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Phase IIa randomized placebo-controlled clinical trial to evaluate the efficacy of bromhexine in combination with standard therapy in the early treatment of COVID-19 in primary care patients

David Ramírez[1,2,], Romina Rojas[1,3], Humberto Fierro[3], Daniela Alejandra Nova[3], Georgina M. Renard[4], Estibaliz Ampuero[5], Jorge Fuentealba[3,6], Ana Martínez[7], Carmen Gil[7], Miguel Reyes-Parada[2,4,*].*

[1]Departamento de Farmacología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.
[2]Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Santiago, Chile.
[3]Center for Advanced Research on Biomedicine (CIAB), Universidad de Concepción, Concepción, Chile.
[4]Centro de Investigación Biomédica y Aplicada (CIBAP), Escuela de Medicina, Facultad de Ciencias Médicas, Universidad de Santiago de Chile, Santiago, Chile.
[5]Laboratorio Neurofarmacología del Comportamiento, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile.
[6]Departamento de Fisiología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.
[7]Centro de Investigaciones Biológicas -Margarita Salas, Consejo Superior de Investigaciones Científicas, Madrid, España.

** Corresponding author: David Ramírez / dramirezs@udec.cl*

Ensayo clínico aleatorizado de fase IIa controlado con placebo para evaluar la eficacia de la bromhexina en combinación con la terapia estándar en el tratamiento temprano de la COVID-19 en pacientes de atención primaria

ABSTRACT

The continuous appearance of SARS-CoV-2 variants with both increased transmission and immune evading potential, in addition to the reluctance of some populations to be vaccinated, supports the search for alternative treatments to protect against the viral infection consequences. Bromhexine is a well-known, over-the counter, expectorant which have garnered interest for the potential prevention and treatment of COVID-19. ***Aim:*** *To evaluate the efficacy of oral bromhexine in patients with mild to moderate symptomatic active SARS-CoV-2 infection.* ***Methods:*** *A randomized, placebo-controlled clinical trial assessing the effectiveness of oral bromhexine in outpatients with active SARS-CoV-2 infection. Adult patients diagnosed with active SARS-CoV-2 infection (n= 36) were randomly assigned (1:1) to receive bromhexine or placebo, with both groups also receiving standard care. The primary efficacy endpoint was viral load reduction (day 4 vs. baseline), while reductions of a series of COVID-19 clinical symptoms were considered as secondary endpoints.* ***Results:*** *No differences in the viral load at different times after the initiation of treatment were observed. In addition, no differences between groups were detected in most of the clinical symptoms evaluated. Remarkably, a significant*

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decrease in the percentage of patients with cough was observed at days 5-7 in the bromhexine group, an effect that was not apparent in the group receiving a placebo. ***Conclusions:*** *Since coughing is one of the primary forms of transmission for SARS-CoV-2, the observed reduction of coughing might be highly beneficial, particularly for patients living in crowded, poorly ventilated, or confined environments.*
Keywords: *Bromhexine; COVID-19; Drug Repositioning; SARS-CoV-2.*

RESUMEN
La aparición continua de variantes del SARS-CoV-2 con mayor transmisión y capacidad para evadir el sistema inmunitario, además de la reticencia de algunas poblaciones a vacunarse, respalda la búsqueda de tratamientos alternativos para proteger contra las consecuencias de la infección viral. La bromhexina es un expectorante de venta libre bien conocido que ha despertado interés por su potencial en la prevención y tratamiento de la COVID-19. ***Objetivo:*** *evaluar la eficacia de bromexina oral en pacientes con infección activa por SARS-CoV-2 sintomática leve a moderada.* ***Métodos:*** *ensayo clínico aleatorizado y controlado con placebo, en el que se evaluó la eficacia de la bromhexina oral en pacientes ambulatorios con infección activa por SARS-CoV-2. Pacientes adultos diagnosticados (n= 36) fueron asignados aleatoriamente (1:1) para recibir bromhexina o placebo, y ambos grupos también recibieron atención estándar. El criterio de valoración principal de eficacia fue la reducción de la carga viral (día 4 vs. línea base), mientras que la reducción de una serie de síntomas clínicos de COVID-19 se consideró como criterio de valoración secundario.* ***Resultados:*** *No se observaron diferencias en la carga viral en diferentes momentos después del inicio del tratamiento. Además, no se detectaron diferencias entre los grupos en la mayoría de los síntomas clínicos evaluados. Notablemente, se observó una disminución significativa en el porcentaje de pacientes con tos en los días 5-7 en el grupo de bromhexina, un efecto que no fue aparente en el grupo que recibió placebo.* ***Conclusiones:*** *Dado que la tos es una de las principales formas de transmisión del SARS-CoV-2, la disminución de tos observada podría ser altamente beneficioso, especialmente para pacientes que viven en entornos abarrotados, mal ventilados o confinados.*
Palabras clave: *Bromhexina; COVID-19; SARS-CoV-2; Reposicionamiento de Medicamentos.*

