ISSN: 2755-0168

Journal of Biotechnology & Bioinformatics Research

SCIENTIFIC Research and Community

Research Article

Open Access

Polifunctional Liposomes with Trace Elements and Vitamins to Prevent SARS-Cov2 Infection

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ABSTRACT

After many months of the SARS-CoV2 pandemic, in the absence of a vaccine or effective drug therapy, we offer a new generation supplement. The supplement is formulated with trace elements and vitamins of undisputed immunostimulating efficacy, included in the individual liposomes, which act as carriers and therefore we define multifunctional, to improve the absorption and bioavailability of the substances used. We focus the discussion on the role of Zinc as an effective immunostimulant and specific antagonist of the SARS-Cov2 virus.

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Received: January 29, 2021; **Accepted:** February 01, 2021; **Published:** February 06, 2021

Keywords: Infectious Diseases, SARS-Cov2, Prevention of Immune Defenses, Polyfunctional Liposomes

Introduction

The SARS-CoV2 virosis, several months after its first manifestation, continues to spread around the world and increase its virulence. To date, millions of people have been infected and several hundred thousand deaths have been attributed as "causa mortis" to SARS-CoV2 syndrome. Thanks to the international adoption of hygiene measures, such as hand washing, the use of face masks and restrictions on interpersonal contacts through domestic segregation, there has been a slowdown in the spread of the pandemic. The reduced incidence of infections has prompted the various governments to review the anti Covid-19 impositions, allowing a partial and gradual return to normality, put in serious crisis by the virus in all production sectors. As widely expected, to the relaxation of the restrictive measures, the world population has responded with behaviors lacking the initial hygiene-sanitary rigor. Restaurants, bars, night clubs have been identified as the meeting places where the virus is presumed to have caused contamination in significant numbers. Numerous drug therapies and several SARS-CoV2 vaccines are being tested around the world. To date, we still do not have effective therapies, in the absence of which it becomes increasingly urgent to adopt treatments capable of correcting the deficiencies in the immune functions of symptomatic subjects affected by the virus, in an analytically proven form, and to reduce the risk of fatal events. In our previous scientific article we proposed a holistic approach aimed at contrasting and fighting infectious diseases with the use of nutraceutical supplements, with recognized immunostimulating efficacy, as adjuvants to the therapies available today [1]. In this work we take up the topic discussed in the previous scientific publication, with a specific study on the usefulness of immunomodulating nutraceuticals and their mechanism of action. We also propose the use of polyfunctional liposomal technology for the administration of these substances, in order to obtain their maximum assimilation.

Coronavirus Pathogenesis

Coronaviruses are large capsule RNA viruses that can generate respiratory diseases of varying severity, from the common cold to fatal pneumonia. Only 7 varieties of Coronavirus are known to cause disease in humans. Three of the seven viral variants cause far more serious and sometimes fatal respiratory infections in humans than other coronaviruses. The SARS-CoV2 coronavirus of severe acute respiratory syndrome (SARS) has been identified as the etiological cause of a severe acute respiratory syndrome outbreak. Coronavirus MERS-CoV was identified in 2012 as the cause of Middle East Respiratory Syndrome (Middle East Respiratory Syndrome [MERS]). SARS-CoV2 is a new coronavirus identified as the cause of coronavirus disease in 2019 (Covid-19) that is speculated to have started in the Chinese city of Wuhan in late 2019 and then spread around the world. The most relevant clinical manifestations of SARS-CoV2 disease are: acute respiratory failure and systemic coagulopathy. Symptoms that, if associated with significant previous morbidity, characterize the mortality recorded following the Covid-19 syndrome. Coronavirus infections begin with binding of the protein S receptor (Spike) which mediates the entry of the virus into host cells. The S proteins of most coronaviruses are cleaved by the host protease into two functional subunits: an N-terminal receptor binding domain (S1) and a C-terminal domain (S2), specific for the attached cell membrane.The interaction among the cell surface receptor and

S1 subunit is the major determinant of coronavirus tropism.

