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EFFECTIVENESS IN HUMANS OF AN ANTIVIRAL DRUG BASED ON CHOLINERGIC AGONISTS WITH SPECIFIC DESIGN AND ROUTE OF ADMINISTRATION AGAINST COVID-19 SYMPTOMS IN A GROUP OF INFECTED PATIENTS VS. CONTROL GROUP

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Abstract

Objective

To establish the therapeutic action of a nicotinic cholinergic agonist agent (CA) composition in the symptoms in a group of human patients infected with SARS-CoV-2 vs. control group.

Methods

Basic Odds Ratio study (95% confidence interval) in 20 patients for intervention group and 15 patients for the control group. The evaluation in the groups was carried out during 15 days assessing the improvement or worsening of each symptom daily.

Results:

Cough, (OR = 0.5), Dyspnea (OR = 0.38), Muscle fatigue (OR = 0.69), Ageusia (OR = 0.27), Anosmia (OR = 0.21) General malaise (OR = 0.62), are less than 1 converting the use of the cholinergic agent in a protective and therapeutic factor showing therefore improvement of these symptoms, after its use, compared to the control group.

Conclusions:

The positive results obtained on the symptoms caused by COVID-19 using cholinergic agonists molecules by delivering a cholinergic agent (CA) composition with special oral and nasal route of administration and specific pharmacological design against COVID-19 in humans infected by SARS-CoV-2 versus the control group, endorse preliminary the nicotinic hypothesis on SARS-CoV-2 and the therapeutic potential of the use of these molecules. Larger multicentrical trials in humans are encouraged.

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HIGHLIGTHS:

- A nicotinic hypothesis for SARS-CoV-2 has been proposed by several authors, which reports an alternative interaction other than glycoprotein S/ ACE2 binding that affects and disrupts nicotinic receptors trough alternative epitopes or extracellular domains linked to nAChrs, a so-called TBS (toxin-binding site). This alternative nicotinic interaction disrupts especially α9 and α7 nAChRs' subunits, impacting the cholinergic system, with implications in the RAAS, the cytokine storm, the hemophagocytic lymphohistiocytosis with immune overresponse through macrophages and showing an interference at the interface between the nervous system and the immune system that affects the vagus nerve and the anti-inflammatory nicotinic pathway that involve acetylcholine and its nicotinic receptors.
- ACE2/S-SARS-CoV-2 RBD plays a key role in infectivity, but the alternative toxin-binding site linked to nAChRs, involving a cholinergic epitope or cryptic epitope, may play a crucial role in severity and mortality observed in COVID-19 disease and must be addressed and targeted urgently being cholinergic agonist molecules one of the best candidates for this purpose.
- Nicotine and other cholinergic agonist agents have been proposed as candidates against SARS-CoV-2 based on clinical evidence, well-based hypotheses and *in silico* molecular docking studies, showing preliminary antagonization properties with SARS-CoV-2/S-Protein.
- For the first time in the context of the COVID-19 pandemic, a nicotinic cholinergic agonist composition has been tested

in humans showing significant effectiveness for the improvement and reduction of the major symptoms of COVID-19 when compared to a control group. This is the first novel drug designed specifically against COVID-19 that shows preliminary positive and significant results in humans.

ABSTRACT

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ABBREVIATIONS

Ach, Acetylcholine; ADAM17, ADAM metallopeptidase domain 17; ACEi, Angiotensin-converting enzyme inhibitors; ARBs, Angiotensin II receptor blockers; CA, Cholinergic Agent; CAR-T therapy, Chimeric Antigen Receptor Therapy; CNS, Central Nervous System; CS, Cholinergic System; CRS, Cytokine release syndrome; DIC, disseminated intravascular coagulation; FP, Fusion Peptide; G-CSF, colony stimulating factor of granulocytes; HLH, hemophagocytic Lymphohistiocytosis; IFN1, Interferon 1; IFN-γ, Interferon gamma; IL-6, interleukin 6; IS, Immune System; LPS, lipopolysaccharides; LTH1, Type 1 lymphocytes helper; LTH2, Type 2 lymphocytes helper; MAOI, inhibitors of monoamine oxidase; NIAID, National Institute of Allergy and Infectious Diseases; NRT, Nicotine Replacement Therapy; nAChRs, Nicotinic acetylcholine receptors; NK, Nuclear factor; NF-Kb, Nuclear factor kappa beta; NMDA, AChRN-methyl-D-aspartate; PRR, pattern recognition receptor; PNS, Peripheral Nervous System; RAAS, Renin-Angiotensin-Aldosterone

System; SARS, Severe Acute Respiratory Syndrome ; STAT, Signal Transducer and Activator of Transcription; RBD, receptor-binding domain; TBS, toxin-binding site; Th, helper T lymphocytes; TNFα, tumor necrosis factor alpha; TNFRI , TNF receptor-1; TNFRII , TNF receptor-2

KEYWORDS

Cholinergic agonists; Nicotine; Nicotinic acetylcholine receptors; COVID-19, SARS-CoV-2; nAChR; Nicotinic hypothesis; Human trial, RAAS (Renin-Angiotensin-Aldosterone System), ACE2; Anti-inflammatory nicotinic pathway, Long Covid, Post-Covid Syndrome

1. INTRODUCTION

1.1 NEED FOR SPECIFIC ANTIVIRALS OR IMMUNOMODULATORS AGAINST COVID-19

Currently, there are no significantly effective or specific antiviral or immunomodulatory drugs against SARS-COV-2 / COVID-19 that are also safe to use in patients. The world's largest randomized study on the therapeutic and pharmacological management of COVID-19 has generated conclusive evidence on the efficacy of newly applied drugs for the treatment of COVID-19. The provisional results of this study called *Solidarity*, coordinated by WHO, were published on October 15th, 2020 (1). More than 12,000 patients had been recruited from 500 participating hospitals around the world. The trial is being carried out in 30 of the 43 countries that have the necessary authorizations to start recruitment. The study found that the four treatments evaluated (Remdesivir, Hydroxychloroquine, Lopinavir / Ritonavir, and Interferon) had little or no effect on overall mortality, initiation of ventilation, and length of hospital stay in hospitalized patients.

To face the current pandemic, various therapeutic strategies have been used for different stages of the disease, although there has not been a very high success rate for patients with critical COVID-19 and the effectiveness rate in less critical patients is very low or nil as the *Solidarity* study shows in part.

One of the strategies is the use of antiretroviral drugs such as Remdesivir (2), Lopinavir, Ritonavir, Favipiravir that interrupt viral replication by inhibiting the RNA polymerase of the virus with inhibitory activity against SARS and MERS. Remdesivir being preliminary the most effective with a powerful anti-coronavirus activity, but with very limited efficacy, although somewhat significant only in severe COVID-19 patients, managing to reduce the duration of the disease by 31%, but with a reduction in mortality of only 3% (p = 0.059; 8,0% in patients treated with Remdesivir vs 11,6% in patients without such treatment). All this according to a clinical trial by the US National Institute of Allergy and Infectious Diseases (NIAID). (3)

The use of corticosteroids for Severe Acute Respiratory Syndrome

(SARS) consequence of viral infection is very controversial. Corticosteroid therapy is not within the WHO protocols for viral pneumonia or respiratory distress due to the high risk of immunosuppression in these patients, although in China glucocorticoid therapy for COVID-19 has been used since the day 6th of the disease until day 12th with a dose of 1-2 mg / Kg / day.

Another strategy is the use of antimalarials such as chloroquine and hydroxychloroquine showing in a study on 2314 healthy contacts that this drug was not associated with lower incidence of SARS-CoV-2 (4). Chloroquine increases the pH of endosomes, which are required mainly for the initial phases of intracellular transport of the virus and also for the final phases before its extrusion by exocytosis. On the other hand, it interferes with the glycosylation of cellular receptors to SARS-CoV-2. Hydroxychloroquine would have a more powerful effect than chloroquine and has an indirect mechanism of

action, related to the inflammatory response towards the virus; however, the reported adverse effects of ventricular arrhythmias due to the QT interval prolongation on the ECG (5) and higher mortality during the hospital stay, have ended up advising partially against this treatment for COVID-19.

The inhibition of inflammatory mediators such as tumor necrosis factor alpha (TNFα) and its receptor, interleukin 6 (IL-6), among others, causes a response interruption in the cascade of immune events towards the virus, such as endothelial permeability and alveolar, which would have a potential benefit in the prevention and treatment of acute respiratory distress caused by coronavirus. The biologics most used in this strategy due to the pandemic have been till now: tocilizumab, sarilumab, anakinra, among others. Tocilizumab is a humanized recombinant monoclonal antibody that inhibits the effect of IL-6, involved in cytokine storm. Sarilumab is an IL-6 inhibitor and its effect on the new Coronavirus is still under study. It acts as an inhibitor of IL-1α and IL-1β and has been used successfully in the past for Macrophage Activation Syndrome and sepsis. But at present, data and there is no enough evidence about the effectiveness of tocilizumab, sarilumab, anakinra and further randomized clinical trials are needed to determine its use as a therapy for COVID-19.

There is also CAR-T therapy (Chimeric Antigen Receptor) that uses cultured and expanded CAR-T T lymphocytes, and administered intravenously to the patient, with a conditioning protocol (lymphodepletion chemotherapy) previously performed; however, this treatment has shown adverse effects similar to cytokine-release syndrome (CRS) and other side effects are commonly developed, including symptoms such as high fever, hypotension, hypoxia, and/or multiorgan toxicity (6).

The administration of antibiotics such as azithromycin has been considered in bacterial infections concomitant to COVID-19 and following the protocols of antibiotic therapy. To date, patients with MERS and severe pneumonia who require ICU and receive this intervention, show no additional benefit in the elimination or clearance of the virus or in the reduction of mortality (7).

