The Method and Results of a Treatment Targeting SARS-CoV-2-Activated Inflammasomes

JONG HOON LEE (science@research.re.kr)
Seoul National University https://orcid.org/0000-0003-1775-5656
Badar Kanwar
Hunt Regional Hospital
Asif Khattak
Hunt Regional Hospital
Jenny Balentine
Hunt Regional Hospital
Chul Joong Lee
Zein Pain Clinic Seongbuk
Sang-Suk Oh
Ewha Womans University

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Abstract

A COVID-19 committee at Hunt Regional Medical Center reviewed the use of dapsone as an inflammasome competitor. The hospital then revalidated its effectiveness by reporting the findings of 44 (22 cases/22 controls) patients with acute respiratory distress syndrome (ARDS) treated with dapsone. All of 17 ARDS Onset patients who received standard COVID-19 treatment, including dapsone, did not die except one patient not taken dapsone after relapsed, whereas 8/20 patients who received standard COVID-19 treatment without dapsone died; the mortality rates were 5.9% and 40%, respectively. Dapsone treats and prevents SARS-CoV-2 ARDS. We confirmed that dapsone clinically treated the onset of ARDS by targeting SARS-CoV-2-activated inflammasomes.

Introduction

Critically ill coronavirus disease 2019 (COVID-19) patients in the intensive care unit (ICU) often have acute respiratory distress syndrome (ARDS)[1]. COVID-19-associated ARDS suggests a role of intravascular pathology in COVID-19. Mean static compliance is higher in COVID-19 ARDS than in classic ARDS, and hypoxemia is not closely associated with lung stiffness, unlike in classic ARDS. Moreover, D-dimer levels are elevated in COVID-19 ARDS, and higher D-dimer levels are correlated with increased dead space ventilation and higher mortality[2]. We reported no prevalence of COVID-19 among Hansen's disease (HD) patients in Korea treated with 4,4'-diaminodiphenyl sulfone (dapsone, DDS)[3]. The inflammasome's role and sound rationale[3,4] prompted a trial in rapidly deteriorating COVID-19 patients. Dapsone is useful in the molecular regulation of Nod-like receptor family pyrin domain-containing 3 (NLRP3), which activates mild cognitive impairment (MCI), Alzheimer's disease (AD)[3,5,6], and SARS-CoV-2-associated adult respiratory distress syndrome (ARDS)[7]. The targeting of NLRP3 itself or up-/downstream factors of the NLRP3 inflammasome by dapsone may be responsible for its observed preventive effects[5], functioning as a competitor[3].

The Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial compared dexamethasone to the standard of care in hospitalized COVID-19 patients. Dexamethasone reduced mortality from 25.7% to 22.9% overall and from 41.4% to 29.3% among patients requiring invasive mechanical ventilation (IMV)[8]. They reported a summary odds ratio of 0.66 (95% confidence interval (CI) 0.48–1.01; $p < 0.001$) for mortality in ICU patients using corticosteroids[9]. However, other repurposed therapies have so far failed to show convincing benefit. Convalescent plasma, tocilizumab, hydroxychloroquine, lopinavir/ritonavir, remdesivir, or interferon-β1a did not benefit patients requiring IMV[10,11].

NLRP3 is a critical component of the innate immune system that mediates caspase-1 activation and the secretion of the proinflammatory cytokines IL-1β/IL-18 in response to microbial infection and cellular damage. Diverse stimuli activate NLRP3. Multiple molecular and cellular events have been shown to trigger its activation. NLRP3 responds to signalling events, including ionic flux, mitochondrial dysfunction, reactive oxygen species production, and lysosomal damage[12].

A COVID-19 committee at Hunt Regional Medical Centre reviewed the use of dapsone as an off-label medication based upon treatment adjuncts and inflammasome theory[3,7]. After RNA virus infection, NLRP3 inflammasomes are activated by the mitochondrial protein mitofusin 2[13]. Mitochondrial antiviral signalling protein (MAVS) with NLRP3 and SAMHD1 (SAM and HD domain-containing deoxynucleoside triphosphate triphosphohydrolase 1) are linked to the NF-kB pathway[14]. Also, NLRP3 is able to regulate inflammasome activity[15]. The following guidelines for COVID-19 ARDS as an indication were established. In COVID-19 patients, clinicians should consider an increase in the oxygen requirement from 6 litres per minute (LPM) to as high as 100% with a high-flow nasal cannula (HFNC) as well as dapsone administration[16]. (Protocol for SARS-CoV-2 ARDS)

Dapsone is a well-known drug for treating leprosy, a chronic, progressive bacterial infection caused by *Mycobacterium leprae*. As a therapeutic and prophylactic drug for inflammasomes caused by SARS-CoV-2, it was administered to patients with worsening ARDS despite standard COVID-19 treatment.

