-COV-2 S protein, napthoquinone, ACE-2, S1 and S2 protein, glocosylation, cell, spike protein, furin protease.

INTRODUCTION

With the spread of infection caused by SARS-CoV-2 S protein and its variants, the humanity has been facing extinction upon earth. In order to extinguish the infection, the different technologies have been applied, but of no avail. The temporary solution does not fit into the bill. The safety of humanity lies in a permanent or lasting solution. There are two types of viruses: good and bad. Bacteriophages (aka phages) are good viruses whereas SARS-CoV-2 S protein and itsvariants are bad viruses. The organisms: bacteria, viruses, fungi and protozoa etc are mainly the part of intestinal microorganisms. Human health depends upon the interaction and homeostasis between different microorganisms. The intestinal microorganism has been found to have abundance of two main species: bacteria and viruses.[1] Phages, being the main members of intestinal viruses, play an important role in regulating intestinal microbiome. The bacteria drug resistance globally has been attributed to the excessive application of antibiotics. As a result, the new treatment procedures, based upon the intestinal microecology, have caught the attention of the scientists worldwide. An advanced knowledge, acquired over time on the composition of intestinal phages and their functions, has contributed heavily not only as a key point of microecology research, but also a new entry point for protecting human health. The human digestive tract exceeds $10^{[14]}$ bacteria. Fujimura[2], K.E. reported on the intestinal microbiome of healthy people mainly *Firmicutes*, *Bacteroids*, *Proteobacteria*, *Actinobacteria*, accounting for over 90% of total number of intestinal bacteria, whereas Dion[3], M.B.et al. studied on phage diversity, genomics and phylogeny, describing phages as the main compnents of

intestinal viromes, having upto $10^{[8]}$ billion virus-like particles per ml of fecal filtrate. In line with their lytic ability, the phages are divided into lytic phages (virulent phages) and lysogenic phages (temperate phages). Phages are further divided on the basis of morphological variation into tailed phages, tailless phages and filamentous phages. They are still further divided into Siphovirida, Myoviridae, Potoviridae. Adsorption, invasion, replication, assembly, maturation and lysis have been registered as the main steps in the lysis of host bacteria by lytic phages.

The human intestine also contains lysogenic phages as the main components. Dutilh, B.E.[4] et al. in their studies have shown that wieldy distribution of intestinal phages across the world, are crASS- like phages. Manrique, P.[5]. et al. and Norman, J.M.[6] et al., working on healthy people as subjects, had indentified 23 core phages, shared by 750% individuals and 132 common phages, shared by 35% individuals.

The different authors studied the main mechanism of action of phages effecting the body health and found their application in the regulation of the composition of intestinal microbiota: predation[7] seesaw effect[8], epithelial defence[9], lygenic conversions[10], phage mediated immune regulation[11] relationship between phages and diseases and clinical application of phages and infectious diseases[12], skin and soft tissue infection[13], oral infection[14], gastrointestinal infection[15], respiratory infection[16], urinary tract infection[17], eye infection[18], ear infection[19], nasal infection[20], sepsis/ bacteremia[21], novel corona virus pneumonia[22], phages and noninfectious diseases[23], inflammatory bowel disease[24], alcoholic hepatitis[25-26], diabetes mellitus,[27],colorectal cancer[28], breast cancer[29], Parkinson disease[30], Schizophrenia[31]. Phages have also found application in PDT Clinical programming.[32]

The **NATURAL ORIGIN vs LAB LEAK** debates merged almost simultaneously with the pandemic. Recently US statements have reignited the controversy. Some scientists have contributed to mudding water. In 2020, a statement of a group of scientists, published in The Lancets[33], called it a conspiracy and claimed that the evidence was overwhelmingly conclusive that the viruses (SARS-CoV-2) had emerged naturally. In the same year (2020) in an article in Nature Medicine[34], another set of scientists claimed that their analysis clearly showed that the virus was not a laboratory construct or a purposefully manipulated one. On 16th march 2023, the scientists unveiled the new data on the possible origin of COVID-19 pandemic, which put a strange, squat omnivore- creature (RACCOON DOG*: Nyctereutes procyonoides* which appears svelte in summer and pack on the pound for winter) squarly in the spotlight (unpublished). The animals were at least occasionally sold at the Human Seafood Wholesale Market, where many virologists suspect that many COVID-19 pandemic may have started. The United States Department of Energy of FBI now believes that the virus most likely leaked from a laboratory Wuhan, China and did not emerge organically. It is not a time to waste time over whether SARS-CoV-2 is a natural origin or lab leak. Instead the joint efforts of world Scientists are required to fight SARS-CoV-2 S protein and its variants and protect the humanity upon earth.