Another strategy corresponds to the antiparasitic ivermectin, a drug used against river blindness, lymphatic filariasis and other neglected tropical diseases. It also has some antiviral effect against single-stranded RNA viruses such as dengue and yellow fever. In early April, Australian researchers reported that Ivermectin in vitro inhibits the replication of SARS-CoV-2 (8) and made two pre-publications on observational clinical studies reporting the apparent usefulness of ivermectin in treating COVID-19 patients who required mechanical ventilation. But none of these studies were peer-reviewed or formally published, and one of them was subsequently withdrawn. However, ivermectin is a positive allosteric modulator of the nicotinic alpha7 acetylcholine receptors (9) and may still offer some benefit in off-label use. This has still to be proven further.

It is therefore urgent to develop and launch specific and effective drugs against COVID-19 that complement and support vaccines, most of which still have to face regulatory approvals, storage and distribution difficulties, and demonstrate its safety on a large scale, its capacity in covering effectively all current or new strains of SARS-CoV-2, its efficacy in prophylaxis, therapeutic effectiveness on reduction of mortality and / or on symptoms as well as the duration of immunity, avoiding unexpected antibody-dependent-enhancement or similar problems, in addition to being viable and accessible to the population in the shortest possible time. The fact that a significant percentage of the population, which varies from around 14% to 60% depending on the country, is either anti-vaccine or reluctant to be vaccinated, and the fact that the FDA

discourages vaccination of certain vaccines for minors, pregnant women, allergy sufferers, cancer and immunosuppressed people, and patients with coagulopathies, increases the need for alternative drugs effective against COVID-19.

This article presents the scientific bases on the use of cholinergic agonist molecules that have been proposed as a therapeutic and prophylactic alternative to the COVID-19 pandemic, nicotine being the most important of them, but not the only one, being cholinergic agonist molecules preliminary effective, fast to produce, inexpensive, and safe at appropriate dosages.

1.2 THE NICOTINIC CHOLINERGIC HYPOTHESIS AND THE USE OF NICOTINIC CHOLINERGIC AGONISTS AGAINST SARS-COV-2

Direct agonist cholinergic drugs have a parasympathomimetic effect by stimulating muscarinic or nicotinic receptors. For the topic at hand, we focus exclusively on direct nicotinic cholinergic agonists. In order to bring more scientific evidence to the nicotinic hypothesis and the effectiveness of cholinergic agonists in COVID-19 disease, a study in humans has been carried out with a new drug that has recently been specifically designed against COVID-19 by the company Niccovid® and that has been preliminarily named Cholinergic Agent (CA).

The drug was designed by a team of researchers based on observations and clinical evidence published in a French peer-reviewed journal (10) by researchers such as Changeux, Félix Rey, and Amoura, who have raised the hypothesis, together with other researchers such as Farsalinos, Poulas, le Houezec, among others (11), that SARS-COV-2 is a nicotinic virus that disturbs the cholinergic system and that it could be addressed prophylactically and therapeutically with cholinergic agonists.

Clinical evidence from hospitals in several countries like China (16) , USA, Spain, Greece, France (10), UK and data from the US CDC (13) including peer-reviewed studies (14,15) and systematic reviews (16,17) found a lower prevalence of COVID-19 among smokers, which it could be attributed to a probable protective factor for nicotine contained in the cigarettes but not from cigarette or smoking itself. This lower prevalence has been corroborated by several studies, including systematic reviews and meta-analysis (17,18) and some researchers from universities and hospitals from Greece (16), France, Wales, the USA and Spain (19) have proposed the use of nicotine as a cholinergic agonist agent. In France, L'Assistance Publique – Hôpitaux de Paris is testing in a large multicentrical-randomized study coordinated by Prof. Zahir Amoura nicotinic drugs as cholinergic agonists against COVID-19 in the form of nicotine patches (20).

While the prevalence of SARS-CoV-2 infections among smokers is much lower than expected, there is also a worse prognosis among smokers that must be hospitalized. This paradox is resolved by considering that, although smoking is not at all a therapeutic option against SARS-CoV-2 and is a high-risk factor for the development of several diseases such as cardiopulmonary diseases, the intake of some molecules contained in cigarettes seems to be, preliminary, a protective factor. In other words, a chronic smoker who intakes large amounts of nicotine and other alkaloids seems to receive some protection from COVID-19 because of these molecules, but at the same time is intaking thousands of toxins and chemicals harmful to health. Thus, we hypothesize that when the protection factor of the cholinergic agonists contained in the cigarette is less than the damage that the cigarette causes in the smoker's body due to the toxicity of the hundreds of chemicals contained in the cigarette (50% of the cigarette is composed of chemicals added to the natural tobacco plant) and the thousands of toxins and dozens of carcinogens derived from its combustion, probably in conjunction with other pre-existing comorbidities, or abrupt cessation of smoking due to hospitalization (nicotine plasma levels decrease close to

zero within 10 hours after nicotine intake cessation) and hospitalized smokers in most cases do not receive NRT's products as alternative to cigarette at hospital, it is logical that the smoker has a more adverse prognosis for COVID-19 once hospitalized. In contrast, in chronic smokers with no or few comorbidities, the protective factor of nicotine and other cholinergic agonists naturally contained in the tobacco plant may offer protection against SARS-CoV-2 despite cigarette damage. Therefore, it is very important to separate the debate between harmful cigarette smoking and the therapeutic potential in other medicinal pathways of cholinergic agonists for the exploration of their therapeutic potential against SARS-CoV-2. For example, Nicotine Replacement Therapy (NRT) offers safe routes of administration in which, due to the lack of combustion and lack of added chemicals, the phenomenon of cigarettes-linked damage or toxicity is not observed (21).

Nevertheless, these nicotinic cholinergic agonist medications are based on NRT and are not specifically designed against COVID-19 / SARS-COV-2, due to their route of administration and pharmacological design, and will most likely offer an interesting but only very limited efficacy against COVID-19 having a slower systemic effect than a rapid neurotropic desired one. In example, the transdermal route of nicotine patches and oral mucosa route of chewing gums that contain nicotine for cigarette addiction replacement therapy (NRT) do not offer rapid and direct stimulation of the neurotropic pathway since they work in a systemic way. Furthermore, the dosage and pharmacokinetics are intended for cessation of smoking and not designed for SARS-CoV-2. In the case of cholinergic agonist drugs designed for NRT, the molecule used is only nicotine, avoiding other equally important cholinergic molecules that act with more specificity and efficacy on alpha7 nAChR subunits, such as i.e. anabaseine (22), among other molecules, which by themselves or in combination can offer, combined in a synergistic mixed composition, a greater therapeutic potential for SARS-CoV-2.

The surprising finding about the low prevalence of COVID-19 in active smokers brought to the table the hypothesis that nicotine may play a preventive or therapeutic role in the management of the current COVID-19 pandemic. The use of tobacco and nicotine in smoked cigarettes is harmful to health - the authors of this article strongly discourage the use of smoked cigarettes as a method of preventing COVID-19 - because cigarettes contain 250 added dangerous chemicals (tar, phenol, catechol, pyrene, benzopyrene, phytosterols, sigmasterol, etc.), of which at least 50 are carcinogenic (pyrene, catechol, nitrosamines, polonium, nickel, cadmium, hydrazine, formaldehyde, nitrogen oxide, etc.) and by the several thousand toxic derivatives produced by cigarette combustion (carbon monoxide, methane, acetaldehyde, acetone, hydrogen cyanidin, toluene, benzene, etc.). Nevertheless, cholinergic agonist molecules as nicotine derived from the tobacco plant or other plants in medicinal and non-smoked therapeutic administration routes do not have a toxicity and are well tolerated (23,24) and safe even at long-term use (25), they do not have relevant or minimal side effects, nor addiction potential or withdrawn symptoms when applied to non-smokers (24), even at high doses (26), nor significant cardiovascular risk (27–30) even in patients with previous cardiovascular and coronary events (31,32) even with concomitant smoking (33) when administered at appropriate therapeutic doses and posology and taking into account the risk-benefit ratio (34). Nicotine itself as a molecule, aside from the cigarette, does not exhibit carcinogenic properties (35) and its medicinally inhaled nasal application does not significantly alter lung function, nor diastolic blood pressure when compared with placebo (36).

Furthermore, the addictive potential of nicotine and other cholinergic agonist molecules in the medicinal form is very low due to a much lower nicotemic peak as most forms of nicotine replacement therapy (NRT) deliver nicotine more slowly

than smoking a cigarette and thus the increase in serum of nicotine levels is more progressive. Compared to smoking, few reinforcing effects are obtained from nicotine patches or spray nasal nicotine delivery (37). In addition, in the particular case of the administration of nicotine, social and psychological factors as well as predisposition, exert an influence effect (38). Psychosocial aspects play a special role in cigarette addiction that is not found in other therapeutic routes of administration of nicotine and other cholinergic agonists. It has been epidemiologically shown that people with the highest rates of stress are also those with the highest rates of smoking (39). This ratio of stress to smoking is largely correlated with alcohol, caffeine, and other legal or illegal addictive substances.

NRT nasal sprays approved by FDA and EMA relieve withdrawal in less time than other NRT products, but compared to cigarette combustion, absorption is slower and serum nicotine levels are lower (40).

The therapeutic medicinal use of nicotine and other cholinergic agonists is being and has been explored, proposed, and studied in memory disorders (41) Parkinson's disease, Alzheimer's disease, cognitive disorders, including antiviral uses for HIV (42,43) or herpes simplex (44).

In silico studies support the hypothesis that nicotine interacts positively in the disruption of SARS-CoV-2 on the human angiotensin-converting enzyme II (ACE2) (45,46) and also in the dysregulation of the nicotinic-cholinergic system by SARS-CoV-2 (11,47).

