Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Medical Hypotheses 144 (2020) 110079

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Ibuprofen, a traditional drug that may impact the course of COVID-19 new effective formulation in nebulizable solution

Néstor H. García[a,*], Daniela J. Porta[a], Roxana V. Alasino[b,c], Sonia E. Muñoz[a], Dante M. Beltramo[b,c]

[a] Instituto de Investigaciones en Ciencias de la Salud—FCM (INICSA-CONICET), Córdoba C 5000, Argentina
[b] Centro de Excelencia en Productos y Procesos de Córdoba (CEPROCOR), Ministerio de Ciencia y Tecnología de Córdoba Pabellón CEPROCOR, Santa María de Punilla, Córdoba CP 5164, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Godoy Cruz 2290, C1425FQB CABA, Argentina

A B S T R A C T

The traditional formulation of ibuprofen is poorly soluble in water, so the administered dose must be 10 times higher than the dose required for a therapeutic effect. The development of a hydrosoluble form of ibuprofen can be a strategy to reach a high concentration in the lungs by using modern inhalation devices. Therefore, the development of an inhalable formulation with high bioavailability in the lungs was the leitmotiv of our investigation. The hypertonic ibuprofen solution to be nebulized (NIH) presents great relevant characteristics: bactericidal, virucidal, mucolytic and has a known anti-inflammatory property. Bactericidal and virucidal effects are related to the physico-chemical properties of Na-ibuprofenate as an amphipathic molecule. It has the capability to insert into the bilayer membranes destabilizing the structure, altering its biological properties and avoiding the duplication or infection. Our preliminary results indicate that the presence of this high ionic strength solution reduces 10 times the amount of ibuprofen necessary to kill bacteria, but also the time to kill $1x10^6$ bacteria, from 4 h (in its absence) to only three minutes (in its presence). That was observed using *Pseudomona aeruginosa, methicillin-resistant Staphylococcus aureus* and Burkholderia *cepacia.* Also, “in vitro" ibuprofen demonstrated virucidal activity against the so-called enveloped virus, a family that includes coronavirus strain (2019-nCoV). We observed too, the markedly reduced local inflammation in the airways after administering NIH lays on its ability to inhibit the enzyme cyclooxygenase and to markedly diminish reactive oxygen species (ROS). Other investigators also showed the importance of actin in the rapid spread of virus infection. Furthermore, reorganization of the actin filaments is a key step in lung inflammation induced by systemic inflammatory responses caused by SARS-CoV-2. These findings suggest that the interaction between actin proteins and S1 is involved in the 2019-nCoV infection and pathogenesis.

Consequently, the possibility of interfering in this interaction could represent a valid hypothesis for the development of promising therapeutic and prevention strategies. In conclusion, we consider that treating people with COVID-19 with NIH may be beneficial and an opportunity to contribute for the current global health emergency.

The importance of ibuprofen in the respiratory tract has been described previously in children with pulmonary cystic fibrosis but besides the beneficial effect observed in this scenario, it is not frequently prescribed, and the main reason is the high dosage required to produce the desired effect in the lungs.

The traditional formulation of ibuprofen is poorly soluble in water, so the administered dose must be 10 times higher than the dose required for a therapeutic effect [1]. In this sense, the distribution rate of currently available traditional dosage forms is limited, leading to poor bioavailability from a high oral dosage [2]. The development of a hydrosoluble form of ibuprofen can be a strategy to reach a high concentration in the lungs by using modern inhalation devices, without the potential systemic effects of high dosages. This approach would allow us to deliver micro or nanometer sized particles to reach a large surface area, such as the pulmonary alveolar. The other benefit of this size is that particles from 0.5 to 3 μm are captured by macrophages through phagocytosis, but not those $<$ 0.25 μm [1]. Having a formulation with these properties would lead to a high potential for the treatment of lung infections, such as pulmonary cystic fibrosis, pneumonia, and bronchiectasis [19].