Peng, M.Y.[35] et al. studied immunological aspects of SARS-CoV-2 infectious and putative beneficial role of vitamin D, covering Covid pathophysiology[36], viral cell entry[37], antiviral activity of vitamin and innate immune response[38], adaptive immune regulation by Vitamin D[39] and modulation of ACE2 and RAS by Vitamin D.[40] The S protein on the surface of viruses is a key factor involved in infection. It is a trimeric class 1 TM glycoprotein responsible for viral entry, and it is present in all kinds of HCoVs, as well as in other viruses such as HIV (HIV, glycoprotein 160, Env), influenza virus (influenza hemagglutinin, HA), paramyxovirus (paramyxovirus F) and Ebola (Ebola virus glycoprotein). Similar to other coronaviruses, the S protein of SARS-CoV-2 mediates receptor recognition, cell attachment, and fusion during viral infection.[41] Currently nonspecific therapeutic or prophylactic has been used clinically to treat SARS-CoV-2 infection. Nonspecific antiviral drug such as remdesivir, chloroquine, favipiravir and alopinavir- ritonavir (Aluvia) have been clinically used to treat COVID-19 in China. Continued strengthening and monitoring of the SARS-CoV-2 S protein is of great importance for subsequent new drug development and protection against COVID-19. Even an universal preventive drug(GSPZ9) against SARS-CoV-2 S protein and its variants has been found as the best option in comparision of the vaccine against the viruses. Latko[42], M.et. al. reported that the surface of SARS CoV-2 is crowded with the glycosylated S protein which bind to the host cell receptor, angiotensin converting enzyme2 (ACE2), mediating viral cell entry. When S protein binds to the host cell receptor(ACE2), TM protease serine to TMPRSS2, a type of TM serine proteases located on the host cell membrane, promotes virus entry into the cell by activating S protein. Once the virus enter the cell, the virus RNA is released. The proteins are translated from the RNA genome and replication and transcription of the viral RNA genome, occur via protein cleavage and assembly of the replicase– transcription complex. Viral RNA is recplicated and structural proteins are synthesized, assembled and packaged in the cell, after which viral protein are released. These proteins are critical to the viral life cycle and provide potential targets for drug therapies. The ACE2 based peptides are the possible inhibitors against SARS-CoV-2 S protein. The SARS-CoV-2 S protein is highly conserved among all human corona viruses (HCoVs) and is involved in receptor recognition, viral attachment and entry in host cell. Due to its indispensible functions, it represents one of the most important targets for SARS-CoV-2 S protein

vaccine and therapeutic research. The literature abounds studies on SARS-Co-2 S protein and its variants and its allied area by different scienentists.

The phages (good viruses) and SARS-CoV-2 S protein and its variants (bad viruses) have been evolved and developed under the laws of nature with the similar type of proteins with the defined functional groupes thereon. Zhu N.[43] et al in 2019 studied a novel coronavirus from patients with pneumonia in China, Describing the epidemic of novel coronavirus disease 2019, caused by a new coronavirus occurred in December 2019 with its spread worldwide, turning into a global pandemic. Soon COVID-19 was discovered to be caused by a coronavirus later, named as severe acute respiratory syndrome coronavirus2 (SARS-CoV-2), which belongs to B coronavirus family. Four of the coronaviruses (229E, NL63,OC43 and HKU) only cause slight symptoms of common cold. On the contrary SARS-CoV, MERS-CoV, SARS-CoV-2 cause severe symptoms and even death, with fatality rates of 10%, 37% and 5% respectively, Wu, C.[44] et al. and Liu, C.[45] et al., working on the analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational method, research and development on therapeutic agents and vaccines for COVID-19 and relative coronavirus diseases, concluded that much work and research need to be done on meeting the challenges of SARS- CoV-2 S proteins.

Inspite of the advances on the vaccination programmes to contain SARS-COV-2 S protein and its variants (bad viruses), the impact has been found as a short-term palliative measure, demanding a better alternative to prevent the infection of bad viruses to humans. Revisitation on known structural details on bacteriophages (phages), SARS-CoV-2 S protein and human lung cell to effect effective lasting solution to contain SARS-CoV-2 S proteins infection, is an essential exercise.

