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REVIEW ARTICLE

ROLE OF AMBROXOL AS A PROPHYLACTIC AGENT AGAINST COVID-19

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Abstract

Currently the world is facing a pandemic disease, namely Coronavirus disease 2019 (COVID-19) that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As finding recent drugs targeting COVID-19 will take a long time, so repositioning currently existing FDA approved drugs for treating this disastrous disease is an acceptable solution. It has been found that for SARS-CoV-2 to be infective, this necessitates splitting of the viral spike glycoproteins by the serine protease "type II transmembrane serine protease TMPRSS2" that has shown to be widely expressed in pulmonary tissues. Thus, TMPRSS2 is suggested to be potential target for antiviral drug design against COVID-19. The mucokinetic drug "Ambroxol" has been reported as a potent inhibitor of TMPRSS2, thus it could represent a therapeutic as well as a prophylactic candidate against SARS-CoV2. This review gives a brief summary about ambroxol's potential role against COVID-19's TMPRSS2.

Keywords: Ambroxol, Coronavirus disease 2019 "COVID-19", mucokinetic drug, Type II transmembrane serine protease "TMPRSS2".

INTRODUCTION

Pathogenic microorganisms are always a major threat to human health[1]. Currently the whole world is facing a pandemic disease, namely Coronavirus disease 2019 (COVID-19) that caused by the RNA virus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The mortality rates have been reported to range from 1% to more than 5%, along with the highly infectious power of the virus[2]. Attempts to evolve recent antiviral drugs are concentrating on elements that can impact viral or host factor(s) that are essential for virus replication[3]. Transmembrane protease serine 2 (TMPRSS2) is one of the promising targets that when inhibited can slow or stop replication of viruses[4]. Cleavage of the viral spike glycoproteins by serine protease causes their activation and facilitates virus-cell membrane fusions leading to host cell entry, replication, and spread. A multi domain type II transmembrane serine protease TMPRSS2 is one of the serine proteases critical for viral infectivity and it has shown to be widely expressed in lung tissues[5,6].

TMPRSS2 has been suggested in new evidence to be involved in other coronavirus such as severe acute respiratory syndrome-related coronavirus (SARS-CoV) as well as Middle East respiratory syndrome (MERS) protein synthesis for infection in many susceptible host cells from various organs[7]. Based on similarities between SARS-CoV-2 and the Middle East respiratory syndrome (MERS), TMPRSS2 was proposed as a potential target for antiviral drug design against COVID-19[8].

As finding new drugs that can inhibit TMPRSS2 and target COVID-19 will take a long time so repositioning some of the existing FDA approved drugs for treating this disastrous disease is an acceptable solution. Repurposing of drugs is a quick answer to the crucial need for a treatment for COVID-19 as it screens through a few number of drugs that have already proven to be safe for human use and are readily available in the market. The FDA approved mucokinetic drug, ambroxol is the active form of another mucokinetic drug bromhexine, either drug is a well-known potent inhibitor of TMPRSS2[9]. Thus, inhabitation of pulmonary TMPRSS2 with ambroxol could be a therapeutic as well as prophylactic strategy against the airborne transmission of SARS-CoV2. Given the relative safety profile of this drug, it seems that this TMPRSS2 inhibitor could represent a prophylactic agent against SARS-CoV-2 infection.

TMPRSS as a prospective target in our fight against COVID-19

The interactions between the spike glycoprotein (S) and its cognate receptor, Angiotensin converting enzyme 2 (ACE2) is the initial step in attachment of SARS-CoV-2 to the target cell[10]. Following receptor engagement, SARS-CoV-2 S is processed by a plasma membrane–associated type II transmembrane serine protease, TMPRSS2 that is crucial to release the viral contents into the cytosol of host cell[11].Corona virus S proteins are typical class I viral fusion proteins, and protease cleavage is essential for activation of its fusion potential[12]. CoV S proteins may be cleaved by one or several host proteases, transmembrane protease serine protease-2,-4 (TMPRSS-2), (TMPRSS-4) depending on the viral strains and cell types[13]. Whether CoVs enter cells through plasma membrane or endocytosis is largely dependent on the availability of these proteases on target cells[14]. However, whether any of these proteases could promote SARS-CoV-2 virus entry remains elusive.