1.3 PATHOPHYSIOLOGY OF SARS-COV-2 AND THE ROLE OF THE POSSIBLE MECHANISM OF ACTION OF NICOTINIC CHOLINERGIC AGONISTS IN COVID-19

SARS-CoV-2 is a zoonotic and neurotropic virus (48,49) which exhibits nicotinic-cholinergic properties (50) with clear interactions with the Renin-Angiotensin-Aldosterone System and implications of an inflammatory over-response activated by cells of the immune system and pro-inflammatory cells such as cytokines, which can cause multiorgan failure (51). The virus can be transmitted by Flügge droplets, aerosols or fomites. The incubation time is usually between the 4th and 5th day and in symptomatic patients, symptoms usually appear on the 11th day. Undiagnosed asymptomatic carriers represent approximately 40% to 45% of all infected and can transmit the virus for a period of approximately 14 days or more (52).

β-coronaviruses have the ability to enter the peripheral nerves and spread through the brainstem, affecting the respiratory and cardiovascular centers. The main neurotropic pathways of entry are through the conjunctival mucosa (53), the central nervous system rich in ACE2 and nAChRs, especially trough the brain stem for which SARS-CoV-2 has a special predilection (nucleus of the solitary tract and nucleus ambiguus), the gustatory mucosa (54) and the olfactory mucosa (55) with a high neuroinvasive and respiratory failure potentials (56), this respiratory infective route being the most common due to the virus's ability to remain in aerosolized microparticles (57).

In the epithelium-endothelium of the olfactory mucosa we found a high expression of ACE2, TMPRSS2 and furin. The virus enters through olfactory receptor neurons of the olfactory mucosa via the cribriform plate and nerve endings of the olfactory bulb, spreading transneuronally from the orbitofrontal cortex, agranular insula, sub-nuclei of the amygdala, piriformis cortex, and entorhinal cortex.

Furin is the enzyme that makes the proteolysis of SARS-CoV-2 glycoprotein S and cleaves it at S1 and S2 (58). ACE2 is the receptor for the S1 subunit of the SARS-CoV-2 glycoprotein S. RBD is the receptor binding domain of the S1 subunit of the SARS-CoV-2 glycoprotein S glycoprotein and is responsible for the binding link between the virus and ACE2. The S1 subunit of the SARS-CoV-2 glycoprotein S is responsible for binding to ACE2. The S2 subunit of the SARS-CoV-2

glycoprotein S is activated by TMPRSS2 and is responsible for membrane fusion. S2 cleavage unfolds FP (Fusion Peptide) to join the virus membrane with the host membrane. TMPRSS2 cleaves the S2 subunit of the SARS-CoV-2 glycoprotein S to make virus-cell fusion at the membrane level (59). CD147 Ig interacts with the S protein of SARS-CoV-2 allowing its entry (60).

CD147 Ig and TMPRSS2 are responsible for the adhesion and internalization of the virus. Jialu Qiao *et al.* (49) found that the CNS is more susceptible to being infected with SARS-CoV-2 due to the high expression of mRNA, CD147 protein and TMPRSS2 in the pituitary, cortex and cerebellum in mice. Ageusia, hypogeusia, anosmia, hyposmia and other neurological manifestations such as dizziness, headache, changes in vision, emotional lability, cognitive impairment and pituitary hypofunction in patients with COVID-19 could respond to neuronal degeneration as a result of the affinity of these brain regions to SARS-CoV-2.

On the other hand, at the alveolar level, SARS-CoV-2 produces an overexpression of CD147 Ig (60), increasing the proliferation induced by TGFbeta1 and myoactin, facilitating fibroblasts to invade the intra-alveolar area and produce a remodeling consistent with pulmonary fibrosis.

SARS-CoV-2 activates NF-Kb (Nuclear factor kappa beta) (61) that controls immune and inflammatory responses through pattern recognition receptors and accumulated AngII. Several studies indicate that nicotine inhibits the production of pro-inflammatory cytokines in macrophages by inhibiting NF-KB 8 that requires ubiquitination of IkB (62) through a modulatory mechanism dependent on the cholinergic system via α7nAChRs (63).

The activation of α7nAChRs, deregulated by SARS-Cov-2, can prevent IkB degradation and p65 nuclear translocation in addition to modulating the signaling pathways of p38 kinase and nuclear factor-κB (64), which would explain why nicotinic cholinergic agonists have a therapeutic potential in monocytes, macrophages, and endothelial cells affected in COVID-19; furthermore, in diabetic and obese patients there is an association with the expression of α7nAChR. All this induces the production of inflammatory cytokines such as TNFα and IL-6 by means of ADAM17 (Metalloprotease) (65) followed by the activation of IL6 AMP (Amplifier).

The proteolytic breakdown of ACE2 is mediated by ADAM17 and regulated by endocyted proteins of the virus (66). Entry of ACE2 raises AngII levels by activating the AngI receptor due to RAAS imbalance (67). A higher amount of ADAM17 also releases TNFα. SARS-CoV-2 upregulates ADAM17 producing a cytokine storm. Elevated TNFα levels facilitate entry of the virus (68). Nicotine has a regulatory effect on TNFα, a downregulation of IL6 and MCP (Monocyte chemotactic Protein 1) and is an inhibitor of pro-inflammatory cells (69). Because TNFα is also necessary for the proper function of the immune system, complete suppression of TNFα over a long period of time can be detrimental with serious implications for human health. Considering the hyper inflammatory syndrome that accompanies severe and critical COVID-19, this pathology is a potential and interesting target for TNFα modulation therapy; thus, the aim is not to suppress it but to regulate or modulate its expression (70).

TNFα binds to receptors on neutrophils (71), endothelial cells, fibroblasts, serum, and synovial fluid, among others. This can lead to increased local activity of the endothelium, release of nitric oxide producing vasodilation, increased vascular permeability, recruitment of inflammatory cells, immunoglobulins and complements, activation of T and B lymphocytes, and activation of platelet adhesion, which can produce a septic shock and disseminated intravascular coagulation (DIC).

The entry and viral replication of SARS-CoV-2 produce increased release of pro-inflammatory cytokines in epithelium and

endothelium, increased exudate, decreased oxygen with difficulties to cross the hemato-alveolar barrier, all of which triggers dyspnea and hypoxemic type II Acute Respiratory Failure with increased innate inflammatory response (macrophages and granulocytes) which generates severe respiratory distress (72). Due to the massive release of cytokines, the inflammatory response and the destruction of pneumocytes II, the rupture of the hypoxemic alveolar cells is induced, which increases blood viscosity and platelet adherence. The activity of α 7nAChR is also decreased, which increases blood viscosity with potential systemic coagulopathy (73).

The mediation of TMPRSS2 and CD147 in interaction with ACE2 triggers piroptosis of the alveolar epithelium. Therefore, the cytokine storm is generated leading to an overproduction of IL-1β, IL-2, IL-6, IL-7, IL-10, IL-12, TFN, G-CSF (colony stimulating factor of granulocytes), inducible INFα and INFγ protein 10, TGF-β, MCP-1, MIP-1α and TNFα, and CCL2 chemokines, CCL3, CCL5, CXCL8, CXCL10, etc. Endothelial viral shedding spreads to the CNS, kidney, heart, adrenals, spleen, lymph nodes, among others (74).

SARS-CoV-2 evades innate immunity with the release of IFN1 (Interferon 1) (75) by inflammatory and epithelial cells in viral infections and binds to the membrane receptor to activate STAT (Signal Transducer and Activator of Transcription) proteins. The virus hacks the union of importins alpha / beta with IFN1 allowing the virus to enter the cell nucleus. This induces the pyroptosis of macrophages and lymphocytes, giving an increase in the inflammatory response, dysregulation of ACE2 by down regulation and an increase in pyroptosis. All this cycle leads to an imbalance of the defense lines in favor of LTH2 (T helper 2 releasing IL-6 and IL-10) and to the detriment of LTH1 (T helper 1 antiviral inhibitor), where an increase in LTH2 is associated with fatal infection and more adverse outcome as it has already been observed for SARs-CoV-1 (76).

The inflammatory response due to this hacking of the virus with alpha / beta importins creates antibodies and, in the most severe cases, non-neutralizing antibodies that unstably bind to the virus. In antibody-mediated immune response the antibody is expected to have a stable neutralizing effect on viral proteins. The mutation of the non-structural protein glycoprotein S of SARS-CoV-2 makes an unstable binding with the receptor of macrophages, which highly express α7nAChRs that are also deregulated by viremia. This unstable antibody-virus union allows the entry of the virus, loss of the antiviral action of innate immunity due to increased pyroptosis and viral replication increasing its infectivity. Pyroptosis in macrophages and T lymphocytes is responsible for cytokine storm, inducing lymphopenia (77), increased antibody-mediated infection, and increased infectivity and viral load.

1.4 CYTOKINE STORM AND COVID-19

There seems to be a clear correlation between the hyperinflammatory cytokine storm syndrome and the high prevalence of mortality (78). Cytokine storm is a collateral effect caused by an inflammatory response to viremia, it consists of a dysregulation of nAChRs' macrophages and is associated with rapid clinical deterioration and severe acute respiratory syndrome (79). The clinical therapeutic approach that has been tested in various countries around the world includes interleukin receptor blockers known as anti-cytokines (80) with the purpose of modulating the COVID-19 cytokine storm. Anakinra blocks IL-1 receptors, emapalumab blocks Interferon gamma (IFN-γ) receptors, tocilizumab (81) and sarilumab block IL-6 receptors. However, this anti-cytokine treatment may look promising, but it still requires many more clinical trials before drawing firm conclusions because giving it to COVID-19 patients too early could worsen viremia and giving it too late would no longer make sense. The anti-cytokine pharmacological strategy is not infallible due to the risk of adverse

reactions and because, ultimately, it seeks to control the cytokine storm without having previously regulated what is triggering the storm.

One of the lesser known but no less important effects of the cytokine storm is that excess TNF prevents the development of T helper lymphocytes and therefore the formation of germinal centers by B and T helper lymphocytes. In the absence of an adequate number of germinal centers, herd immunity is affected as there is no enough memory of the immune system and this will allow the reinfection of those who manage to recover from COVID-19. Vaccines probably create germinal centers by the induced immune response, but that does not guarantee that it is possible to acquire or develop immortal antibodies against COVID-19.