After its first identification in 2019[1], severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused an outbreak of COVID-19 that quickly spread throughout the world, being characterized as pandemic by the World Health Organization (WHO) early in 2020. Since then, more than 670 million cases of infection had been registered and more than 6.8 million infected people died (webpage: https://l1nq.com/PoEpc - data until March 2023 and retrieved February 2024). Even though the use of multiple vaccines helped to mitigate the effects of COVID-19 pandemic on public health,

the continuous appearance of SARS-CoV-2 variants with both increased transmission and immune evading potential[2,3], as well as the insufficient immune response exhibited by some immunocompromised individuals[4], in addition to the reluctance of some population to be vaccinated[5], support the search for other treatment options to protect against SARS-CoV-2 infection consequences, especially in high-risk populations.

Due to the urgent need for effective treatments for symptomatic COVID-19 patients, repurposing existing drugs was adopted early in the pandemic (see for example "The WHO Solidarity PLUS Trial" - https://acesse.dev/E8tdP – retrieved February 2024). While most efforts focused on approved antiviral agents (with some successful examples[6,7,8]), non-antiviral drugs have also been assessed[9]. In this context, a recent study using human–SARS-CoV-2 interactome data and network pharmacology, allowed the identification of some human targets (e.g. Transcription factor Dp-1 (TDP1), Geminin (GMNN) and Cytochrome P450 3A4 (CYP3A4) that interact with SARS-CoV-2 proteins[10], as well as a handful of old drugs (e.g. disulfiram, bromhexine) that might serve as potential therapies.

Bromhexine, a mucoactive drug marketed since 1963, is commonly used as an over-the-counter expectorant for treating upper respiratory infections in both adults and children[11]. Recently, bromhexine and its metabolite ambroxol have gained interest for COVID-19 prevention and treatment due to their interactions with lung cell receptors[12,13]. There is evidence showing that bromhexine is a potent inhibitor of the transmembrane protease serine 2 (TMPRSS2)[14], and other proteins[15] that play key roles in SARS-CoV-2 mechanisms for viral invasion and infection[16]. Based on this, early in the pandemic, this drug was proposed as a treatment or prophylactic agent for COVID-19 patients or those at high risk of infection[12,17,18,19].

Several clinical trials have investigated bromhexine's efficacy, yielding controversial results[20,21,22,23]. These discrepancies may be due to variations in pandemic timing (predominant viral variants, vaccine availability), patient types (hospitalized or outpatients), clinical outcomes measured (mortality, ventilation needs, viral load, respiratory symptoms), and the presence or absence of placebo-controlled groups[24]. Despite these considerations, the safety profile, low-cost and high availability of bromhexine, in addition to the still existing need to find efficacious and affordable treatments in the current scenario of the pandemic and for future similar events that threaten public health, merit the assessment of the clinical efficacy of bromhexine to ameliorate the symptoms of COVID-19 or suppress the transmission of SARS-CoV-2 variants.

In the present work, we report the results of a placebo-controlled randomized clinical trial, in which the efficacy of oral bromhexine was tested in outpatients, mostly vaccinated with two doses, with mild to moderate symptomatic COVID-19.

Materials and Methods

This study is a prospective, randomized, Phase IIa trial, which followed a double-blind, placebo-controlled design with parallel groups, conducted at a single center, in Concepción city, Chile. The study period began on June 09, 2022 and ended on July 08, 2022. The study duration for each patient was 28 days.

The primary objective was to evaluate the efficacy of bromhexine plus standard of care (SOC), referred to as active treatment, versus placebo plus SOC (control) administrated to SARS-CoV-2 infected patients. The primary efficacy endpoint was viral load reduction in the treated group as compared to the control group. In addition, differences in the evolution of a series of clinical symptom in both groups were assessed as secondary endpoints (see below for details).

