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

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Abstract

Background Clinicians considered dapsone administration to treat SARS-CoV-2 inflammasome. Dapsone is helpful in the molecular regulation of Nod-like receptor family pyrin domain-containing 3 (NLRP3).

Objective To study the targeting of NLRP3 itself or up-/downstream factors of the NLRP3 inflammasome by dapsone must be responsible for its observed preventive effects, functioning as a competitor.

Methods This is a randomized controlled trial (RCT). We set out to use objective criteria of improvement, such as A. a reduction in the FIO2 requirement and B. a decrease in the progression of hypoxia. We treated the patients with standard COVID-19 acute respiratory distress syndrome (ARDS) treatment with dapsone. The RCT results were analyzed.

Results ARDS progression was blocked in 17 of 19 total patients at the first period. The 44 (trial 22/ control 22) subjects were analyzed during the second period. The chi-square statistic is 5.1836. The p-value is .02280. (RR 0.21, OR 0.1) Fisher's exact test statistic value is 0.0433. (The result is significant at $p < .05$) (RR 0.15, OR 0) It is significant at the ARDS onset stage.

Conclusion There was a significant difference in dapsone treatment results in the ARDS-onset group. We confirmed that dapsone clinically treated the onset of ARDS by targeting SARS-CoV-2-activated inflammasomes. Like chemically reacting substances, inflammasome and dapsone compete, proving that it is effective in early ARDS.

ClinicalTrials.gov Identifier NCT04918914

Introduction

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 helpful 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 25.7–22.9% overall and from 41.4–29.3% among patients requiring invasive mechanical ventilation[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 invasive mechanical ventilation[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. However, aberrant activation of the NLRP3 inflammasome has been linked with several inflammatory disorders, including cryopyrin-associated periodic syndromes, AD, type 2 diabetes, and atherosclerosis. 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]. Although ARDS is a complication of SARS-CoV-2 infection, it is not a viral-replication-associated condition or a condition that causes tissue injury[13]. Instead, ARDS results from dysregulated hyperinflammation in response to viral infection[14].

A COVID-19 committee at Hunt Regional Hospital 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[15]. 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[16] and NLRP3 to regulate inflammasome activity[17].

Dapsone is a well-known drug for treating leprosy, a chronic, progressive bacterial infection caused by the bacterium *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. Those who do not take DDS are 2.55% and those who take DDS are 1.05%[18], so this is epidemiological data consistent with the theory. South Korea's Sorokdo National Hospital reported that about 10,000 Hansen's disease patients were not infected from 1 January 2020 to 15 April 2021. (Supplement S1)

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[19].

Methods

This study was proposed by an observer who developed the theory of the inflammasome competitor[3], and the clinical study was conducted in collaboration with medical staff at Hunt Regional Hospital when 100% of patients died from COVID-19 ARDS. It was a case series for medical staff at Hunt Regional Hospital. Still, a cross-sectional study was conducted to treat COVID-19 ARDS by the medical staffs, and it was also a randomized controlled trial of COVID-19 ARDS onset patients to the observer. Statistical analysis was performed according to the observer's point of view.

Hunt Regional Hospital 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. This is case series with or without intervention; cross-sectional study. 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 cases of ARDS 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. The cross-sectional study observed the result of medical treatment from 21 December to 29 December 2020.

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 analyzed the data for statistical effectiveness.

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)[20], 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[21]. The venous methemoglobin level should be checked every day, and a mild methemoglobin level of 2–10%[22] is tolerated well. Dapsone should be discontinued if the level reaches 15 or above.

Results

An off-label medication at the first period

Patients started to feel better within 24 hours after the administration of dapsone with standard COVID-19 therapy. 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 (FIO_2), intermittently requiring bilevel positive airway pressure (BiPAP) and barely having an O_2 saturation (SaO_2) 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 FIO_2 requirement reduced to 50% within 48 hours, and the other patient had his/her FIO_2 need reduced to 80%. Encouraged by an objective response, we started to administer

dapsone to other patients. We did not observe any improvement in mechanically ventilated patients. Nineteen patients with severe, worsening ARDS and imminent death due to SARS-CoV-2 infection were treated. According to our first 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 (decreased FIO_2). (Table 1)

Table 1
A total of 19 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)	6	0	1
Aggravated (FIO administered via an HFNC of 95–100% and/or BiPAP)	4	0	6
Severe (requiring mechanical ventilation)	0	2	0
Total: 22	10	2	7

Cross-sectional study at the second period

We stopped prescribing dapsone for two weeks, determined the prescribing guidelines for dapsone[23], and started treating again. We prescribed dapsone in the second period, including the hiatus (22 cases) after the first period. The results of the first (off-label medication) and second (cross-sectional) period were almost identical.