When binding of the S1 receptor occurs, a conformational change is activated in the S2 subunit, such as to expose its hidden fusion peptide for insertion into the cell membrane. This event is followed by the packing of the two heptad repeats (example of a structural design consisting of a repetitive pattern of seven amino acids) into the three monomers in a six-helix beam fusion core. This close juxtaposition of the viral and cell membranes allows the fusion of the lipid bilayers so that the viral nucleocapsid is thus transported into the cytoplasm Fig 1. [2-8].

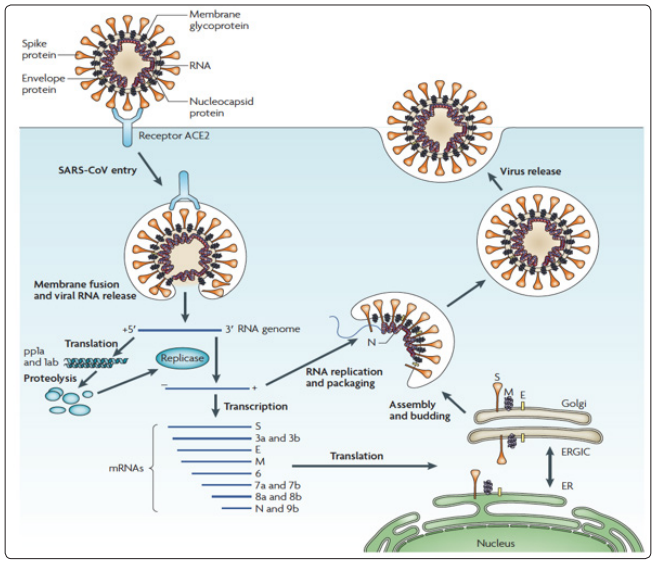


Figure 1: Schematic diagram showing the replication cycle of coronavirus

The most evident clinical manifestations observed in patients with SARS-CoV2 are: cough, fever, chest pain, worsening of dyspnoea, headache, anosmia, ageusia, gastrointestinal disorders. The serious and peculiar endothelial commitment deriving from viral contamination is manifested by the appearance of arterial hypertension, arterial and venous thromboembolism, organ failure (pulmonary, renal, hepatic, cardiac) [2-4]. Endothelial dysfunction is one of the major pathological determinants of SARS-CoV2 syndrome.

The SARS-CoV2 coronavirus accesses host cells via binding of its spike glycoprotein to the angiotensin converting enzyme 2 (ACE2), sialic acid receptor, transmembrane serine protease 2 (TMPRSS2), and metalloproteinase inducer of the extracellular matrix (CD147); cathepsin B and L also participate in the virus entry. All these factors are expressed in endothelial cells, so endothelial dysfunction is a common feature of the clinic expressed by all patients in conditions of medium-important severity Fig.2 [5-8].

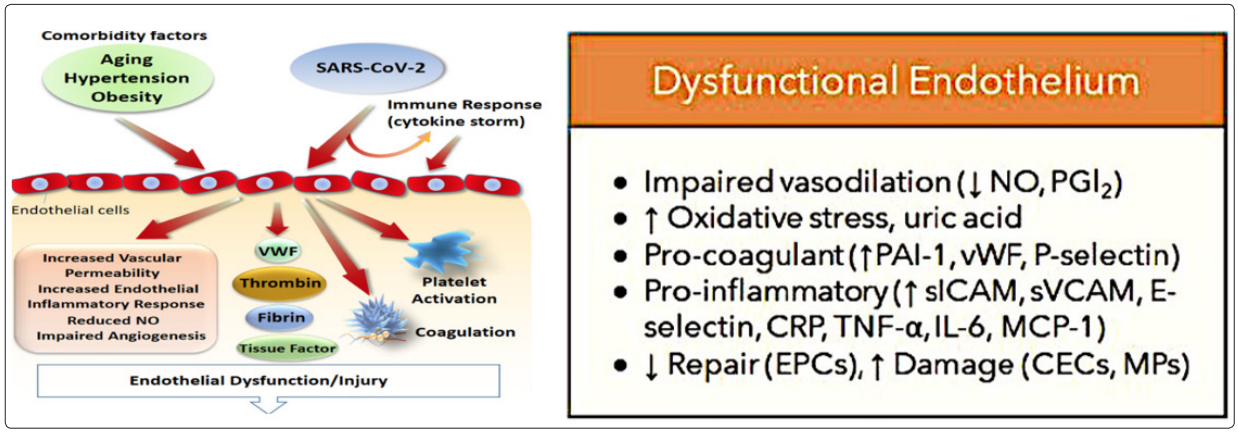


Figure 2: Schematic representation of the effects of coronavirus entry within endothelial cells