These pathologic characteristics of SARS-CoV-2-activated inflammasomes are intense, rapid stimulation of the innate immune response that triggers the activation of the NLRP3 pathway. Affected patients develop ARDS, such as refractory hypoxia, which is invariably fatal. Hypoxia's leading cause is severe inflammation leading to pulmonary microangiopathy, with relentless clotting unchallenged due to a state of fibrinolysis[17]. Faced with the COVID-19 crisis and seeing innocent people dying, we felt it was ethical to try dapsone on several COVID-19 patients at Hunt Regional Hospital.

Results

All of 17 ARDS Onset patients who received standard COVID-19 treatment, including dapsone, did not die except one patient not taken dapsone after relapsed (Supplement 2, Case 1), whereas 8/20 ARDS Onset patients who received standard COVID-19 treatment without dapsone died; the mortality rates were 5.9% and 40%, respectively. Our most prominent cases in the first period were in two patients on an HFNC with 50 LPM flow and 100% fraction of inspired oxygen (FIO2), intermittently requiring bilevel positive airway pressure (BiPAP) and barely having a O2 saturation (SaO2) in the 87-91% range for several days. These patients' clinical conditions worsened slowly every day, nearly requiring intubation. With dapsone administration, one patient had his/her FIO2 requirement reduced within 48 hours, and the other patient had his/her FIO2 need reduced, too. Encouraged by an objective response, we started to administer dapsone to other patients affected with COVID-19. We did not observe any improvement in mechanically ventilated patients. In this study, 36 of 43 (83.7%) patients with severe, worsening ARDS and imminent death due to SARS-CoV-2 infection were treated. A patient who was released from the ICU after dapsone administration in a critical condition in the first period was hospitalized again and died while receiving standard COVID-19 treatment before the 2nd treatment period. The chi-square statistic reflects patient mortality.

First-period results

Patients started to feel better within 24 hours after the administration of dapsone with standard COVID-19 therapy. The criterion for ARDS onset was the requirement of FIO_2 via simple nasal cannulation of up to 15 L/min. There was a decrease in FIO_2 requirements in 6 patients and no worsening in 1 patient. The criteria for exacerbated ARDS cases were FIO_2 administered via an HFNC of 95-100% and/or BiPAP. There were decreases in FIO_2 requirements in 4 patients and no worsening of FIO_2 in 6 patients. The criterion for severe cases of ARDS was the need for mechanical ventilation. No response was observed in 2 patients. In this study, 19 patients with severe, worsening ARDS and imminent death due to SARS-CoV-2 infection were treated. According to our research results, ARDS progression was blocked in 17 of 19 total patients, and two patients' conditions progressed to requiring mechanical ventilation. ARDS improved in 10 patients (Supplement 1).

Second-period results

We prescribed dapsone in 44 cases in the second period, including the hiatus after the first period.

The criterion for ARDS onset was the requirement of FIO_2 via simple nasal cannulation of up to 15 L/min. The criteria for aggravated ARDS cases were FiO_2 administered via an HFNC of 95-100% and/or BiPAP. The criterion for severe cases of ARDS was the need for mechanical ventilation. This report is the result of medical treatment from 21 December to 29 December 2020.

Almost all patients started to feel better within 24 hours. The progression of ARDS in onset and aggravated cases was stopped. We stopped prescribing dapsone for two weeks, determined the prescribing guidelines for dapsone[16], and started redosing again. The results of the first period[18] and the second period (current report) were almost identical.

Table 1: The results of treating ARDS-onset cases indicate that there is decreased FIO_2 in 7 patients and no worsening in 1 patient. The results of treating aggravated ARDS cases were as follows: decreased FiO_2 in 6 patients and no worsening in 3 patients. The results of treating severe ARDS cases were as follows: no response in 2 patients. There were three deaths in the aggravated ARDS group treated with dapsone. At the same time, there were no deaths in the ARDS-onset dapsone-treated group. There was a large difference in treatment results in the ARDS-onset group when dapsone was prescribed compared with when dapsone was not prescribed.

Table 2: Simultaneously, in the same hospital ICU, we examined results of standard COVID-19 treatment of ARDS without dapsone. There were eight deaths in the ARDS-onset group not treated with dapsone, with progressing hypoxia, and almost all of these patients died before reaching the criteria for aggravated ARDS.

Statistics

We performed the statistical analysis of the results presented in Tables 1 and 2. The chi-square statistic, p-value and statement of significance appear beneath the table. We described each variable of interest,

provided sources of data and details of assessment methods (measurement) and defined the comparability of assessment methods because there was more than one group (Supplement 1). The results are as follow.

The comparison was made assuming that only the case of decreased FIO_2 was influential in the entire dapsone (+) group and dapsone (-) group, which applied to only the ARDS-onset stage.

We performed the statistical analysis of the results presented in Tables 3 and 4. The chi-square statistic, p-value and statement of significance appear beneath the table. To judge whether the effects of this dapsone treatment (name: Soon-Joe treatment) are meaningful, we also performed Fischer's exact test.

With the chi-square test, based on decreased FIO2 and no further progression of hypoxia, the addition of dapsone to standard COVID-19 treatment was more effective in ARDS-onset and aggravated ARDS cases than in severe ARDS cases.

