Chapter

Interplay between Inhaled Ibuprofenate, SARS-CoV-2, Vasoplegic Pulmonary Vascular Dysfunction, Pneumonia and CARDS

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Abstract

In this manuscript, we will describe the possible mechanisms of action of inhaled sodium ibuprofenate in hypertonic saline formulation-NaIHS, focusing primarily on vasoplegic pulmonary vascular dysfunction leading to severe pneumonia and Coronavirus Disease 2019-associated acute respiratory distress syndrome. We will address the anti-inflammatory, immunomodulatory and antiangiogenic therapeutic effects of NaIHS, which together would exert their action through the negative modulation of local inflammatory mediators, pro-inflammatory cytokines and inflammatory pathways. In such a manner, NaIHS may reverse pulmonary vasoplegia and may thereby restore hypoxic pulmonary vasoconstriction, correcting the uncoupling of the ventilation-perfusion ratio and vasoplegic intrapulmonary shunting and, above all, it may reverse severe hypoxaemia. We will also describe the potential virucidal effects of NaIHS on Severe Acute Respiratory Syndrome-Coronavirus 2. Likewise, we will mention the evidence obtained from the potential adjuvant treatment with NaIHS in two observational cohort studies done in Argentina, the most recent of them with 5146 patients, concluding that NaIHS reduces mortality by 48.7%, although randomised clinical trials are still needed to confirm these data.

Keywords: NaIHS, SARS-CoV-2, pulmonary vascular dysfunction, virucidal effect, severe pneumonia and CARDS

1. Introduction

Coronavirus Disease 2019 (COVID-19) sepsis with a respiratory starting point is a vascular pathology of immuno-inflammatory aetiology secondary to Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) infection, characterised mainly by endothelitis with generalised endothelial dysfunction, occlusive

thromboinflammation, massive cytokine release, renin-angiotensin system (RAS) imbalance, metabolic dysfunction with oxidative stress and reactive oxygen species (ROS) release and respiratory or multi-organ dysfunction [1, 2].

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) derived from propionic acid and poorly soluble in water, acting as a reversible and non-selective inhibitor of cyclooxygenase 1 and 2 (COX) [3], with still poorly understood COX-independent effects [3]. The hydrosoluble NaIHS formulation administered by inhalation bears the following characteristics: high ionic strength and micrometric or nanometric size, thus achieving a high concentration in the pulmonary biophase since, being hydrosoluble, NaIHS requires a dose 10 times lower compared to traditional orally administered ibuprofen to achieve its therapeutic effect and would also have a high pulmonary volume of distribution, thus obtaining a high therapeutic safety and equal pharmacological effect at very low NaIHS doses of 150 mg/day, distributed in 3 aerosolisations of 50 mg every 8 hours (with the possibility of increasing the dose if necessary), using special nebulisation devices that avoid aerosolisation and contagion of health personnel [4].

NaIHS has a history of successful therapeutic use in chronic lung diseases such as cystic fibrosis, idiopathic pulmonary fibrosis, bronchiectasis and chronic obstructive pulmonary disease (COPD); thanks to its anti-inflammatory, immunomodulatory, mucolytic, antiangiogenic and bactericidal effects [4]. In addition, NaIHS has a virucidal effect in vitro on viruses enveloped with a bilayer membrane, including the Coronaviridae family [4].

After the SARS-CoV-2 pandemic declaration in March 2020 by the World Health Organisation (WHO), in June 2020 [4], a group of researchers: Doreski, PA; Garcia, NH; Beltramo, DM et al. in Argentina developed the idea of using NaIHS in moderate and severe COVID-19 pneumonia [4], in the modality of compassionate use and covered by the Declaration of Helsinki [4, 5]. Accordingly, it was approved for emergency use in several Argentine provinces and its phase II clinical trial (Pegasus trial) was approved in July 2021 by the Drug, Food and Technology National Administration of Argentina (ANMAT) [4–6]. Two observational multi-centre cohort studies of heterogeneous populations have been conducted in Argentina with NaIHS: the first study analysed data from 383 patients (15% mechanically ventilated), drawing statistically significant conclusions from both groups studied about decreased mortality, shorter hospital stay, improvement of clinical signs and rapid reversal of hypoxaemia [5]; and the second study, recently in May 2022, analysed data from 5146 patients, conducted with the approval of the Institutional Review Board of the National Bureau of Economic Research (NBER) of Harvard and Columbia Universities, concluded that NaIHS reduces mortality by 48.7% [6]. However, some studies are still needed to confirm these emerging data.