Cross-sectional study: The results of treating ARDS-onset cases were as follows: 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. (Table 2)

Table 2
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

Simultaneously, in the same hospital ICU, we examined the 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. (Table 3)

Table 3
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

There was a significant difference in treatment results in the ARDS-onset group when dapsone was prescribed (dapsone group) compared with when dapsone was not prescribed.

Statistics: the chi-square test and Fisher's exact test

We performed the statistical analysis of the results presented: The chi-square statistic is 5.1836. The p-value is .022801. The result is significant at $p < 0.05$. Relative Risk = 0.21. Odds Ratio = 0.1. (Tables 4). With the chi-square test, based on decreased FIO_2 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.

Table 4
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 $p < .05$.			

Fisher's exact test statistic value is 0.0433 on the category of decreased FIO_2 and no further progression of hypoxia. The result is significant at $p < 0$ .05. Relative Risk = 0.15. Odds Ratio = 0. (Table 5) After a cross-sectional study, two researchers recovered after taking dapsone at COVID-19 ARDS onset. The treatment results of two people were dataized as the third period.

Table 5
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 $p < .05$.			

Mortality of the ARDS-onset stage at the third period

Because a significant difference in ARDS treatment results was observed in the onset group when dapsone was prescribed and when dapsone was not prescribed, all data from all periods were collected (Fig. 1). The ARDS-onset mortality rates were 0% (with dapsone) and 40% (without dapsone). One patient died without dapsone treatment after hospital discharge. The mortality rate was 0% because all studied patients were survived at the onset stage in the dapsone group. (Table 6)

Table 6
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) with Dapsone	*6 (1)*	7	2	0	0	0	1	1	0	0(1)/17
ARDS onset (death) without Dapsone		8			8 (8)			4		8/20

The observer compared the 17 participants who received the dapsone intervention (M = 1.7, SD = 2.6268, 95% CI 0.349–3.051) compared to the 20 participants in the control group (M = 2.8, SD = 3.7947, 95% CI 1.024–4.576).

Randomized controlled trial (RCT) demonstrated a significantly lower mortality score: The t-value is -1.5, and the p-value is 0.075475. The result is significant at $p < 0.10$. ARDS onset with dapsone group survived more 90% than ARDS onset without dapsone group. Moreover, the chi-square statistic is 5.8108. The p-value is 0.015928. (Significant at $p < 0.05$) (Relative Risk = 0.15. Odds Ratio = 0.09.) ARDS onset (with dapsone) survived more 90–95% than the ARDS onset (without dapsone) group. (Supplement S4. Randomized controlled trial of ARDS mortality)

Discussion

The focus of this study is a period when treatment effects change dramatically, so patients with COVID-19 ARDS onset must be carefully analyzed. Only COVID-19 ARDS onset patients meet random allocation criteria for the observer. Here is why: For 19 cases in the first period, the dapsone trial did not classify the patient's ARDS condition as mild or severe. The observer observed the treatment progress, and medical staffs reported the results, so it was a random allocation for ARDS onset. In the second cross-sectional study, patients were randomly allocated because the hospital manager determined when the administration was prohibited or when it could be administered. For 22 cases in the second period, the dapsone trial did not also classify the patient's ARDS condition as mild or severe. In the third period, two medical staffs were infected while treating, corresponding to random allocations. After the trial was completed, 65 subjects were classified by the observer.

In Aracaju, Sergipe state

In Aracaju, Sergipe state, from 17 March to 30 June 2020, it has 571,149 inhabitants. It is an endemic area of leprosy, with 15 cases per 100,000 people. There were 14,814 Covid-19 cases in this area. There were 378 lepers, four were diagnosed with Covid-19, and four died. It is strange that if we compare the Covid-19 prevalence, it is 2.6% for residents and 1.1% for leprosy patients. The mortality rate for residents was 1.93%, but it was 100%[18].

In our t-test study (t-value is -1.5, and the p-value is 0.075475 and significant at $p < 0.10$.), out of 378 leprosy patients, a minimum of 68 were actually infected with Covid-19 which four were dead. The Covid-19 prevalence of leprosy patients is above 18% and 5.8% mortality. The sixty-four persons have survived because of a dapsone prescription. It is an interpretation based on our findings.

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 histologic pattern of the peripheral lung 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[24]. These observations indicated pulmonary vascular disease in patients with COVID-19 ARDS[25].

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 and 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[26].