The 22 patients who received dapsone were divided into onset and aggravated (onset + aggravated) and severe (severe) groups. With Fisher's exact test, based on decreased FIO_2 and no further progression of hypoxia, dapsone was useful, and the results were statistically significant. The results with Fisher's exact test were the same as those with the chi-square test.

The mortality of the ARDS-onset stage

Because a large difference in ARDS treatment results was observed in the onset group when dapsone was prescribed and when dapsone was not prescribed, all data from periods 1, 2, and 3 were collected. The ARDS-onset mortality rates were 5.9% (with dapsone) and 40% (without dapsone) in Table 5.

Based on the combination of the chi-square statistic, Fisher's exact test, and mortality results, the effect of dapsone was the best in ICU patients at the ARDS-onset stage. The mortality rate was 5.9%, and one patient died after hospital discharge. All studied patients were included in this statistical analysis. Since then, the dose of dapsone has been reduced, and dapsone has continued to be taken after discharge.

Discussion

Pathophysiology of SARS-CoV-2-associated ARDS

Refractory progressive respiratory failure has been the primary cause of death in the COVID-19 pandemic. In patients who died from COVID-19–associated or influenza-associated respiratory failure, the peripheral lung's histologic pattern was diffuse alveolar damage with perivascular T-cell infiltration. The lungs from patients with COVID-19 also showed distinctive vascular features, consisting of severe endothelial injury associated with the intracellular virus and disrupted cell membranes. Histologic analysis of pulmonary vessels in patients with COVID-19 showed widespread thrombosis with microangiopathy[19]. These observations indicated pulmonary vascular disease in patients with COVID-19 ARDS[20] or it was easy to judge the result than the process.

By studying moderately and severely ill COVID-19 patients, we found active NLRP3 in peripheral blood mononuclear cells and postmortem patient tissue. Inflammasome-derived products, such as active caspase-1 and IL-18, were observed in the sera, as well as interleukin-6 (IL-6) and LDH, markers of COVID-19 severity. Higher IL-18 and caspase-1 are associated with disease severity and poor clinical outcome[7]. It appears that IL-6 levels in COVID-19 patients are not exceptionally high in the broader context of ARDS[21].

COVID-19 ARDS onset can present with relatively preserved aeration on chest CT imaging despite severe respiratory hypoxemia. In some patients, this early, high-compliance phenotype evolves into a low-compliance phenotype with poor aeration. We described the first clinical presentations as low elastance, high compliance, and preserved aeration (L-type) and the second as high elastance, low compliance, and poor aeration (H-type)[22]. The inhomogeneous phenotypes of COVID-19-associated ARDS illustrate important clinical descriptions and may have diverse therapeutic implications.

Transient bulbar palsy through medulla oblongata may be the origin of ARDS in COVID-19

Extrapulmonary manifestations have been reported, including neurologic complications, such as stroke and encephalopathy; anosmia; cardiac complications, such as acute coronary syndrome, myocarditis, arrhythmia, and Takotsubo cardiomyopathy; renal complications, such as acute kidney injury; hepatic complications, such as transaminitis; and gastrointestinal complications, such as diarrhoea, nausea, vomiting, and anorexia[20]. With the data on these diverse complications, the relationship of the virus, its direct cytotoxic effects, its effects on the renin-angiotensin-aldosterone systems, its impact on the endothelium, and the various manifestations of COVID-19 disease was explored[23].

In vitro human brain organoid experiments and in vivo SARS-CoV-2 mouse model hybrid investigations revealed possible evidence for the neuroinvasive capacity of SARS-CoV-2 and an unexpected consequence of direct infection of neurons by SARS-CoV-2[24]. In hybrid experiments, no consensus on the evidence for CNS infections has been reached.

Brainstem involvement, especially pre-Bötzinger complex involvement[25,26], could explain the respiratory failure and sudden high death rate of COVID-19 ARDS patients. It could also explain the sudden recovery of cases 1, 2, 3, and 4 (within 24-48 hours) after taking dapsone in the ARDS-onset and aggravated stages in the second phase. Dapsone treats and prevents transient bulbar palsy through the medulla oblongata. Dapsone also has a therapeutic effect on COVID-19-associated ARDS, as evidenced by several patients' recovery within 24 - 48 hours of taking dapsone. Therefore, the causal relationship is clear.

The solubility of dapsone varies over an extensive range depending on the solvent used (e.g., water, 0.2 mg/mL, methanol, 52 mg/mL). Dapsone has been considered a difficult-to-handle compound for experimental investigations, especially using living cell assays[27]. According to inflammasome competitor theory[3], the treatment's preventive effect will not appear if dapsone does not reach the proper concentration. After the ingestion of a single 50- to a 300-mg dose of dapsone, maximal serum

concentrations are reached between 0.63 and 4.82 mg/L[28]. A doctor should administer dapsone after thoroughly testing it.

An inflammasome competitor theory

After progressive dyspnoea and worsening desaturation, we tend to see a pattern portending fatal outcome, with no further standard treatments to offer. According to an inflammasome competitor theory, we started oral dapsone 100 mg to target the NLRP3 inflammasome[3].

