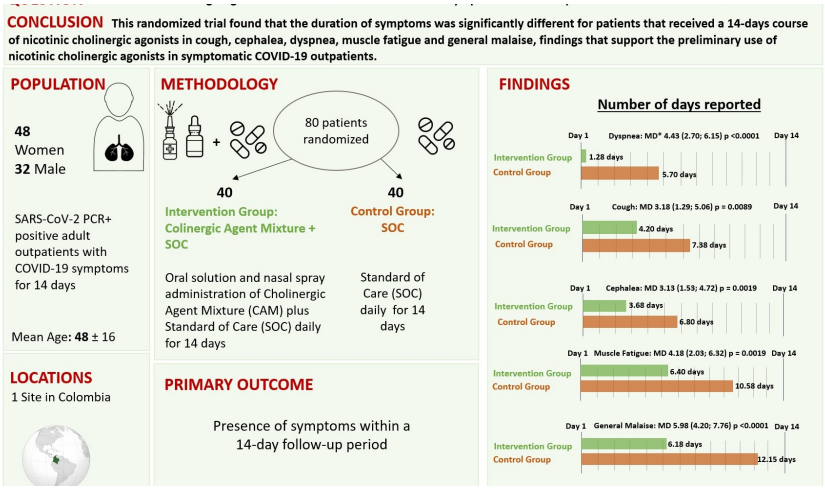

CHOLINERGIC AGONISTS AGAINST COVID-19 IN HUMANS. RESULTS FROM A RANDOMIZED OPEN LABEL PILOT TRIAL

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Funding: The author(s) received no specific funding for this work.

Potential competing interests: The author(s) declared that no potential competing interests exist.

Abstract

Background:

Since the SARS-CoV-2 pandemic emerged in December 2019, it has triggered 4.4 million deaths and strained health systems across the world. Yet more than a year and a half since the pandemic emerged, therapeutic drugs to treat COVID-19 disease are limited.

Objective

To investigate the therapeutic potential of a nicotinic Cholinergic Agonists Mixture (CAM), delivered daily as oral drops and as nasal spray, in alleviating ten common COVID-19 related symptoms in 80 symptomatic human adults with confirmed SARS-CoV-2.

Methods

This randomized open-label pilot trial recruited 80 symptomatic adults with confirmed SARS-CoV-2 infection after RT-PCR+ test less than five days. Participants were recruited from databases of several

Colombian hospitals and were randomly assigned to the control group, which received the Standard of Care (SOC) treatment (outpatient treatment), or the intervention group, which received SOC combined with the Cholinergic Agent Mixture (CAM + SOC). Both groups received their treatment for a total of 14 days. The duration of symptoms was compared across the 14-day period.

Results:

This study found statistically significant reductions in symptom duration for 5 out of 10 symptoms, including dyspnea (reduction of 4.43 days [95% CI: 2.70 ; 6.15], p <0.0001), cough (reduction of 3.18 days [95% CI: 1.29 ; 5.06], p=0.0089), cephalea (reduction of 3.13 days [95% CI: 1.53 ; 4.72], p= 0.0019), muscle fatigue (reduction of 4.18 days [95% CI: 2.03 ; 6.32], p=0.0019) and general malaise (reduction of 5.98 days [95% CI: 4.20 ; 7.76], p <0.0001).The study found no significant reductions in the duration of the following symptoms: fever, ageusia, anosmia, chest tightness, and nasal congestion.

Conclusion:

In comparison to the control group, the intervention group witnessed statistically significant and clinically relevant reductions in the duration of 5 out of 10 common COVID-19 disease symptoms within two weeks.

This includes a reduction of approximately 4.4 days in the duration of dyspnea, a symptom that appears to be strongly correlated to severe COVID-19 disease and admission to Intensive Care Units.

Further studies are needed to confirm these preliminary findings and to evaluate whether this specific nicotinic cholinergic agonists mixture could have implications for public health.

CHOLINERGIC AGONISTS AGAINST COVID-19 IN HUMANS. RESULTS FROM A RANDOMIZED OPEN LABEL PILOT TRIAL

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GRAPHICAL ABSTRACT

HIGHLIGHTS:

- This study is the first randomized trial evaluating the real-life impact of specifically formulated nicotinic cholinergic agents as adjuvant therapy in the treatment of out care symptomatic adult patients with confirmed COVID-19.
- This randomized open-label study revealed that a unique mixture of nicotinic cholinergic agonists significantly reduced the duration of common COVID-19 symptoms, including dyspnea, cough, cephalea, general malaise and muscle fatigue.
- Dyspnea appears to be associated with severe COVID-19, hospitalization, and death, being a relevant significative predictor symptom for ICU admission.
- These preliminary findings indicate that the specific mixture of nicotinic cholinergic agonists could reduce COVID-19 disease severity, with implications for public health.