Therefore, the development of an inhalable formulation with high bioavailability in the lungs was the leitmotiv of our investigation.

Considering the characteristics of available ibuprofen formulations, a solution with hypertonic ibuprofeno solution to be nebulized (NIH) was developed with great relevant characteristics: it is bactericidal, virucidal, mucolytic and has a known anti-inflammatory property. This formulation was developed for the treatment of serious lung conditions, such as cystic pulmonary fibrosis, pneumonia and bronchiectasis, because complications due to opportunistic infections are of great importance in these lung pathologies. Additionally, since administration

* Corresponding author at: Boulevard de la Reforma, Pabellón Biología Celular, Ciudad Universitaria, X5000 Córdoba, Argentina.
E-mail address: garcia.nestor@conicet.gov.ar (N.H. García).

https://doi.org/10.1016/j.mehy.2020.110079
Received 29 June 2020; Accepted 3 July 2020
Available online 07 July 2020
0306-9877/

via inhalation was decided, it overcomes the limitations of the currently available administrative routes to achieve a better therapeutic action at a very low dose [4].

From insoluble to soluble

Recently Irvine et *al.* [18] explained the challenge of ibuprofen solubilization in water. These researchers describe the solubility of this drug as the major problem. So, different technologies particle engineering or crystallization were applied in improving formulation strategies. Finally, an attempt has been made to improve the delivery of this drug into the pulmonary route for achieving better therapeutic action at a very low dose, thus overcoming the limitations of currently available delivery routes. Nanotechnology is the science that recently appeared to solve this problem. In this case, several strategies have been developed; however, the appropriate delivery of ibuprofen to the lung using nanocarriers such as liposomes, dendrimers, emulsions or polymers has been achieved, because there is an inability to reach enough lung deposit [3].

From anti-inflammatory to viricidal and bactericidal effect of Ibuprofen

The bactericidal effect was possibly confounded with the anti-inflammatory effect initially. During the 80′s, Ibuprofen's ability to limit bacterial damage from "in vivo" was evaluated [11–13]. In 1990 Konstan et al. [14] studied Ibuprofen's ability to limit lung damage induced by Pseudomonas infection in rats. This early report was the cornerstone to investigate ibuprofen in lung diseases but focus on the anti-inflammatory properties. Later, preclinical and clinical studies demonstrated the beneficial effect of high dose of ibuprofen. In 1995, Michael W. Konstan et al. [2], showed that high doses of ibuprofen in oral form, decreased the progression of lung disease in patients with cystic fibrosis and mild lung disease. Consequently, Lands et al. [15] investigated the safety and efficacy of oral high-dose ibuprofen in children with CF in a randomized, multicenter, double-blind, placebo-controlled trial along 2 years. This study demonstrated that a slowdown in the predicted impaired vital capacity was observed in patients on high-dose ibuprofen. Collectively, these clinical trials document the benefits and relative safety of long-term use of high-dose ibuprofen in pediatric CF patients by attributing these benefits to the modulating properties of neutrophils [15]. At the same time Elvers et al. [16] evaluated the effect of ibuprofen on the "in vitro" growth of six bacterial strains, and observed that ibuprofen inhibited the growth of Gram-positive species.

¿But how ibuprofen has an antibacterial activity? The bactericidal and virucidal effects are related to the physico-chemical properties of Na-ibuprofenate as an amphipathic molecule. It has the capability to insert into the bilayer membranes destabilizing the structure, altering its biological properties and avoiding the duplication or infection of bacterias or viruses.

Interestingly, this interaction is strongly reinforced and stabilized in the presence of a high ionic strength solution, such as hypertonic sodium chloride. The presence of this high ionic strength solution reduces 10 times the amount of ibuprofen necessary to kill bacteria, but also the time to kill $1x10^6$ bacteria, from 4 h (in its absence) to only three minutes (in its presence). That was observed using *Pseudomona aeruginosa, methicillin-resistant Staphylococcus aureus* and Burkholderia *cepacia.* Other investigators have reported similar results [20]. But the effect does not end here, also "in vitro" ibuprofen demonstrated virucidal activity against the so-called enveloped virus, a family that includes coronavirus strain (2019-nCoV) (Table 1).