Different Therapeutic Approaches Used To Date

A number of non-specific therapies have recently been used for the treatment of severe acute respiratory syndromes: some are based on in vitro research on antiviral activity (against SARS-CoV2 or related viruses) others on the basis of limited clinical experience. A specific drug has not yet been synthesized.

Among the most used should be mentioned:

Chloroquine - Clinical data available in vitro and limited.

Hydroxychloroquine - Has positive effects but burdened by collateral manifestations, sometimes important.

Lopinavir; Ritonavir - Efficacy not proven, even more recent data have not been able to confirm their efficacy.

Remdesivir - Several clinical trials are ongoing to verify its clinical applicability.

Azithromycin - Antibiotic belonging to the Macrolide family, associated with therapies to prevent any respiratory superinfections of bacterial origin.

Tocilizumab - Use based on a theoretical mechanism, rather limited preliminary efficacy data.
Hyperimmune Plasma - Plasma extracted from patients in remission from Covid-19 syndrome. Experimental use is being studied. Very satisfactory preliminary results.

High-dose corticosteroid therapy:
Dexamethasone - Used for patients with septic shock secondary to acute respiratory distress syndrome.

The use of NSAIDs in patients with SARS-CoV2 symptoms is being studied by the FDA. Concern has been expressed about the potential worsening of symptoms following their use. Confirmatory clinical data is currently lacking.

According to the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC) and the FDA, there are currently no drugs or vaccines that have been shown to be effective for treating or preventing SARS-CoV2 virosis [9]. In general, drug treatment is not recommended for young, healthy patients with mild symptoms and no underlying concomitant conditions [10].

Multifunctional Nutraceutical Adjuvants and Immunity
In the absence of pharmacological therapies and vaccines of proven efficacy for the treatment of SARS-CoV2 infections, a strong attention is needed to the prevention and use of alternative therapies to increase the immune response of subjects at risk. The defense mechanisms of innate and adaptive immunity are very complex, they can be described as organized in three main clusters: physical barriers of the host (skin, mucinous secretions), innate immune response, acquired immune response.

Within the normal healthy population, there are inter-individual variations in many immune functions due to gene configuration, age, sex, smoking habit, physical activity, alcohol consumption, diet, stress, etc.

Nutritional status is an important factor contributing to immunocompetence.
It has been recognized that it has profound interactions with the maintenance of a basal state of health and the initiation of infectious mechanisms. In the last decade substantial research has focused on the role of nutrition and above all on the contribution of the function of trace elements and vitamins to the optimal functioning of the immune system. Therefore, the effective strengthening of the immune system allows us to overcome and fight the aggressions of pathogens. This strategy is particularly useful for those who are more susceptible to infectious diseases, such as the elderly, children, and iatrogenic immunodeficients. Today we have many methods to improve the performance of the immune system: 1) using natural substances that experience and clinical trials have shown in all their effectiveness and safety, 2) through the use of drugs. In particular, we know the effectiveness of many plant extracts and some macro and microelements, which serve to prevent infections and support the functionality of our immune system. The list is truly extensive and we cannot fully examine it in this speculative context. Among the numerous beneficial factors, vitamins A, B6, B12, C, D, E, folic acid and the trace elements iron, zinc, copper and selenium act in synergy to support the protective activities and the correct functioning of the immune system [11-28].