ABSTRACT

Background:

Since the SARS-CoV-2 pandemic emerged in December 2019, it has triggered 4.4 million deaths and strained health systems across the world. Yet more than a year and a half since the pandemic emerged, therapeutic drugs to treat COVID-19 disease are limited.

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To investigate the therapeutic potential of a nicotinic Cholinergic Agonists Mixture (CAM), delivered daily as oral drops and as nasal spray, in alleviating ten common COVID-19 related symptoms in 80 symptomatic human adults with confirmed SARS-CoV-2.

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This randomized open-label pilot trial recruited 80 symptomatic adults with confirmed SARS-CoV-2 infection after RT-PCR+ test less than five days. Participants were recruited from databases of several Colombian hospitals and were randomly assigned to the control group, which received the Standard of Care (SOC) treatment (outpatient treatment), or the intervention group, which received SOC combined with the Cholinergic Agent Mixture (CAM + SOC). Both groups received their treatment for a total of 14 days. The duration of symptoms was compared across the 14-day period.

Results:

This study found statistically significant reductions in symptom duration for 5 out of 10 symptoms, including dyspnea (reduction of 4.43 days [95% CI: 2.70 ; 6.15], p <0.0001), cough (reduction of 3.18 days [95% CI: 1.29 ; 5.06], p=0.0089), cephalea (reduction of 3.13 days [95% CI: 1.53 ; 4.72], p= 0.0019), muscle fatigue (reduction of 4.18 days [95% CI: 2.03 ; 6.32], p=0.0019) and general malaise (reduction of 5.98 days [95% CI: 4.20 ; 7.76], p <0.0001).The study found no significant reductions in the duration of the following symptoms: fever, ageusia, anosmia, chest tightness, and nasal congestion.

Conclusion:

In comparison to the control group, the intervention group witnessed statistically significant and clinically relevant reductions in the duration of 5 out of 10 common COVID-19 disease symptoms within two weeks. This includes a reduction of approximately 4.4 days in the duration of dyspnea, a symptom that appears to be strongly correlated to severe COVID-19 disease and admission to Intensive Care Units.

Further studies are needed to confirm these preliminary findings and to evaluate whether this specific nicotinic cholinergic agonists mixture could have implications for public health.

ABBREVIATIONS

ACE2, angiotensin-converting enzyme II; CAM, Cholinergic Agent Mixture; CNS, Central Nervous System; ICU, Intensive Care Unit; I+SOCG, intervention group; mMRC, modified Medical Research Council scale; NRT, Nicotine Replacement Therapy; nAChRs, nicotinic acetylcholine receptors; RBD, receptor-binding domain; ROF, rating-of-fatigue; SOC, Standard of Care treatment; SOCG, control group; TBS, toxin-binding site; VAS, Visual Analogue Scale

KEYWORDS

Cholinergic agonists; Cholinergic system; COVID-19; Nicotine; Nicotinic acetylcholine receptors; nAChR; SARS-CoV-2; nAChR; Human trial; Anti-inflammatory cholinergic pathway, Long Covid, Post-Covid Syndrome

1. INTRODUCTION

Several lines of evidence have suggested that nicotinic cholinergic agonists could protect against COVID-19 disease. Clinical evidence including peer-reviewed studies 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 (1–5). A nicotinic hypothesis on SARS-COV-2 (1,6) and on the probable effectiveness of cholinergic agonists in COVID-19 disease has been proposed (2,7) according to clinical observations and peer-reviewed studies (4,8–11). This controversial nicotinic hypothesis (4,12) is currently under debate. A highly conserved cryptic epitope in the S1-SARS-CoV-2 (aa 365-390) that is involved in the disruption of α7-nAChR has been described (13–15) involving an interaction between SARS-CoV-2 glycoprotein S and a extracellular domain of the alpha9 and alpha7 subunit of nAChR, forming a "toxin binding site" region with the nAChRs (16). The Spike protein of SARS-CoV2 has sequences homologous to snake venom neurotoxins with nicotinic acetylcholine receptor antagonist

activity (1,17).This activity is thought to be involved in paralysis and autonomic nervous system dysfunctions including the anti-inflammatory cholinergic pathway of the vagus nerve regulating macrophage activity (18). Recent computational modulations showed an interaction between SARS-CoV-2 and nAChR and the disruption in the anti-inflammatory response of the cholinergic system (19). This interaction between the alpha 7 subunits and the SARS-CoV-2 glycoprotein S1 was disrupted when cholinergic agonists and molecules such as acetylcholine, carbamylcholine, cytisine, epibatidine, galantamine, nicotine, succinylcholine and varenicline were docked.