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)[27]. The inhomogeneous phenotypes of COVID-19-associated ARDS illustrate important clinical descriptions and may have diverse therapeutic implications.

Transient bulbar palsy might 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[25]. 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[28].

In vitro human brain organoid experiments and in vivo SARS-CoV-2 mouse model hybrid experiments revealed possible evidence for the neuroinvasive capacity of SARS-CoV-2 and an unexpected consequence of direct infection of neurons by SARS-CoV-2[29]. (Fig. 2)

Brainstem involvement, especially pre-Bötzinger complex involvement[30, 31], 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 the recovery of several patients 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[32]. 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[33]. A doctor should administer dapsone after thoroughly testing it.

An inflammasome competitor thesis

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 reducing the FIO_2 requirement and a decrease in the progression of hypoxia.

As in the debate between the inflammatory hypothesis[34, 35] and the regression hypothesis[36] for Alzheimer's disease, in 17 out of 19 patients receiving treatment targeting the NLRP3 inflammasome, the progression of ARDS was blocked, whereas 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[37]. 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 dispersal from the lungs into the vagus nerve via pulmonary stretch receptors, eventually reaching the brainstem[38, 39]. 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.

Suggested 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[40]. 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[41]. NRP1 is also expressed in the CNS, including olfactory-related regions such as the olfactory tubercles and paraolfactory gyri[42]. Furthermore, NRP1 is a host factor for the entry of SARS-CoV-2 into the brain through the olfactory epithelium[43]. 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, specifically respiratory syncytial virus[16, 44]. SAMHD1 links to the NF-kB pathway[16]. Now, we can correlate ACE2, NRP1, and SAMHD1 with the neurologic complications of COVID-19.

South Korea's government provide dapsone to leprosy patients. They provided dapsone accessible to all leprosy patients (dapsone 100 mg–1000 tablets/bottle to 3814 of 9134 leprosy patients). They reported that HD patients in Korea had no reports of outbreaks of respiratory infectious diseases between 2002 and 2021.04.30[3].

Dapsone is a small molecule with anti-inflammatory and immunosuppressive properties as well as antibacterial and antibiotic properties. Dapsone passes through the BBB[45, 46], and high-dose sulfadiazine results in an effective CSF concentration in humans[47]. Dapsone binds to myeloperoxidase and regulates the production of hypochlorite. It reduces the inflammatory response of cells[3]. 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[48–50]. 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 thioester sulfur to sulfur radical cations ($MetS^{\bullet+}$)[51]. 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]. Control for reversing protein ubiquitylation was the subject of our study. The reversibility of ubiquitination by 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[52]. The conjugation of ubiquitin can be reversed by DUBs, which reflect additional regulation of ubiquitin[53]. 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, 54]. 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, 52]. 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[55]. The nucleophilic properties of DDS also compete with NLRP3. ORF8b activates NLRP3 through the interaction of the AT-rich repeat domain of NLRP3[3].

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, suggesting neuronophagia in the olfactory bulb, substantia nigra, a dorsal motor nucleus of the vagal nerve, and the pre-Bötzinger complex in the medulla[56]. 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[57]. 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[58]. Brainstem involvement could explain sudden respiratory failure[56].

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[59]. The redox properties of DDS dependent on amine and sulfone moieties explain the oxidation mechanism of DDS by electron transfer[60]. (Fig. 3) 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 have the potential to alleviate the course of COVID-19. There are enough theoretical clinical data to warrant a pilot study in deteriorating patients with COVID-19[3, 61–63]. Among patients hospitalized with onset to aggravated COVID-19-associated ARDS, the use of dapsone will improve clinical status within one day compared with standard care alone[61].

Conclusion

The statistical processing suggests the evidence that dapsone should be effective at the ARDS onset stage as an inflammasome competitor. Inflammasome and dapsone are competing like chemically reacting substances. Even if medical care with dapsone cannot remove inflammasome, it is time to treat it by reducing reaction probability.

Abbreviations

BiPAP	**bilevel positive airway pressure**
BP	**blood pressure**
FIO_2	the fraction of inspired oxygen
HFNC	high-flow nasal cannula
LPM	litre per minute
NC	pulse rate
P	positive end-expiratory pressure
PEEP	percentage of saturated haemoglobin
95–100%	respiratory rate
R	saturation of O_2
SaO_2	temperature
T	

Declarations

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Author contributions

BK treated SARS-CoV-2-associated ARDS with standard treatment and dapsone. JB classified and analyzed the data and created Tables 3 and 4. AK, REK and CS also examined the treatments individually and designed the care model. CJL performed the comparative statistical analysis. JL designed this study and the methodology and wrote this manuscript.