2. Therapeutic effects of NaIHS and pathophysiological mechanisms of SARS-CoV-2 involved in pulmonary circulation

2.1 Anti-inflammatory and immunomodulatory therapeutic effects.

2.1.1 Pulmonary vasoplegia

In a recently published article, we have postulated nitric oxide (NO) overproduction as the most important possible determinant in the production

of pulmonary vasoplegia, through overexpression and dysregulation of NO-generating enzymes involving indoleamine-2-3-dioxygenase-1 (IDO) and inducible NO synthase (iNOS) [7], producing NO from the amino acids L-tryptophan as substrate in the former and from L-arginine in the latter, respectively. This would occur at the endothelial and mononuclear levels [7]. NO is the main vasoplegic mediator of experimental sepsis, with NO overproduction in COVID-19 possibly being the main responsible for pulmonary vasoplegia and a further determinant of endothelial injury by oxidative stress from ROS generation [7]. The rapid reversal of hypoxaemia observed in the studies mentioned in the introduction may be caused by the therapeutic effect of NaIHS [7], which has led us to consider, on one hand, a pharmacokinetic link with gaseous NO, given that in anaerobiosis, its elimination half-life is as short as 1 to 5 seconds and the production of injury is concentration-dependent in the micromolar range, due to its hyperproduction [7]; and on the other hand, a pharmacodynamic link, since patients with severe vasoplegic pneumonia and COVID-19-associated acute respiratory distress syndrome (CARDS) may respond favourably to NaIHS therapeutics [7], whereas inhaled vasodilator therapeutics such as NO, prostaglandins, prostacyclins and milrinone may lead to a marked deterioration [12]. Patients with acute respiratory distress syndrome (ARDS) and pulmonary hypertension may favourably respond to inhaled vasodilators, although more studies are still needed [12]. Pathophysiologically, pulmonary vasoplegia involving large heterogeneous lung areas presents in about 80% of the cases as severe vasoplegic pneumonia or CARDS [12], favourably responding to NaIHS [7], 100% oxygen supplementation, high flow nasal cannula (HNFC), continuous positive airway pressure (CPAP) and non-invasive mechanical ventilation (NIV) or requiring early invasive mechanical ventilation (MV) or extracorporeal membrane oxygenation (ECMO) [12], on a case-by-case and personalised basis. Pulmonary vasoplegia may lead to pulmonary vascular dysfunction with loss of physiological sympathetic hypoxic pulmonary vasoconstriction (HPV), producing a decoupling of the ventilation-perfusion (V/Q) ratio and a significant right-to-left vasoplegic intrapulmonary shunting with mixed venous blood of approximately 50% or higher [1, 12]. Furthermore, pulmonary vasoplegia may lead to an increase in pulmonary blood flow that may not allow for the time necessary for adequate alveolar-capillary gas exchange, generating severe hypoxaemia and acute respiratory failure [1]. This is to say that alveolar ventilation is preserved, with preserved lung compliance and low lung elastance, but pulmonary perfusion is altered, leading to inadequate gas exchange [12] and furthermore, as a consequence of the above, a significant increase in physiological dead space. It should be clearly stated that the differential diagnosis between severe vasoplegic pneumonia and CARDS is extremely difficult, given the possibility of overlap between both pathological entities, with highly heterogeneous clinical, gasometric (normo- or hypercapnic) and imaging presentation [1, 12]. Lung imaging studies evidence the coexistence of predominantly vasoplegic areas and minor vasoconstricted areas [1], as a consequence of the possible hyperproduction of NO in the predominantly vasoplegic areas [7], which may override the RAS imbalance in the vasoconstricted areas [1]. RAS imbalance would lead to down-regulation of angiotensin-converting enzyme 2 (ACE2) of the RAS axis with vasodilator effect and up-regulation of angiotensin II (Ang II) of the RAS axis with vasoconstrictor effect [1], thus promoting a pathological increase in the vasoconstrictor effect of RAS with increased circulating Ang 2 [1].