However, the present study was based on a novel synergistic combination of nicotine with cotinine, anatabine, anabasine, as well as s-allyl-cysteine and 6-gingerol in lower proportions, which has not previously been suggested.

COVID-19 disease has been associated with inflammatory changes in the brain and the choroid plexus (20) as well with several cognitive disorders even after viral clearance and regardless of disease severity including anxiety, depression (21), memory loss, attention and executive function deficit (22), being psychiatric disorders such as schizophrenia, where α7-nicotinic acetylcholine receptor deficiency plays an important role (23), an associated risk-factor for worse or fatal outcome in COVID-19 (24). Cholinergic agonist molecules such as nicotine used therapeutically are safe and well tolerated (25,26) and even at long-term use (27). Nicotine does not have relevant or minimal side effects and has been approved by FDA (28) and several other drug regulation authorities in the context of smoking cessation. Nicotine nasal spray it is used and has been studied among pregnant women as Nicotine Replacement Therapy (NRT) (29). This applies also for non-smokers, concomitant smokers or to smokers with cardiac disease (26,30–38). Nicotine itself as a molecule, aside from the cigarette, does not exhibit carcinogenic properties (39) and its medicinally inhaled nasal application does not significantly alter lung function, nor diastolic blood pressure when compared with placebo (40). Cotinine is a non-addictive and safe molecule (41) with pharmacokinetic properties suitable for therapeutic use with anxiolytic, antidepressant, antipsychotic and anti-neuroinflammatory properties (42). Cotinine behaves as a nAChR positive allosteric modulator and its neurobehavioral effects significantly differ from those from nicotine alone (43). Anabasine has a higher and more specific agonist effect on the nicotinic α7-nAChR subtype than other nicotinic agonist molecules and has been noticed to ameliorate memory and cognitive deficits (23) attenuating also nicotine withdrawal (44) like in the case of anatabine (45). Anatabine has a powerful anti-inflammatory effect and prevents IL-1b production, reducing pro-inflammatory cytokine production such as IL-6, IL-1b and TNF-α in the plasma (46), biomarkers that are associated predictors for severity and death in COVID-19 (47). 6-gingerol has been found to have various pharmacological effects including anti-inflammatory, analgesic, antipyretic, angiogenic, antioxidant (48,49), and neuroprotective properties (50). In animal models it also facilitates healthy glucose regulation for diabetes (51,52), a comorbidity associated to a higher risk and a more severe outcome for COVID-19 (53). S-allyl-cysteine has antioxidative, cardiovascular and neuronal degeneration protection properties (54–56) and has been proposed as a candidate for inhibiting SARS-CoV-2 (57).

The present randomized open-label trial pilot 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 has been preliminarily named Cholinergic Agent Mixture (CAM). The administration of the novel combination of ingredients in CAM under 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 (58–61). Indeed, the nasal spray has the advantage of offering better and efficient absorption (62), and a higher excitatory effect of cholinergic agonist molecules than patches or tablets. Thus, the therapeutic strategy of CAM use is to provide a slower sustained systemic effect by the oral route with the administration of oral drops in combination with a rapid neurotropic and excitatory effect by nasal spray administration, adapting dosage according to the patient's profile and needs.

2. METHODS

2.1 Study design

This randomized-open trial was conducted in the city of Bogotá in Colombia, in 80 adult patients that tested positive for SARS-CoV-2 through PCR-RT test. Patients were recruited from several official databases of Colombian hospitals and randomized through sequential and consecutive assignment following their status as a symptomatic SARS-CoV-2 positive patients in the last 5 days. To reduce bias, the health care professionals who monitored the patients' symptoms were blinded to the group that was assigned to the patient or to the medicine they were giving.

The intervention group (I+SOCG) consisted of 40 infected patients. Following a positive COVID-19 RT-PCR test within 5 days, patients received Standard of Care (SOC) plus a daily dose of CAM nasal spray (0.128 mL, on each nostril) every twelve hours and CAM oral drops (0.560 mL, in 175 mL of water every 3 times per day).