This article emphasizes that COVID-19 is not only a disease that affects the ACE2 receptors, but that it clearly impacts the nAChR receptors, highlighting the role of the cholinergic system and the nicotinic cholinergic receptors in the current pandemic. Nicotine is a direct nicotinic cholinergic agonist that regulates alpha7 subunits, which has been shown to be widely effective in animal models in modulating inflammation and cytokine production by macrophages.

1.5 ACE2, THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM, THE VAGAL REFLEX AND THE CORRELATION WITH THE INTERFACE BETWEEN THE NERVOUS SYSTEM AND THE IMMUNE SYSTEM.

The ACE2 receptor is affected by SARS-CoV2 causing cytopathic damage and pyroptosis in cells that express this enzyme (82,83). Once the binding and fusion with ACE2 is completed, the virus manages to internalize itself, raising angII levels and activating the angI receptor due to RAAS imbalance with the overexpression of ADAM17 and TNFα, establishing a vicious inflammatory cycle.

When the levels of expression of ACE2 and ADAM17 or their functions are altered, blood pressure can be affected in its regulation from the central nervous system, as well as the regulation of neuro-inflammatory processes (83). Fudim *et al.* (84) have pointed out that << Notably, as part of a feedback loop, hyperactivation of the vagus nerve via the nicotinic acetylcholine receptor (nAChR) downregulates the expression or activity of ACE2, which could prevent viral infection. In summary, the interaction of SARS-CoV with ACE2 could present more than merely an entry point for the virus into the human body but be a nidus for a dysregulation of the potent renin angiotensin system, with detrimental effects on cardiovascular regulation and parasympathetic tone> >.

The nervous system and the immune system have an interface that involves the vagus nerve and the anti-inflammatory nicotinic pathway with acetylcholine and its nicotinic receptors (85).

Activation of immune cells to produce pro-inflammatory cytokines is common in infections of viral origin. The vagus nerve and the CNS can be activated by these proinflammatory cytokines, affecting via afferent CNS processing centers such as the respiratory centers. In the efferent pathway, the body can activate the vagus nerve to fight inflammatory processes by inhibiting the overproduction of cytokines (86) in macrophages.

The role of ACh is not only a modulator of immune cells, but it is the main neurotransmitter of the vagus nerve and is in charge of the nervous regulation of the functions associated with homeostasis and organs. Some regulatory T lymphocytes can mimic the nervous system through the synthesis of ACh in order to mitigate or regulate an eventual overproduction of cytokines by macrophages. This ACh mechanism is carried out thanks to the specificity and efficiency of the α7 subunits of the nicotinic receptors, which are very important regulators in inflammatory processes (87,88).

SARS-CoV-2 dysregulates ACE2 and α7nAChR receptors in human macrophages (89) by increasing or promoting

cytokine storm by hindering the regulatory role of the cholinergic anti-inflammatory pathway on the production of inflammatory cytokines. It is well known that in animal models the stimulation of the vagus nerve promotes the production of ACh by inhibiting inflammation (90) and that, on the contrary, vagotomy in rodents under mechanical ventilation increases the alveolar damage associated with the production of IL-6 (91). In addition, stimulation of the vagus nerve can cope with endotoxins, attenuating the inflammatory response (92), helping to improve lesions at the alveolar level and regulating hyperinflammation at the endothelial level associated with coagulopathies and thrombotic risk in some COVID-19 presentations. In line with Changeux, Amoura, Rey and Miyara <<According to the nicotinic hypothesis, it should be noted that the hematopoietic deficiency of α7nAChR increases the platelet reactive state, which could explain the thrombogenic presentation of Covid-19>> (10).

Fudim *et al.* (84) point out in a very interesting way that <<Diminished cardiac vagal activity is found in patients with pulmonary and cardiometabolic disease. This has been found to predispose patients to develop and die of critical illness. On the contrary, patients with an increased vagal tone might be protected from a cytokine release syndrome. The observation of lower rates of symptomatic COVID-19 infections in active smokers potentially suggests that active nicotine exposure activates the cholinergic anti-inflammatory pathway, previously shown to be protective in various infectious illnesses, despite the deleterious effects of cigarette use. Furthermore, a milder COVID-19 disease course in children, who have a naturally higher vagal tone, even in an infectious setting, could support the significance of the cholinergic anti-inflammatory pathway uniquely in COVID-19 patients.>>

These physiological and clinical evidences strengthen the nicotinic hypothesis about SARS-CoV-2 published by several authors whose references we have already mentioned. The role of nicotine as a direct nicotinic cholinergic agonist is a pharmacological agent with regulatory potential against COVID- 19 with an increasingly solid scientific basis.

1.6 NICOTINE AND SARS-COV-2

Nicotine is an alkaloid found in various plants, especially in the Nicotiana sp. leaf, and it is only one of the 7,000 chemical components released by the burning industrial cigarette and therefore, a single molecule cannot be comparable to the cigarette in its entirety and nor is it the cause of the multiple ravages of smoking on human health. That is why it is necessary to highlight that the effects of nicotine in the body are always related to the dosage, the route of administration and the chemical compounds with which it reacts. The inhaled route with smoke inhalation is the most widespread in the world and represents many health risks due to all the chemical residues resulting from cigarette combustion. However, nicotine administered without the combustion pathway has other effects.

Thanks to NRT (Nicotine Replacement Therapy), alternative ways of administering nicotine have been experimented among smokers who wish to quit. Such alternatives include patches, chewing gums, sublingual tablets, among others, but this approach remains specifically targeted for industrial cigarette smokers.

Our approach to nicotine and other direct nicotinic cholinergic molecules for antiviral, anti-inflammatory and immunomodulatory prophylactic and therapeutic purposes is based on the exploration of the pharmacokinetics of nicotine and other cholinergic agonists in an intranasal and oral route of administration that are radically different from the smoked route and in completely safe doses, having a modulating role in the Cholinergic System (CS) via the anti-inflammatory cholinergic pathway, the Immune System (IS) and the Central Nervous System (CNS).

The nicotinic cholinergic system is one of the major modulators of the immune response and of the stress axis (hypothalamus - pituitary - adrenal). The endogenous ACh agonist (Acetylcholine) and the exogenous nicotine agonist for any nAChR open ion channels in the receptor, allowing cation flow and inducing a wide variety of biological responses. The acetylcholine receptor modulates the interactions between the nervous system and the immune system.

The absorbed nicotine diffuses at high speed, not so much in the transdermal route (93,94) - which takes an hour or more - but more quickly and with more bioavailability, especially transnasally, and then quickly leaves the plasma to concentrate on related structures such as lipids and nAChRs. According to Benowitz (93) << The time course of nicotine accumulation in the brain and in other body organs and the resulting pharmacologic effects are highly dependent on the route and rate of dosing >>. Pharmacological subunit clearance assays revealed that presynaptic nAchRs include the alpha 7 subunit and that nAChRs present in CNS enhance rapid excitatory transmission, revealing a likely mechanism for the CNS and behavioral effects of nicotine (95). For this reason, we propose a therapeutic and prophylactic route of nasal administration that mimics the entry route of the virus through the nasal mucosa to increase transmission and a rapid excitatory response with higher bioavailability and rapid arrival to the CNS, a phenomenon that is not seen with NRT products like chewing gums or transdermal patches.

Nicotine has a high power of action on the nAChRs of alveolar macrophages and on the central nAChRs of macrophages associated with the CNS and the bronchial tree. SARS-CoV-2 blocks the cholinergic system by dysregulation of nAChRs, inhibiting the nicotinic cholinergic anti-inflammatory pathway, triggering hemophagocytic lymphohistiocytosis, viral sepsis, and lung damage (96). In addition, it can infect terminal areas of the afferent or efferent fibers of the vagus nerve causing a down regulation of ACE2 producing local inflammation by interruption of the cholinergic pathway. Experimental studies indicate that direct stimulation of the efferent vagus nerve in response to endotoxin exposure in rodents had an inhibitory effect on TNF reducing both systemic inflammation and mortality (92).

As we have already described, the decrease in the activity of the vagus nerve could be enhanced by nAChR dysregulation, especially the alpha7 subunits, caused by SARS-CoV-2, generating a state of hyper-inflammation, against which the use of cholinergic agonists is a proposed therapeutic route with scientific basis on physiological and therapeutic mechanisms. The activity of the vagus nerve is clearly decreased by SARS-CoV-2 and is itself decreased in patients with obesity and diabetes, affecting the body's immunity and anti-inflammatory capacity (97). As Changeux *et al.* (10) already observed, this fact could explain why patients with diabetes and obesity infected by SARS-CoV-2 have a worse prognosis. It is interesting to add that nicotine’s properties in the improvement of inflammatory processes linked to obesity and ulcerative colitis have been reported (98,99). In addition to that we have to note that α7nAChR are present in interstitial and alveolar macrophages in mices’ lungs, having induced-obesity in mice an impact in the number of α7nAChR cells in alveolar and interstitial macrophages that may affect the cholinergic anti-inflammatory pathway (100).

Computational modeling studies found that nicotine has a binding affinity at certain terminal amino acid residues in the binding site pocket of ACE2 with antagonistic effect. According to Kumar *et al.* (45) << On the other hand, nicotine docked with ACE2 in the presence or absence of SARS-CoV-2. Nicotine established a stable interaction with negatively charged Asp368 of sACE2, which in turn binds with amino acids like Thr362, Lys363, Thr365, Thr371, and Ala372. In the presence of nicotine, INS1 and sACE2 showed a reduced binding affinity score of -12.6 kcal/mol (Vs -15.7 kcal/mol without nicotine), and a lowered interface area of 1933.6 Å2 (Vs 2057.3Å2 without nicotine). The neuronal nicotinic acetylcholine receptor

(nN-AChR) and angiotensin-converting enzyme 2 (ACE2) receptor showed 19.85% sequence identity among themselves. Following these receptors possessed conserved Trp302 and Cys344 amino acids between them for nicotine binding. However, nicotine showed a higher binding affinity score of -6.33 kcal/mol for the sACE2-INS1 complex than the sACE2 alone with -5.24 kcal/mol. A lowered inhibitory constant value of 22.95μM recorded while nicotine interacted with the sACE2-INS1 complex over the sACE2 alone with 151.69 μM. In summary, nicotine showed a profound binding affinity for the sACE2-INS1 complex than the sACE2 alone paving for the clinical trials to validate its therapeutic efficacy as a bitter compound against the SARS-CoV-2 virulence.>>.