Using an "in silico" approach, Ibuprofen has been predicted as an entry inhibitor of Ebola virus [5]. This prediction has then been experimentally confirmed [6,7], showing that Ibuprofen reduced 50% of virus title in Vero cells. This virucidal effect has also been reported "in vitro" for Zika virus (ZIKV), where Ibuprofen plunged AXL expression, a ZIKV entry cofactor. This data highlights the worth of drugs with a high safety profile, which may even be indicated during pregnancy, to prevent ZiKV infection. Although by different mechanisms, clear viral replication regulatory effects have also been shown "in vitro" and in humans for other viruses like HSV-1, HSV-2, rotavirus, rhinovirus [8–10].

Table 1
Effect of Na-Ibuprofenate on the vitro infective capacity of HSV, BVDV, VSV and MV.

Virus	Virus Characteristics		Infective capacity title after Ibuprofen treatment	
	Family	Genome	Size (nM)	TCID mL-l
HSV	Herpes	dsDNA	120–200	> 6.9
BVDV	Flavi	ssRNA	50–70	> 6.5
VSV	Rhabdo	ssRNA	70–170	> 7.7
MV	Paramyxo	ssRNA	150–300	> 5.0

Table 2
NIHM decreases reactive oxygen species (ROS) in human (expressed as ΛU), * = $p < 0.05$.

Patient	ROS Pre-NIHM	ROS Pro-NIHM
85314	2019	353
85315	698	329
85320	1035	513
85315b	762	583
85314b	703	426
85321	1577	887
Mean	**1043**	**441***
STD	**252**	**48**

The markedly reduced local inflammation in the airways after administering NIH lays on its ability to inhibit the enzyme cyclooxygenase and to markedly diminish reactive oxygen species (ROS). (Table 2) These data are consistent with previous publications [16].

On the other hand, the mucolytic properties of NIH was detected in healthy volunteers, and also in patients with COPD, a disease characterized by significant chronic lung inflammation [17]; this response shows the effectiveness of the compound in pathologies with respiratory intervention and the broad spectrum demonstrated in the treatment of conditions with similar characteristics.

From oral to inhaled and safe ibuprofen

The safety of inhaled NSAIDs has been previously demonstrated [18], evaluating the response of inhaled sodium metaphosphate and adenosine monophosphate in the absence of prostaglandins. They used inhaled L-aspirin, indomethacin and sodium salicylate in asthmatic patients. In this report, none of the inhaled NSAIDs changed FEV_1 in sensitive patients. In another study, Tamaoki et al., reported that inhaled indomethacin decreased fluid and mucus in the respiratory tract after 14 days of treatment without alterations in lung function. No adverse effects were observed [19]. Other investigators found similar results, inhalted AINES are safe [20]. Further confirmation of these findings can be found in a recent Cochrane review [20]. Our laboratory evaluated the effect of hypertonic ibuprofen in healthy adults and we found an increase on FEV1/FVC ratio suggesting a bronchodilator effect, perhaps intrinsic to the anti-inflammatory effect (unpublished data), during this experiment, serum ibuprofen increased from 0 to 1.09 ± 0.7 μg/ml and also the reactive oxygen species decreased from 1043 ± 252 to 441 ± 48 ΛU determined 30 min after the nebulization, demonstrating in spite of the low serum concentration, this presentation still maintains the anti-inflammatory property. We also evaluated the effect of hypertonic ibuprofen using compassion and in COPD patients, after 4 months of treatment, their FEV1, and the walking test improved compared to basal, while the daily oxygen

requirement was reduced. Another patient, with idiopathic pulmonary fibrosis, after 1.5 years of treatment, he improved his FEV_1, and the ground glass opacity observed CT scan disappeared after 4 months of treatment. Although these may be anecdotal findings, they may also indicate the beneficial effects of inhaled ibuprofen.