The control group (SOCG) consisted of 40 infected patients and was administered with only SOC following a positive SARS-CoV-2 RT-PCR test within 5 days.

SOC was prescribed in both groups by physicians as a part of their standard treatment to mitigate COVID-19 symptoms (see table 1 for SOC medications).

Both groups were monitored on a daily basis to assess the evolution of symptomatic COVID-19 disease, based on a range of scales: binary scales for absence or presence for cephalea, cough, ageusia, anosmia, chest tightness and nasal congestion; mMRC scale for dyspnea (63), updated Gunnar Borg's for muscular fatigue (64,65), and Visual Analogue Scale (VAS) for general malaise, an analogue scale to sick building syndrome (66) and to rating-of-fatigue (ROF) (67).

Inclusion criteria:

- RT-PCR positive for SARS-CoV-2 within five days.
- Outpatient under ambulatory care (non-hospitalized)
- Symptomatic patient

Exclusion criteria:

- Patient under in-ward hospital care, even if they have positive antigens and / or RT-PCR+ test.
- Decline in study participation.
- Asymptomatic patient even if having a RT-PCR+ test for SARS-CoV-2.
- Under 18 years old.

2.2. Participants

The study population consisted of 80 adult Colombian patients (48 women and 32 men) who attended various official hospital services in Colombia and were diagnosed with SARS-CoV-2 infection confirmed by real-time reverse transcription polymerase chain reaction (RT-PCR) testing performed on nasopharyngeal swab specimens (See Fig 1).

Fig 1.

Study flow diagram: Intervention Group (I+SOCG): with Cholinergic Agent Mixture (CAM) and Standard of Care (SOC). Control Group (SOCG): control group with only Standard of Care (SOC). (See table 1 for detail).

2.3. Intervention

The CAM treatment, consisting of combined administration of oral drops and nasal spray on a daily basis to the intervention group, is mainly composed of cholinergic nicotinic agonist agents, although some non-cholinergic agonists agents with known antiviral activity with synergistic interactions were also added in the CAM composition. The technical dossier of the composition with its formulation and safety profile was submitted to the Colombian food and drug administration (INVIMA) and its application was accepted. The application is currently in the process of approval for definitive health registration.

The Standard of Care (SOC) administered to both groups was prescribed by the treating physicians prior to the study and included various drugs such as antipyretics, NSAIDs, ivermectin, among others (see Table 1).

2.3 Demographics, comorbidities, medications, and endpoints

The following demographic and clinical data were collected: gender, age, date of suspected infection, date of RT-PCR+ for SARS-CoV-2, smoking status, previous comorbidities, SOC's prescribed drugs for COVID-19 symptoms. Following COVID-19 related symptoms were daily measured as endpoints in both groups: fever, cough, dyspnea, muscle fatigue, cephalea, ageusia, anosmia, chest pressure, general malaise, and nasal congestion.

The following comorbidities present prior to SARS-CoV-2 infection such as cardiovascular disease, hypertension, hypothyroidism, obesity, among others, were also documented in two groups (See table 1).

Following medications prescribed as SOC such as salbutamol, NSAIDs, antihistamines and others were reported in both groups (See table 1).Medications prescribed previously to SARS-CoV-2 infection for ongoing comorbidities were also collected in both groups such as NSAIDs, corticosteroids, antipyretics, among others (See Table 2).

In the intervention group (I+SOCG) discomfort manifestations after CAM's oral drops intake and CAM's nasal spray administration were also reported to assess tolerance and possible side-effects of CAM (see Table 3).

The evaluation in both groups, where the interventional group (I+SOCG) was administered with CAM + SOC and the control group (SOCG) was administered with only SOC, was carried out for 14 days assessing the improvement or worsening of each symptom on a daily basis. At day 0 an initial measurement of symptoms without the administration of CAM in the intervention group (I+SOCG) and in the control group (SOCG) was performed where both groups were already under SOC treatment. In days 1 to 14 the measurement of the evolution of each symptom with SOC + CAM for group I+SOCG and without CAM but with SOC for group SOCG was also equally assessed.

The following symptoms were measured binarily (yes/no) for absence or presence of the symptom: fever (≥38º), cephalea, cough, ageusia, anosmia, chest tightness and nasal congestion. Dyspnea was calculated from 0 to 4 according to the modified mMRC (Medical Research Council) scale, which is useful in dyspnea evaluation in physical activities of daily living (63) and in COVID-19 (68,69) . Muscular fatigue was 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 (64,65).