Our study tries to find initial evidence in humans on whether these *in silico* observations are of clinical relevance for the medicinal use of nicotine with other cholinergic agonists against SARS-CoV-2.

NAChRs are present at the neuromuscular junction, skeletal muscle, ganglion neuron dendrites, nerve cell surface, bronchoalveolar fluid, CNS, PNS, lung and bronchial epithelium, endothelium, immune system, and in macrophages, especially the alpha7nAChR subunit (101), including alveolar cells.

SARS-CoV-2 is a nicotinic virus because it causes a dysregulation of the cholinergic pathway through the nAChRs. The alpha7nAChR subunit is found at the interface between the immune system and the nervous system and has a protective and positive role in inflammation and immunomodulation by reducing levels of pro-inflammatory cytokines, chemokines and regulating the activation and differentiation of immune cells that are important for maintaining immune homeostasis (102). This interface is expressed in monocytes producing cytokines and activated by ACh for down regulation of proinflammatory cytokines. The nAChRs would play a key role in exacerbating the pathogenesis of Severe Acute Respiratory Syndrome (SARS) when SARS-CoV-2 interferes with the regulatory functions of dendritic cells and macrophage-dependent monocytes (103).

The alpha7nAChR subunit is abundant in alveolar macrophages and nervous system and airway associated macrophages. An acute nicotinic effect mediated by receptors has been identified in alveolar macrophages with anti-inflammatory therapeutic potential in animal models, which is curiously reversed by alpha-bungarotoxin (104), a toxin which is also contained in the SARS-CoV-2 genomic sequence.

The α7 subunits are expressed in macrophages and their expression plays an important anti-inflammatory role in vagal nerve signaling (85). Thus, nicotine exerts anti-inflammatory effects on macrophages that can be offset by selective α7 antagonists (88). Selective α7nAChR agonists have been shown to be effective in reducing macrophage cytokine production and inflammation in animal models of pancreatitis (105) and ulcerative colitis problems (106).

SARS-CoV-2 produces a dysregulation of ACh (Acetylcholine) through the nAChRs in bronchoalveolar fluid, alveolar macrophages and nervous system and airway associated macrophages. This causes HLH (hemophagocytic lymphohistiocytosis) from an ineffective LTH1 response and consists of a hyper inflammatory syndrome due to abnormal activation of the immune system after the proliferation of Natural Killer (NK) cells, macrophages and CD8 T lymphocytes. The clinical picture includes a severe increase in IL6 and ferritin. Hyperferritinemia is a poor prognostic marker for COVID-19 patients because it reveals an up regulation of macrophages that express CD163, which are responsible for these alarming levels of ferritin. Traditionally, hemophagocytic lymphohistiocytosis is an entity that occurs in infants and children, although it is a rare disease that could also occur at any age. However, HLH in adults is the result of severe viremia. The SARS-CoV-2 virus is responsible for cases of secondary HLH due to hyper production of NK, alveolar macrophages and

nervous system and airway associated macrophages, and CD8 T lymphocytes in patients with severe and critical COVID-19.

As we have seen, SARS-CoV-2 blocks ACh (Acetylcholine) by efferent pathways of the vagus nerve, affecting communication with macrophages and α7nAChR. This results in an inhibition of the cholinergic anti-inflammatory pathway of macrophages and α7nAChR-dependent cytokines. The result is secondary hyperinflammation due to the inability to modulate TNF-α downwardly.

Modulation of ADAM17 (TNF-α converting enzyme) (107) by cholinergic agonists could have a beneficial and protective effect against COVID-19 (108). Additionally, nicotine and cholinergic agonists (109) have a regulatory effect on the RAAS axis.

1.7 SARS-COV-2 AND NACHR SUBUNITS:

Farsalinos *et al.* identified an interaction between aa381-386 of the SARS-CoV-2 glycoprotein S and aa189-192 of the extracellular domain of the alpha9 subunit of nAChR, a region that forms the core of the "toxin binding site" from the nAChRs (89). The authors also identified an interaction very similar to the interaction between α9 nAChR and α-bungarotoxin and a similar interaction was observed between α7 pentameric nAChR and SARS-CoV-2 glycoprotein S in addition to an interaction between the binding domain of ligands of a pentameric α7 of the nicotinic receptor and the S1 subunit of the SARS-CoV-2 glycoprotein S.

According to Carlson *et al.* (110) << Excitotoxic neuronal death mediated by N-methyl-D-aspartate (NMDA) glutamate receptors can contribute to the extended brain damage that often accompanies trauma or disease. Both the inflammatory cytokine tumor necrosis factor-alpha (TNF-α) and nicotine have been identified as possible neuroprotective agents to NMDA assault. We find that TNF-alpha protection of a subpopulation of cultured cortical neurons to chronic NMDA-mediated excitotoxic death requires both the activation of the p55 / TNFRI, but not p75 / TNFRII, and the release of endogenous TNF-alpha. Nicotine protection to NMDA was mediated through an alpha-bungarotoxin-sensitive receptor. When coapplied, neuroprotection to NMDA by either TNF-alpha or nicotine was abolished but could be recovered with alpha-bungarotoxin. These results suggest that the cytokine TNF-alpha and alpha-bungarotoxin-sensitive nicotinic neurotransmitter receptors confer neuroprotection through potentially antagonistic pathways. >>

Certain neurotoxins from snakes and the rabies virus (111) bind to nicotinic-cholinergic receptors (111). Nicotine and cholinergic agents, especially anabaseine and several other cholinergic agonist molecules other than nicotine, inhibit the release of ADAM17 which prevents the excessive release of TNF-α offering protection against endotoxic shock.

A recent paper by Alexandris *et al.* (112) showed through computational modulations the clear interaction between SARS-CoV-2 and nAChR and the disruption in the anti-inflammatory response of the cholinergic system. The study found a clear interaction between the alpha 7 subunits and the SARS-CoV-2 glycoprotein S1 when it joined cholinergic agonists and molecules such as Acetylcholine, Carbamylcholine, Cytisine, Epibatidine, Galantamine, Nicotine, Succinylcholine and Varenicline developing the following hypothesis: <<we have built a hypothesis that SARS-CoV-2 Spike glycoprotein, bearing a "toxin-like" sequence in its RBD, could bind to the toxin-binding domain of the α-subunit of the nAChRs [...] It is possible that cholinergic agonists/antagonists (i. e., nicotine, cystine, epibatidine, and varenicline) could impede the interaction between human nAChRs and SARS-CoV Spike RBD. The coordination of nicotine and the rest agonists/antagonists is driven by a highly conserved group of amino acids in their respective structures, identically

recognized by the LBD located on the nAChRs structure>>.

The study presented here provides significant evidence in humans for preliminary validation of the use of cholinergic agonists in SARS-CoV-2 infected humans as proposed in the *in silico* study made by Alexandris *et al*. and according to the nicotinic hypothesis proposed by the aforementioned authors.

1.8 MECHANISM OF ACTION AND DESIGN OF ANTIVIRAL, ANTI-INFLAMMATORY AND IMMUNOMODULATORY CHOLINERGIC AGONIST (CA) COMPOSITION AGAINST SARS-CoV-2

Based on the above, Niccovid® has designed a composition called preliminary CA (Cholinergic Agent) with 16 main active ingredients based on nicotine along with several other cholinergic molecules whose mechanism of action and pharmacodynamics is specifically designed to combat SARS-CoV-2 to prevention, prophylaxis and probably also for the recovery of patients suffering from Post-Covid Syndrome.

The design of the CA drug is not only intended as an antiviral but also as an anti-inflammatory and immunomodulator. These qualities potentially give it a therapeutic effect against SARS-CoV-2 dangerous mutations and against current or new strains as well. If such new strains may appear, they should not *a priori* outdate the mechanism of CA's therapeutic action.

The drug not only contains nicotine but also specific cholinergic agonist molecules that exhibit nicotine replacement properties and more specifically regulate α9 and α7nAChRs and stimulate a wide variety of nicotinic acetylcholine receptors (nAChR), such as neuromuscular receptors (α12β1γδ or α12β1γε) and which are inhibited by the snake venom peptide α-bungarotoxin. In addition, these other molecules may be more specific and effective than nicotine against cognitive disorders and neuroinflammation, problems observed in a large group of patients affected by COVID-19. Several of these molecules exhibit substitutive as well as synergistic properties with nicotine and its enhancing effects, a priori increasing its pharmacokinetic and therapeutic activity, acting as allosteric modulators in nAChRs, improving brain plasticity, stimulating Akt and inhibiting GSK3β in the hippocampus, promoting axonal growth and behavioral recovery after a central nervous system injury, and have modulating properties of the serotonin and dopamine system differentiated from nicotine, facilitating the release of neurotransmitters and the expression of synaptic proteins, also possessing a longer half-life , which provides a therapeutic potential greater than the use of nicotine alone. Some of these cholinergic molecules also function as inhibitors of monoamine oxidase (including MAO-A and MAO-B), an enzyme that catalyzes the production of hydrogen peroxide. Thus, they could, through a different pathway (as an oxidative stress reducer as an MAOI agent), as an oxidoreductase agent, contribute to regulate or inhibit mitochondrial reactive oxygen species in cellular oxidative stress in COVID-19 patients. This is because reactive mitochondrial oxygen species, free radicals and free oxygen molecules can bind to any molecular group causing various dangerous reactions like over-inflammation. These enzymes related to oxidative stress could be inhibited in individuals who have coronavirus thanks to the CA composition designed by Niccovid®. The therapeutic application of CA could reduce reactive mitochondrial oxygen species thanks to its indirect activity of MAO and catecholamines. In addition, and very importantly, most of the cholinergic agonist molecules contained in CA other than nicotine have a lifespan which is over 10 times longer than nicotine, providing an extended protection potential.