The hypothesis

The new pathogen belonging to a family of viruses with a lipid envelope in its structure. Using confocal microscopy and molecular biology tools, [21], studied how the human coronavirus OC43 enters the susceptible cell, visualizing that the virus uses endocytosis and that the internalization process of vesicles that require actin, so it can play an important role in early entry events during human coronavirus infections [22]. Other investigators also showed the importance of actin in the rapid spread of virus infection [26]. Furthermore, reorganization of the actin filaments is a key step in lung inflammation induced by systemic inflammatory responses caused by SARS-CoV-2 [23]. These findings suggest that the interaction between actin proteins and S1 is involved in the 2019-nCoV infection and pathogenesis. On the other hand, the angiotensin-converting enzyme 2 (ACE2) is the functional receptor for the spike glycoprotein of the SARS-CoV-2, therefore it would play a crucial role in the entry of the virus into the cell to cause the final infection [24]. Consequently, the possibility of interfering in this interaction could represent a valid hypothesis for the development of promising therapeutic and prevention strategies.

Nebulized hypertonic ibuprofen has antibiotic, viricidal and anti-inflammatory effects that are expected to prevent viral replication in the respiratory tract, minimize bacterial superinfections and reduce inflammatory cytokines which appear to be the major problems in COVID-19 [25]. Ibuprofen with the highest FDA performance side effect profile, would be a promising adjunctive treatment for COVID-19 patients with moderate-severe respiratory symptoms. Additional research is needed to further define its efficacy and safety in treating COVID-19.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This paper represents a collaborative effort among members of Córdoba Center for Products and Processes (CEPROCOR), the National Council for Scientific and Technical Research (CONICET) and the private pharmaceutical company Luar Chemistry (Química Luar ®). We wish to acknowledge Jessica Buck for her technical assistance with English grammar and spelling.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.110079.