General malaise for measuring its intensity was calculated from 0 to 10, a commonly usual assessment method similar to Visual Analogue Scale (VAS) in sick building syndrome (66) and to rating-of-fatigue (ROF) (67).

To proceed with the analysis, the dichotomization of symptoms in the non-binary scales was categorized as follows: dyspnea (mMRC score ≤ 1 versus mMRC score ≥ 2) (70–72); muscle fatigue (absence -0- versus presence -1 to 10-) (67,73); general malaise (absence -0- versus presence -1 to 10-).

The raw data were compiled in a table using Microsoft Excel by trained health professionals.

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2.5 Statistical Analysis

Qualitative variables are reported as absolute frequencies and percentages while quantitative variables are reported as means and standard deviations. In order to compare the expected value of quantitative variables in the I+SOCG vs the SOCG the t-test (assuming heterogenous variances) was used, whereas for qualitative variables the chi-square test for independence was used. Although most quantitative variables cannot be considered as normal (outcomes of interest like number of days with a given symptom), the t-test was still used in place of non-parametric alternatives, since t-test is highly robust in non-normality conditions (74).

Effect measures were used to quantify the differences between the two groups using the mean difference (estimated assuming heterogenous variances) and odds ratios with their corresponding 95% confidence intervals.

In this study multiple symptoms were assessed as outcomes of interest and baseline demographics, comorbidities and Standard of Care (SOC) treatments for COVID-19 were also evaluated in order to determine the comparability between intervention and control groups. Thus, multiple comparison adjustment was done using the false discovery rate approach proposed by Benjamini *et al.* (75), setting a global significance level of 5%.
All statistical analyses were done in software R version 4.2.0 (76).

2.6 Ethics committee approval

This study was 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 participants enrolled in the research, which is widely described in the justification of the study protocol and informed consent.

3. RESULTS

Both groups had the same number of patients (n=40) and were analogous in terms of age (I+SOCG: 45.2 ± 18.0 years; SOCG: 50.5 ± 13.1 years) and gender (I+SOCG: 47% male; SOCG: 32.5% male). The ethnicity all participants was white (88% of Colombian population is white) and their smoking status was also assessed, 0% of the participants were current smokers nor former recent smokers in either group.
In comparative terms, any of the prior referred comorbidities did not differ significantly (see Table 1) in both groups, being hypertension (27.5% in the intervention group and 37.5% in the control group) and chronic obstructive pulmonary disease (12.5% in the intervention group and 25% in the control group) the most common ones, except for cardiovascular disease which was present in the interventional group (25%) and absent in the control group (p=0.0121). Of the 14 medications prescribed as SOC in both groups for COVID-19 symptoms mitigation no significant differences were observed in both groups in the prescribed drugs except for antipyretics that were more present in the control group (p=0.0013), (See table 1). Also, no significant differences were found in any of the medications prescribed for comorbidities prior to COVID-19 in both groups (See Table 2) being angiotensin-converting enzyme inhibitors (15.0% in the intervention group and 12.5% in the control group) and angiotensin II receptor blockers (5.0% in the intervention group and 10.0% in the control group) the most common used medications.
When evaluating in days the presence of COVID-19 associated symptoms at the final day of the study (day 14) a statistically significant reduction of symptoms after adjusting p- values in the intervention group was observed in dyspnea (reduction of 4.43 days [95% CI: 2.70 ; 6.15], p <0.0001), cough (reduction of 3.18 days [95% CI: 1.29 ; 5.06], p=0.0089), cephalea (reduction of 3.13 days [95% CI: 1.53 ; 4.72], p= 0.0019), muscle fatigue (reduction of 4.18 days [95% CI: 2.03 ; 6.32], p=0.0019) and general malaise (reduction of 5.98 days [95% CI: 4.20 ; 7.76], p <0.0001) (See table 1 and Fig 2). No significant differences were found in the remaining symptoms (see Table 1).

No adverse effects were reported in the application of oral drops of the Cholinergic Agent Mixture (CAM) in the intervention group that took CAM. The nasal spray administration of the CAM produced mild transient effects such as nasal or oropharyngeal itching, among others, which lasted an average of 7 minutes (SD 4) and no major complications were observed (see table 3).

Fig. 2.

Boxplots for symptoms with significant p-value representing the presence of each symptom in number of days for each group (intervention group = I+SOCG; control group = SOCG) during the 14 days of the evaluation.