Other aspects of TMPRSS and COVID-19 interrelationship

Another interesting point in the context of TMPRSS and COVID-19 is that TMPRSS2 has been found to be androgen-regulated[15] and so we are wondering, whether there is any relationship between TMPRSS2 and the male to female discrepancy in COVID-19 given the fact that COVID-19 requires TMPRSS2 to enter into the lung cell. Is there more TMPRSS2 in the male lung as a result of androgen versus the female lung? Away from viral infections, TMPRSS2, actually, has a widely recognized role in the pathogenesis of prostate cancer[16]. Because of its oncogene driving function, TMPRSS2 now serves as a canonical readout of androgen receptor (AR)-dependent transcription in prostate cancer models and tissues[17]. Up till now, it is not known if TMPRSS2 expression in the normal human lung is regulated by androgens in physiological settings or not. If the answer is yes, thenTMPRSS2 expression in lung tissue might account for male preponderance of COVID-19.

TMPRSS2 is also expressed in a number of different cells, including not only the lung but also the gastrointestinal system. TMPRSS2 is highly expressed in the gastrointestinal (GI) tract, particularly by intestinal epithelial cells (IECs), the cardinal target cells for many human enteric viruses[18]. Obvious GIT symptoms including abdominal pain and diarrhea have been observed in 20 to 50% of patients with COVID-19 and sometimes precede the development of respiratory illness[19]. Does this expression vary between individuals and could it account for variability in clinical presentations of COVID-19?!Expression of TMPRSS in the enterocytes has been proved where it has been found to facilitate SARS-CoV-2 spike fusogenic activity and promote theviral entry into host cells[20].

Pharmacology of ambroxol

Ambroxol (2-amino-3,5-dibromo-N-[trans-4-hydroxy-cyclohexyl] benzylamine), is the N-desmethyl active metabolite of the prodrug bromhexine. Both are widely used as over the counter mucoactive agents for treatment of acute or chronic respiratory diseases associated with viscid mucus since 1978 with excellent records of safety[21].

When administered orally, ambroxol has a bioavailability of 79%[22]. It is metabolized by cytochrome P450 3A4 in the liver with its elimination half-life is approximately 10 h, and a total clearance of 660 ml/min[22,23]. Ambroxol has a good lipophilicity and low polar surface area (PSA 58 Å2), predicting a good CNS penetration[24]. On distribution to tissue, the highest concentration is found in the lungs. In human lung tissue, ambroxol has been achieved15- to 20-fold higher concentrations than in the blood[21]. Moreover, the pharmacokinetics of Ambroxol has not been shown to be affected by age or gender to a clinically relevant extent, therefore dose adjustment is not indicated[23]. For adults, the normal expectorant dose ranges from 75-100 mg/day. For pregnant women experiencing premature delivery, 1000 mg IV doses are used to assist fetal lung maturation, while a dose of 30 mg/kg is used in neonates for managing fetal respiratory distress syndrome[24].

Clinical experience assembled from observational studies and randomized clinical trials suggest that ambroxol is a well-tolerated and safe therapeutic for bronchopulmonary diseases, with a well-balanced and favorable benefit-risk profile in adults as well as in the pediatric population. The most common adverse effects are mild and self-limiting GIT disturbances. The risk of serious cutaneous adverse reactions reported by the Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicines agency (EMA) with ambroxol is extremely low[21,23]. As a mucolytic it reduces the mucus viscosity and crosslinking by disrupting the secretions' polymer networks via slicing disulfide bonds, depolymerizing mucopolysaccharides, dissolving proteins, and breaking down DNA filaments and actin[25].

Ambroxol is presumed to stimulate the secretion of surfactant and mucus, thus promote normalization of mucus viscosity in the secretions. However, recent systematic reviews provide proof of a generalized benefit using ambroxol for a range of issues, including secretolytic activity (promoting mucus clearance), anti-inflammatory (through inhibition of pro-inflammatory mediators generation by leukocytes) and antioxidant activity(via direct scavenging of reactive oxygen species) as well as local anaesthetic effect (through neuronal sodium channels inhibition)[21,24,26]. In addition, antiviral, antibacterial and antifungal properties for ambroxol have been recently described and published. Ambroxol at 1 to 60 mg/mL concentrations has been shown to exhibit antimicrobial effects on *C. albicans*, *Candida parapsilosis* and *Pseudomonas aeruginosa*[27]. Direct and indirect anti-infectious properties of ambroxol have been concluded such as; increasing bioavailability of antibiotics, increasing the concentrations of immunoglobulins A and G and its ability to suppress virus multiplication[28,29,30]. It is worth to mention that the use of ambroxol as an adjuvant in treatment of respiratory tract infection (RTIs) with biofilm-producing pathogens such as *Pseudomonas aeruginosa* and *Candida albicans* is of a special