2.1.2 Main pathophysiological mechanisms that may lead to NO hyperproduction and possible therapeutic response using NaIHS

IDO overexpression and activation with consequent activation of the IDO-kynurenine pathway may lead to NO hyperproduction in anaerobiosis [2]. Such overexpression and activation may be due to the positive modulation of the following inflammatory pathways through COX2 [17], nuclear factor kappa light chain enhancer of activated B cells (NF-kB) [2], signal transducer and activator of transcription 3 (STAT3) [2], toll-like receptor 4 (TLR4) [2] and the pro-inflammatory cytokines interferon gamma (IFN-γ) [2, 17], being this one the most important [2, 17], interleukin-1 beta (IL-1β) [17], tumour necrosis factor alpha (TNF-α) [17] and IL-6 [2]. NaIHS may indirectly inhibit IDO [17] by negatively modulating all the inflammatory pathways and pro-inflammatory cytokines mentioned above [8].

RAS imbalance would occur through the down-regulation of ACE2 of the anti-inflammatory RAS axis and the up-regulation of Ang II of the pro-inflammatory RAS axis [1]. ACE2 down-regulation would promote iNOS overexpression and induction, decreased expression of endothelial synthase (eNOS), which would exert a regulatory function on iNOS and endothelial protective effect, and increased vascular smooth muscle reactivity to NO [1]. Ang II up-regulation would also promote iNOS overexpression and induction, through the activation of NF-kB, which would interact with the Ang type 1 receptor (ATR1) and activate nicotinamide adenine dinucleotide phosphate complex 2 (NADPH-oxidase) and, also through (ATR1), generating oxidative stress and ROS [8]. In addition, RAS imbalance would increase the release of pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6 and others, which would lead as well to iNOS induction and overexpression [1, 8]. NaIHS may reverse RAS imbalance through direct inhibition of COX2, NF-kB and transforming growth factor-beta 1 (TGF-β1) [8], and it may thereby restore ACE2 up-regulation of the anti-inflammatory RAS axis and Ang II down-regulation of the pro-inflammatory RAS axis [13]. It should be noted that ibuprofen does not produce ACE2 overexpression in both uninfected and SARS-CoV-2 infected patients and does not promote viral entry, but only restore the RAS imbalance caused by COVID-19 [19].

Possible inhibition of the vasopressor response by blocking calcium entry into vascular smooth muscle cells, generated by iNOS induction [10]. iNOS induction and overexpression would be triggered by activation of the inflammatory pathways COX2 and NF-kB and by the pro-inflammatory cytokines IL-1β, TNF-α and IL-6 [10]. NaIHS may restore the vasopressor response by reversing the blockade of calcium influx, through negative modulation of the inflammatory pathways and the pro-inflammatory cytokines mentioned above [8].

The mononuclear pro-inflammatory cytokine storm syndrome would be amplified by a positive feedback loop between STAT3, NF-kB and IL-6 [9], further amplifying the immuno-inflammatory response and thus promoting iNOS over-expression and induction [9]. NaIHS would prevent the generation of the positive feedback loop mentioned above, through the negative modulation of all its components [8].

The Warburg effect would be activated as a consequence of anaerobic cellular metabolism and possible positive modulation by M1 macrophages, via the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/

AKT/mTOR) pathway [11]. Hypoxaemia generates mitochondrial dysfunction and oxidative stress, forcing cells to opt for an alternative metabolic pathway (Warburg effect) to more rapidly and efficiently obtain the energy needed [11]; that is to say that cellular aerobic glycolysis (Krebs or tricarboxylic acid cycle) is blocked and the Warburg effect would be activated, characterised by impaired aerobic glycolysis with lactate production, fatty acid synthesis and glutaminolysis and in this way, the Warburg effect would promote iNOS induction and overexpression [11]. NaIHS may reverse hypoxaemia [7] and may negatively modulate the PI3K/AKT/mTOR pathway [14], and it may thereby inhibit the Warburg effect [7].

In conclusion, NaIHS may indirectly inhibit IDO and iNOS enzymes and may thus prevent the hyperproduction of NO as a generator of pulmonary vasoplegia, as explained in each case above [7]. NaIHS may reverse pulmonary vasoplegia and restore HPV and may thus correct the V/Q ratio and vasoplegic intrapulmonary shunting [7] but most of all, it may be able to reverse severe hypoxaemia [7] and acute respiratory failure where possible in severe vasoplegic pneumonia and CARDS.