	Overall patients (n = 80)	I+SOCG Intervention Group (n = 40)	SOCG Control Group (n = 40)	Measure Effect (Confidence Interval 95%)	P-Value
Age (years)	47.8 ± 15.9	45.2 ± 18.0	50.5 ± 13.1	5.3 (-1.6 ; 12.2)	0.3523
Male	32 (40.0%)	19 (47.5%)	13 (32.5%)	1.9 (0.75 ; 4.72)	0.4569
Current smoker	0 (0.0%)	0 (0.0%)	0 (0.0%)	---	---
Recent former smoker	0 (0.0%)	0 (0.0%)	0 (0.0%)	---	---
Hypertension	26 (32.5%)	11 (27.5%)	15 (37.5%)	0.64 (0.24 ; 1.65)	0.8124
Diabetes mellitus	1 (1.3%)	1 (2.5%)	0 (0.0%)	1 (0.12 ; 77.80)	1.0000
Cardiovascular Disease	10 (12.5%)	10 (25%)	0 (0.0%)	12.9 (1.57 ; 494.6)	0.0121*
Chronic kidney disease	7 (8.75%)	6 (15%)	1 (2.5%)	3.34 (0.79 ; 31.03)	0.3405
COPD	15 (18.8%)	5 (12.5%)	10 (25.0%)	0.44 (0.12 ; 1.40)	0.4569
Liver disease	2 (2.5%)	1 (2.5%)	1 (2.5%)	0.49 (0.10 ; 10.03)	1.0000
Pheochromocytoma	0 (0.0%)	0 (0.0%)	0 (0.0%)	---	---
Hyperthyroidism	10 (12.5%)	5 (12.5%)	5 (12.5%)	1 (= 0.25 ; 4.03)	1.0000
Gastrointestinal disorders	3 (3.75%)	2 (5.0%)	1 (2.5%)	1 (0.22 ; 13.58)	1.0000
Drug abuse	0 (0.0%)	0 (0.0%)	0 (0.0%)	**----**	---
Obesity	4 (5.0%)	2 (5.0%)	2 (5.0%)	0.65 (0.16 ; 6.11)	1.0000
Other diseases	8 (10.0%)	5 (12.5%)	3 (7.5%)	1.29 (0.40 ; 6.84)	1.0000
Standard of Care (SOC) for COVID-19					

• Salbutamol	13 (16.3%)	2 (5%)	11 (27.5%)	0.15 (0.02 ; 0.63)	0.0690
• Acetylsalicylic acid	21 (26.3%)	7 (17.5%)	14 (35.0%)	0.40 (0.13 ; 1.13)	0.3523
• NSAIDs	16 (20.0%)	11 (27.5.0%)	5 (12.5%)	2,59 (0.83 ; 9.25)	0.3559
• Azithromycin	10 (12.5%)	4 (10.0%)	6 (15.0%)	0.64 (0.15 ; 2.51)	1.0000
• Nebulizers	3 (3.8%)	1 (2.5%)	2 (5.0%)	0.32 (0.07 ; 4.65)	1.0000
• Ivermectin	11 (13.8%)	7 (17.5%)	4 (10.0%)	1.87 (0.50 ; 7.99)	0.8446
• Enoxaparin	1 (1.3%)	1 (2.5%)	0 (0.0%)	1 (0.12 ; 77.80)	1.0000
• Unfractionated heparin	0 (0.0%)	0 (0.0%)	0 (0.0%)	**----**	---
• Hydroxychloroquine	0 (0.0%)	0 (0.0%)	0 (0.0%)	**----**	---
• Glucocorticoids	8 (10.0%)	4 (10.0%)	4 (10.0%)	0.78 (0.25 ; 3.99)	1.0000
• Oxygen therapy	2 (2.5%)	1 (2.5%)	1 (2.5%)	0.49 (0.10 / 10.03)	1.0000
• Antihistamine	5 (6.3%)	5 (12.5%)	0 (0.0%)	5.56 (0.67 ; 235)	0.2328
• Anti-influenza drugs	9 (11.3%)	8 (20.0%)	1 (2.5%)	4.73 (1.14 ; 41.53)	0.1350
• Antipyretics	32 (40.0%)	7 (17.5%)	25 (62.5%)	0.13 (0.04 ; 0.36)	0.0013*
• Antibiotics	4 (5.0%)	1 (2.5%)	3 (7.5%)	0,23 (0.06 ; 2.90)	0.9516
• Vitamin supplements	1 (1.25%)	1 (2.5%)	0 (0.0%)	1 (0.12 ; 77.80)	1.0000
COVID-19 related symptoms	Overall patients (n = 80). Number of days reported	I+SOCG Intervention Group (n = 40) Number of days reported	SOCG Control Group (n = 40) Number of days reported	Measure Effect (Confidence Interval 95%)	P-Value
Fever	1.8 ± 3.2	1.15 (± 2.31)	2.43 (± 3.18)	1.28 (-0.11 ; 2.66)	0.2443
Cough	5.8 ± 4.6	4.20 (± 4.33)	7.38 (± 4.29)	3.18 (1.29 ; 5.06)	0.0089*
Cephalea	5.2 ± 3.9	3.68 (±3.08)	6.80 (± 4.12)	3.13 (1.53 ; 4.72)	0.0019*
Ageusia	3.7 ± 5.1	3.03(± 4.38)	4.38 (± 5.74)	1.35 (0.89 ; 3.59)	0.4569
Anosmia	2.6 ± 3.9	2.50 (±3.91)	2.63 (± 3.95)	0.13 (-1.59 ; 1.85)	1.0000
Chest tightness	2.9 ± 4.0	2.23 (±3.17)	3.48 (±4.72)	1.25 (-0.51 ; 3.01)	0.3559
Nasal congestion	3.9 ± 3.9	3.33 (±4.05)	4.53 (±3.64)	1.20 (-0.49 ; 2.89)	0.3559
Dyspnea	3.5 ± 4.5	1.28 (±1.63)	5.70 (±5.31)	4.43 (2.70 ; 6.15)	<0.0001*