interest[30]. This explains its benefits in the prophylaxis and treatment of upper or lower RTIs associated with impaired mucus secretion or transport. Its efficacy has been detected in more than 100 clinical observational, uncontrolled, or randomized, controlled, double-blind trials on 15.000 adult and pediatric patients with various forms of acute and chronic upper and lower respiratory tract diseases[24,31].

Use of ambroxol as a prophylactic and/or therapeutic agent against Covid19

Since the entry of COVID 19 virus into cells was reduced by camostat mesylate, a TMPRSS2 inhibitor, Hoffman *et al*., suggested that TMPRSS2 could be a potential therapeutic target for COVID-19. The IC50 of camostat mesylate is 4.2 nM[11]. In Japan camostatmesylate, is currently approved only for treatment of chronic pancreatitis, with very little available data on the drug's risk profile and no experience with its use in children, pregnant women, multimorbid, and other high-risk patients. In addition, being a non-selective TMPRSS2 inhibitor, means that it has greater and more severe side effects[32]. The FDA-approved mucokinetic agent ambroxol, the active form of bromhexine hydrochloride, is a well-known potent selective inhibitor of TMPRSS2 with IC50 equal to 0.75 Mm (a level far below the IC50 of camostat mesylate) holding promise for this drug as a potential anti-COVID-19 treatment[13]. Bromide compounds, especially aromatic bromide compounds, show a relatively high binding affinity for serine-containing peptide sequences, proteins, and enzymes and this effect is due to a selective TMPRSS2inhibition by bromhexine[33]. Since epithelisin is expressed primarily in the apical surface of airway epithelial cells, thus inhibition of pulmonary TMPRSS2 with ambroxol could represent a prophylactic/therapeutic strategy against COVID19 transmission[13].

Studies assessing potential role of ambroxol as a TMPRSS2 inhibitor in COVID-19

An "*in vitro*" study showing that a TMPRSS2 inhibitor Camostat inhibited SARS-CoV-2 from entering cells[14], encouraged conducting studies on ambroxol and its prodrug" bromhexine". Indeed, ambroxol has been shown to inhibit rhinovirus infection of human tracheal epithelial cell cultures by a number of mechanisms[34]. One of these mechanisms (reducing acidic endosomes) might apply against SARS-CoV-2. According to researchers from IBM, computer simulations indicate that Ambroxol might inhibit SARS-CoV-2's main protease[35]. Ambroxol has been also shown to up-regulate endogenous protease inhibitors. Ambroxol's up-regulation of these defensive molecules has been shown to increase the survival of mice exposed to influenza virus[36]. The daily dosage reported to be most effective was found to be 10mg/kg, which for humans would be equivalent to 0.8 mg/kg[37]. Ambroxol may be also an ACE2 binding agent according to an artificial intelligence drug target screening done by researchers at the School of Basic Medicine Sciences at Peking University[38]. In addition, ambroxol has been found to inhibit SARS-CoV-2 replication in an *in vitro* screening of a number of approved drugs, according to a pre-print paper released several days ago[39]. The concentration of ambroxol found to inhibit the replication of SARS-CoV-2 falls in the range that can be achieved in lung tissue with therapeutic doses[40] indicating that ambroxol may have antiviral effects against SARS-CoV-2 at concentrations achievable with normal dosages. Currently, there is an epidemiologic study underway in China evaluating ambroxol's potential in the treatment of COVID-19, sponsored by Boehringer Ingelheim[41]. Clinical trials assessing the possible antiviral effect of ambroxol's prodrug, namely bromhexine, in COVID-19 patients are also currently going on in a number of countries[42,43] and we are waiting for results to be released. These clinical trials are based on preliminary promising results of bromhexine in inhibiting SARS-CoV-2[44,45]. The use of ambroxol at adequate dose to selectively inhibit the TMPRSS2, resulting in prevention of viral entrance via TMPRSS2-specific pathway, could be a new hope for an effective treatment of Covid-19.