The CA composition has been designed to have specific anti-inflammatory properties through the effect of some of its

molecules in the reduction of TNF-α levels in the brain, reduction of IL-6 levels and in the prevention of STAT3 and NFκB phosphorylation induced by lipopolysaccharides (LPS) or TNF- α in SH-SY5Y, HEK293, human microglia, and human blood mononuclear cells.

On the other hand, other molecules of non-cholinergic origin were introduced into CA for modulating the oxidative stress of nicotine and do also have anti-inflammatory, antifungal, antioxidant, neuroprotective and gastroprotective properties (the enteric system is also affected by SARS-CoV-2), facilitating, some of them, the healthy regulation of glucose in diabetics. The mentioned supporting molecules for the cholinergic agonist molecules contained in CA would provide synergies and extra effectiveness through a differentiated pathway of the Renin-Angiotensin-Aldosterone axis by regulating the ACE enzyme and iron (serous ferritin values are overexpressed in many severe COVID-19 patients) in addition to having analgesic, antipyretic, chemopreventive, angiogenic, and antiemetic properties. The have also known properties against certain types of pulmonary fibrosis and exhibit antioxidants properties also, and they do mediate in the modulation of the NF-κB activation cascade.

Although the aforementioned nicotinic hypothesis proposed by several authors has been based exclusively on the study of the nicotine molecule against SARS-CoV-2, we believe that a drug with a mixture of several molecules with the properties described and by a combined administration of oral drops and especially of a nasal spray, would have a much greater effectiveness in the preventive and therapeutic management of COVID-19. The administration of CA in pulverized intranasal aerosol or nasal spray has been specifically designed to achieve an excitatory and therapeutic effect much faster and more directly on arrival at the CNS than other routes such as sublingual or transdermal. The intranasal liquid spray solution can reach the higher centers through the olfactory receptor neurons of the olfactory mucosa via the cribriform plate and the nerve endings of the olfactory bulb. In mice, for example, there is evidence of the expression of nicotinic acetylcholine receptors on nasal trigeminal nerve endings that do innerve solitary chemosensory cells being those ones autoregulated by cholinergic receptors. Moreover, the nasal spray has the advantage of offering better absorption and higher bioavailability of cholinergic agonist molecules than patches or tablets and prevents the exhalation of viral particles or other particles unlike, for example, in the case of a theoretical nebulization administration with inhalation. Thus, the pharmacokinetic strategy of CA use is to provide a slow systemic effect by the oral route with the administration of oral drops in combination with a rapid neurotropic effect by nasal spray administration, adapting dosage according to the patient's profile and needs.

2. METHODS

2.1 Ethics committee approval

This study has been verified and evaluated by the Cediff Biomedical Research Ethics Committee, stating that the protocol complies with the ethical standards described in the national and international regulations related to biomedical research. The risk-benefit ratio was found favorable by the Ethics Committee for the subjects participating in the research, which is widely described in the justification of the study, protocol, and informed consent. This study has been endorsed by Ethics Committee guaranteeing its adherence to the following international standards related to biomedical research on human subjects: Nuremberg Code (International Tribunal of Nuremberg) 1947. Declaration of Helsinki. World Medical

Association, 1964 and later revisions; Belmont Report, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research 1979; Universal Declaration on Bioethics and Human Rights, UNESCO 2005. Likewise, it adheres to the following national Colombian regulations: Resolution 8430 of 1993, Resolution 3823 of 1997, Resolution 2378 of 2008 and all the guidelines and updates issued by Colombian regulatory agency (INVIMA) or the Colombian Ministry of Health and Social Protection in relation to the subject.

2.2 Study design

The composition to be studied is made up of cholinergic agonist (CA) agents and other non-cholinergic agonist agents with synergistic interactions and with a specific route of administration. CA has been patented (2 patents were applied under numbers #2013130.6 and #2013131.4 at Uk's Patent Office which form the basis for international protection) and its design, dosage and route of administration have been made specifically to combat COVID-19 in form of oral drops and nasal spray.

Group A: infected patients (20 patients): After a positive COVID-19 PCR test, patients receive a daily dose of CA nasal spray (1 puff on each nostril every twelve hours) and oral drops (9 drops in 175 mL of water every 6 hours).

Group B: control patients (15 patients): a control group of 15 subjects has been established also after a positive COVID-19 PCR test.

The same daily monitoring evaluation was done in both groups to assess the evolution of the disease based on scientifically supported medical scales for each symptom.

Ethnicity: the subjects were Colombian from white ethnicity (88% of Colombian population is white).

Inclusion criteria:

- positive patient for COVID-19 by PCR with results no more than 5 days old.
- outpatient under ambulatory care (non-hospitalized)
- Recruitment: patients were randomly recruited from a database of patients diagnosed with positive PCR tests for SARS-CoV-2 from several hospitals in Colombia.

Exclusion criteria:

- patient under in-ward hospital care, even if they have positive antigens and / or PCR.
- Decline in study participation.
- Under 18 years old.

2.3 Data collection

Group A received CA nasal spray and CA oral drops daily in the indicated dose according to the following criteria:

Clinical data: Date of suspected infection, date of onset of symptoms, date of PCR + or positive antigen for COVID-19, previous comorbidities (heart disease, kidney disease, lung disease, smokers <10 cigarettes / day, smokers > 10 cigarettes / Day, non-smokers, liver disease, pheochromocytoma, hyperthyroidism, hypertension, diabetes mellitus, drug dependence, other diseases.

Pharmacological data: ACEi, ARBs, hydroxychloroquine, heparin, beta-blockers, enoxaparin, salbutamol, statins, benzodiazepines, interferon therapy, antidepressants, corticosteroids, azithromycin, ivermectin, opiates, colchicine.

2.4 The following symptoms and tolerance to CA measured:

Symptoms:

- fever, cough, dyspnea, muscle fatigue, headache, ageusia, anosmia, chest pressure, general malaise, nasal congestion.

CA tolerance: side-effects and their time duration:

- Nasal itching, oropharyngeal discomfort, dizziness, nausea, headache, slight increase in heart rate, runny nose, lacrimation, malaise.

Measurements were made according to internationally accepted standard medical criteria and scales under the design of an expert in epidemiology.

Group B did not receive CA and was used as an epidemiological control group to compare the efficacy of CA who have gone through the disease without treatment or with some already existing treatment protocols as i.e. NSAIDs and/or ivermectin among others.

The evaluation in groups A, B was carried out for 15 days assessing the improvement or worsening of each symptom on a daily basis, with day 0 being the day of measurement of symptoms without the administration of CA and taking days 1 to 14 the measurement of the evolution of each symptom with CA for group A and without CA for group B.

2.5 Analysis plan

A basic OR study analysis was performed by analyzing the information collected with Microsoft SPSS software. Statistically significant tests were considered if the p-value was less than 0.05 (IC 95%); as well as a concept of the tendency of some inconclusive outcome will be given.

2.6 Methods and clinical scales for evaluation of each symptom

1. Fever calculated in:

- - "Yes", I have a fever
- - "No", I don't have a fever

For statistical purposes we have converted "Yes"/"No" to "1"/"0" in order to calculate the following values:

Day 0 (before starting treatment). CA treatment from Day 1 to Day 14

2. Dyspnea calculated from 0 to 4 according to the modified mMRC (Medical Research Council) scale, within the indirect scales, was initially used to study pneumoconiosis, but has since been modified to measure dyspnea. Of English origin, it has prognostic value. Previously, the MRC scale ranged from 1 to 5, but now the ATS recommends a scale that is incorporated into the BODE and which mainly measures the magnitude of the task that causes the patient to experience shortness of breath. The grades are:

- “0” Dyspnea occurs only with great physical effort
- “1” Dyspnea occurs when walking fast on the flat or when climbing a gentle slope
- “2” Dyspnea makes it impossible to keep up with other people of the same age
- “3” You have to stop and rest when walking ~100 m or within a few minutes of walking on the flat
- “4” The dyspnea prevents the patient from leaving the house or appears with activities such as dressing or undressing

3. Headache (cephalea) calculated in:

- - "Yes", I have a headache

- - "No", I don't have a headache

For statistical purposes we have converted "Yes"/"No" to "1"/"0" in order to calculate the following values:

Day 0 (before starting treatment). CA treatment from Day 1 to Day 14

4. Muscular fatigue calculated according to:

Muscle fatigue calculated from 0 to 10 according to Gunnar Borg's updated scale devised in the 1980s and improved a few years ago, a system for assessing intensity based not on value measurements but on the patient's own perception of effort. This is a fatigue scale that measures the perception of fatigue from 1 to 10, which was originally intended as a way of assessing medical damage, obtaining a standardized response to the sensations of pain, so different in each patient. In sports and, in particular, in exercise tests, the rating of perceived effort (RPE), measured by Borg's rating of the scale of perceived effort (RPE scale), is a quantitative measure of the frequent use of perceived effort during physical activity. In medicine, this is used to document the patient's exertion during a test, and sports coaches use the scale to assess training intensity and competition. This scale is especially used in the clinical diagnosis of choking and dyspnea, chest pain, angina, and musculoskeletal pain. The CR-10 scale is most appropriate when there is a predominant sensation arising from a specific area of the body, for example, muscle pain, quadriceps pain or fatigue or from lung responses.

The Borg scale can be compared with other linear scales such as the Likert scale or a visual analogue scale. The sensitivity and reproducibility of the results are generally very similar, although the Borg can exceed the Likert scale in some cases.

