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Importance of antiviral H_2S in treatment protocols for COVID-19

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Резюме

Aim. To propose a new type of antiviral treatment for COVID-19, pending the rollout of the developed vaccines and bypassing vaccine resistance of the new upcoming mutated virus variants. Aiming for prophylaxis and early therapy, the search focused on small molecules or repurposed, safe, oral and inexpensive drugs, also suitable for low-income countries. **Methods.** A search in peer-reviewed literature for preclinical antiviral mechanisms highlighted at last two clinical studies for further detailed clinical analysis: 1) High dose N-acetylcysteine (NAC) was successfully applied in very severe COVID-19-pneumonia; 2) The discovery of serum level H_2S (hydrogen sulfide) as a prognostic host factor. **Results.** Combining of these two findings resulted in a step-by-step approach with 3 perspectives that describes how H_2S works in viral respiratory diseases, how H_2S targets at least three vulnerabilities in the SARS-CoV-2 virus; finally, how H_2S can be generated and with which drugs. More than 3 dozen successful, clinically well-documented applications have already been found. Conclusion. By using NAC as the H_2S donor, the generated endogenous antiviral H_2S reactivates the collapsed innate immunity, providing a therapy regimen for COVID-19. Further randomized controlled trials are warranted, considering antiviral H_2S for inclusion in some master trial protocols.

Key words: H_2S, N-acetylcysteine, antiviral, COVID-19.

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Важность противовирусных препаратов, содержащих H_2S, в протоколах лечения COVID-19

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Abstract

Целью исследования явилось предложение нового типа противовирусной терапии для больных COVID-19 в ожидании выведения на рынок разработанных вакцин в обход устойчивости к вакцинам новых мутаций вируса. Поиск средств для профилактики и ранней терапии был сосредоточен на низкомолекулярных препаратах или перепрофилировании безопасных пероральных недорогих препаратов, подходящих для применения в т. ч. в странах с низким уровнем доходов. **Материалы и методы.** При поиске публикаций в рецензируемых журналах рассматривались работы о механизмах защиты от вируса, описанных у животных. Для дальнейшего подробного клинического анализа обнаружены 2 клинических исследования на следующие темы: 1) успешное применение высоких доз N-ацетилцистеина (NAC) при очень тяжелой пневмонии COVID-19; 2) подтверждение прогностической роли сывороточного уровня H_2S (сероводорода) в организме хозяина. **Результаты.** После объединения результатов указанных исследований поэтапно описаны 3 основных аспекта – как H_2S работает при вирусных респираторных заболеваниях; как H_2S воздействует на уязвимости вируса SARS-CoV-2 по крайней мере 3 типов; как и под действием каких лекарств вырабатывается H_2S. Обнаружено более 3 десятков успешных примеров применения этого механизма с подробными клиническими данными. **Заключение.** При использовании NAC в качестве донора H_2S эндогенный противовирусный H_2S реактивирует врожденный иммунитет, который противодействует COVID-19. С учетом того, что противовирусный H_2S может быть включен в некоторые основные протоколы клинических испытаний, необходимы дальнейшие рандомизированные контролируемые исследования.

Ключевые слова: H_2S, N-ацетилцистеин, противовирусное средство, COVID-19.

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The coronavirus disease 2019 (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has developed since December 2019 and caused a global pandemic with more than one million fatalities globally. Hallmarks of this type of coronavirus compared to older types are a high infectivity and more severe inflammation.

One year deep into the COVID-19 pandemic we only found dexamethasone in the treatment on evidenced grounds [1].

No other antiviral drug candidates have evidence, based trial results [2 – 4], neither in the guidelines. The current vaccines need also extra time for the logistics and obtaining a durable antibody response is also unclear, but in the mean, time the danger of a SARS-CoV-2 mutant remains [5]. Aside from timely steroids, supportive care, anticoagulants and ventilator support, there is no validated evidence for antiviral therapy, so we must consider well-designed studies, while waiting for a jab.

Aims. To identify treatments that can be used to treat mild and moderate cases of COVID-19 early and prevent spikes in hospitalizations that could overwhelm fragile and already overburdened health systems in low income countries. Here we propose a new treatment option in line with viral expert professor *David Fedson* [6].