References

[1] Bolten D, Lietzow R, Tuerk M. Solubility of ibuprofen, phytosterol, salicylic acid, and naproxen in aqueous solutions. Chem Eng Technol 2013;36:426–34.
[2] Klueglich M, Ring A, Scheuerer S, et al. Ibuprofen extrudate, a novel, rapidly dissolving ibuprofen formulation: relative bioavailability compared to ibuprofen lysinate and regular ibuprofen, and food effect on all formulations. J Clin Pharmacol 2005;45:1055–61.
[3] Manrique J, Martinez F. Solubility of ibuprofen in some ethanolþwater cosolvent mixtures at several temperatures. Lat Am J Pharm 2007;26:344–54.
[4] Muñoz AJ, Alasino RV, Garro AG, Heredia V, García NH, Cremonezzi DC and Beltramo DM. High Concentrations of Sodium Chloride Improve Microbicidal Activity of Ibuprofen against Common Cystic Fibrosis Pathogens Pharmaceuticals. 2018;11(2):47.
[5] Veljkovic V, Goeijenbier M, Glisic S, Veljkovic N, Perovic VR, Sencanski M, Branch DR, Paessler S. In silico analysis suggests repurposing of ibuprofen for prevention and treatment of EBOLA virus disease [version 1; peer review: 2 approved]. F1000Res 2015;4:104. https://doi.org/10.12688/f1000research10.12688/f1000research.6436.1.
[6] Zhao Y, Ren J, Harlos K, et al. Toremifene interacts with and destabilizes the Ebola virus glycoprotein. Nature 2016;535(7610):169–72.
[7] Paessler S, Huang C, Sencanski M, Veljkovic N, Perovic V, Glisic S, et al. Ibuprofen as a template molecule for drug design against Ebola virus. Front Biosci (Landmark Ed). 2018 Jan;1(23):947–53.
[8] Allen SJ, Mott KR, Ghiasi H. Inhibitors of signal peptide peptidase (SPP) affect HSV-1 infectivity in vitro and in vivo. Exp Eye Res 2014;123:8–15.
[9] Guerrero CA, Murillo A, Acosta O. Inhibition of rotavirus infection in cultured cells by N-acetyl-cysteine, PPARγ agonists and NSAIDs. Antiviral Res 2012;96(1):1–12.
[10] Gwaltney Jr JM, Winther B, Patrie JT, Hendley JO. Combined antiviral-anti-mediator treatment for the common cold. J Infect Dis 2002;186(2):147–54.
[11] Sordelli DO, Cerquetti MC, El-Iawil G, et al. Ibuprofen modifies the inflammatory response of the murine lung to Pseudomonas aeruginosa. Eur J Resp Dis. 1985;67:118–27.
[12] Lee CC, Sugerman HJ, Tatum JL, Wright TP, Hirsh PD, Hirsch JI. Effects of ibuprofen on a pig Pseudomonas ARDS model. J Surg Res 1986;40(5):438–44.
[13] Harvey CF, Sugerman HJ, Tatum JL, Sielaff TD, Lee EC, Blocher CR. Ibuprofen and methylprednisolone in a pig Pseudomonas ARDS model. Circ Shock. 1987;21(3):175–83.
[14] Konstan MW, et al. Effect of high-dose ibuprofen in patients with cystic fibrosis. N Engl J Med 1995;332:848–54.
[15] Shah PN, Marshall-Batty KR, Smolen JA, et al. Antimicrobial activity of ibuprofen against cystic fibrosis-associated Gram-negative pathogens. Antimicrob Agents Chemother 2018;62(3):e01574–1617.
[16] Husain MA, Sarwar T, Rehman SU, Ishqi HM, Tabish M. Ibuprofen causes photocleavage through ROS generation and intercalates with DNA: a combined biophysical and molecular docking approach. PCCP 2015;17(21):13837–50.
[17] Kantor TG. Ibrupofen. Ann Intern Med 1979;91(6):877–82.
[18] Wang M, Wisniewski A, Pavord I, Knox A, Tattersfield A. Comparison of three inhaled non-steroidal anti-inflammatory drugs on the airway response to sodium metabisulphite and adenosine 5'-monophosphate challenge in asthma. Thorax 1996;51(8):799–804.
[19] Tamaoki J, Chiyotani A, Kobayashi K, Sakai N, Kanemura T, Takizawa T. Effect of indomethacin on bronchorrhea in patients with chronic bronchitis, diffuse panbronchiolitis, or bronchiectasis. Am Rev Respir Dis 1992;145(3):548–52.
[20] Llewellyn-Jones CG, Johnson MM, Mitchell JL, Pye A, Okafor VC, Hill SL, et al. In vivo study of indomethacin in bronchiectasis: effect on neutrophil function and lung secretion. Eur Respir J 1995;8(9):1479–87.
[21] Owczarek K, Szczepanski A, Milewska A, et al. Early events during human coronavirus OC43 entry to the cell. Sci Rep 2018;8(1):7124.
[22] Doceul V, Hollinshead M, van der Linden L, et al. Repulsion of superinfecting virions: a mechanism for rapid virus spread. Science 2010;327(5967):873–6.
[23] Du L, Zhou J, Zhang J, et al. Actin filament reorganization is a key step in lung inflammation induced by systemic inflammatory response syndrome. Am J Respir Cell Mol Biol 2012;47(5):597–603.
[24] Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci 2020:1–5.
[25] Nile Shivraj Hariram, et al. COVID-19: Pathogenesis, cytokine storm and therapeutic potential of interferons. Cytokine Growth Factor Rev. 2020;53:66–70. https://doi.org/10.1016/j.cytogfr.2020.05.002.
[26] Doceul V, et al. Repulsion of superinfecting virions: a mechanism for rapid virus spread. Science 2010;327(5967):873–6. https://doi.org/10.1126/science.1183173.