Furthermore, the dapsone dose was increased to 200 mg PO daily because the theory is that SARS-CoV-2-activated inflammasomes and dapsone compete for DNA binding at the molecular level. We have used dapsone so far in a total of 19 patients. We established objective criteria for improvement, such as a reduction in the FIO2 requirement and a decrease in hypoxia progression.

As in the debate between the inflammatory hypothesis[29,30] and the regression hypothesis[31] for Alzheimer's disease, in 17 out of 19 patients receiving treatment targeting the NLRP3 inflammasome, the progression of ARDS was blocked. In contrast, in 2 patients, no response to dapsone treatment was observed, and their conditions progressed to requiring mechanical ventilation. Dapsone passes the BBB very well. Dapsone is a newly proven drug for the treatment and prevention of AD[5].

The findings for dapsone are essential. SARS-CoV-2 might gain entry to the CNS through the olfactory bulb to invade the brainstem[32]. Moreover, SARS-CoV-2 CNS access might also occur from the peripheral circulation through BBB compromise. Another possible SARS-CoV-2 CNS entry route could be its dispersal from the lungs into the vagus nerve via pulmonary stretch receptors, eventually reaching the brainstem[33,34]. Brainstem involvement, especially pre-Bötzinger complex involvement, could explain the respiratory failure and high death rate of COVID-19 patients and the sudden recovery of patients after taking dapsone. (Figure 1)

This study also suggests potentially mesoscopic effects depending on the details of the geometry. The size of this experiment is in the range of picometers to nanometres. It is useful for understanding quantum mechanics and relativity theory and attracts much attention by showing new phenomena. The time-independent equations of the Schrödinger equation represent the potential (voltage) formed between the electron and the nucleus. Voltage is formed among the nucleus and the electron clouds because their distances are not relatively long. The Dirac equation is expressed when it satisfies when a particle with a stationary mass of m moves freely without being influenced by the outside[35]. However, the outside is a state in which two- or one-electron oxidation is actively working in the mesoscopic area.

Many inflammasomes are generated and interact, and the cation environment in the body is also rapidly changing. The effects of medications administered to patients are often adverse, and it was assumed that the physical energy must be mutually transferred to the energy of electromagnetic force. A magnetic/electric field may vary the gravitational field according to experiments[36,37]. The gravitational

redshift/blueshift, the bending of light by gravity and Shapiro time delay showed that gravity has an action on the photon[38].

These various mesoscopic effects appear directly as a pathology caused by SARS-CoV-2-activated inflammasomes. Still, the intermediate reaction process makes it very difficult to identify even in very sophisticated in vivo in vitro experiments. No conclusions can be drawn in relation to the neurologic features of COVID-19[25]. There is no consensus on the consequences of CNS infections[24]. The initial ultrarapid immune responses could be a prognostic factor[26]. Therefore, enthusiastic medical staff should first believe in the results of treating and reporting on patients with SARS-CoV-2-associated ARDS. This study presents simple scientific data and very in-depth scientific theory rather than diverse experimental studies in mesoscopic areas.

Inflammasome treatment mechanism

The receptor-binding domain of the S protein on the surface of SARS-CoV-2 interacts with the ACE2 receptor (ACE2) in host cells[39]. It is now well-established that the entry of SARS-CoV-2 into host cells is facilitated by its neuropilin-1 (NRP1), a transmembrane receptor that lacks a cytosolic protein kinase domain[40]. NRP1 is also expressed in the CNS, including olfactory-related regions such as the olfactory tubercles and paraolfactory gyri[41]. Furthermore, NRP1 is a host factor for the entry of SARS-CoV-2 into the brain through the olfactory epithelium[42]. SARS-CoV-2 evokes a response that needs strong induction of a subclass of cytokines, including type I and, obviously, type III interferons and a few chemokines, such as the response to influenza A virus and, specifically, respiratory syncytial virus[14,43]. SAMHD1 links to the NF-kB pathway[14]. Now, we can correlate ACE2, NRP1, and SAMHD1 with the neurologic complications of COVID-19.

Japanese encephalitis virus (JEV) is a common cause of acute and epidemic viral encephalitis. JEV infection is associated with microglial activation, resulting in proinflammatory cytokines, including IL-1β and IL-18. It is a signalling pathway that leads to the activation of caspase-1 for the maturation of both IL-1β and IL-18 in NLRP3. The depletion of NLRP3 results in a reduction in caspase-1 activity and subsequent production of these cytokines. Reactive oxygen species (ROS) production and potassium efflux mediate the two danger signals that link JEV infection to caspase-1 activation and subsequent IL-1β and IL-18 maturation[44]. Patients with JEV had an 11-fold elevation in CNS IL-8 compared to controls, and dapsone stopped seizures[45,46]. The population of Korea has suffered severely from SARS-CoV (2002), influenza A virus subtypes H1N1 (2009), MERS (2015), and SARS-CoV-2 (2020). The Korea Centers for Disease Control and Prevention (KCDC) and the Korean Hansen Welfare Association (KHWA) supply dapsone to Hansen's disease (HD) patients. The KCDC provided dapsone free to all HD patients (dapsone 100 mg–1000 tablets/bottle to 3814 of 9134 HD patients). Of note, the KHWA reported that HD patients in Korea had no reports of any outbreaks of respiratory infectious diseases between 2002 and 2020[3].