Materials and Methods

The natural course of corona infection is usually mild, but there are known risk factors that can cause deterioration, suggesting that a host factor is disturbed by these factors, such as hypertension, diabetes, obesity and older age. Screening for viral protection in animals, like the *in vitro* and preclinical studies by *C.Casola* and coworkers (below, [10 – 13]), brings hydrogen sulfide (H_2S) as a possible antiviral option to our attention.

In this article, we investigate why H_2S supplementation may be beneficial for the treatment of COVID-19 disease. Next, it is important that dissecting the breakdown of N-acetylcysteine (NAC) can provide us with insights to modulate the level of H_2S.

A three-step viewpoint examines whether H_2S:

- may be an antiviral host factor;
- may be generated from N-acetylcysteine;
- may act multi-targeted in SARS-CoV-2 infection.

Viewpoint 1. H_2S is an antiviral host factor

Recently a clinical study defined serum H_2S a prognostic factor in COVID-19 [7].

In severe COVID-19 pneumonia cases (n = 74) the authors found that **for mortality**:

- low serum levels of H_2S on day 1 had the best trade-off for sensitivity and specificity;
- decrease in serum level H_2S from day 1 to day 7 greater of 36% as the best discriminator;
- mortality after 4 weeks was 32% *vs* 4.1% for suboptimal *vs* optimal level H_2S;
- serum H_2S was negatively associated with IL-6, Procalcitonin and CRP;
- the 4 weeks survivors are those who consume less of this H_2S.

This evidence suggests that the reduction of H_2S bioavailability may be considered as an indicator of enhanced pro-inflammatory response and that the administration of exogenous H_2S may be viewed as a pharmacological strategy to restore H_2S plasma levels in order to counteract the severe consequences of COVID-19 infection [7].

This also may give rise to a first statement that H_2S is a Host Factor in COVID-19.

About H_2S. H_2S is endogenously produced out of sulfur amino acids (SAA) like cysteine, and it's level is very strictly regulated. After all, H_2S was previously known as a poison.

H_2S is produced from L-cysteine by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and from 3-mercaptopyruvate (3MP) also mercaptopyruvate sulfurtransferase (3MST) produces H_2S, which is produced from cysteine and α-ketoglutarate by cysteine aminotransferase (CAT). SAA and their derivatives transport sulfur through successive oxidation reactions, which then also release H_2S, which once produced, can travel significant distances within and between different cell types, and can act as an autocrine and paracrine messenger. H_2S permeates all membranes freely as a gasotransmitter (like NO and CO) and unlike classical regulators of signal transduction, it acts independently of transmembrane receptors [8]. Produced in mammalian tissues, H_2S acts as biological mediator and signals many important physiological processes in humans. Figure 1 streamlines clearly the sulphur redox reactions [9].

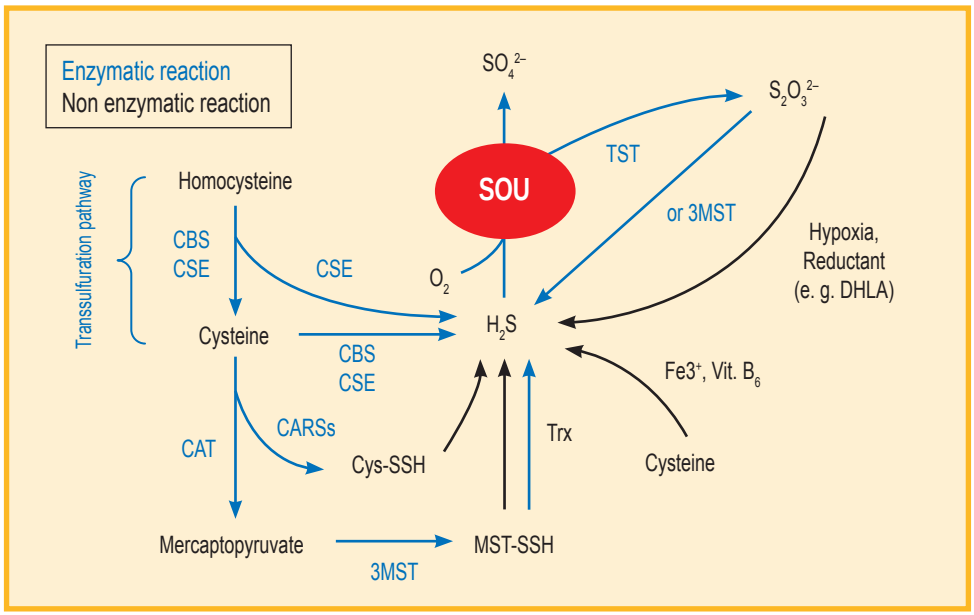

Figure 1. H_2S release (*E.Marutani* [9])