5. Cough calculated in:

- "Yes", I have a cough

- "No", I don't have a cough

For statistical purposes we have converted "Yes"/"No" to "1"/"0" in order to calculate the following values:

Day 0 (before starting treatment). CA treatment from Day 1 to Day 14

6. Ageusia calculated in:

- "Yes", I have ageusia
- "No", I don't have ageusia

The patient is asked about the sensation of taste and the flavors he feels with the food (sweet, spicy, salty, etc.).

For statistical purposes we have converted the "Yes"/"No" to "1"/"0" in binary to calculate the following values:

Day 0 (before starting treatment). CA treatment from Day 1 to Day 14

7. Anosmia calculated in:

- "Yes", I have anosmia, I don't smell "Coffee" and/or "Vinegar"
- "No", I don't have anosmia, I smell correctly "Coffee" and/or "Vinegar"

The patient is asked about the sensation of smell and the smells he feels when smelling coffee and vinegar. The coffee and vinegar anosmia test used for COVID-19 patients in South Korea and other countries, such as by the Argentinean Ministry of Health, was used in this study.

For statistical purposes we have converted the "Yes"/"No" to "1"/"0" in binary to calculate the following values:

Day 0 (before starting treatment). CA treatment from Day 1 to Day 14

8. Chest tightness calculated in:

The patient is asked if he feels "tightness in the chest or thorax".

- "Yes", I have chest tightness
- "No", I don't have chest tightness

For statistical purposes we have converted "Yes"/"No" to "1"/"0" in order to calculate the following values:

Day 0 (before starting treatment). CA treatment from Day 1 to Day 14

9. General malaise:

There are several scales for measuring the intensity of “general malaise" which are normally calculated in a similar way to VAS from 0 to 10.

"General malaise" calculated in:

- 0, I have no general malaise
- to
- 10, maximum general malaise, extremely intense.

10. Nasal congestion:

The patient is asked if he feels "nasal congestion".

Nasal congestion calculated in:

- "Yes", I have nasal congestion
- "No", no nasal congestion

For statistical purposes we have converted "Yes"/"No" to "1"/"0" in order to calculate the following values:

Day 0 (before starting treatment). CA treatment from Day 1 to Day 14

3. RESULTS

3.1 Descriptive statistics.

Within the intervention group, 55.0% correspond to the male gender while 45.0% to the female gender, the mean age is 42 years with a deviation of 17.39 years. For the control group, 80% corresponded to female gender with a mean age of 53.7 years.

Within the medical history, following diseases were present in the intervention A Group as follows: hypertension 30.0%; kidney disease 10.0%, lung disease 10.0%, heart disease 30.0%, liver disease 5.0%, pheochromocytoma 0.0%, hyperthyroidism 15.0%, gastrointestinal disease 10.0%, drug dependence 0.0%, diabetes mellitus 0.0%, obesity 5.0%.

Within the medical history, following diseases were present in the B control group as follows: hypertension 33.0%; kidney disease 6.7%, lung disease 0.0%, heart disease 0.0%, liver disease 0.0%, pheochromocytoma 0.0%, hyperthyroidism 6.7%, gastrointestinal disease 0.0%, drug dependence 0.0%, diabetes mellitus 0.0%, obesity 0.0%.

The number of smokers of <10 cigarettes / day and smokers > 10 cigarettes / day was zero in the A group as well in the B control group (Colombia has a low prevalence of smokers of 7.0% according to official data) which avoids biases in the study for this specific case.

Regarding the previous use of medications, patients were told to not suspend any medication for their previous comorbidities nor the treatments as NSAIDs or others that were prescribed for COVID-19 symptoms treatment by their physicians. This applies for both groups.

Following medications for ongoing comorbidities were reported in group A (intervention group): use of beta blockers 10.0%, ACEi 5.0%, ARB 10%, salbutamol 10.0%, statin 5.0%, enoxaparin 5,0%, levothyroxine 5.0%, ASA 10%, corticosteroids 10%, opiates 15.0%.

Following medications for mitigating COVID-19 symptoms, that were prescribed by head physicians to the patients, were reported in group A: azithromycin 12.5%, ivermectin 20.0%, amoxicillin 5,0%, NSAIDs 40.0%, hydroxychloroquine 0.0%, chloroquine 0.0%, dexamethasone 5.0%, cetirizine 15%, loratadine 5,0%

Following medications for ongoing comorbidities were reported in group B (control group): use of beta blockers 0.0%, ACEi 6,7%, ARB 0.0%, salbutamol 0.0%, statin 0,0%, enoxaparin 0,0%, levothyroxine 0.0%, ASA 20%, corticosteroids 0.0%, opiates 0.0%.

Following medications for mitigating COVID-19 symptoms, that were prescribed by head physicians to the patients, were reported in group B: azithromycin 13.3%, ivermectin 20.0%, amoxicillin 0.0%, NSAIDs 86.7%, hydroxychloroquine 0.0%, chloroquine 0.0%, dexamethasone 0.0%, cetirizine 0.0%, loratadine 0.0% , prednisolone 6.7%.

Likewise, the discomforts that occurred after the use of CA in the research subjects were evaluated, finding the following effects. Nasal itching 81.3%, oropharyngeal discomfort 62.5%, dizziness 18.8%, nausea 43.8%, headache 50%, slight increase in heart rate 6.3%, rhinorrhea 25%, lacrimation 75%, general malaise 25%.

No discomforts were observed among any patient in the oral drops intake. In the more intrusive but more therapeutical designed route of administration trough nasal spray application the duration of discomforts remained only for a few minutes (4.3 minutes as average for all discomforts) -as expected- and each of them was taken into account as the difference between the moment when it was presented and the moment when it was resolved, this is shown in table 1.

Table1. **CA's DISCOMFORT IN NASAL SPRAY ADMINISTRATION**

	N	Minimum duration in minutes	Maximum duration in minutes	Median duration in minutes	SD	
Nasal itching	*14*	*1,00*	*13,00*	*8,7857*	*3,01735*	
Oropharyngeal discomfort	*14*	*,00*	*13,00*	*4,3571*	*3,69214*	
Dizziness	*6*	*1,00*	*7,00*	*2,3333*	*2,33809*	
Nausea	*7*	*0,00*	*7,00*	*3,2857*	*2,81154*	
Cephalea	*11*	*,00*	*7,00*	*3,4545*	*2,80584*	
Slight increase in heart rate	*1*	*3,00*	*3,00*	*3,0000*	*.*	
Rhinorrhea	*4*	*0,00*	*2,00*	*1,2500*	*,95743*	
Lacrimation	*13*	*1,00*	*13,00*	*8,5385*	*4,82382*	
General malaise	*6*	*1,00*	*8,00*	*3,6667*	*2,42212*	

An analysis was carried out through the calculation of the OR and the corresponding expected impact for each variable that were evaluated in the study, the OR values with their 95% confidence intervals, presented in table 2.

Table 2. Evaluation between intervention group and control group

Variable	OR	P value
Fever	1,45	0,12
Cough	0,5	0,05
Dyspnea	0,38	0,14
Muscle fatigue	0,69	0,25
Cephalea	6.0	0,8
Ageusia	0,27	0,069
Anosmia	0,21	0,05
Chest tightness	1,0	0,25
General malaise	0,62	0,25
Nasal Congestion	1,0	0,65

In relation to the OR values, the variables Cough, (OR = 0.5) Figure 1, Dyspnea (OR = 0.38) Figure 2, Muscle fatigue (OR = 0.69) Figure 3, Ageusia (OR = 0.27) Figure 4, Anosmia (OR = 0.21) Figure 5, General malaise are observed (OR = 0.62) Figure 6, are less than 1, making the use of CA a protective factor, thus showing improvement in these symptoms, after its use compared to the control group; fever, chest tightness, nasal congestion, although they present an OR value greater than 1, they also present confidence intervals that cross 1, this being inconclusive that they do not favor the reduction of said symptoms, Figures 7,8 & 10. For its part, the OR of cephalea is 6.0 with a confidence interval between 0.64-56.5. This particular symptom, although it is presented as a risk factor, the literature reports that its presence is associated with a decrease in the complication of COVID-19 patients (113,114), this premise being then favorable to the intervention branch. The expected impact is the difference in the distance of change or difference that occurs at the time of measuring the event in days, that is, it defines whether there is a change in the experimental group vs. the control group in the reduction of each of the events (symptoms). P value confirms that there is a statistically significant difference between the intervention group and the control group.

3.2. Figures

Figure1. Graphic compares CA vs control in terms of expected impact on cough duration in days. OR: 0.5.

Figure 2. Graphic compares CA vs control in terms of expected impact on dyspnea duration in days. OR: 0.38.

Figure 3. Graphic compares CA vs control in terms of expected impact on muscle fatigue duration in days. OR: 0.69.

Figure 4. Graphic compares CA vs control in terms of expected impact on Ageusia duration in days. OR: 0.27.

Figure 5. Graphic compares CA vs control in terms of expected impact on Anosmia duration in days. OR: 0.21.

Figure 6. Graphic compares CA vs control in terms of expected impact of General Malaise duration in days. OR: 0.62.

Figure 7. Graphic compares CA vs control in terms of expected impact on Chest tightness duration in days. OR: 1.00

Figure 8. Graphic compares CA vs control in terms of expected impact on Fever duration in days. OR: 1.45

Figure 9. Graphic compares CA vs control in terms of expected impact on Cephalea. OR: 6.00

Figure 10. Graphic compares CA vs control in terms of expected impact on Nasal Congestion. OR: 1.00

4. DISCUSSION

4.1 Observations around the results

It is interesting to note that the intervention group (A) had a higher number of male subjects (55.0%) in comparison with the control group (B) (20%). Men are more affected by SARS-CoV-2 and do have a higher-associated risk factor for death and ITU admission according to meta-analysis (115). In addition, Group A subjects in the intervention group had more comorbidities than those group B (control group) such as hypertension 30.0%; kidney disease 10.0%, lung disease 10.0%, heart disease 30.0%, liver disease 5.0%, hyperthyroidism 15.0%, gastrointestinal disease 10.0%, obesity 5.0%, in the group A being those comorbidities significantly lower in the group B such as per in this second group being hypertension 33.0%; kidney disease 6.7%, lung disease 0.0%, heart disease 0.0%, liver disease 0.0%, pheochromocytoma 0,0%, hyperthyroidism 6.7%, gastrointestinal disease 0.0%, and obesity 0.0%.