CONCLUSIONS

As the world observes the alarming levels of spread and severity of COVID-19, plan of actions to battle this outbreak are in great need. Drug repurposing is a fascinating alternative drug discovery approach because there is the advantage of accessibility, decreased cost of development, availability of toxicity data and the availability of options for combination studies. The available background pharmacological knowledge for such compounds may also reduce concerns regarding adverse effects in patients. Using ambroxol that selectively suppresses TMPRSS2-specific viral entry seems to be a weapon against SARS-CoV-2. We propose the use of ambroxol in prophylaxis against COVID-19 (since it might inhibit the viral attachment to its target receptor) as well as a treatment against COVID-19. Furthermore, a combination with hydroxylchloroquine, that is considered an effective endosomal protease inhibitor, inhibiting cathepsin B/L, could be a favourable combination for the therapy of COVID-19 cases. On the basis of this review, it seems thatTMPRSS2couldbe a potential and attractive target to be seriously considered for SARS-CoV-2 antiviral therapy. Ambroxol (or bromohexine) could be a promising candidate for immediate use as either drug is a TMPRSS2 inhibitor already approved by the FDA.The scientific testing of ambroxol is deeply encouraged.

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AUTHOR'S CONTRIBUTION

Baraka AM: writing original draft, literature survey. **El-Hadidy WF:** methodology, formal analysis, conceptualization. Both authors revised the article and approved the final version.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. Pamuk S. The black death and the origins of the 'great divergence' across Europe, 1300–1600. Eur Rev Econ Hist 2007; 11: 289-317. *https://doi.org/10.1017/S1361491607002031*
2. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese center for disease control and prevention. JAMA 2020; 323: 1239- 42. *https://doi.org/10.1001/jama.2020.2648*
3. Zhirnov OP, Matrosovich TY, Matrosovich MN, Klenk HD. Aprotinin, a protease inhibitor, suppresses proteolytic activation of pandemic H1N1v influenza virus. Chem Chemother 2011; 21(4):169-74. *https://doi.org/10.3851/imp1715*
4. Meyer D, Sielaff F, Hammami M, Böttcher-Friebertshäuser E, Garten W, Torsten Steinmetzer T. Identification of the first synthetic inhibitors of the type II transmembrane serine protease TMPRSS2 suitable for inhibition of influenza. virus activation. Biochem J 2013; 452(2):331-43.*https://doi.org/10.1042/bj20130101*
5. Lukassen S, Chua RL, Trefzer T, Kahn NC, Schneider MA, Muley T. *et al*. SARS- CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells, EMBO J 2020; 18;39(10):e105114. *https://doi.org/10.15252/embj.20105114*
6. Zmora P, Hoffmann M, Kollmus H, Moldenhauer AS, Danov O, Braun A, *et al*. TMPRSS11A activates the influenza A virus hemagglutinin and the MERS coronavirus spike protein and is insensitive against blockade by HAI-1. J Biol Chem 2018; 293:13863-73. *https://doi.org/10.1074/jbc.RA118.001273*
7. Limburg H, Harbig A, Bestle D, Stein DA, Moulton HM, Jaeger J, *et al*. TMPRSS2 is the major activating protease of influenza A virus in primary human airway cells and influenza B virus in human type II pneumocytes. J Virol.2019; 93: e00649-19. *https://doi.org/10.1128/JVI.00649-19*
8. Shen LW, Mao HJ, Wu YL, Tanaka Y, Zhang W. TMPRSS2: A potential target for treatment of influenza virus and coronavirus infections. Biochimie 2017; 142: 1-10.*https://doi.org/10.1016/j.biochi.2017.07.016*
9. Habtemariam S, Nabavi SF, Ghavami S, Cismaru CA, Berindan-Neagoe I, Nabavi SM. Possible use of the mucolyticdrug, bromhexine hydrochloride, as a prophylactic agent against SARS-CoV-2 infection based on its action on the Transmembrane Serine Protease 2. Pharmacol Res 2020; 157:104853. *https://doi.org/10.1016/j.phrs.2020.104853*
10. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020;181, 281–92.e6. *https://doi.org/10.1016/j.cell.2020.02.058*