Рис. 1. Механизмы образования H_2S (*E.Marutani* [9])

H_2S-antiviral evidence in preclinical studies. Anti-viral and anti-inflammatory effects

In four preclinical studies the group of *C.Casola* [10 – 13] uncovered a critical protective role of H_2S in RSV infection *in vitro* and *in vivo*, by modulating innate inflammatory responses and viral replication.

They used enzyme blocking, donor H_2S and knock-out models and they reduced the viral replication and chemokine secretion by modulation of transcription factors nuclear factor (NF)-ϰB and the interferon regulatory factor (IRF)-3. They uncovered two mechanisms that were at play. Firstly donor H_2S treatment drastically reduced the secretion of the cytokines IL-6, TNF-α and G-CSF, as well as the chemokines IL-8, RANTES, IP-10, MCP-1 and MIP-1β from infected cells. Secondly airway epithelial cells infected with RSV then display a decreased ability to generate endogenous H_2S and enhanced degradation of H_2S, indicating that viral infection leads to changes in H_2S cellular homeostasis or even to H_2S depletion. These well-designed preclinical studies provided a solid foundation for the antiviral and anti-inflammatory effects of H_2S. Therefore, additional studies were expected to elaborate on this.

Currently the rationale for using H_2S in COVID-19 is supported in several other studies, such as *X.Yang* [14], *M.B.Evgen'ev* [15], and *V.Citi* [16].

The antiviral activity of a series of H_2S releasing molecules and reference H_2S donors (e.g., GYY4137 and sodium hydrosulfide), was first tested and this preliminary screen showed that most of the sulfur molecules provided a significant antiviral effect.

A disulfide compound (XM-01) was selected for further evaluation on both enveloped and non-enveloped viral strains, such as RSV, influenza virus (A/WSN/33 strain) and rotavirus. As observed in previous GYY4137 studies, XM-01 showed antiviral effects on enveloped viruses. Remarkably, no activity was observed on non-enveloped viruses such as rotavirus. Since the antiviral activity may be due to viral membrane changes, GYY4137 and its analogues may be useful against enveloped viruses especially at the time of viral entry into the host cell. Recently, a paper hypothesized that H_2S may exhibit antiviral activity against SARS-CoV-2 by interfering with the ACE2 receptor and TMPRSS2 [14].

Viewpoint 2. N-Acetyl cysteine generates H_2S

The above preclinical research leads us to suggest that it may be useful to generate H_2S in mammals. Previous research suggests that NAC is able to do this: *R.C.Zanardo* [17] using intravital microscopy in animals found anti inflammatory effects at the leuko-endothelial interface induced not only by donor H_2S but also by NAC. Inhibition of the CSE enzyme reversed all these NAC effects. This suggested, for the first time to our knowledge, that NAC could generate H_2S. *D.Ezerina* [18] confirmed this hypothesis about NAC, by dissecting in NAC the antioxidant effect apart from the H_2S-generating potential. NAC-derived cysteine has been shown to be desulfurated to generate hydrogen sulfide which is then oxidized in mitochondria to sulfane sulfur species. These sulfane sulfur species would be the actual substances responsible for mediating the antioxidant and cytoprotective effects we previously attributed to NAC. A different degradation pathway for NAC may be used, via cysteine and 3 MP, to generate H_2S and sulfane sulfur species such as persulfides [18 – 21]; later *K.Zuhra* [22] confirmed this finding and also *P.K.Yadav* [23] suggest NAC, serving as a source of cysteine, could support MST activity.

It may be appropriate to restore cysteine and H_2S levels immediately after exhaustion by the SARS-CoV-2 infection, in order to maintain the antiviral and anti-inflammatory effects.