The study was conducted in accordance with Good Clinical Practice guidelines and according to the Revised Declaration of Helsinki[25]. All participants provided written informed consent, whose protocol was endorsed and approved by the Scientific Ethical Committee of the Concepción Health Service (SSC-CEC 21-12-56, approved April 2022), Chile.

Participants

Recruitment of participants was performed at the Guillermo Grant Benavente Hospital in Con-

cepción (Chile). Subjects who presented symptoms associated with COVID-19 and attended the Staff Medicine Unit for PCR diagnosis were invited to participate in the recruitment program for the study. All participants provided written informed consent. Additionally, all volunteers had received at least one dose of standard vaccine.

Eligible subjects were men or women aged 18 years or older diagnosed with active SARS-CoV-2 infection confirmed by PCR testing for viral RNA in the presence of compatible symptoms (fever, cough, dyspnea or respiratory distress, sore throat, body or muscle pain, fatigue, headache, chills, nasal congestion, loss of taste or smell, nausea or vomiting, and diarrhea). Symptomatic patients had to have one or more of the clinical manifestations in the last 72 h, with mild or moderate severity. Exclusion criteria were patients with high severity of illness such as: need for hospitalization, respiratory rate 30 breaths per minute, heart rate >100 beats per minute, moderate-severe dyspnea, chest-pleuritic pain, persistent hemoptysis, oxygen saturation <94% determined by pulse oximetry, systolic blood pressure 90 mmHg or diastolic blood pressure 60 mmHg, signs of disseminated intravascular coagulation, chest radiograph with bilateral infiltrates, condensations, foggy vitreous opacities, need for oxygen therapy, symptoms of shock, patients presenting with or suspected of having reinfection with SARS-CoV-2; patients with a disease that could complicate or affect the study results (active infectious disease, uncontrolled respiratory disease, ischemic heart disease, heart failure, atrial fibrillation, severe renal failure, active or undergoing treatment for malignant neoplasm, immunosuppression, severe obesity), patients with hypersensitivity to bromhexine or any of the excipients, pregnant women, lactating women, patient on treatment with drugs with known antiviral potential, participation in a clinical trial in the last month.

A total 48 subjects between 24 and 61 years of age of both sexes, which tested positive for COVID-19, were recruited for the trial. Finally, 36 patients completed the study protocol until at least the seventh day. Twelve of the initially recruited patients dropped out of the study due to various personal reasons unrelated to the disease or inherent to the study.

Randomization and intervention

Participants were randomly assigned (Microsoft Excel®) in a 1:1 ratio to the bromhexine group, which received the investigational treatment together with SOC, or to the placebo group, which received placebo plus SOC (paracetamol, NSAIDs, hydration, inhalers and antibiotics under moderate clinical conditions). The primary endpoint was viral load, while a series of COVID-19 clinical symptoms were considered secondary endpoints.

Patients randomized to active treatment received bromhexine, 3 daily doses of 16 mg/10 mL (48 mg/day) for 14 days plus SOC therapy. Five 100 mL vials (16 mg/10 mL) were provided to each patient. Since the effective daily dose of the active product with viral load reduction capacity was unknown, the maximum labeled dose of the marketed product (16 mg/10 mL 3 times daily equivalent to 48 mg/day or 30 mL/day) was used for 14 days. No increase in the dose of bromhexine was allowed. Each patient recorded daily the absence or presence of symptoms. Body temperature was also recorded with a digital surface (axillary) thermometer, while a finger pulse oximeter was used for assessment of oxygen saturation and heart rate. SOC for SARS-CoV-2 infection included paracetamol 500 mg (1-4 times daily), non-steroidal anti-inflammatory drugs (NSAIDs), symptomatic treatment and hydration for mild COVID-19. In moderate disease and only in case of suspected bacterial coinfection/superinfection, the following therapeutics schemes were implemented: azithromycin (500 mg/24 h for 3 days) plus amoxicillin (1 g / 12 h for 7 days); amoxicillin-clavulanate (875-125 mg / 8 h for 7 days); levofloxacin 500 mg/12 h on the first day and then 500 mg/24 h for 4 days. Other treatments when necessary included bronchodilators or inhaled corticosteroids in patients with asthma or chronic obstructive pulmonary disease, low doses of systemic corticosteroids in patients requiring oxygen therapy, and antithrombotic prophylaxis in immobilized patients or patients with risk factors for thrombosis.