11. Matsuyama S, Nao N, Shirato K, *et al*. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proc Natl Acad Sci USA 2020; 117,7001–3. *https://doi.org/10.1073/pnas.2002589117*
12. Ou X, Zheng W, Shan Y, Mu Z, Dominguez SR, Holmes KV, Qian Z . Identification of the fusion peptide-containing region in beta coronavirus spike glycoproteins. J Virol 2016; 90, 5586–600. *https://doi.org/10.1128/jvi.00015-16*
13. Bertram S, Dijkman R, Habjan M, Heurich A, Gierer S, Glowacka I, *et al*. TMPRSS2 activates the human coronavirus 229E for cathepsin-independent host cell entry and is expressed in viral target cells in the respiratory epithelium. J Virol 2013; 87, 6150–60. *https://doi.org/10.1128/jvi.03372-12*
14. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, *et al*. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181, 271–280.e8. *https://doi.org/10.1016/j.cell.2020.02.052*
15. Sharifi N, Ryan CJ. Androgen hazards with COVID-19. Endocr Relat Cancer 2020; 27(6):E1-E3. *https://doi.org/10.1530/ERC-20-0133*
16. DaiC, Heemers H, Sharifi N .Androgen signaling in prostate cancer. Cold Spring Harbor Perspectives in Medicine 2017; 7 a030452. *https://doi.org/10.1101/cshperspect.a030452*
17. Cai H. Sex difference and smoking predisposition in patients with COVID-19. Lancet Respiratory Medicine. 2020;8(4):e20. *https://doi.org/10.1016/S2213-2600(20)30117-X*
18. Weiss SR, Leibowitz JL, Coronavirus pathogenesis. Adv Virus Res 2011; 81, 85–164. *https://doi.org/10.1016/b978-0-12-385885-6.00009-2*
19. Pan LM, Yang M, Sun P, Wang Y, Yan R, Li J, *et al*. Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: A descriptive, cross-sectional, multicenter study. Am J Gastroentero 2020;115, 766–73 . *https://doi.org/10.14309/ajg.0000000000000620*
20. Zang R, Gomez Castro MF, McCune BT, Zeng Q, Rothlauf PW, Sonnek NM, *et al*. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. Sci Immunol 2020 ;5(47):eabc3582.*https://doi.org/10.1126/sciimmunol.abc3582*
21. Cazan D, Klimek L, Sperl A, *et al*. Safety of ambroxol in the treatment of airway diseasesin adult patients. Exp Opin Drug Safety 2018; 17(12):1211–24. *https://doi.org/10.1080/14740338.2018.1533954*
22. Gupta PR. Ambroxol - Resurgence of an old molecule as an anti-inflammatory agent in chronic obstructive airway diseases. Lung India 2010; 27:46-8. *https://doi.org/10.4103/0970-2113.63603*
23. Kantar A, Klimek L,Cazan D, Sent A, Mesquita M. An overview of efficacy and safety of ambroxol for the treatment of acute and chronic respiratory diseases with a special regard to children. Multidiscip Resp Med 2020; 15(1): 511.*https://doi.org/10.4081%2Fmrm.2020.511*
24. Malerba M, Ragnoli B. Ambroxol in the 21st century: pharmacological and clinical update. Expert Opin Drug Metab Toxicol 2008; 4: 1119–29. *https://doi.org/10.1517/17425255.4.8.1119*
25. King M, Rubin BK. Mucus-controlling agents: past and present. Respir Care Clin N Am 1999; 5:575–94. PMID: 10565882
26. Balsamo R, LanataL, Egan CG. MucoactiveDrugs. Eur Respir Rev 2010; 19(116):127-33. *https://doi.org/10.1183/09059180.00003510*
27. Li X, Zhao Y, Huang X, Yu C, Yang Y, Sun S. Ambroxolhydrochloride combined with fluconazole reverses the resistance of candida albicans to fluconazole. Front Cell Infect Microbiol 2017; 7:124. *https://doi.org/10.3389/fcimb.2017.00124*
28. Yang B, Yao DF, Ohuchi M, *et al*. Ambroxol suppresses influenza-virus proliferation in the mouse airway by increasing antiviral factor levels. Eur Respir J 2002; 19:952-8.*https://doi.org/10.1183/09031936.02.00253302*
29. Li F, Yu J, Yang H, *et al*. Effects of ambroxol on alginate of mature *Pseudomonas aeruginosa* biofilms. Curr Microbiol 2008;57:1-7. *https://doi.org/10.1007/s00284-008-9142-8*
30. Plomer M, de Zeeuw J. More than expectorant: new scientific data on ambroxol in the context of the treatment

of bronchopulmonary diseases. J Int Crit Care 2017 ; 3 (3) : 37. *https://doi.org/10.21767/2471-8505.100096*