This can be achieved in two different ways:

a) Taurine suppletion:

- In the breakdown of cysteine the CSE enzyme is strongly boosted by taurine.
- Taurine increases the expression of the H_2S-synthesizing enzymes CBS and CSE, and thereby it contributes strongly to increase (doubling) the endogenous H_2S level in a human RC trial [24, 25]. An animal study supports this principle [20]. This option is only apt if there is sufficient substrate (cysteine): otherwise consider option b).

b) N-acetylcysteine suppletion:

- Importantly the (extracellular located) NAC in itself should not be considered a powerful antioxidant: its power is the targeted replenishment of the intracellular glutathione (GSH) stock in deficient cells and it is unlikely to be effective in cells already packed with GSH. This intracellular GSH stock is available for the formation of cysteine. And, if necessary, this cysteine can be further desulphurized to produce H_2S combined with a so-called sulfansulfur (the latter is the actual antioxidant).To investigate the antiviral therapy against the SARS-CoV-2 virus, we opt to use this endogenously produced H2S, by administering the prodrug NAC. It is possible now to conclude that treatment of cells with the Cys-prodrug NAC triggers endogenous H_2S production.

Viewpoint 3. H_2S acts multi-targeted in SARS-CoV-2 infection

3a. The Innate Immunity and Amino acids

Suppression-Exhaustion-Suppletion. The components of the innate immune system act as first responders for the detection and clearance of viral infections. But many viral infections evade the host innate immune response, sometimes resulting in a complete Host Shut-Off (reviewed in detail [26]).

Then the virus will hijack the host's defenses by total capture of the cellular translation machinery for its own use.

In addition, the virus bypasses the type I interferon (IFN-I) response, which normally promotes an antiviral state in both the infected and neighboring cells, limiting viral replication and inducing apoptosis to protect the organism from virus spread. Indeed, coronaviruses have evolved multiple strategies to escape and to counteract innate detection and IFN-I production. Such efficient

strategies allow the virus to replicate and disseminate in infected individuals without encountering the initial host defense. While many arms of the innate immune response are potently activated by COVID-19, in comparison to other respiratory viruses, SARS-CoV-2 infection drives a lower antiviral transcriptional response. This is marked by low IFN-I and IFN-III levels and elevated chemokine expression, suggesting that some aspects of the innate immune response to COVID-19 might actually benefit from a more careful amplification [27].

The secretion of both *IFN-α* and *-β* were all reduced by donor H_2S [13].

Also, significant dysregulation of monocytes and macrophages seems to be a feature of severe COVID-19, apart from a decrease in T-cell levels, a significantly higher neutrophil-to-lymphocyte ratio (NLR) and depleted peripheral NK-cell counts. The number of total T-cells, $CD4^+$ and $CD8^+$ T-cells were dramatically reduced in COVID-19 patients and correlated negatively with survival [28]. COVID-19 patients show significantly higher levels of the exhausted marker PD-1 [29]. T-cell numbers in patients were negatively correlated to serum IL-6, IL-10, and TNF-α concentration, but H_2S can positively impact these concentrations. It reduced IL-6 and TNF-α-induced NF-ϰB activation. H_2S also potentiates T-cell activation [30].

On the other hand, SARS-CoV-2 infection also caused a rapid depletion of the sulfur-containing amino acids (SAA) as a function of oxidant stress or inflammation-induced proteolysis. Cysteine and taurine levels then tended to decrease [31], especially with moderately high IL-6. In contrast, oxidized forms of SAA (methionine sulfoxide, cystine) increased [31] All together, this will reduce the bioavailability of cysteine as a substrate for H_2S. Likewise NAC treatment safely replenishes whole blood GSH and T-cell GSH in HIV-infected persons with a depletion of SAA [32, 33]. GSH is a stock for cysteine. A rapid response of changes in Cys levels is observed within hours of NAC supplementation [34].

3b. NLRP3 Inflammasome

A Potential Drug Target In COVID-19. Although, innate immune mechanisms such as optimal activation of the NLRP3 inflammasome plays an important role in antiviral host defenses, its aberrant activation and downstream mediators often lead to pathological tissue injury during infection [35]. Also, infection with SARS-CoV is known to induce a storm of pro-inflammatory cytokines, especially IL-1β, IL-6, and TNF. These play an important role in the progression of tissue inflammation causing acute respiratory distress syndrome ARDS and often leads to death. Hydrogen sulfide inhibits NLRP3 inflammasome activation and reduces cytokine production [36].