Dapsone is a small molecule with anti-inflammatory and immunosuppressive properties as well as antibacterial and antibiotic properties. Dapsone passes through the BBB[47,48], and high-dose sulfadiazine results in an effective CSF concentration in humans[49].

Cell mechanism

Dapsone binds to myeloperoxidase and regulates the production of hypochlorite. It reduces the inflammatory response of cells[3].

Molecular mechanism

The nucleophilic/electrophilic region of DDS interacts with amino acids by molecular bonding. Neurotoxicity, aggregation, and free radical formation are initiated by the methionine (Met) residue at position 35 in the Aβ C-terminal domain[50-52]. Two-electron oxidation of bicarbonate is mediated by hydrogen peroxide after the generation of peroxymonocarbonate ($HCO4^-$). The bicarbonate/carbon dioxide pair stimulated one-electron oxidation. Carbonate radical anions ($CO3^{\bullet-}$) mediate one-electron reactions to promote one-electron oxidation to efficiently oxidize Met residue thioesther sulfur to sulfur radical cations ($MetS^{\bullet+}$)[53]. DDS has a structure that can competitively reduce the positively charged sulfur radical production rate because it has a similar structure to methionine sulfoxide[3].

Ubiquitin mechanism

Control for reversing protein ubiquitylation was the subject of our study. The reversibility of ubiquitination by the action of deubiquitinating enzymes (DUBs) serves as a significant regulatory layer within the ubiquitin system. The human genome encodes approximately 100 DUBs, and DUBs have implicated pathologies, including neurodegeneration and cancer[54]. The conjugation of ubiquitin can be reversed by DUBs, which reflect additional regulation of ubiquitin[55]. The covalent attachment of ubiquitin to substrates has generated a repurposed drug (dapsone) capable of competing with pathogenic targets in Ub-conjugating targeting chimaeras[3,56]. The nucleophilic properties of dapsone compete with ubiquitin (Ub), similar to DUBs. Before loading Ub onto the substrate, the Ub-activating (E1)/Ub-conjugating (E2)/E3 ligase acts at each stage of the ubiquitination process. Enzymes can carry Ub via a thioester linkage, which allows vigorous favourable attack of the substrate nucleophile. Dapsone can compete with the ubiquitination cascade. The identical mechanism can potentially ubiquitinate cysteine thiols and hydroxyls on serines, threonines, leucines, and tyrosines[3,54].

Inflammasome mechanism

Dapsone noncovalently binds/interacts with the minor groove of DNA. The DDS-DNA interaction/binding relative binding energy is −6.22 kcal mol-1, estimated using in silico studies. Docking analysis further revealed that dapsone preferentially binds to the AT-rich region of DNA[57]. The nucleophilic properties of

DDS also compete with NLRP3. ORFb activates NLRP3 through the interaction of the AT-rich repeat domain of NLRP3[3]. (Figure 2)

Dapsone is a proper treatment with a favourable response in most dermatologic patients[58]. Patients who showed remission or improvement after three months[58] were significantly older than patients with stable or progressive disease. Additionally, remission after three months was associated with a substantially lower dose of dapsone than improvement only[58]. A better prognosis when responding at lower doses and the fact that dapsone is more effective in elderly individuals means that it is also related to the recent NLRP3 and genetic link. Data on active NLRP3 inflammasomes in peripheral blood mononuclear cells and postmortem inflammasome-derived products in moderately and severely ill COVID-19 patients were critical in preliminary rationale studies. Higher levels of IL-18 and caspase-1 have been associated with disease severity and poor clinical outcome[7]. There is evidence supporting a causal link between low expression of the interferon receptor gene IFNAR2 and high expression of the gene encoding tyrosine kinase 2 (TYK2) in life-threatening disease. These findings identify robust genetic signals relating to key host antiviral defence mechanisms and mediators of inflammatory organ damage in COVID-19[59].

Dapsone was added in the desperate attempt to stop the decline in these patients' conditions. Excluding the patients whose conditions progressed to requiring mechanical ventilation, the rapid recovery of ARDS patients within 24 hours of being treated with dapsone provides evidence supporting the use of this very new, meaningful clinical treatment in the ICU. Activated microglia were found adjacent to neurons by pathologic studies, which suggests neuronophagia in the olfactory bulb, substantia nigra, a dorsal motor nucleus of the vagal nerve, and the pre-Bötzinger complex in the medulla[60].

Transient global amnesia is a rare clinical syndrome in which a sudden onset of anterograde amnesia recovers within 24 hours. Although the underlying pathophysiology is uncertain, focal hippocampal ischaemia, venous congestion, migraine-related mechanisms, hypoxic-ischaemic events, epilepsy, and metabolic stress may be involved[61]. Serum neurofilament light chain (NFL) is a specific biomarker of neuronal injury. NFL was higher in patients with COVID-19 than in the comparator groups. Higher NFL levels were associated with short-term outcomes, indicating that neuronal injury is common in critically ill patients[62]. Brainstem involvement could explain sudden respiratory failure[60].