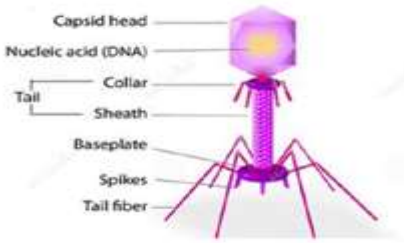
Understanding of bacteriophages (good viruses), SARS-CoV-2 S protein(bad virus) and human lung cell structurally and characteristically has helped give us leads on the lasting solution to deal with SARS-CoV-2 S protein and its variants effectively.

Bacteriophages (phages), which are good in nature and act as body defences against unwelcome bacteria causing various disease, unlike SARS-CoV-2 S protein, are free to latch onto the host (bacteria), pierce the host body and release DNA into, infect and kill the host.

SARS-CoV-2 S protein structurally and characteristically cannot self- infect the host (humans) unlike bacteriophages. It assumes dormancy outside and inside the human body. Its dormancy is broken when S protein gets cleaved into S1 and S2 proteins, by furin protease attached to the receptor ACE2 of lung cell.

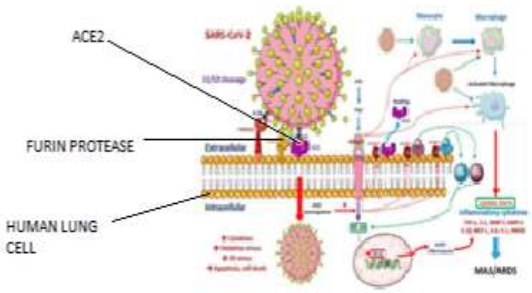
Revisitation on the phage, SARS-CoV-2 S protein and human lung cell structures for better understanding of the subject

The structures(1,2) of the phage, and the possible pathophysiological pathways after the entry of SARS-CoV-2 S protein into the human lung cell have been drawn from the studies by Mansour, N.M.J.[46] and Peng, M.Y.[35] Wang, M.L.[47] reported that currently no specific therapeutic or prophylactic has been used to treat and prevent SARS-CoV-2 S protein infection. Non specific anti viral drug such as remdasivir, chloroquine, flavipirvir and lopinavir-rotenavir (aluvia) has been clinically used to treat COVID 19 in China, showing effectiveness in vitro. The experience shows that their effectiveness in vivo had been far less than expected in China, demanding a more effective and universal preventive drugs against SARS-CoV-2 S protein and its variants.



Structure 1: Typical Bacteriophage structure.

Sourced from: Mansour, N.M.J.[46] Food Microbial. 1, 22-24 (2017)



Structure 2: Possible pathophysiological pathways after the entry of SARS-COV- 2.

Sourced from: Peng, M.Y[35] etal. International Journal of MolecularSciences. 22,525, (2021)

The structure 1 of bacteriophage (aka phage) composed of Capside head, DNA, Collar, Sheath, Base plate, Spike, Tail fiber. Out of these parts, the spike attaches the host (bacteria), infect and kill the host. Nature has protected human body, incorpotating phages as the part of its scheme in the human system to protect the body from the bacterially defined diseases like HIV (HIV glycoprotein 160), influenza virus (hemagglutinin, HA), paramyxovirus (paramyxovirus F) and E.bola (Ebola virus glycoprotein). The Structure 2 of SARS-CoV virus comprises of glycoprotein S, M-protein, Hmagglutinin-

esterase-dimer (HE), E-protein, Envelope and RNA- N protein. In the same structure the human lung cell defined with furin protease attached with the receptor (ACE2), showing SARS--CoV 2 S protein, entering into the host cell involving furine protease attached with the receptor.

Analysis of these structures showed that the phage, SAR-SCo-V-2 S protein and human lung cell are all made up of the structurally and characteristically defined similar proteins, which have minimum 18 of – C=O and -OH groups, - in addition to N-terminus and C- terminus, having amino and carboxyl groups with the capacity to hydrogenbond with any substance which has the complementary functional groups to hydrogenbond. This is the principle on which this whole study has been founded upon.