Muscle Fatigue	8.5 ± 5.3	6.40 (±4.82)	10.58 (±4.96)	4.18 (2.03 ; 6.32)	0.0019*
General Malaise	9.2 ± 5.0	6.18 (±4.13)	12.15 (±3.99)	5.98 (4.20 ; 7.76)	<0.0001*

Table 1

Demographic, clinical characteristics of patients and symptoms outcome. Effect measures reported are mean differences and odds ratios for quantitate and qualitative variables correspondingly.

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	Overall patients (n = 80)	I+SOCG Intervention Group (n = 40)	SOCG Control Group (n = 40)	OR (95% Confidence Interval)	P-Value
Angiotensin-converting enzyme (ACE) inhibitors	11 (13.75)	6 (15.0%)	5 (12.5%)	1.23 (0.33 ; 4.77)	1.0000
Angiotensin II receptor blockers	6 (7.5%)	2 (5.0%)	4 (10.0%)	0.37 (0.11 ; 2.64)	0.6712
Beta blockers	5 (6.3%)	5 (12.5%)	0 (0.0%)	5,56 (0.67 ; 235)	0.0647
Salbutamol	5 (6.3%)	1 (2.5%)	4 (10.0%)	0.18 (0.05 ; 2.06)	0,3556
Benzodiazepines	0 (0.0%)	0 (0.0%)	0 (0.0%)	----	----
Antidepressants	1 (1.25%)	0 (0.0%)	1 (2.5%)	0 (0.01 ; 8.22)	1.0000
Azithromycin	0 (0.0%)	0 (0.0%)	0 (0.0%)	----	----
Ivermectin	0 (0.0%)	0 (0.0%)	0 (0.0%)	----	----
Colchicine	0 (0.0%)	0 (0.0%)	0 (0.0%)	----	----
Hydroxychloroquine	0 (0.0%)	0 (0.0%)	0 (0.0%)	----	----
Unfractionated heparin	0 (0.0%)	0 (0.0%)	0 (0.0%)	-----	----
Enoxaparin	2 (2.5%)	1 (2.5%)	1 (2.5%)	0.49 (0.10 ; 10.03)	1.0000
Statins	4 (5.0%)	4 (10.0%)	0 (0.0%)	4.32 (0.52 ; 192)	0.1238
Interferon	0 (0.0%)	0 (0.0%)	0 (0.0%)	----	----
Glucocorticoids	2 (2.5%)	1 (2.5%)	1 (2.5%)	0.49 (0.01 ; 10.03)	1.0000
NSAIDs	1 (1.3%)	0 (0.0%)	1 (2.5%)	0 (0.01 ; 8.22)	1.0000
Opiates	5 (6.3%)	5 (15.5%)	0 (0.0%)	5.56 (0.67 ; 235)	0.0646
Levothyroxine	5 (6.3%)	2 (5.0%)	3 (7.5%)	0.47 (0.13 / 3.75)	1.0000

Table 2

Non-SOC medications related to existing comorbidities previous to SARS-CoV-2 infection.