An alternative way to help us prevent or treat SARS-CoV-2-associated ARDS is to use an inflammasome competitor to reduce the prevalence rate. The molecular properties of dapsone, including electron density and its Laplacian delocalization index, have provided a specific mechanism for chemical bonding and atomic and molecular details[63]. The redox properties of DDS dependent on amine and sulfone moieties explain the oxidation mechanism of DDS by electron transfer[64]. As it applies to electrons, we can understand the various neuropathologic findings of COVID-19, including its mysterious sensory manifestations.

Early indications of dapsone's effects show that it may potentially alleviate the course of COVID-19. There are enough theoretical clinical data to warrant a pilot study in deteriorating patients with COVID-

19[3,18,46,65]. Among patients hospitalized with onset to aggravated COVID-19-associated ARDS, the use of dapsone, alone or with adjuvants, will improve clinical status within one day compared with standard care alone[18].

Declarations

Competing interest statement

The authors declare no competing interests.

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Tables

Table 1. A total of 22 patients were treated with standard COVID-19 therapy (with dapsone)

Category	Decrease in FIO_2	Progression of hypoxia	No further progression of hypoxia
Onset (FIO_2 requirement via simple nasal cannulation of up to 15 L/min)	7	0 (0 deaths)	1
Aggravated (FIO administered via an HFNC of 95-100% and/or BiPAP)	6	3 (3 deaths)	3
Severe (requiring mechanical ventilation)	0	2 (2 deaths)	0
Total: 22	13	5	4

Table 2. A total of 22 patients were treated with standard COVID-19 therapy (without dapsone)

Category	Decrease in FIO_2	Progression of hypoxia	No further progression of hypoxia
Onset (FIO_2 requirement via simple nasal cannulation of up to 15 L/min)	8	8 (8 deaths)	4
Aggravated (FIO_2 administered via an HFNC of 95-100% and/or BiPAP)	1	1	0
Severe (requiring mechanical ventilation)	0	0	0
Total: 22	9	9	4

Table 3. The chi-square statistic of 44 patients treated with standard COVID-19 therapy (with dapsone)

Study 3	Decreased FIO_2	Others	Row total
Dapsone (+) onset	7 (4.29) [1.72]	1 (3.71) [1.98]	8
Dapsone (-) onset	8 (10.71) [0.69]	12 (9.29) [0.79]	20
Column total	15	13	28 (total)

The chi-square statistic is 5.1836. The p-value is .022801. The result is significant at $p < .05$.

Table 4. Fisher’s exact test of 44 patients treated with standard COVID-19 therapy (with dapsone)

Study 2-4	Decreased FIO_2 + no progression	Progression	
Dapsone (+) onset + aggravated	17	3	20
Dapsone (+) severe	0	2	2
	17	5	22 (grand total)

Fisher's exact test statistic value is 0.0433. The result is significant at $p < .05$.

Table 5. Mortality at the ARDS-onset stage from Hunt Regional hospital in Greenville, TX

	Decrease in FIO_2			Progression of hypoxia			No further progression of hypoxia			Mortality
Period	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	
ARDS onset (death)	*6 (1)*	7	2	0	0	0	1	1	0	1/17
ARDS onset (death)		8			8 (8)			4		8/20

Methods

Hunt Regional Hospital in Greenville, TX, ethically approved this clinical patient treatment based on the World Medical Association's Declaration of Helsinki. The patients provided written informed consent (or their parents or guardians). We administered medicines in compliance with medical and pharmacy laws with informed consent. For every patient admitted with COVID-19 ARDS, the medical doctor treated the patient with standard COVID-19 treatment. We started oral dapsone 100 mg to target NLRP3 inflammasomes.

We set out to use objective criteria of improvement, such as A. a reduction in the FIO_2 requirement and B. a decrease in the progression of hypoxia.

Protocol for administration

Written informed consent should be obtained, and potential side effects should be explained. The common side effects are haemolytic anaemia (in patients with G6PD deficiency)[69], methemoglobinemia, and allergic reaction. The patients should also be informed that currently, G6PD is a send-out test and can take up to 5-7 days. Cimetidine 400 mg orally TID will now be administered to counter dapsone methemoglobinemia side effects[70]. The venous methemoglobin level should be checked every day, and a mild methemoglobin level of 2-10%[71] is tolerated well. Dapsone should be discontinued if the level reaches 15 or above.

In the Hunt Regional Hospital ICU, the doctors treating COVID-19 patients change approximately every eight days. The hospital stopped prescribing dapsone after the first period for two weeks (standard COVID-19 treatment group without dapsone (22)) and rigorously revalidated its effectiveness. Furthermore, during this period (standard COVID-19 treatment group with dapsone (22)), 44 (22/22) patients with ARDS in the intensive care unit were re-examined. Therefore, we analysed the data for statistical effectiveness.