3c. The vascular compartment

ARDS ACE-2 expression endothelioitis coagulation. H_2S modulates leukocyte-mediated inflammation. In the leuko-endothelial interface it interferes with leukoadhesion and leukoinfiltration [17]. In COVID-19 this could have some relevance for the complicating ARDS [37] or SIRS and other complicating vascular problems like myocardial infarction, stroke, thrombo-embolia [38, 39].The ACE2 receptor is not only necessary for the viral entry in the AECs, but the ACE2 receptor is also expressed in the vascular system [40].

SARS-CoV infection reduces ACE2 expression in lung cells and that loss of pulmonary ACE2 function is associated with acute lung injury. As virus-induced ACE2 downregulation may be important for disease pathology, then on the contrary ACE2 upregulation by H2S (as shown by Lin [41]) may attenuate the acute lung injury. At last, a deficiency of H_2S-producing enzymes results in hypertension, and administration of H_2S donors lowers blood pressure and protects against organ damage in the experimental setting [42].

3d. Pharmaceutical aspects of N-Acetylcysteine

Disulfide bonds. High Dosing: NAC is used safely for some 30 years in case of acetaminophen intoxication.

N-Acetylcysteine may be applied as tablet, intravenously or by nebulization. Its mostly mentioned anti-oxidant effect was dissected in 2018 from its H_2S generating effect in a breakthrough study [18, 19], later confirmed by two other studies.

NAC easily penetrates cells where it is deacetylated to yield L-cysteine thereby promoting GSH synthesis. Therefore, NAC works per se in the extracellular environment and as a precursor of GSH inside cells. Accordingly, all its intracellular effects are mediated by GSH replenishment [43].

NAC has mucolytic properties on sputum by breaking disulfide bonds in the mucus, and H_2S shows only antiviral effects on enveloped viruses, which use a fusion protein for the cell entry [16].

The ability to break disulfide bonds may be important to this fusion process, as the SARS coronavirus peak S2 domain is flanked by cysteine residues C822 and C833 and this domain is required for membrane fusion activation. FP2 has some effect on the membrane sequence.

Two cysteines (C822 and C833) within FP2 are considered an internal disulfide bond, giving this domain a loop structure. It is questionable whether these disulfide bonds will resist the local H_2S and/or NAC in the membrane fusion region [44].

A.L.Lai et al. tested whether such a disulfide bond could play a role in the FP2-mediated membrane ordering. They found that in the presence of 5 mM dithiothreitol (DTT), a reducing agent that removes disulfide bonds, the membrane-ordering effect of FP2 was abrogated [45].

Not only at the cell entry (ACE2 receptor) but also in the cytosol it makes sense to consider a same antiviral effect. The RNA-dependent RNA polymerase (RdRp), also named nsp12 may be a target in SARS-CoV-2. In the cytosol this is the central component of the coronaviral replication and transcription machinery.

Recently *Y.Gao et al.* [46] were able to identify an N-terminal β hairpin (D29 to K50) which inserts into the groove clamped by the NiRAN domain and the palm subdomain in the RdRp domain and forms a set of close contacts to