The poor performance of vaccination against SARS-CoV-2 S protein, inspite of enough development of antibodies to neutralize SARS-CoV S protein has been noticed worldover. The sciences on phages (as a reference) SARS-CoV-2 S protein and the human lung cell equipped with the furin protease attached with the receptor (ACE2) need to be revisited in order to find the lasting solution of SARCS-CoV-2 S protein. The phages (good viruses), SARS-CoV-2 S protein (bad viruses) and human lung cell defined with furin protease attached with the receptor (ACE2) are made up of the structurally and characteristically defined similar protein structures, having minimum 18 binaries of –C = O and – OH groups and a N-terminus and C- terminus group with its capacity to hydrogenbond with the chemical defined with its complementary functional groups, say, -OH and-C = O ones. Out of 30 trillion human cells, the human lung cells are only defined with furin protease attached with the receptor (ACE2), a weak point at which the SARS-CoV-2 S protein attaches in the human body to infect. SARS-CoV-2 S protein is a mysterious bad virus with the defined qualities. Firstly, it in its native state is dormant with no apparent infection activity, subject to the cleaving of S protein into S1 and S2 proteins. Secondly the heavily glycosylated spike protein has the evasive property to evade antibodies, elicited on vaccination, but the functional groups thereon the glycosylated spike protein –C=O and –OH remain active.

The scientists worldover missed these natural qualities of SAR-CoV-2 S protein, while envolving and developing the concept of vaccination against virus. As a result the poor performance of vaccination has resulted in. The napthoquines defined with the comlementary functional group: -OH and –C=O to those of the similar proteins, the phages, SARS-CoV-2 S protein and the furin protease attached with the receptor (ACE2) of human lung cell are the best option to contain SARS-CoV-2 S protien. The underlying principle, on which the present study has been based upon, has been developed to prevent the occurrence of the virus infection, rather to elicit antibodies development, simply disallowing the marriage of SARS-CoV-2 S protein with furin protease, and ultimately cleaving of S protein to S1 and S2 proteins.

The antiviral activities of napthoquinone against the phages could be found as 99.99 (%log reduction) at 100%, 90%, 80% and 70% napthoquinone concentration, suggesting similar fate of SARS-CoV-2 S protein with napthoqqinone.

Underlying principle of this study

The scientists worldover had been quick in the envolution and development of vaccines against SARS-CoV-2 S protein, without understanding the natural qualities of SARS-CoV-2 S protein endowed with. SARS-CoV-2 S protein is itself ineffective in infecting the host. Its effectiveness to infect the host becomes noticeable subject to S protein cleaving into S1 and S2 protein on its attachment with furin protease attached with ACE2. The vaccination against SARS-CoV-2 S protein responded poorly, but results in the overproduction of antibodies to neutralize the spike protein. The poor performance of vaccination against SARS-CoV-2 S protein has been noticed in recent time in different countries worldover due to the endowed with evasive property of glycosylated spike protein to evade antibodies.

In contrast to above concept of vaccination, a preventive concept has been developed to meet the challenges of SARS-CoV-2 S protein. The underlying principles of this study has been based upon the binding affinity of the proteins, SARS-CoV-2 S protein, having 18 binaries of –OH and –C=O groups and N- terminus and –C terminus group, complementary to -C=O and –OH functional groups thereon the structure of napthoquinone- an universal preventive drug (GSPZ9).

Naphthoquinone and antiviral activity

Naphthaquinones constitute a class of organic compounds structurally related to naphthalene. The naphthoquinone atovaquinona, an analog of ubiquinone (coenzyme Q_{10}) commonly used to treat pneumocystis pneumonia and malaria, inhibits complex III in cancer cell lines and breast cancer stem cell, by competitive inhibition of active site of ubiquinone. VitaminK is an example of a naphthoquinone. They have antibacterial and anitumour effect. Naphthaquinone are not wide spread but are found in; Bignoniaceae, Kigelia pinnata, Tabebuia spp. Vitamin K is an essential for the functionality and sustainability of human body. Vitamin K dependent blood coagulating factor are X, IX, VII, II, protein S, which have been found as lifelines of human system. Sawhney[48-50] in his studies on vitamin K has found the complementarity of vitamin K and relentless sun high intensity UVB as the defined natural strategy at tropics in determining Homo sapien skin pigmentary order, vitaminK defieciency as the precipitating factor for causing Homo sapien(HES,[51]leucoderma) skin depigmentary disorder in human at tropics. The HES, the

human deficiency of vitamin K biosynthesining bacteria in their gut microbiome, has been treated with vitamin K like chemicals. Vitamin K with their threshold blood circulating organic compound in human system has no ability to neutralize SARS-CoV-2 S protein as this naphthoquinone derivatives do not have the complementary groups like – OH and –C=O groups thereon its structure, an essential of the underlying principle on which the preventive nature of the drug against SARS- CoV-2 S protein and its variants, has been based upon.