Data availability

The authors declare that all main data generated or analyzed during this study supporting the findings are available within the article and its supplementary information files. Extra data that support the findings of this study are available from the corresponding author upon reasonable request. The full detailed study or the clinical protocol for the clinical trial is provided as a separate file. Pages 1 in the Protocol for SARS-CoV-2 ARDS describe the key objectives of the clinical trial to provide the pre-specified outcomes reported in this manuscript. Source data are provided with this paper.

Methods references

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Figures

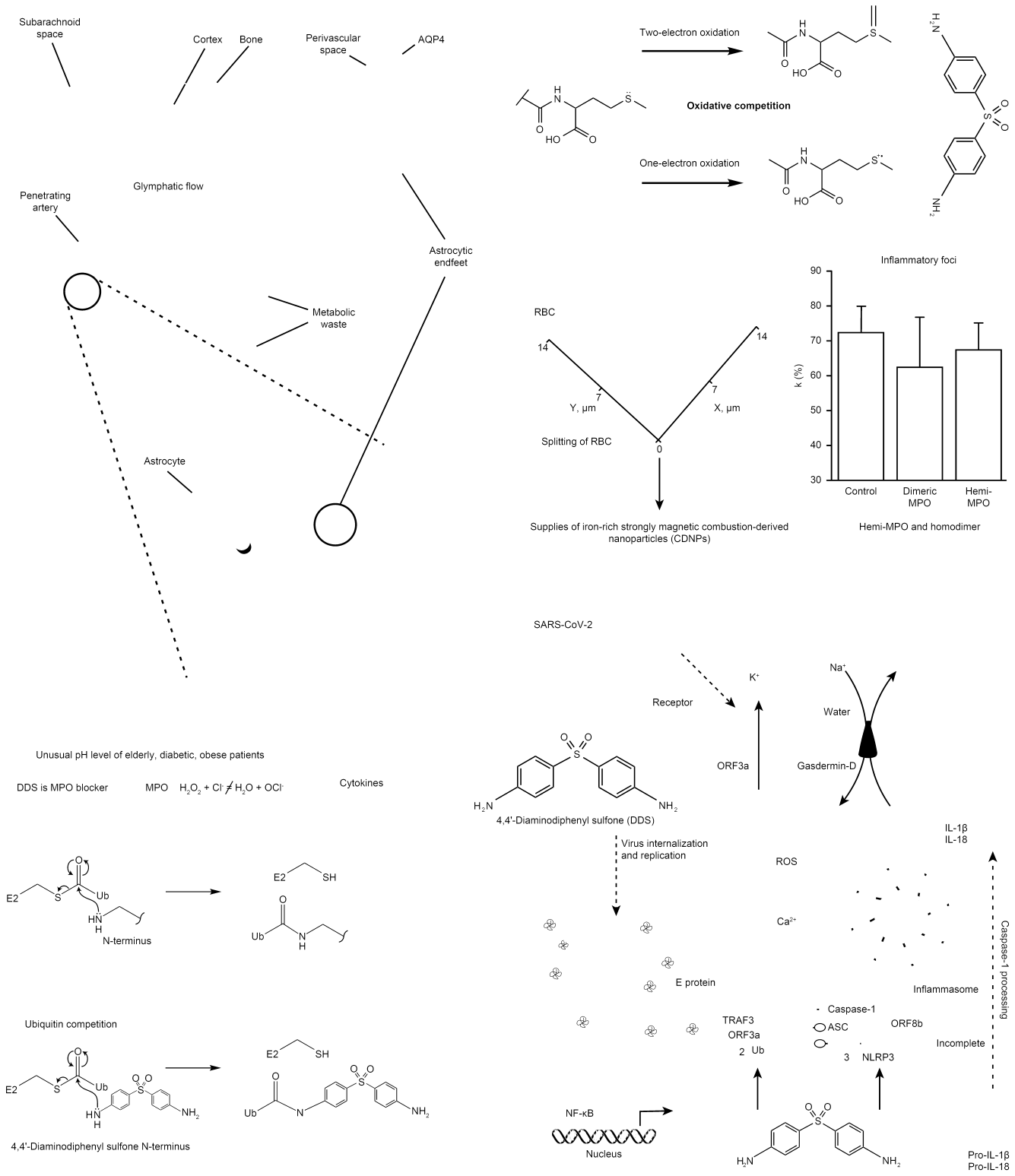

Figure 1

The perivascular space in the brain consists of a single or double layer of invaginated pia, forming an interstitial fluid-filled space representing an extension of the extracellular fluid space around the intracranial vessels as they descend into the brain parenchyma. Human sensory stimuli affect the breathing sensation via the cerebral cortex and hypothalamus. The abnormal muscular sensation is also a contributor to dyspnoea. The respiratory muscles are not intentionally activated in healthy breathing.