31. Scaglione F, Petrini O. Mucoactive agents in the therapy of upper respiratory airways infections: fair to describe them just as mucoactive? Clinical Medicine Insights: Ear, Nose and Throat 2019; 12:1-9. *https://doi.org/10.1177/1179550618821930*
32. Depfenhart M, Danielle de Villiers D, Lemperle G, Markus Meyer M, Di Somma S. Potential new treatment strategies for COVID-19: is there a role for bromhexine as add-on therapy? Intern Emerg Med 2020; 26 : 1–12. *https://doi.org/10.1007/s11739-020-02383-3*
33. Lucas JM, Heinlein C, Kim T, Hernandez SA, Malik MS, True LD, *et al*. The androgen-regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasis. Cancer Discov 2014; 4(11):1310–25. *https://doi.org/10.1158/2159-8290.cd-13-1010*
34. Yamaya M, Nishimura H, Nadine LK, Ota C, Hiroshi Kubo H, Nagatomi R. Ambroxol inhibits rhinovirus infection in primary cultures of human tracheal epithelial cells. Arch Pharm Res 2014; 37(4):520-9. *https://doi.org/10.1007/s12272-013-0210-7*
35. Huynh T, Wang H, Luan B. In silico exploration of the molecular mechanism of clinically oriented drugs for possibly inhibiting SARS-CoV-2's main protease. J Phys Chem Lett 2020; 11(11):4413-20. *https://doi.org/10.1021/acs.jpclett.0c00994*
36. Kido H, Okumura Y, Yamada H, Mizuno D, Higashi Y, Yano M . Secretory leukoprotease inhibitor and pulmonary surfactant serve as principal defenses against influenza A virus infection in the airway and chemical agents up-regulating their levels may have therapeutic potential. Biol Chem 2004;385(11):1029-34. *https://doi.org/10.1515/bc.2004.133*
37. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 2016; 7(2): 27–31. *https://doi.org/10.4103%2F0976-0105.177703*
38. Min L, Jin W. Prospect of ambroxol in the treatment of COVID-19. The Chinese J Clin Pharmacol 2020 *https://doi.org/10.1016%2Fj.jpha.2020.12.001*
39. Touret F, Gilles M, KarineBarral K, Nougairède A, Decroly E, de Lamballerie X, Bruno Coutard B. *In vitro* screening of FDA approved chemical library reveals potential inhibitors of SARS-CoV-2 replication. European Virus Arch 2020 4; 10(1):13093. *https://doi.org/10.1038/s41598-020-70143-6*
40. FelixK , Pairet M, Zimmermann R. The Antioxidative Activity of the Mucoregulatory Agents: Ambroxol, Bromhexine and N-acetyl-L-cysteine. A Pulse Radiolysis Study. Life Sci 1996;59(14):1141-7.
41. https://www.boehringer-ingelheim.com/covid-19/global-support-program/china/relief-efforts-in-china.
42. Use of Bromhexine and Hydroxychloroquine for Treatment of COVID-19 Pneumonia. *https://clinicaltrials.gov/ct2/show/NCT04355026*
43. Evaluating the efficacy and safety of bromhexine hydrochloride tablets combined with standard treatment/ standard treatment in patients with suspected and mild novel coronavirus pneumonia (COVID-19). *https://www.smartpatients.com/trials/NCT04273763*
44. Depfenhart M, Lemperle G, Meyer M, Rautenbach M, Bertossi D, de VilliersD . A SARS-CoV-2 prophylactic and treatment; a counter argument against the sole use of chloroquine. AJBSR.2020. *https://doi.org/10.34297/AJBSR.2020.08.001283*
45. Maggio R , Corsini GU. Repurposing the Mucolytic Cough Suppressant and TMPRSS2 Protease Inhibitor Bromhexine for the Prevention and Management of SARS-CoV-2 Infection. Pharmacol Res 2020; 157:104837. *https://doi.org/10.1016%2Fj.phrs.2020.104837*