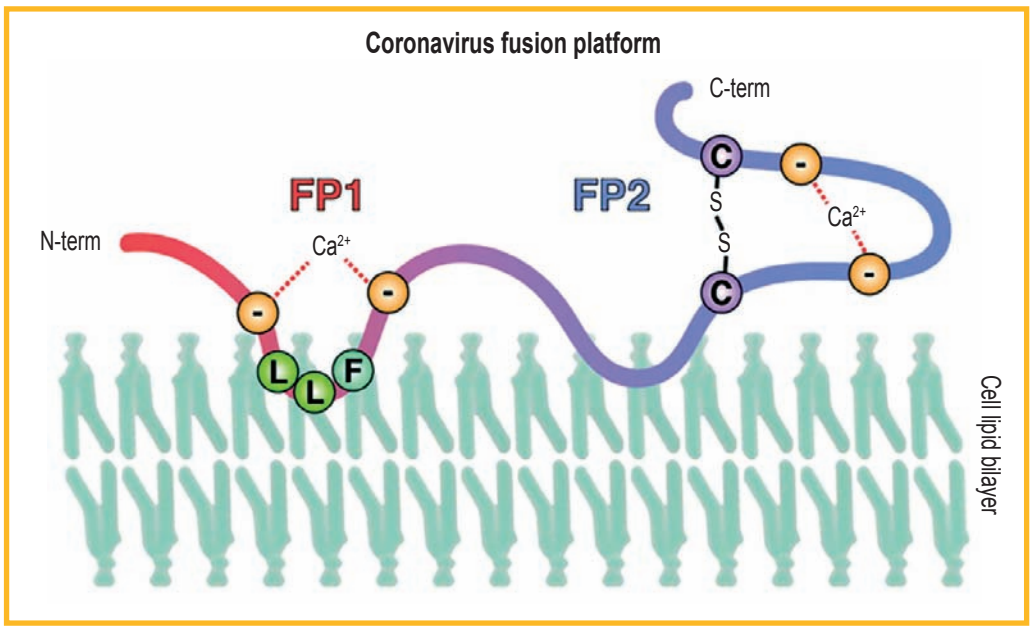

Figure 2. Receptor – Fusion peptide – target for NAC and H_2S [45]

Рис. 2. Рецептор – пептид слияния, мишень NAC и H_2S [45]

stabilize the overall structure. In the absence of DTT they showed C301 to C306 and C487 to C645 to form disulfide bonds. But – in the presence of DTT – chelated zinc ions were present in the same location as that observed in SARS-CoV. So, it is doubtful whether the disulfide bonds in the nsp12-nsp7-nsp8 complex of the SARS-CoV-2 virus will resist the local effects of H_2S and/or NAC.

Note: H_2S functions as a gasotransmitter and is not bothered by membranes.

3e. Timing of the treatment

Initial virus load and subsequent inflammation. Timing of treatment is important when this NAC-Taurine model is combined with other antiviral treatments (e.g. Tocilizumab; Dexametasone); their sequence is depending on the stage of the disease [47]. In one trial, only high-dose NAC was given and also started too late without clinical benefit [48].

SARS-CoV-2 infection initially has a very high viral load at the onset of symptoms, so the early antiviral component (e.g. NAC orally or per nebulizer) is important. If symptoms are worsening around 7 – 10 days, adding dexametasone to NAC should be considered to reverse the progressive inflammation.

One thing should be emphasized with the NAC/H_2S administration:

- only start at an incipient viral infection or a nearby viral threat;
- especially with an encapsulated virus. Chronic intake of NAC/H_2S is not recommended.

Viewpoint 4. Is H_2S clinical relevant in COVID-19?

4a. H_2S a prognostic factor in severe COVID-19?

A Greek study was published defining serum H_2S a prognostic factor in COVID-19 [7].

In COVID-19 pneumonia cases ($n = 74$) authors found that **for mortality**:

- Low serum levels of H_2S on day 1 had the best trade-off for sensitivity and specificity;
- A decrease in level H_2S from day 1 to day 7 greater of 36% as the best discriminator;
- The mortality after 4 weeks was 32% *vs* 4.1% for suboptimal *vs* optimal level H_2S;
- Serum H_2S was negatively associated with IL-6, Procalcitonin and CRP;
- The 4 weeks survivors are those who consume less of this H_2S.

This lead to considerations for exogenous H_2S supplementation as treatment strategy.

4b. Checking some risk factors in COVID-19, related to low H_2S

Risk factors in COVID-19 are known in general. Nevertheless, a number of risk factors for a serious course have been found that may suggest a relationship with H_2S levels:

- ***Gender risk***: more men then women affected: Firstly it may relate to the ACE2 gene, only located on the X chromosomes. In addition: estrogen boosts expression of CSE in the vasculature, so boosting generation of H_2S [49];
- ***Hypertension:*** a deficiency of H_2S-producing enzymes [42];
- ***Diabetes***: the lower H_2S blood values [50];
- ***Obesity***: blood levels of H_2S were twice as low *vs* normal [51];
- ***Advanced age***: the efficiency of glutathione synthesis and glutathione tissue levels decline with age. This age-related deficit in GSH can be corrected with supplemental NAC [52]. From that GSH stock, cysteine generates H_2S;
- ***Young patients, ages 2 – 15 yrs:*** Multisystem inflammatory syndrome in children (*MIS-C*) is a serious condition that appears to be linked to *coronavirus* disease 2019 (*COVID-19*) with inflammation in multiple organ systems and features of *Kawasaki* disease following to SARS-CoV-2 infection; in the acute phase, plasma H_2S is low [53];

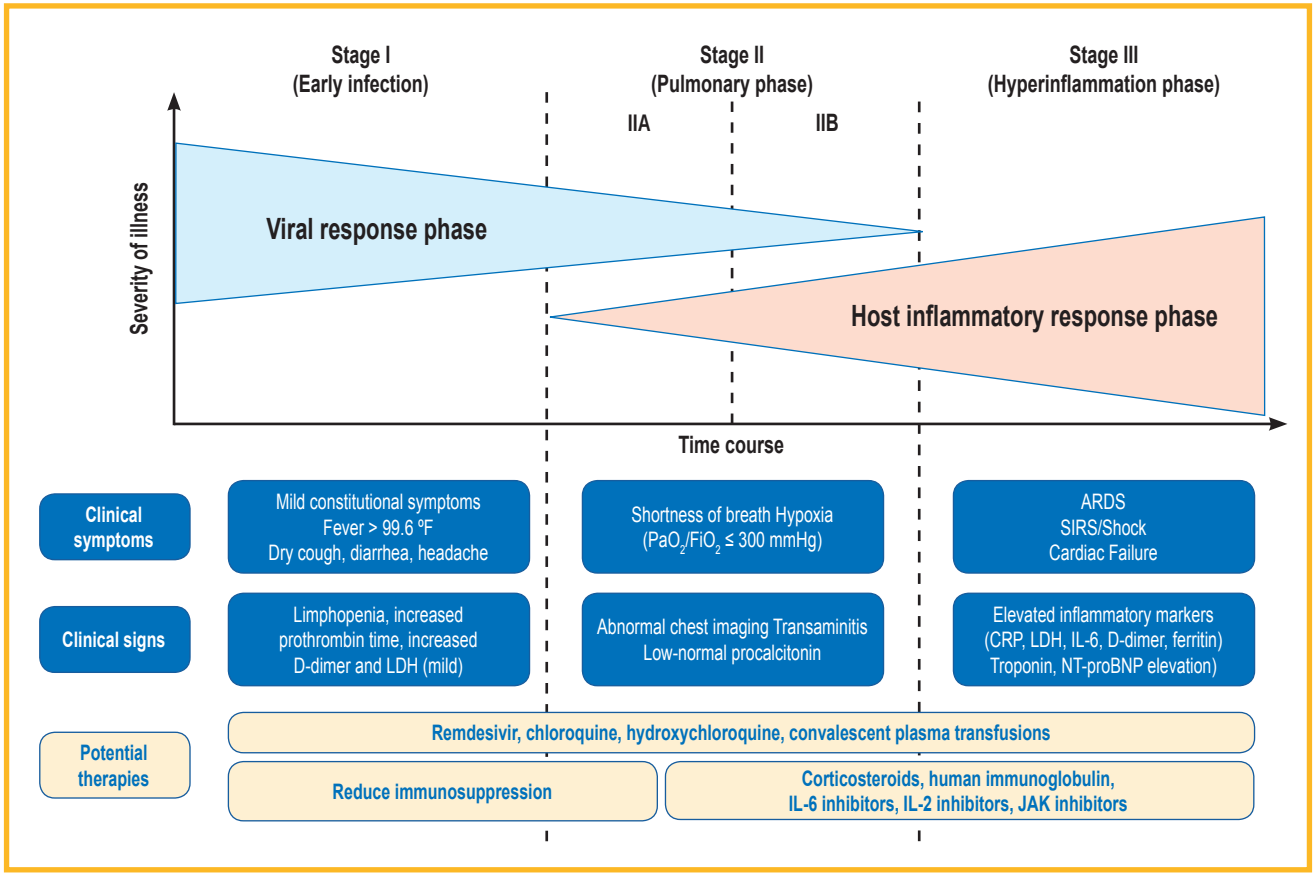

Figure 3. The Timing of Treatment in COVID-19 [47]
Рис. 3. Сроки проведения терапии COVID-19 [47]

- ***Beneficial course in infants and toddlers***: taurine in breast milk or added to bottled milk, increases the H_2S synthesis [21].