Competing interest statement

The authors have no conflicts of interest to declare.

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Data availability

The authors declare that all primary data generated or analyzed during this study supporting the findings are available within the article and its supplementary information files. Additional data that support the

findings of this study are available from the corresponding author upon reasonable request. The complete 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.

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Figures

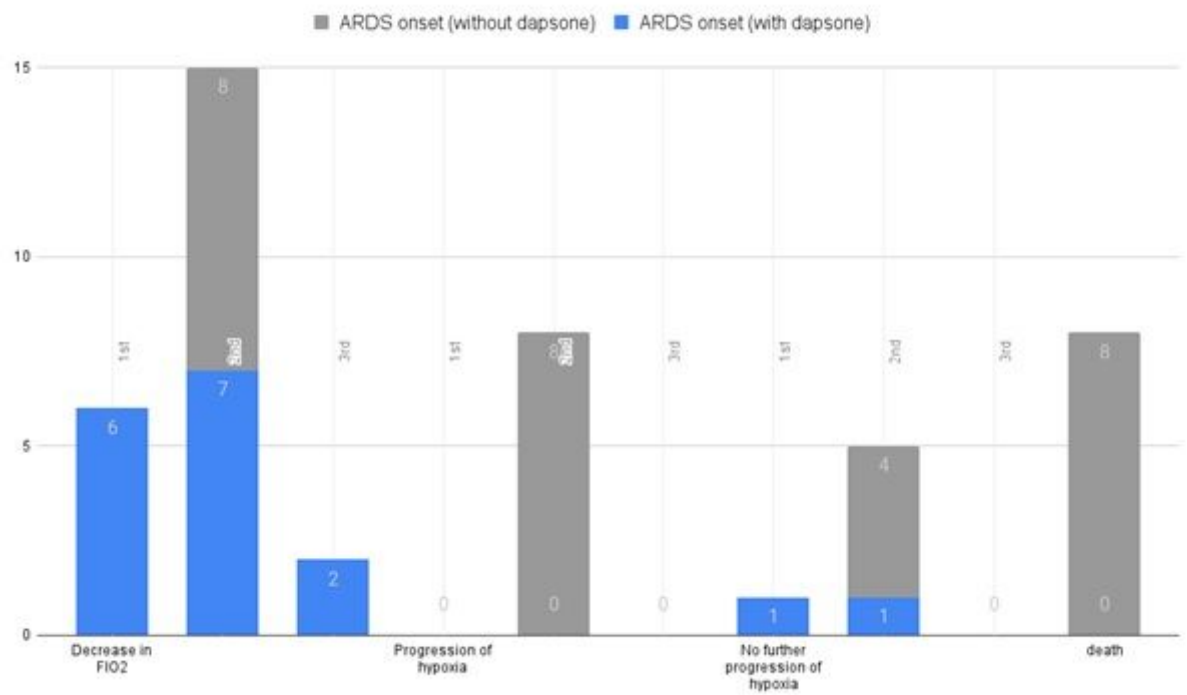

Figure 1

ARDS onset data from periods 1, 2, and 3. The mortality rate was 0% because all studied patients were survived at the onset stage in the dapsone prescribed group. Because of a significant difference in ARDS treatment results: 0% (with dapsone) and 40% (without dapsone), all data from periods 1, 2, and 3 were collected. In the 2nd period, eight patients who did not use dapsone died by the progression of hypoxia. The total number of deaths is eight.

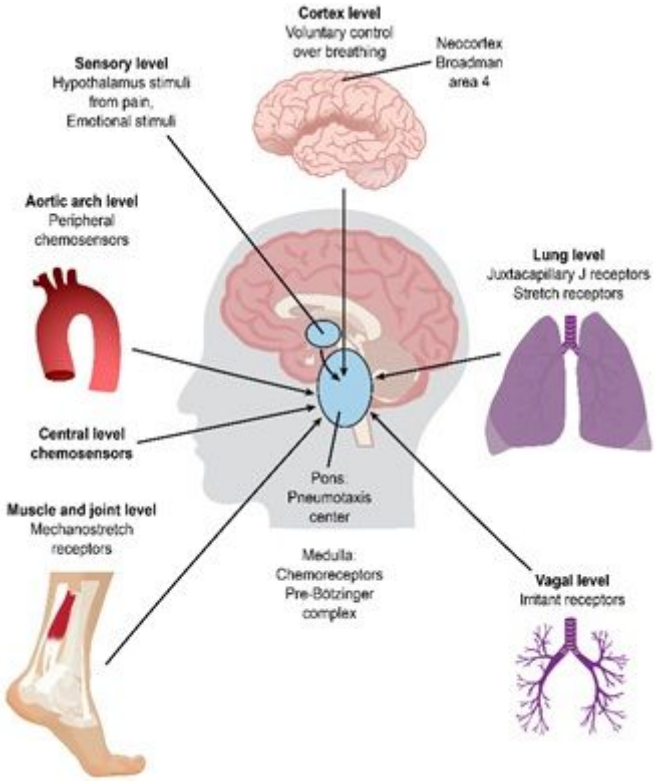

Figure 2

The perivascular space in the brain. 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[64-66].