Study procedures

The study included a screening (baseline) visit, where eligibility criteria were confirmed, a complete medical history was taken, a rapid SARS-CoV-2 antigen test was performed, a salivary sample was collected for a SARS-CoV-2 PCR test, a fasting peripheral blood sample was drawn for laboratory analysis, informed consent was signed, and a diary study medication were given. Patients were instructed on how to take the assigned medications and fill out the self-report diary of symptoms, which described in plain language the criteria for hospitalization.

Telephone contacts were made on days 1, 4, 7 and 14 after starting treatment. At the end of the study, on day 28, patients visited the primary care center. Saliva samples for the SARS-CoV-2 PCR assay were collected on days 4, 7 and 14 at the patients' homes, due to limited medical visits in quarantined patients. In all telephone contacts, pulse oximetry, heart rate and temperature data recorded by the patient with the study material provided were recorded. Questions about the occurrence of new symptoms and the severity of symptoms were assessed on a numerical rating severity scale (NRS) from 0 to 10 points (0= no symptoms, 10= most severe symptoms imaginable). Symptoms recorded on the diary card, as well as concomitant non-prescribed drugs, were reported to the physician during telephone calls. In addition, the investigator asked patients whether they had experienced any adverse events since the last study contact and, if so, recorded them on the "Adverse Event" page of the case report form and described the event. All adverse events were followed up until resolution or chronicity.

Viral load

Viral load was determined by detecting a highly conserved epitope region within the SARS-CoV-2 pathogenic viral RNA strain, the N1 nucleocapsid protein (N-protein), in saliva samples at baseline and on days 4, 7 and 14 after initiation of treatment. These analyses were performed by the Prevegen laboratory (Concepción, Chile).

Virus RNA lysis and extraction was performed with the Viral Nucleic Acid Extraction Kit II (GENEAID). For amplification, the extracted RNA was used as a template in the RT-PCR reaction. The TaqMan technology-based RT-PCR uses the FAM fluorophore to detect the N1 (nucleocapsid) region of SARS-CoV-2. Kit reagents included the use of primers (Table 1) and probes for N1 (Probes 2019-nCoV Kit 500 rx catalog:10006606 , IDT DNA), GoTaq®polymerase (Probe 1-Step RT-qPCR System cod: A6120, Promega) and as positive controls the plasmid containing the complete SARS CoV-2 virus nucleocapsid gene (2019-nCov_N catalog: 10006625 , IDT DNA) and the plasmid containing a portion of the human Hs_RNP30 gene (Hs_RPP30, catalog: 10006626, IDT DNA).

To monitor viral load, a baseline PCR was performed to confirm the positive diagnosis, then 3 PCRs were taken on days 4, 7 and 14 of treatment. The reactions were carried out on the MIC qPCR cycler thermocycler kit from Biomolecular System, MIC PCR software V.2.10.0. The estimated platform sensitivity and specificity are 95% (CI: 1.047-0846) and 100% (CI:1-1), respectively.

Viral load was estimated as the number of amplification cycles in which the fluorescent signal crosses this threshold (cycle thresholds, Ct) to detect the gene coding for Protein N in a single PCR reaction. An RT-PCR for SARS-CoV-2 was considered positive in the presence of a Ct value below 37. A higher number of cycles means a lower viral load. Viral load was defined as "high" for Ct values 25, "medium" for Ct values between 25 and 30, and "low" for Ct values 30.

Efficacy endpoints

The primary efficacy endpoint was viral load reduction (day 4 vs. baseline) in the active treatment group (bromhexine plus SOC) compared to the control group (placebo plus SOC). Secondary criteria were the assessment of analytical efficacy combined with standard treatment in PCR negativization, from baseline (viral load reduction, Ct value>37), in the two study arms at 7 and 14 days of treatment. These endpoints were used to evaluate the clinical efficacy of active treatment (bromhexine+SOC) in reducing the proportion of patients presenting symptoms of COVID-19 disease in the asymptomatic patient group, to evaluate the clinical efficacy of Bromhexine+SOC in reducing symptoms of COVID-19 disease. Also, to evaluate the efficacy of Bromhexine+SOC

Table 1. Primers included in the 2019-nCoV CDC USA kit.