We also emphasize that most of these same substances such as Iron, Zinc, Copper, Folic Acid, Vit.B6 and Vit. B12, as well as being essential for optimal functioning of the immune system, are key elements for hematopoiesis. A particularly interesting aspect is the possible antithrombotic effect that some of these principles have demonstrated. In fact, the association between folic acid, vitamin B6 and vitamin B12 controls the metabolism of homocysteine, preventing its excessive accumulation in the blood. Hyperhomocysteinemia is a recognized independent risk factor for cardiovascular disease. [29-33]. After describing the usefulness linked to the intake of some trace elements and vitamins to strengthen the immune system, we want to focus on the element Zinc (Zn) for its important implications in the functioning of the immune system.

Zinc (Zn), an Antiviral Element of Fundamental Importance
Many works have described the immunomodulating power of zinc salts [38-43]. The growing interest in the fundamental role that zinc plays in the state of well-being and homeostasis of the immune system has recently stimulated the creation of two international organizations dedicated exclusively to the study of zinc biology: Zinc-Net and The International Society for Zinc Biology [34]. Among the many works examined, we recall two authors, Inga Wessels and Coll [36]. Anatoly V. Skalny and Coll, who presented a detailed description of the role of zinc in immune system enhancement and as an antiviral inhibitor of the SARS-CoV2 virus [37]. The authors analyze the multiple molecular mechanisms of the antiviral activity of zinc against the Covid-19 syndrome, accompanied by their clear and effective graphic representation.

In particular, the role of zinc in virus-induced vascular complications has so far been insufficiently discussed. Interestingly, most of the risk groups described for SARS-CoV2 syndrome are at the same time clusters associated with zinc deficiency. Since this trace element is essential to preserve the natural barriers of tissues such as the respiratory epithelium, preventing the entry of pathogens, for a balanced function of the immune system and the redox system, its deficiency can probably be added to the factors that predispose the individuals to infection and harmful progression SARS-CoV2. Finally, due to its direct antiviral properties, it can be assumed that the administration of Zinc is beneficial for the majority of the population, especially for those with a non-optimal concentration of this trace element, mainly due to factors of nutritional origin.

It has been suggested that zinc can prevent virus fusion with the host cell membrane, decrease viral polymerase function, alter protein translation and processing, block the release of viral particles, and destabilize the viral envelope. Low-dose zinc supplementation along with small concentrations of zinc ionophores pyrithione or hinokitol (natural monoterpene found in the wood of the Cupressaceae family trees) reduced RNA synthesis in influenza, poliovirus, picornavirus, virus equine arteritis and SARS-CoV2 by directly inhibiting the RNA-dependent RNA polymerase of the virus. Fig.3