2.1.3 Vasodilation and pulmonary oedema

The activation of local inflammatory mediators such as prostaglandins (mainly prostaglandin E2), prostacyclins (prostaglandin I2), bradykinins and the

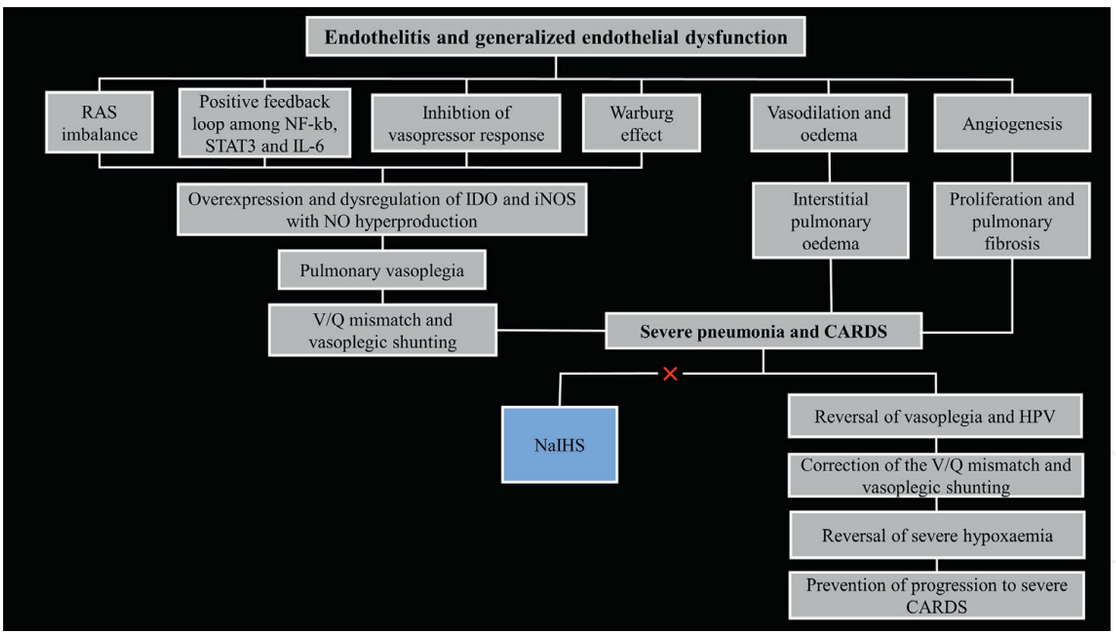

Figure 1.
Potential therapeutic effects of NaIHS on pulmonary vascular dysfunction in COVID-19. Endothelitis and generalised endothelial dysfunction caused by the respiratory SARS-CoV-2 infection may lead, at the pulmonary level, to NO hyperproduction, by the possible overexpression and dysregulation of iNOS and IDO [7]; through the following mechanisms such as RAS imbalance [1, 8], positive feedback loop among NF-kB, STAT3 and IL-6 [9], the loss of the vasopressor effect [10] and the activation of the Warburg effect [11]; NO hyperproduction may cause pulmonary vasoplegia and the loss of HPV [7], this pulmonary vascular dysfunction may lead to the V/Q mismatch and the vasoplegic pulmonary shunting [12]. This alteration of gas exchange added to vasodilation with pulmonary oedema and angiogenesis may all together [5, 7, 12] produce severe vasoplegic pneumonia and CARDS. One arm shows preliminary evidence after the treatment with NaIHS, which may revert pulmonary vasoplegia and HPV, correcting the V/Q mismatch, vasoplegic shunting and severe hypoxaemia [7]; and it may also prevent the progression of severe pneumonia and CARDS. The other arm shows preliminary evidence without treatment with NaIHS.

aforementioned NO produces vasodilation with increased capillary permeability and mixed interstitial pulmonary oedema, at the onset of severe vasoplegic pneumonia and CARDS, showing the characteristic tomographic pattern of ground-glass opacity [5, 7].

NaIHS, by direct inhibition of COX-2, would inhibit all of the above-mentioned local inflammatory mediators and thus prevent vasodilation and pulmonary oedema [5, 7]. In the particular case of bradykinin, it is worth mentioning that NaIHS, through the aforementioned COX2-dependent mechanism, may produce two effects: on one hand, it may lead to down-regulation of the bradykinin B2 receptor [18] and on the other hand, it may promote ACE2 up-regulation [13]; hence, NaIHS may prevent bradykinin action, negatively modulating the des-arginine 9-bradykinin (des-Arg 9-BK) pathway and producing down-regulation of the bradykinin B1 receptor [1].

2.1.4 Pulmonary angiogenesis

In severe vasoplegic pneumonia and CARDS, angiogenesis is found, which is of great importance in the progression to the proliferative and fibrotic stages of CARDS and also in the subsequent post-COVID-19 pulmonary fibrotic sequelae [5]. NaIHS, through direct inhibition of COX-2, may negatively modulate vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) [15] and by COX-independent effect, it may inhibit hypoxia-inducible factor-1 alpha (HIF-1α) [16], thereby inhibiting angiogenesis and pulmonary fibrosis see **Figures 1** and **2**. [15, 16].