Primers	Decription	Sequence (5′ - 3′)
2019-nCoV_N1-F	Forward	gaccccaaaatcagcgaaat
2019-nCoV_N1-R	Reverse	tctggttactgccagttgaatctg
2019-nCoV_N1-P	Probe	accccgcattacgtttggtggacc
2019-nCoV_N2-F	Forward	tt1caaacattggccgcaaa
2019-nCoV_N2-R	Reverse	gcgcgacattccgaagaa
2019-nCoV_N2-P	Probe	acaatttgcccccagcgcttcag
human RNAseP-F	Forward	agatttggacctgcgagcg
human RNAseP-R	Reverse	gagcggctgtctccacaagt
human RNAseP-P	Probe	ttctgacctgaaggctctgcggc

on the need for medical care, hospitalization, oxygen therapy or mortality up to day 28 from the start of treatment, ad to evaluate the safety of Bromhexine+SOC administered in SARS-CoV-2 infected patients.

Statistical analysis

The analysis of the primary efficacy endpoint was performed by ANOVA for repeated measurements with Bonferroni adjustment for multiple comparisons, which was applied to the comparison of viral load between the study groups at baseline, day 4, day 7, and day 14.

For the other parameters, the student t-test (two-tailed) for quantitative variables (e.g. age, temperature or heart rate) or Fisher's exact test for qualitative variables (e.g. cough, fatigue, headache) were used to assess differences between the two groups or for intragroup comparisons. In all cases, statistical significance was set at $p < 0.05$.

Results

Forty-eight symptomatic patients diagnosed with COVID-19 infection were recruited in a primary healthcare center after a rapid SARS-CoV-2 antigen-test. The patients were randomly assigned to two groups, Standard of Care (SOC) plus placebo or SOC plus bromhexine treatment. Twelve patients (7 in the control group and 5 in the treated group) dropped out of the study due to diverse reasons unrelated either to the disease or to the treatment. Demographic and clinical characteristics (Table 2) as well as the viral load confirmed by qPCR analysis (Figure 1), were similar in both groups at the beginning of treatment (baseline; data are shown for those patients that completed the study).

As shown in Figure 1, in all patients the viral load decreased during the course of the study (14 days), and those treated with bromhexine did not show significant differences on the reduction of viral load as compared to the placebo control group.

As illustrated in Table 3, which shows the clinical symptoms evaluated at day 7, no statistical differences between groups of treatment were observed when assessing the effects of bromhexine upon symptoms of COVID-19.

Nevertheless, when analyzing the presence of individual symptoms within each group during both treatments, some significant differences were observed. Specifically, in the bromhexine group a significant decrease (as compared with day 1) in the percentage of patients reporting cough was observed after 5-7 days of SOC+bromhexine treatment, an effect that was not apparent in the control group (Figure 2, Table 4). No differences were observed in other symptoms.

Table 2. Demographic and clinical characteristics in patients treated with SOC or treated with SOC+Bromhexine at day 1.

	SOC+placebo group (n= 17)	SOC+Bromhexine group (n= 19)	p value
Demographic characteristics			
Age	35.88 2.639	34.79 1.409	0.7090[a]
Gender (male/female)	6/11	6/13	>0.9999[b]
Baseline clinical characteristics			
Temperature (C)	36.66 0.202	36.4 0.2101	0.3834[a]
Heart rate	89.94 3.124	84.74 3.423	0.2733[a]
O_2 saturation (%)	98.35 0.2564	98.79 0.09609	0.1057[a]
Cough, n (%)	15 (88)	18 (95)	0.5929[b]
Dyspnea, n (%)	1 (6)	4 (21)	0.3420[b]
Dysphagia, n (%)	13 (76)	15 (79)	>0.9999[b]
Myalgia, n (%)	9 (53)	13 (68)	0.4955[b]
Fatigue, n (%)	9 (53)	13 (68)	0.4955[b]
Headache, n (%)	10 (59)	11 (58)	>0.9999[b]
Chills, n (%)	9 (53)	4 (21)	0.0819[b]
Rhinorrhea, n (%)	17 (100)	17 (90)	0.4873[b]
Anosmia, n (%)	7 (41)	4 (21)	0.2814[b]
Nausea/vomiting, n (%)	2 (12)	4 (21)	0.6617[b]
Diarrhea, n (%)	0 (0)	2 (11)	0.4873[b]

[a] student t-test. [b] Fisher´s exact test.