The good viruses like phages and bad viruses like SARS-CoV-2 S protein and its variants are bioconstructed with the similar type of proteins, having 19 binaries of –C=O and -OH groups present on the structures, causing their binding through hydrogenbonding of proteins with the complementary –OH and –CO= groups which acts as a protein buffer like skin buffer against UVR upon earth, against SARS-CoV-2 S protein virus, preventing it to attach furin protease.

Thus the napthoquinone with –OH and –C=O groups is an indispensable chemical to neutralise the SARS- CoV-2 S protein and its variants.

It has been demonstrated experimentally seeing the naphthoquinone effects on good virures: PH1 X174 Phage (ATCC13706) and MS2 Phage, ATCC 1559 following Bacteriophage Plague Inhibition Essay. The results showed 99.99% neutralization of these phages at 100%, 90%. 80%, 70% naphthoquinone concentration. Since the good viruses (phages) and had viruses (SARS-CoV-2 S protein and its variants) are made up of the structurally and characteristically defined similar proteins, the bad viruses would meet the phage like fate with napthoquinones.

Poor response of vaccination against SARS- Cov-2 S protein and its variants

The concept of vaccine development against SARS-CoV-2 S protein and its variants, revolves around the packaging of COVID-19 RNA genome in adenovirus, its (adenovirus) fusion with the human plasma membrane, the entry of RNA genome into the cell and its subsequent self translation into their proteins, the releasing of the proteins outside the cell, to be identified by immune cells as antigen as a response of which the production of antibodies against the virus protein occurs to fight SARS-CoV-2 S protein and its variants. The vaccines so produced and applicable against RNA viruses had not been universal. There are 5 Types of antibodies: IgG, IgM, IgA, IgD, and IgE which are distributed and function differently in the body. As a response to the antigen (the proteins as above), all antibodies are not produced on vaccinations. Production of one or two antibodies on vaccination is a sign of poor performance of vaccines. Secondly with mutation of the virus, the failure of vaccines to fight virus has been reported in China, which has been facing heavily the SARS-CoV-2 and its variants, in addition to USA, Japan, UK etc. Secondly thr spike protein endowed with glycosylation has the inherent property of evading the antibodies, elicited on vaccination to fight the virus. Five antibodies, IgG, IgM, IgA, IgD, and IgE, have not responded to the proteins (antigens) produced, consequent to the antiviral vaccination.

Vaccines against SAR-CoV-2 S protein have no ability to prevent the attachment of the virus to the furin protease attached with the receptor (ACE2) available at the surface of human lung cell, basic to the initiation of lung cell infection like napthoquinone which has the functional groups, complementary to the functional groups thereon the proteins, to hydrogenbond leaving far less room to the SARS-CoV-2 S protein to attach.

In contrast, the napthoquinone- a preventive drug, which is launched under the study as an universal preventive drug (GSPZ9), meets the challenges of SARS-COV-2 S protein and its variants.

Nature of SARS-CoV-2 S protein

To-day we are barraged with a bewildering array of alphanumerics: H3N2, H2N2, H1N1 and of course the unforgettable SARS-CoV-2. These combinations are complex enough to serve as highly secure passwords to enter our bodies operating system, lulls us into complacency, terrorise us with dejuvu. The ongoing COVID-19 pandemic already has resulted in about eight million deaths. All these viruses have mutated into less lethal avatars, which are gallivanting across the globe, resulting in several million infections and more than a million deaths every year.

Antibiotics do not work on viruses. On the other hand, they do an incredibly sinister job of fostering antibiotic resistance, which is growing to become a monumental problem in healthcare.