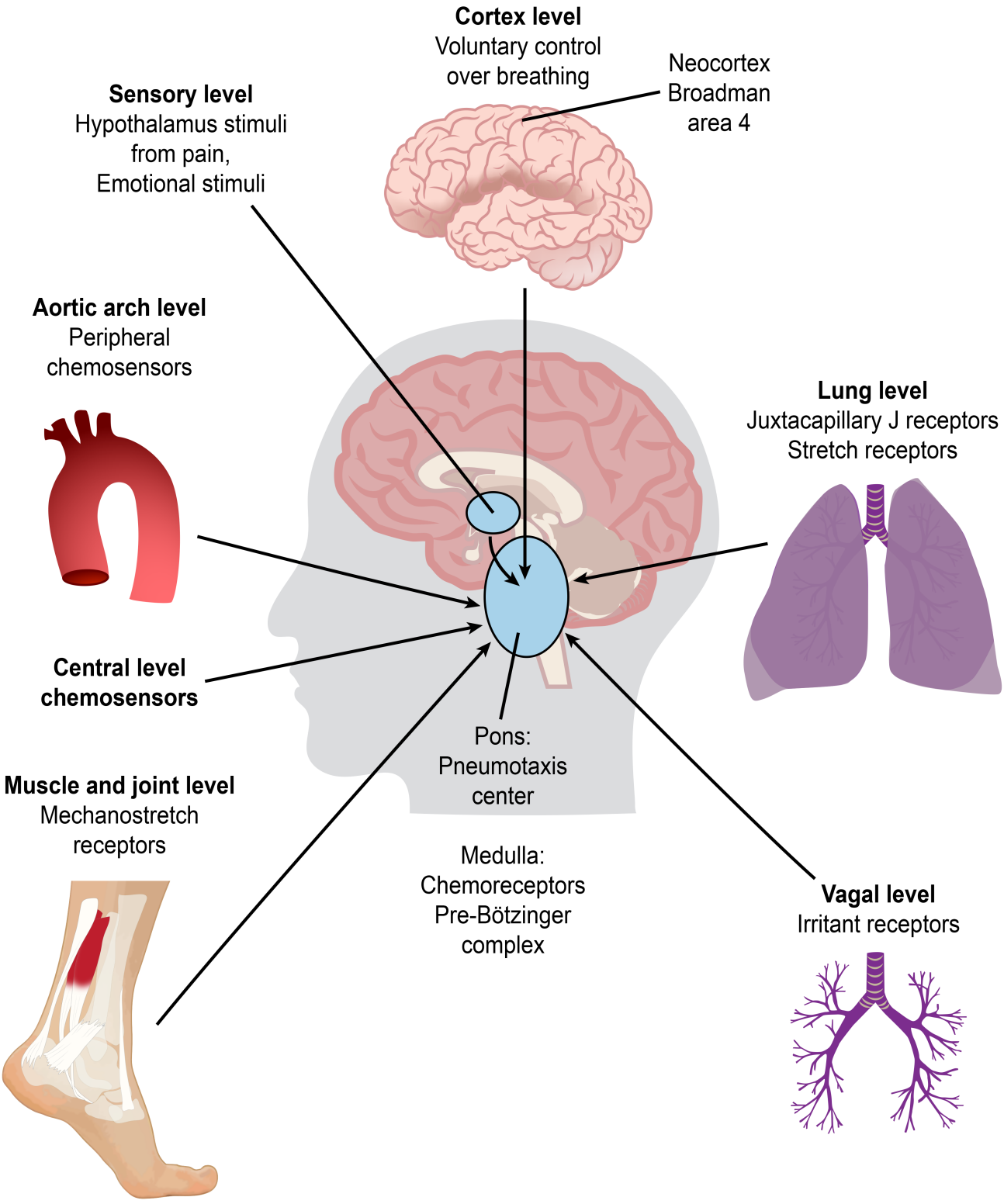

Figure 2

DDS binds to myeloperoxidase and regulates the production of hypochlorite, thereby reducing the cellular circumstance. The topological properties of DDS, such as electron density and its Laplacian delocalization index, the negative potential of the vicinity of O and O atoms is susceptible to severe electrophilic attack. The nucleophilic/electrophilic region of DDS interacts with amino acids by molecular bonding. DDS has a structure that can reduce the sulfur radical production rate by electron charge

transfer because they are structurally similar to methionine sulfoxide. Proteins contain many nucleophilic sites capable of attacking a ubiquitin (Ub)-conjugating enzyme (E2)–Ub thioester linkage and undergoing ubiquitination. The best-described sites are the amine-containing internal lysine residues and the free amine of the polypeptide backbone's N-terminus. Ub is activated by a Ub-activating (E1) enzyme, using energy from ATP hydrolysis, and passes to a Ub-conjugating (E2) enzyme. Ub can then be passed to a substrate protein, specified by the distinct E3 ligase that binds both the substrate and the E2. DDS can compete with the ubiquitination cascade. Nucleophilic properties of DDS compete with NLRP3. ORF activates NLRP3 through direct interaction of the leucine-rich repeat domain of NLRP3.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplement1.docx
- Supplement2.docx
- STROBEchecklistCriticalCareDAPSONENaturefinal.docx
- ProtocolforSARSCoV2ARDS.docx