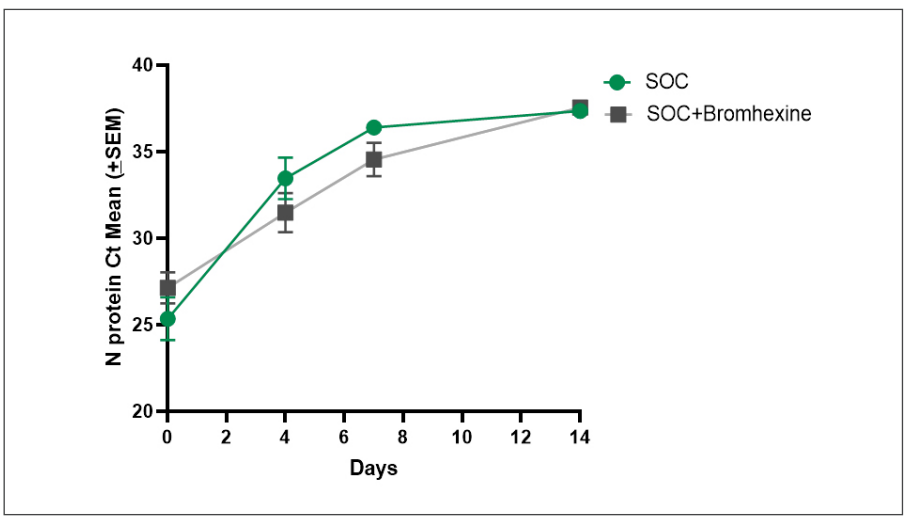

Figure 1: *Evolution of Ct values of N Protein at follow-up in the SOC+placebo group and SOC+Bromhexine group.*

Table 3. Clinical characteristics in patients treated with SOC+placebo or treated with SOC+Bromhexine at day 7.

Clinical characteristics at treatment day 7	SOC+placebo group (n= 15)	SOC+Bromhexine group (n= 19)	*p* value
Temperature (C)	35.91 0.1225	35.88 0.09538	0.8572[a]
Heart rate	79.53 3.349	78.68 3.625	0.8676[a]
O_2 saturation (%)	98.27 0.2667	98.79 0.1636	0.0907[a]
Cough, n (%)	10 (67)	10 (53)	0.7338[b]
Dyspnea, n (%)	1 (7)	2 (10)	>0.9999[b]
Dysphagia, n (%)	3 (20)	6 (32)	0.6974[b]
Myalgia, n (%)	0 (0)	3 (16)	0.2380[b]
Fatigue, n (%)	2 (13)	3 (16)	>0.9999[b]
Headache, n (%)	3 (20)	5 (26)	>0.9999[b]
Chills, n (%)	0 (0)	0 (0)	>0.9999[b]
Rhinorrhea, n (%)	6 (40)	8 (42)	>0.9999[b]
Anosmia, n (%)	3 (20)	2 (10)	0.6343[b]
Nausea/vomiting, n (%)	1 (7)	1 (5)	>0.9999[b]
Diarrhea, n (%)	1 (7)	1 (5)	>0.9999[b]

[a] student t-test. [b] Fisher´s exact test.