The S of SARS-CoV-2 S protein consists of an extracellular N- terminus, a transmembrane (TM) domain anchored in the viral membrane and a short intacellular C- terminal segment. Normally S exists in a metastable, perfusion and conformation. The spike protein is glycosylated, a kind of camouflage to evade the surveillance of the host immune system during the viral entry in human lung cell. SARS-CoV-2 S protein consists of a single peptide (amino acids 1-13) located at N-terminus and S1 subunit and S2 subunit. During the viral infection, the target lung cell furin proteases activates the S protein by cleaving it into S1 and S2 subunits, which is necessary for activating the membrane fusion domain for viral entry into target cell. The cleavage site of SARS-CoV is known wheras that of SARS-CoV-2 S protein has yet been known. The binding of viruses particles to the cell receptor on the surface of the host cell is the initiation of virus infection. Thus the receptor recognition is an important determinant of viral entry and a drug design target

RBD situated in the S1 subunit binds to the cell receptor (ACE2) in the region of amino-peptidase N which contains NTD and CTD. The S2 subunit, composed successively of FP, HR1, HR2, TM domain fusion (CT), is responsible fusion and entry of the virus into the lung cell and subsequent human infection.

MATERIALS AND METHODS

In the light of the underlying principle of this study, the experimental designs were curved out and experimented upon in order to generate data on the permanent solution on the fight against SARSCoV-2 S protein, basing upon that all the viruses (good and bad) and human cells are made up of the similar proteins with the inherent qualities with the binding affinities to the substances defined with the complementary functionals groups.

Viruses which have been evolved and developed under the natural programing, are of two types good and bad. Bacteriophages (aka phages) are good phages whereas SARS-CoV-2 S proteins and its variants are bad viruses. The naphthoquinone – an universal preventive drug (GSPZ9) against SARS-CoV-2 S protein and its variants, would neutralize equaly the phages and SARS-CoV-2 S proteins and its variants.

The phages: PHI X 174 Phage ATCC 13706 and MS2 Phage ATCC 15597 were choosen for testing antiviral activity of naphthoqinone, following Bacteriophage Phlague Inhibition Essay at 37 ˚C +- 1 C against these good viruses in order to determing the fate of bad viruses: SARS-CoV-2 S Proteins and variants. The strains of test organisms were revived as instructed by ATCC

Summary of test method

1. The soft agar overlay was prepared to a final concentration of 0.70% w/v agar,1 % w/v glucose, 1% w/v $CaCl_2$ and 1% w/v $MgSO_4$ and autoclaved at 120^0 C for 20 minutes.
2. Plague inhibition essay

- 490 ul of the naphthoquinone solution was inoculated with 10 ul of bacteriophage (1.0 x 10^{10} pfu/ml) of PH1 X 174 Phage (ATCC 13706 or MS2 Phage ATCC 15597 and inoculated overnight at 4^0 C
- 500 µl of host culture was added in above solution and inoculated at 37º C for 20 minutes
- The bacteria/ virus/ naphthoquinone mixture was then added to 3 ml soft agar overlay and immediately poured over pre-made agar plates (28%w/v agar)
- The plates were allowed to set for 15 minutes at room temperature, inverted and incubated overnight at 37ºC. nutrient broth and deionized water were used as negative controls.
- After incubation, the plaques were counted and percentage inhibition and log reduction were recorded.

The above test performed at 100% was repeated at 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% and 10% naphthoquinone concentration. The first five concentrations (100%, 90%, 80%, 70% and 60%) had neutalised 1.0x 10^{10} pfu/ml completely of the good viruses (PHI X 174 Phage ATCC 13706 and MS2 Phage ATCC 1559).

What naphthoquinone did to good viruses at these first five concentrations, would be the fate of SARS-CoV-2 S protein and its variants as both viruses (good: Bacteriophages) and (bad: SARS-CoV-2 S protein and its variants) have been evolved and developed out of the similar proteins under laws of nature.

RESULTS

The data based calculations gave the leads to prepare oral dose and intranasal cream against SARS-CoV-2 S proteins and its variants. The 5mg naphthoquinone tablet to be taken orally in a day with water or milk to buildup the naphthoquinone concentration in human blood and braceup the human system to fight and neutralize the incoming bad viruses before they attack the target : human lung cell. The $8x10^{-2}$ % $^{w/w}$ naphthoquinone in cream base is recommended to apply twice a day intranasally to stop the entry of bad viruses beyond intranasal level.