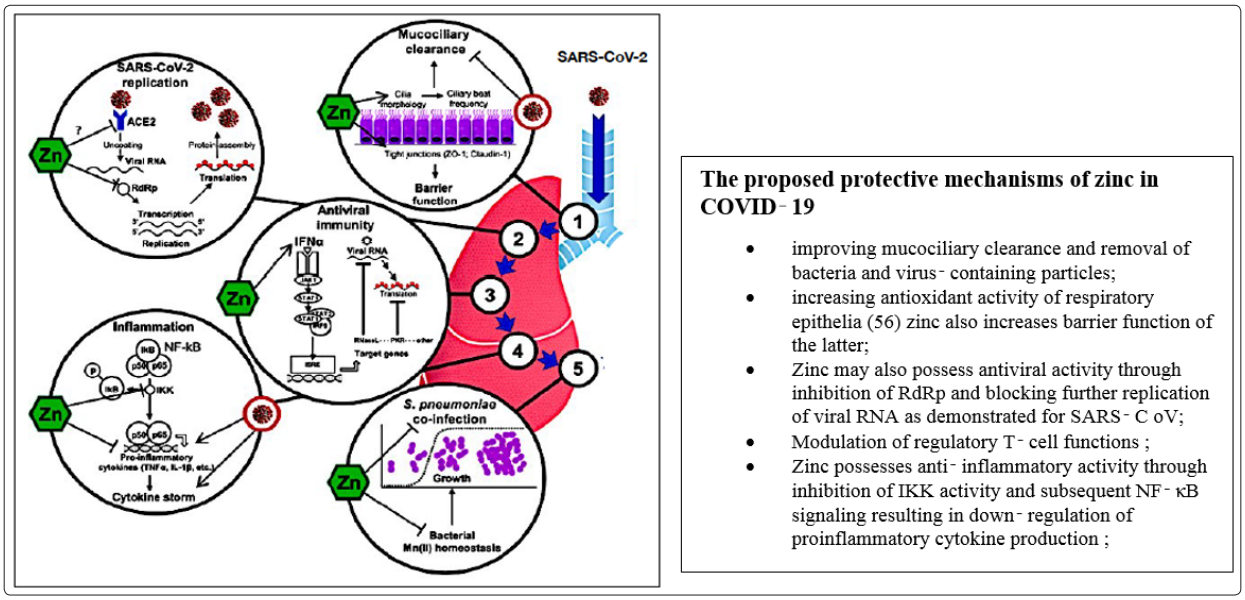
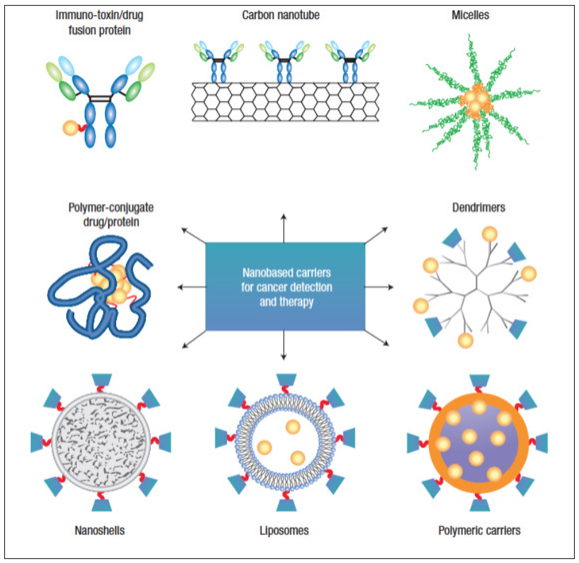
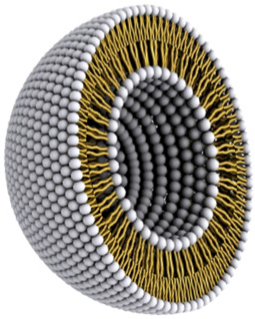


Figure 3: The proposed protective mechanisms of zinc in SARS-CoV2

The effective modulation of the immune system increases significantly with the administration of micronutrients and vitamins, when administered in the form of polyvalent complexes a synergistic effect is obtained. Unfortunately, most of the food supplements on the market have the disadvantage of being poorly absorbed and even less assimilated to fully express all their beneficial effects [44]. Pharmaceutical technology offers us various solutions to overcome the limits of the reduced assimilation of these substances. In particular, many vitamins and plant extracts have insufficient bioavailability and must be administered following precise indications.

Among the various technological options available including liposomes, polymer conjugates, micelles, dendrimers, carbon and metal based nanoparticles and other different carriers, we have adopted liposomal technology, because it is the most widespread and used and its ease of production and versatility of usage are known. Liposomes consist of a double layer of phospholipids with an internal cavity that can contain and transport various substances in solution, such as drugs or active ingredients Fig. 4 [45-46].





Some delivery systems *Liposome*

Figure 4: Schematic Representation of Most Delivery Systems Technologies

The use of liposomes as transporters is significant for pharmacologically active substances that have a low bioavailability index (such as some anticancer agents, antibiotics, etc.). They allow to reduce the concentration of drugs and improve their bioavailability with reduction of side effects. The Argentine company Lipotech S.A. (www.lipotech.com.ar) has obtained the most innovative results and has produced multifunctional liposomes, capable of carrying several substances simultaneously within the same liposomes.