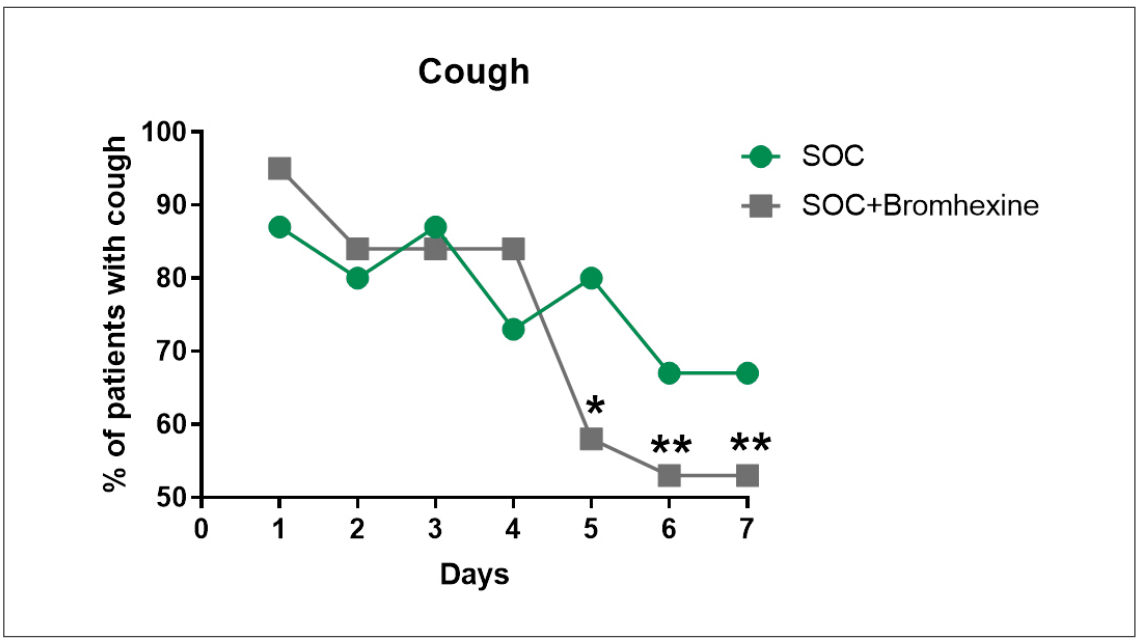

Figure 2: *Evolution of cough in the SOC+placebo and SOC+Bromhexine groups. *p 0.05; **p 0.005 (vs day 1; Fisher's exact test).*

Table 4. Comparison day 1 versus days 5-7 in the number of patients with/without cough in the SOC group and the SOC+Bromhexine groups.

	SOC + placebo		*P* value	SOC + Bromhexine		*P* value
	Day 1	Day 7		Day 1	Day 7	
with Cough (without cough)	13 (2)	10 (5)	0.3898	18 (1)	10 (9)	0.0078**
	Day 1	**Day 6**		**Day 1**	**Day 6**	
with Cough (without cough)	13 (2)	10(5)	0.3898	18 (1)	10 (9)	0.0078**
	Day 1	**Day 5**		**Day 1**	**Day 5**	
with Cough (without cough)	13 (2)	12(3)	>0,9999	18 (1)	11(8)	0.0188*

* p 0.05; ** p 0.005 (vs day 1; Fisher's exact test).

Discussion

The present clinical study compared the efficacy of SOC plus bromhexine versus SOC plus placebo, in the treatment of outpatients with mild to moderate symptomatic COVID-19. Both groups were comparable at the beginning of the study at their demographic and clinical characteristics.

Regarding the primary end point, no differences in the viral load at different times (4, 7 or 14 days) after the initiation of treatment, were observed between treated and control groups. In addition, no differences between groups were also detected in most of the clinical symptoms evaluated. Even though the relatively small sample size is a limitation of the present study, our results are in agreement with those obtained in a recent study[23] which considered a similar patient population, although in an open-label study. Even though with different study designs (e.g. hospitalized patients or prophylactic use, placebo-controlled or open-label), a few other studies have also shown a lack of association between bromhexine treatment and the reduction of viral load or clinical symptoms, as compared with SOC[20,22,26].

At the time of the study (2023), more than 90% of the population in Chile had completed a two-doses scheme of vaccination[27,28], while Omicron (XBB 1.5) was the most common variant[29]. In this scenario, it is not completely surprising that after seven days of treatment, all patients in both groups exhibited viral loads values below the limit of detection, which correlated with a clear amelioration of the clinical symptoms. Remarkably, a significant intragroup difference was detected regarding cough. Thus, when compared with the first day of treatment, a significant decrease in the percentage of patients with cough was observed at days 5-7 in the group treated with SOC+bromhexine, an effect that was not apparent in the control (SOC+placebo) group. Even though our data do not allow us to determine the exact mechanism associated with this effect, it is likely that it is more related with the well-known antitussive action of bromhexine[11,30,31], rather than a truly antiviral effect. Beyond these considerations, and bearing in mind that coughing is one the main forms of transmission for SARS-CoV-2 and other coronaviruses[32], this effect might be beneficial in particular in crowded, poorly ventilated or confined environments

In conclusion, although the present findings do not support the use of bromhexine as an antiviral

for treating outpatients with mild-to-moderate COVID-19, they suggest that it may be used as an addition to reduce the disease transmission

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Competing Interests

The authors declare no conflicts of interest.

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