DISCUSSION

The human system has been revealing about the nature of SARS-CoV-2 S protein and the body conditions, which help the virus to infect the humans- a fact which has not been given due couriderations by the working scientists, while developing the vaccination against the viral infection. In human system, there 68 trillion cells, out of which there are 30 trillion human cells which are characteristically and structurally varying slightly. The only human cell, equipped with furin protease attached with the receptor (ACE2), invites and welcomes the virus to infect human. It is the inherent mechanism endowed with in the framework of the lung cell, which has been identified as responsible for the viral infection in human, and not the SARS-CoV-2 S protein itself. In its native state, SARS-CoV-2 S protein remains dormant and does not infect humans as such. Its dormancy is broken subject to cleaving of its S protein int S1 and S2 proteins, which causes its activation and energisation to infect humans. On its entry into the human system, the virus evades the body defences including antibodies as its spike protein has been endowed with the heavily glycosylation, which helps it evade antibodies. Consequently SARS-CoV-2 S protein accesses to the target, furin protease attached with the receptor (ACE2) where on attachment with furin protease S protein is cleaved into S1 and S2 protein. RBD situated in the S1 subunit binds to the cell receptor,(ACE2) in the region of amino- peptidase defined with NTD and CTD domains, whereas S2 subunit composed successively of FP, HR1, HR2, TM and CT domains.is responsible for fusion to

cell membrane and mediation of virus entry into the lung cell, causing human infection.

As a corollary, the receptor recognition is an important determinant of virus entry and a drug design target: the vaccination programme launched worldwide against SARS-CoV-2 S protein, elicits antibodies to contain the virus, but the spike protein evolved and developed, had been glycosylated in order to evade antibodies, elicited by vaccination, casting doubts on the reasonable performance of vaccination technique and concept.

In order to target drug design against SARS-CoV-2 S protein, the functionalities of the similar proteins were revisited. There are two types of viruses: good and bad Bacteriophages are good viruses whereas SARS-CoV-2 S protein and its variants are bad viruses, but both viruses had been evolved and developed with similar proteins. Further analysis revealed that the proteins having 18 binaries of -C=O and -OH functionalities thereon and a N – terminal and C terminus group have the inherent ability to neutralise the chemical structures defined with the complementary -OH and -C=O groups thereon. Several structures were examined and analyzed on the complementary nature of their functional groups to those of the proteins. Ultimate choice fell upon naphthoquinones defined with the complementary -C=O and -OH groups.

The experiments, following Bacteriophage Plague Inhibition Essay, were carried out against two phages: PHI X 174 Phage ATCC 13706 and MS2 Phage ATCC 15597 with naphthoquinone as the neutralizing candidate. In order to determine the fate of SARS-CoV-2 S protein and its variants under the influence of naphthoquinone, the results recorded showed 99.99 (% log reduction) neutralization of phages at 100%, 90%, 80%, 70% and 60% naphthoquinone concentration. This means that like phages, napthoquinone would neutralize 99.99 (% log reduction) SARS-CoV-2 S protein reducing the virus capacity to infect the host (human) to naught in order to meet the challenges of SARS-CoV-2 S protein and its variants, SARS-CoV-2 S protein has been fought intranasally and orally. The 5 mg naphthoquinone oral dose twice a day, and intranasal naphthoquinone in cream base, have been prepared and standardized with no side effects whatsoever upon humans. The blood circulating naphthoquinone volume acts as an additional defence, like natural defences, against SARS-CoV-2 S protein. Firstly SARS-CoV-2 S protein would be neutralized at intranasal level, when applied with naphthoquinone cream intranasally. The oral doses of naphthoquinone in due course of time would build up the blood volume. With the escape of SARS-CoV-2 S protein to the inside body, the blood circulating naphthoquinone steps in as a body defence, and hydrogenbonds the proteins of spike protein and neutralizes them, making it 100% ineffective to attach to furin protease. The blood circulating naphthoquinone would also generate a protein buffer by interacting with the furin protease attached with the receptor (ACE2), leaving no room or space for SARS-CoV-2 S protein to attach. If the union of SARS-CoV-2S protein and furin protein occurs by chance, the blood circulating naphthoquinone would intervene and de couple the weakly hydrogen bonded players and hydrogenbond both the players strongly, stopping SARS-CoV-2 S protein and furin protease to remarry. The naphthoquinone with the capacity to prevent the SARS-CoV-2 S protein infection with the prevention of the union of the SARS-CoV-2 S protein and furin protease attached with the receptor ACE2 in sharp contrast to vaccination stands promisingly justified.

Thus a universal preventive naphthoquinone drug(GSPZ9) against SARS-CoV-2 S protein and its variants is the far better option compared to vaccination which has responded poorly.

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