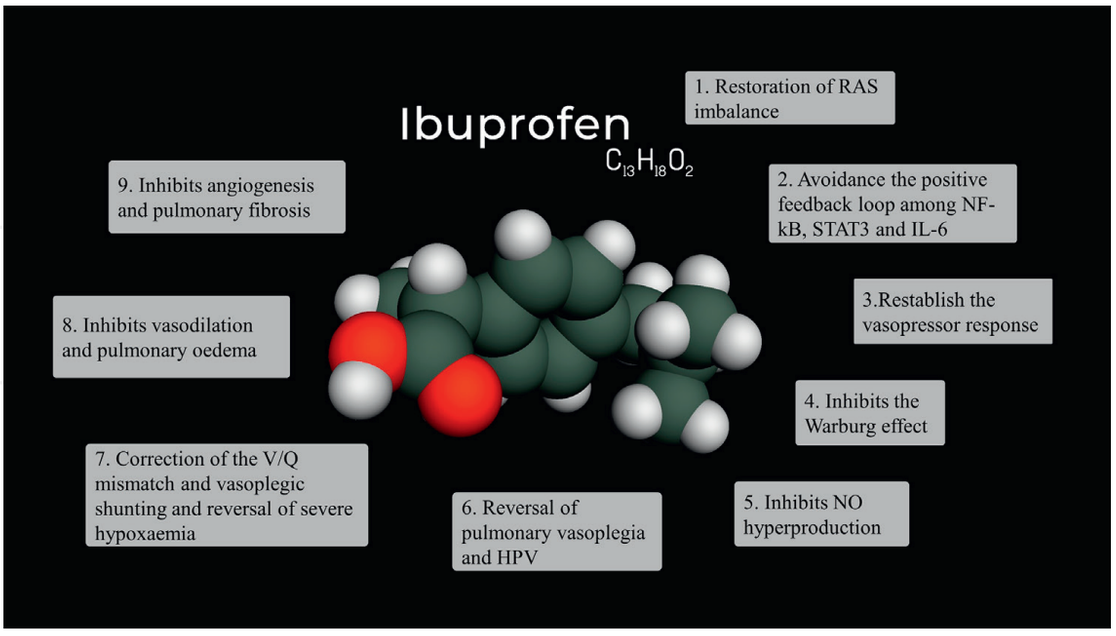

Figure 2.
Potential therapeutic effects of NaIHS on vasoplegic pneumonia and CARDS.
1. *Restoration of RAS imbalance [8, 13];* **2.** *Inhibition of positive feedback loop [8] among NF-kB [8], STAT3 [8] and IL-6 [8];* **3.** *Restoration of the vasopressor response [7];* **4.** *Inhibition of Warburg effect [7, 14];* **5.** *Prevention of NO hyperproduction [7];* **6.** *Reversal of pulmonary vasoplegia and restoration of HPV [7];* **7.** *Correction of the V/Q mismatch and vasoplegic shunting and thus, reversal of severe hypoxemia [7];* **8.** *Inhibition of vasodilation and pulmonary oedema [5, 7] and* **9.** *Inhibition of angiogenesis [15, 16] (**Table 1**).*

Therapeutic targets	Mechanisms of action	Therapeutic effects
1. RAS imbalance	Inhibition of COX2-dependent effect	Inhibition of COX2, NF-kB and TGF-β1
2. Positive feedback loop among NF-kB, STAT3 and IL-6	Inhibition of COX2-dependent effect	Inhibition of NF-kB, STAT3 and IL-6
3. Inhibition of the vasopressor response	Inhibition of COX2-dependent effect	Inhibition of COX2, NF-kB, IL-1β, TNF-α and IL-6
4. Warburg effect	Inhibition of COX2-dependent effect	Inhibition of PI3K/AKT/mTOR
5. IDO	Inhibition of COX2-dependent effect	Indirect inhibition of IDO by Inhibition of COX2, STAT3, NF-kB, TLR4, IFN-γ, IL-1β, TNF-α and IL-6
6. Pulmonary vasoplegia	Inhibition of COX2-dependent effect	Indirect inhibition of iNOS and IDO by inhibition of COX2, STAT3, NF-kB, TLR4, TGF-β1, IFN-γ, IL-1β, TNF-α, IL-6 and PI3K/AKT/mTOR
7.Vasodilation and interstitial pulmonary oedema	Inhibition of COX2-dependent effect	Inhibition of prostaglandins, prostacyclins, NO and bradykinins
8. Angiogenesis	Inhibibition of COX2-dependent and COX-independent effects	Inhibition of VEGF, bFGF and HIF-1α
9.V/Q mismatch and vasoplegic pulmonary shunting	Inhibition of COX2-dependent effect	Indirect inhibition of iNOS and IDO by inhibition of COX2, STAT3, NF-kB, TLR4, TGF-β1, IFN-γ, IL-1β, TNF-α, IL-6 and PI3K/AKT/mTOR