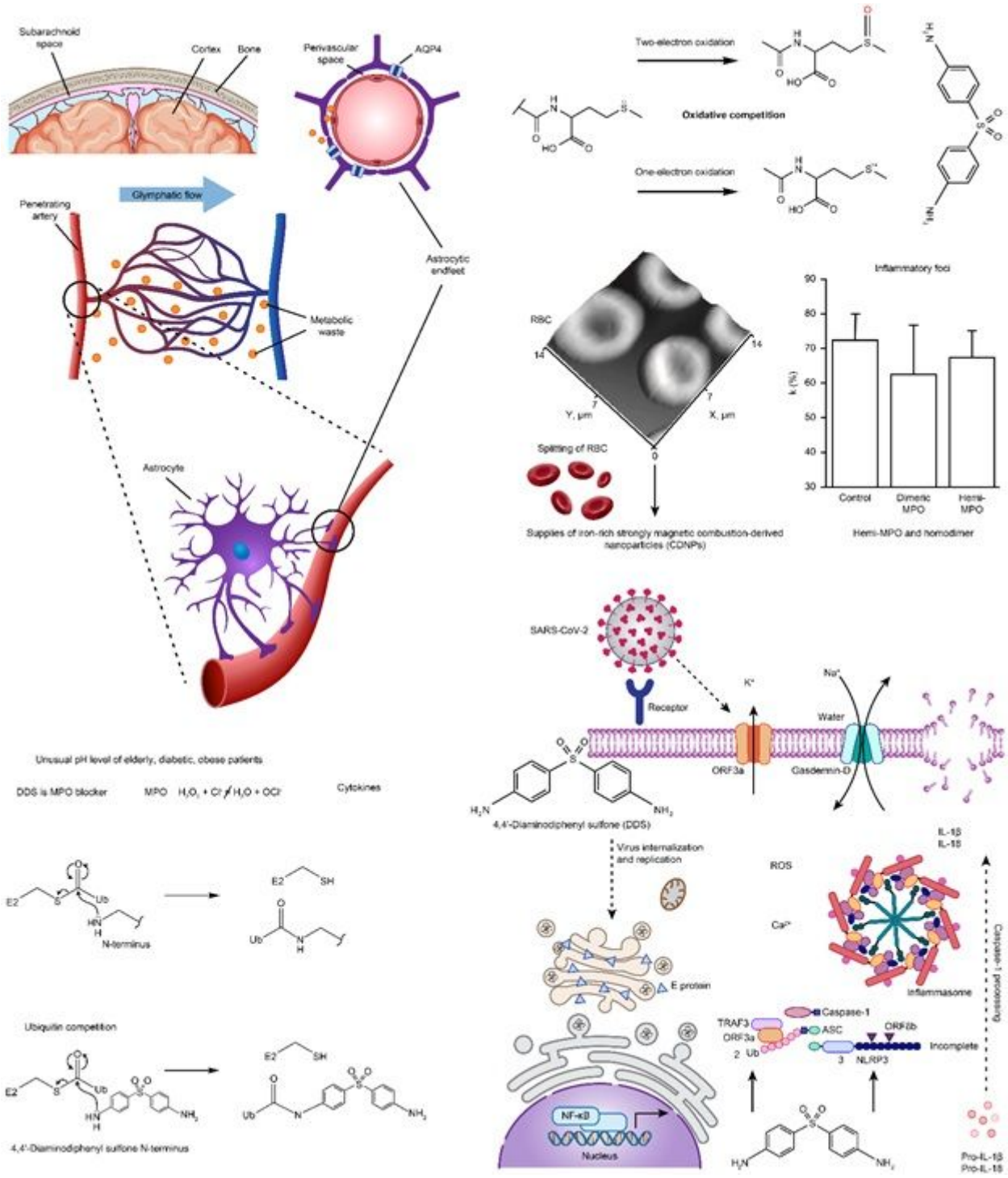

Figure 3

Nucleophilic properties of DDS compete with NLRP3. 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

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- SupplementaryMaterial.docx