Despite the fact that the intervention group (A) had 64% more male subjects and had also a higher number of patients with comorbidities associated to a worse prognostic outcome for COVID-19, the CA drug still showed to be a protective factor for cough, dyspnea (probably the worst indicator for COVID-19), muscle fatigue, ageusia, anosmia, and general malaise with an OR are less than 1, without ruling out the possibility that the drug may be also effective in larger-sample studies for fever, chest tightness, and nasal congestion.

Although for the purposes of this study only the symptoms showed in the Table 1 were specifically measured for safety,

adverse effects and tolerance measurement purposes, it should be mentioned offhand that several patients reported unexpected benefits (better concentration, better focus, improved allergies, improved intestinal transit, increased energy) which correlated with the known and reported benefits of nicotine in non-smoked medicinal administration pathways.

4.2 The need of a specific drug against COVID-19 with a potential for covering new strains and other existing or to appear beta-coronaviruses

In the absence of effective drugs to fight COVID-19 the validation of the nicotinic hypothesis in humans is of critical relevance, since the CA drug is of rapid development, low cost and exhibits tolerance and safety in agreement with other drugs of the same category.

New strains of SARS-CoV-2 with several mutations of concern (116,117) in the spike protein have been discovered in November 2020 in the UK and South Africa and lately another one in Brazil and are spreading very fast in several countries. This could potentially compromise current COVID-19 vaccines as is already the case with many other viruses. As noted in the introduction, CA has been designed specifically against the SARS-CoV-2 virus that causes COVID-19 disease. However, due to its particular mechanism of action and its other anti-inflammatory and immunomodulatory properties it could be of potential application in all current existing strains and in other future strains of SARS-CoV-2 covering most likely future appearances of new coronaviruses, as well as existing coronaviruses, such as those of the common cold. This should be studied further. In fact, and accordingly to Lagoumintzis *et al.* (118) a “toxin-like” epitope on the Spike Glycoprotein has been reported having interestingly protein complexes that involve a vast part of the “toxin-like” sequences of SARS-CoV-1 and SARS-CoV-2 Spike glycoproteins and toxin binding site of human α7 nAChR. Probably, too much attention has been driven to the RBD of ACE2-S Glycoprotein, ignoring that one thing is the binding and infectivity capacity of the virus through the RBD of ACE2 and another thing is the interactions with epitopes that do not interfere with ACE2 but can be determinants in the lethality and severe progression of the disease. If we were to direct our focus beyond the binding interactions of betacoronaviruses with ACE2 by focusing more on the disruptive interactions of this family of viruses with other epitopes, we would surely find that the key to reducing the lethality and severe forms of disease by betacoronaviruses such as the common cold, SARS-CoV-1 and SARS-CoV-2, should not only be directed to a design to avoid only binding with the RBD of ACE2 but towards the development of new drugs capable of antagonizing alternative disruptions in epitopes such as those related to nAChR. In view of the appearance of probable severe mutations and new strains or new betacoronaviruses, a convenient strategy would be, therefore, to tackle the complications derived from the disruption of the cholinergic and immune system by modulating hyperinflammation and immune deregulation processes, which would fight lethality and complications due to viremia through an alternative mechanism, in an independent way, making less relevant the capacity of infectivity and mutagenesis of such viruses.

4.3 The long Covid: a coming public health’s issue challenge

It is also important to point out the therapeutic potential to be explored of CA in the cases of "Long Covid". In the absence of more conclusive studies, preliminary data from studies published to date (119–123) indicate that between 10% to 53% of those infected with SARS-CoV-2, including especially also healthy youth and adults with no history of comorbidities and mostly not hospitalized, who passed the COVID-19 in a mild manner reaching a prevalence of 80% in the sequelae at 3

months in this subgroup according to a study by Göertz *et al.* (124), suffer preliminary neurological sequelae such as mental fog, fatigue, headache, shortness of breath, muscle pain and moderate damage to heart, lung, kidney, liver and pancreas. According to a CDC Morbidity and Mortality Weekly Report (125), 35% of patients had not returned to their usual state of health when interviewed 2/3 weeks after the test. Although it is still early to have clear scientific evidence on the impact of long-Covid, it is very likely that persistent symptoms exist for weeks, months or even years in a significant number of patients. This phenomenon was described by the WHO and by two studies (126,127) showing persistent symptoms as impaired exercise capacity and health for 24 months in the case of survivors of SARS-CoV-1 in 2003. About 40% of people recovering from SARS-CoV-1 had to deal with symptoms of chronic fatigue even 3.5 years after they had overcome the illness. Although it has yet to be determined, the ravages of Long-Covid should not be underestimated and are a source of logical concern. Either the endocytosis that SARS-CoV-2 performs on ACE2 and its downregulation becomes more or less chronic or prolonged in patients with Long-Covid affecting the RAAS axis and its ability to return to homeostasis of the lung, kidney, heart, or other organs; or the involvement in the CNS by the neurotropism of SARS-CoV-2 is at the base of the symptoms of Long-Covid; or because the alteration, short-circuiting and disruption of the immune system through the SN and Immune System interface with macrophage interactions or by alteration of the cholinergic anti-inflammatory system are partially disrupted or weakened in COVID-19 patients, it is necessary and urgent to address a therapeutic approach for this Long-Covid or Post-Covid Syndrome that will most likely affect millions of people. In this context, cholinergic agonists should be further studied and, if their therapeutic action during infection is further confirmed and validated, the same therapeutic action could also be valid to address the therapeutic management of Long-Covid symptoms since the same mechanism of action that offers therapeutic advantages during infection could offer a promise of accelerated recovery in Long-Covid symptoms. This should be further examined.

5. CONCLUSION

Despite the limitation of study in the sample of patients, the positive results obtained on the symptoms caused by COVID-19 through the use of cholinergic agonists molecules in humans infected by SARS-CoV-2 versus the control group, endorses preliminary the nicotinic hypothesis on SARS-CoV-2 and the therapeutic potential of the use of these molecules. Larger multicentrical trials in humans are encouraged in the light of the previous existent evidences as i.e. clinical evidence on lower prevalence of smokers, the nicotinic hypothesis on SARS-CoV-2, the well-known anti-inflammatory and immunomodulatory properties of nicotine when administered medicinally and non-smoked, the preliminary evidences of the nicotine's therapeutic potential and other cholinergic molecules trough medicinal administration in several other diseases, the safety and well-known tolerance of NRT's products approved by regulatory agencies as over-the-counter products -even for pregnant women-, *in silico* studies on the interactions between ACE2 and nAChRs with SARS-CoV-2 and on the antagonization of cholinergic agonists, and in the light of the results of this study.

Tolerance to the drug was good, mild side effects lasted only a few minutes as expected in liquid nasal application of cholinergic agonists that enhance the vagal reflex, and it correlates with the tolerance and safety observed in other drugs such as NRTs.

This is study in humans shows evidence on the therapeutic effect against COVID-19 of cholinergic agonist molecules trough a specific pharmacological design and with combined oral and nasal administration routes, and preliminarily validates the clinical observations of the low prevalence of smokers observed among COVID-19 infected patients,

explaining the paradox about smoking being itself a dangerous and not recommended tool to combat COVID-19 but that nicotine and/or other cholinergic agonists molecules could offer a promising therapeutic tool when administered in a medicinal way and imitating most common viral entry route through the olfactory mucosa. The study endorses also the hypotheses about COVID-19 being a nicotinic virus and the therapeutic use of cholinergic agonist agents to cope with it as well as the *in silico* evidence that has shown that several cholinergic molecules have the capacity to antagonize with SARS-CoV-2 either in the RBD of the ACE2, or at the "Toxin-like-domain" in disruptive interaction with the nAChRs, or by the regulation of hyperinflammatory processes linked to malfunctioning of the RAAS by viremia, the regulation the cholinergic anti-inflammatory system or of immune dysfunctions linked to failures in the interface of the ADAM17/TNF-a modulation. However, larger multicenter studies should be conducted to gather more clinical and scientific evidence.

LIMITATIONS OF THE STUDY

Sample size: The study results are limited to the number of patients included in it, 35 (20 for intervention group and 15 for control group). It is suggested that the same study be conducted with larger multicentrically studies that include higher number of patients in both groups.

AUTHOR'S STATEMENTS

The authors hereby declare:

- The authors of this study do not promote the use of the smoked cigarette on the contrary, they advise against it under any circumstances, and they are not promoting or encouraging the use of alternative products such as electronic cigarettes for harm-reduction or smoking cessation products as NRT's for fighting COVID-19. This is another discussion.
- It is of interest to the authors to point out that although the smoked cigarette and its added components and smoked route of administration are clearly harmful, the tobacco plant and cholinergic agonist molecules are of therapeutic interest in the COVID-19 disease if they are treated and explored in a scientific and medicinal way, leaving aside controversies and stigmatizations that may well apply to the industry and the consumption of cigarettes, but should not be mixed with the therapeutic potential of medicinal drugs based on such molecules with a scientific pharmacological design and from a pure medical perspective for the benefit of human health.

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CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

León Higuera J.G.: Methodology, Investigation, Conceptualization, Supervision; **Restrepo Guerrero F.H:** Software, statistical analysis; **León García M.:** review & supervision; **Politi M.:** review, supervision**; Mendive F.**: project advisor, review & editing; **Angulo Ceballos O.:** Conceptualization, Supervision, Writing - original draft, Writing-, review & editing.

DECLARATION OF COMPETING INTEREST

The authors report no declarations of interest.

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