1. *Restoration of the RAS imbalance [8, 13] by inhibition of COX2 [8], NF-kB [8], and TGF-β1[8];* **2.** *Avoidance of the positive feedback loop among STAT3 [8], NF-kB [8] and mainly IL-6 [8];* **3.** *Restoration of the vasopressor response by inhibition of NF-kB [8], COX2 [8], TNF-α [8], IL-1β [8] and IL-6 [8];* **4.** *Inhibition of the Warburg effect via inhibition of PI3K/AKT/mTOR [14];* **5.** *Indirect inhibition of IDO [17] by inhibition of COX2 [8], NF-kB [8], STAT3 [8], TLR4 [8], IFN-γ [8], IL-1β [8], TNF-α [8] and IL-6 [8];* **6.** *Reversal of pulmonary vasoplegia by indirect inhibition of iNOS and IDO [7] by inhibition of COX2 [8], NF-kB [8], STAT3 [8], TLR4 [8], TGF-β1 [8], IFN-γ [8],IL-1β [8], TNF-α [8] and IL-6 [8];* **7.** *Inhibition of vasodilation and interstitial pulmonary oedema by inhibition of COX2 [7, 8]: inhibition of prostaglandins [5], prostacyclins [5], NO [7], bradykinins [18] and, by up-regulation of ACE2 [13] inhibition of bradykinins [1];* **8.** *Inhibition of angiogenesis by inhibition of COX2-dependent effect [15]: VEGF and bFGF [15] and by inhibition of COX-independent effect [16], inhibition of HIF-1α [16] and* **9.** *Correction of the V/Q mismatch and the vasoplegic pulmonary shunting by indirect inhibition of iNOS and IDO [7] by inhibition of COX2 [8], STAT3 [8], NF-kB [8], IFN-γ [8], TGF-β1 [8], IL-1β [8], TNF-α [8], IL-6 [8] and PI3K/AKT/mTOR [14].*

Table 1.
Potential therapeutic effects of NaIHS on the main vascular pathophysiological mechanisms secondary to SARS-CoV-2 infection.

3. NaIHS potential virucidal effects on SARS-CoV-2

The binding of the Spike glycoprotein of the SARS-CoV-2 to the pulmonary ACE2 receptor anchored to the plasmatic membrane of type 2 pneumonocytes requires proteolytic cleavage by the host of the S1 and S2 subunits, with the fusion of both membranes and subsequent viral endocytosis.

3.1 Mechanisms that might prevent SARS-CoV-2 endocytosis

Kringle-containing transmembrane protein 1 (KREMEN1) receptor: It has been recently described as one of the new receptors for SARS-CoV-2 but being these

receptors less efficient compared to the classical ACE2 receptor [27]. It has been hypothesised that the binding of protein S and KREMEN1, with subsequent internalisation of SARS-CoV-2, might depend on the activation of protein S by transmembrane protease serine 2 (TMPRSS2) or cathepsin L [27]. NaIHS may inhibit the Wingless/Int-1/beta-catenin (Wnt/β-catenin) pathway [14], given its agonist effect on peroxisome proliferator-activated receptor gamma (PPAR-γ) and through this effect [14], it might negatively modulate KREMEN1 gene expression [20]. In addition, NaIHS might inhibit the action of the KREMEN1 receptor through possible negative modulation of TMPRSS2 [8, 13, 19] and cathepsin L [8], which will be further developed.

AXL tyrosine kinase coreceptor: The ACE2 receptor, typically described for SARS-CoV-2 binding, in the absence of inflammation is only present in the lung at a density of less than 0.1% and, in the presence of inflammation, the resulting downregulation may further reduce its density, leading to the suggestion of other receptors and co-receptors for SARS-CoV-2, such as AXL [28]. By the direct inhibition of COX2-dependent effect, NaIHS may negatively modulate the synthesis and expression of the AXL coreceptor and may also induce its proteolytic degradation [21].