Multifunctional Liposomal Technology

Lipotech's multifunctional liposomal technology has provided us with a product that contains 6 substances: Zinc, Selenium, Folic

acid, Vit. C, Vit.B6 and Vit.B12 contained in the individual liposomes. We tested the morphological (Average Size [A], Zeta Potential [B]) and qualitative characteristics of the liposomes, with investigations conducted at the Micrometrics accredited center. Fig. 5

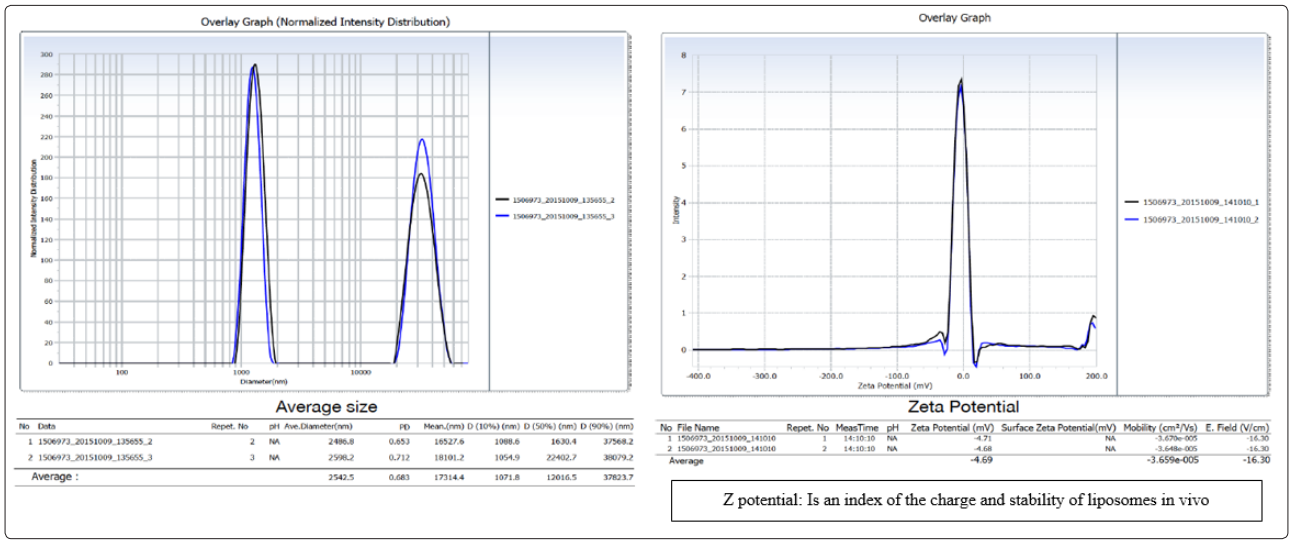


Figure 5: Small Amount of Sample Dispersed In 20ml Filtered DI Water and Sonicated for 2 Minutes

Subject of Study

The product was used on 70 patients to test its effectiveness in preventing SARS-CoV2 virus infection.

Materials and Methods

70 patients with different solid-type oncological pathologies, for whom the administration of components of the Vitamin B family does not produce metabolic effects in favor of neoplastic growth, coming from different regions of Northern Italy (where the greatest number of cases of SARS-CoV2 pure syndrome or associated with more or less important comorbidities), all subjects at risk for known reduced immune activity, belonging to various age groups, were admitted to the study, following an open protocol that essentially considered the "status" of well-being general (Karnofsky Status Index) and the absence of general respiratory symptoms related to SARS-CoV2 infection.

We used the liposomed product with micronutrients and vitamins encapsulated in a vegetable coating. Patients were treated by taking a single capsule a day containing the vitamin mix and specific trace elements. All patients signed the informed consent, communicating the side effects and any intolerances encountered during treatment to a single referent. Each patient was advised to gather information on their daily health status by noting the various daily skills deemed improved, based on individual daily experiences.

Results and Discussion

All 70 patients have easily overcome the period of "lock down" imposed by the national government, due to the suspension of medical control services linked to the follow up of their oncological pathologies. The encouraging results obtained with this purely preliminary empirical work, which demonstrated the safety of use and the absence of side effects, stimulate us to confirm its efficacy on a wider range of patients at risk and with a more rigorous protocol than also include a control group. Based on the preliminary, however very encouraging results of the study, we believe that the experimentation should be implemented on a larger scale and on the use of analytical and statistical methodologies such as to give numerical confirmation of the optimal clinical evidence obtained.

Disclosure

The authors report no conflicts of interest in this work.

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