	Number of days with discomfort (Mean and SD) over 14 days of CAM nasal administration
Nasal Itching	8.50 (±4.20)
Oropharyngeal discomfort	6.30 (±4.30)
Dizziness	0.80 (±1.39)
Nausea	1.43 (±2.14)
Headache	3.05 (±2.91)
Slight increase in heart rate	0.40 (±1.33)
Hypotension	0.20 (±0.90)
Lacrimation	7.02 (±5.39)
General Malaise	2.77 (±3.71)

Table 3

Discomfort after Cholinergic Agent Mixture (CAM) in nasal spray delivery administration in the intervention group (I+SOCG). No discomforts were observed in the oral drop ingestion of CAM. Average for each discomfort in number of days on 14 days. The aggregated duration of discomfort in minutes after application of CAM in nasal spray delivery for all symptoms was 7 minutes with an SD of 4 minutes.

4. DISCUSSION

This study is the first randomized trial evaluation of a specific combination of cholinergic nicotinic agents as adjuvant therapy in the treatment of out care symptomatic adult patients with confirmed COVID-19. Tolerance to the drug was good with no relevant nor long-lasting side effects. CAM drug showed to be a protective factor reducing significatively the number of days for cough, cephalea, dyspnea, muscle fatigue and general malaise.

According to previous published meta-analysis, the most prevalent COVID-19 symptoms in the severe COVID-19 disease group were cough (70.5%), fever (64.1%) and fatigue (44.5%); in the ICU group these were cough (67.2%), fever (62.9%) and dyspnea (61.2%) (77). The highest risk among comorbidities for ICU admission were hypertension and cardiovascular disease, both predictive for both severe disease and even more strongly associated to ICU admission (77). In our study we found that, although the intervention group had a significantly higher presence of cardiovascular disease ($p=0.0121$), the evolution of symptoms was significantly positive when compared to control group. Interestingly, being cough and fatigue predictive signs for severe COVID-19 and cough and dyspnea for ICU admission (77), we found in our study that CAM seems to be a significant factor for remission and improvement of these three symptoms. If confirmed in larger studies, CAM could be a candidate of public health relevance in the remission of cough, fatigue and dyspnea, and its administration could have a beneficial impact on the reduction of these three predictor symptoms for severe disease or ICU admission.

Since dyspnea is the only predictive symptom with significance for hospitalization and admission to the ICU according to meta-analysis (77), we wish to highlight, following the results obtained in this study in the

reduction of days in the intervention group, the importance of the beneficial effects of CAM on dyspnea symptom, being 1.28 days (±1.63) in the intervention group and 5.70 days (±5.31) in the control group (measure of effect 4.43 [2.70 ; 6.15]; adjusted p-value <0.0001).

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CAM drug may be a valid candidate for further clinical research being of rapid development, low cost, easy storage, and exhibiting preliminary a good tolerance and safety in agreement with other drugs of the same category. CAM should be explored as a candidate for an over-the-counter product in the prevention or treatment of COVID-19 out care patients or also as an inward patient's hospital product under oral, nasal and/or nebulized administration.

Because Long-Covid is also an important Public Health issue (78–84) that could be probably described as the sequalae following the intoxication and disruption of the cholinergic system, cholinergic agonist combinations such as CAM should be further studied to elucidate if they can also be of use in the recovery of Long-Covid patients.

5. CONCLUSION

In an outpatient treatment setting, the novel combination of Cholinergic Agent Mixture (CAM) in nasal spray and oral drops led to statistically and clinically significant improvements at day 14 from randomization in a composite clinical endpoint. Importantly, CAM reduced the duration of dyspnea by 4.43 days (95% CI: 2.70; 6.15, p <0.0001), as well as four other symptoms including cough, general malaise, muscle fatigue and cephalea. These findings are encouraging because dyspnea, cough, and fatigue are highly prevalent and strongly linked with severe disease, hospitalization and death. Larger randomized studies are needed to confirm our results and to further evaluate the public health implications of CAM.

LIMITATIONS OF THE STUDY

Sample size: The study results are limited to the number of patients included in it, 80 (40 for intervention group and 40 for control group). This has been a randomized open-label trial pilot study. It is suggested that the same study be conducted with larger multicenter studies that include higher number of patients in both groups with double blind randomized trials with placebo group.

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.

FUNDING STAMEMENT

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors have not obtained any funding from the tobacco industry, nor from the vaping industry nor NRT-related products companies.

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. Two international (PCT) patent applications have been filed relating to the formulation of CAM. But none of the authors are applicants or related to them.

ACKNOWLEDGEMENTS

Not applicable

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