NaIHS may inhibit in vitro disintegrin and metalloproteinase domain 17 (ADAM 17) [13], by the direct inhibition of COX2, avoiding the excision of ACE2 from the pulmonary membrane and preventing dysregulation of the RAS towards inflammation, contributing to the protective anti-inflammatory effect mentioned above [13].

NaIHS may inhibit in vitro TMPRSS2 [8, 13, 19] through an indirect COX2-dependent anti-androgenic effect [8], which may inhibit hepatic glucuronidation of testosterone, via inhibition of UDP-glucuronosyltransferase and UGT2B15 and thus lead to transcriptional repression and expression of testosterone [8]. This decrease in testosterone would be related to the inhibition of TMPRSS2 transcription and expression, given that TMPRSS2 expression is regulated by androgens [8], being frequent in males with hypogonadism and mainly in those over 60 years of age [8]. Moreover, NaIHS might indirectly inhibit poly (ADP-ribose) polymerase 1 (PARP1) by a COX2-independent effect, since PARP1 inhibition may negatively modulate TMPRSS2 transcription and expression [19].

NaIHS may prevent the reorganisation of actin filaments, impeding the interaction between the viral S1 protein and host actin [4] by the inhibition of the synthesis and release of the cytokine IL-1βand the negative modulating of the rock kinase A (RhoA) pathway, through a COX2-dependent effect [22]. NaIHS may also inhibit the activation of Rho GTPase and its alternatively spliced isoform Ras-related C3 botulinum toxin substrate 1b (RAC1b), through a COX-independent effect [5].

NaIHS may negatively modulate the protease cathepsin L by the inhibition of the synthesis and release of cytokine IL-6 [8] and through the possible negative modulation of all the components of the positive feedback loop aforementioned, among IL-6 [8], NF-kB [8] and STAT3 [8], preventing protein S cleavage and subsequent endosomal viral endocytosis [8].

3.2 Mechanisms that might inhibit SARS-CoV-2 replication

SARS-CoV-2 uses the host cellular interactome for its replication.

NaIHS inhibits in silico [23] and may inhibit in vitro [24, 25] the SARS-CoV-2 main protease (Mpro).

NaIHS may inhibit in silico PTGS2EI gene expression by direct inhibition of prostaglandin-endoperoxide synthase 2 (PTGS2) and it may inhibit in silico the expression of 8 important proviral factors: TYMP, PTGS2, C1S, CFB, IFI44, XAF1, CXCL2 and CXCL3 (**Figure 3**) (**Table 2**) [26].

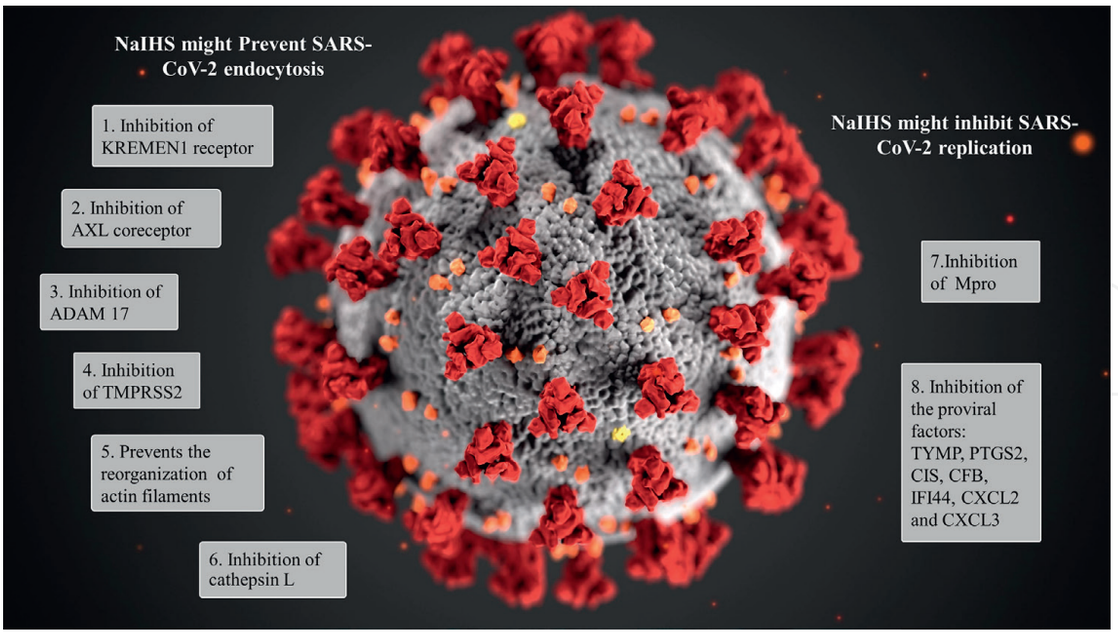

Figure 3.
Potential virucidal effects of NaIHS that might avoid endocytosis and replication bySARS-CoV-2.
1. *Inhibition of KREMEN1 receptor [8, 13, 14, 19, 20];* **2.** *Inhibition of AXL coreceptor [21];* **3.** *Inhibition of ADAM 17 [13];* **4.** *Inhibition of TMPRSS2 [8, 13, 19];* **5.** *Inhibition of the reorganisation of actin filaments [4, 22];* **6.** *Inhibition of cathepsin L [8];* **7.** *Inhibition of Mpro in silico [23] and in vitro [24, 25];* **8.** *Inhibition of the pro-viral factors TYMP, PTGS2, CIS, CFB, IFI44, CXCL2 and CXCL3 [26].*

Therapeutic targets	Mechanisms of action	Therapeutic effects
1. KREMEN1 receptor	Inhibition of COX2-dependent and COX-independent effects	Prevention of SARS-CoV-2 endocytosis
2. AXL coreceptor	Inhibition of COX2-dependent effect	Prevention of SARS-CoV-2 endocytosis
3. ADAM 17	Inhibition of COX2-dependent effect	Prevention of SARS-CoV-2 endocytosis
4. TMPRSS2	Inhibition of COX2-dependent and COX-independent effects	Prevention of SARS-CoV-2 endocytosis
5. Actin	Inhibition of COX2-dependent and COX-independent effects	Prevention of SARS-CoV-2 endocytosis
6. Cathepsin L	Inhibition of COX2-dependent effect	Prevention of SARS-CoV-2 endocytosis
7. Mpro	Inhibition in silico and in vitro	Inhibition of SARS-CoV-2 replication
8. Pro-viral factors: TYMP, PTGS2, CIS, CFB, IFI44, XAF1, CXCL2 and CXCL3	Inhibition in silico	Inhibition of SARS-CoV-2 replication

1. *KREMEN1 receptor: NaIHS inhibits the Wnt/β catenin pathway [14] and through this effect, it might negatively modulate KREMEN1 gene expression [20] and might also inhibit the action of the KREMEN1 receptor through possible negative modulation of TMPRSS2 [8, 13, 19] and cathepsin L [8];* **2.** *AXL coreceptor: the direct inhibition of COX2, may negatively modulate the synthesis and expression of the AXL corecetor [21] and may also induce its proteolytic degradation [21];* **3.** *ADAM 17: inhibition by the direct inhibition of COX2 [13];* **4.** *TMPRSS2: indirect inhibition by antiandrogenic effect (COX2-dependent effect) [8, 13] and it might indirectly inhibit PARP1 (COX-independent effect) [19];* **5.** *Actin: by the inhibition of the synthesis and release of IL-1β and the negative modulation of the RhoA pathway (COX2-dependent effect) [22] and, the inhibition of the RhoGTPase and RAC1b (COX-independent effects) [5];* **6.** *Cathepsin L: negative modulation by the inhibition of IL-6 [8] and the inhibition of the positive feedback loop among IL-6 [8], NF-kB [8] and STAT3 [8];* **7.** *Mpro inhibition in silico [23] and in vitro [24, 25];* **8.** *Inhibition in silico of the proviral-factors: TYMP, PTGS2, C1S, CFB, IFI44, XAF1, CXCL2 and CXCL3 [26].*

Table 2.
NaIHS potential virucidal effect on SARS-CoV-2.

4. Conclusion

With the ongoing emergence of new variants and sub-variants of concern due to immune escape secondary to SARS-CoV-2 mutations, not only are vaccines and their booster doses necessary and vital but also the urgent need for effective medical treatments, which are currently very few and controversial. Therefore, in addition to testing new drugs against SARS-CoV-2, we believe it is essential to reuse known drugs such as ibuprofen, with over 50 years of experience in clinical practice, effective, economical, universally accessible and therapeutically safe.

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Conflict of interest statement

The authors declare that they have no conflicts of interest to this chapter.

Statement on ethics approval and consent

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