4c. Successful Case reports (n = 37):

- *H.Ibrahim* [54] described 10 consecutive patients with severe COVID (10 on ventilator and 9 on ECMO) with good clinical response on high dose NAC, with corresponding decrease in CRP and ferritine. No mortality here, quite unexpected (expected 37.4% [55]). Steroids were also administered.
- *V.V.Gaynitdinova* [56]; In a RC trial intravenous NAC 1,200 – 1,500 mg/day (n = 24) was compared to standard (hydroxychloroquine, azithromycine, enoxaparine and dexametasone; and tocilizumab when CRP ⩾ 60 mg/l). NAC safely and effectively attenuated oxygenation, CT patterns and inflammation parameters in stage II hospital COVID pneumonia, effects were ascribed to glutathione suppletion.
- *R.I.Horowitz* [57] described in 2 COVID patients immediate improvement after suppletion with glutathione (GSH) and NAC. Worth to mention: NAC is a direct precursor of GSH. Glutathione inhibits viral replication in mice [58].
- *C.Puyo* [59] succesfully treated one critical ill COVID patient with high dose NAC intravenously at 75 mg/kg over 4 hours, then 35 mg/kg over 16 hours, followed by 17 mg/kg over 24 hours on Day 2. Also HCQ was given on day 1 and day 2. Detailed clinical parameters showed fast improvement, although a complicating
- Trombo embolic activation was seen.

4d. Trials in progress (as off augustus-2020):

Additional research on NAC in COVID-19 is started in 3 clinical trials (USA and China):

- NCT04419025; Boston Cambridge Health Alliance;
- NCT04279197; Shanghai ShuGuang Hospital;
- NCT04374461; Memorial Sloan Kettering Cancer Center NY.

Caution! If only NAC is started and too late (later then 7 – 10 days after the onset of symptoms) and no course of steroids is added around that time, then the clinical result will be negative (*J.C.G. de Alencar* [47]). *H.K.Siddiqi* [46] proposed a logical sequence in medication.

4e. NAC used in other (NON-Corona) viral states

Influenza and HIV-1:

- In influenza NAC was effective with a lower incidence and lower burden of disease in a predominant elderly cohort [60]. Oral N-acetylcysteine did not prevent viral infection, but with equal rate of seroconversion strongly reduced the incidence of clinically apparent H1N1 disease (NAC 25% *vs* 75% placebo).
- Severe H1N1 Influenza pneumosepsis was treated with high dose NAC (100 mg/kg) continuous 3 days.

It showed (twice) a fast improvement in weaning, oxygenation and CRP [61]. However different influenza strains show different effects of NAC [62].

- In HIV-1 patients: Lower concentration in blood of cystine, tryptophan and methionine were suppleted with oral NAC [63]. The concentrations of cysteine and glutathione increased in mononuclear cells of patients with HIV infection [64].

Sadly no H_2S was measured in these studies.

Discussion

This review reveals a role for endogenous hydrogen sulfide (H_2S) as a fundamental defense mechanism against viral infections by three steps:

1) H_2S is acting as an antiviral host factor;

2) Endogenous H_2S can be generated by N-acetylcysteine (NAC) and taurine;

3) H_2S acts multi-targeted in SARS-CoV-2 infection.

- Based on the studies reviewed above (*in vitro, in vivo*, preclinical and clinical), H_2S emerged as a host factor for viral infections, while in addition the serum H_2S level was attributed a role as a prognostic marker of COVID-19 pneumonia.

In corona the natural clinical course is usually in 80% mild; in 15% a desaturation requiring hospitalization occurs and in 5% ICU care is needed. This primary natural healing process suggests that an antiviral host factor is active here, but that some risk factors are disturbing that natural course: gender risk, diabetes, obesity, advanced age, in contrast to beneficial course in infants and toddlers. These were already checked in paragraph 4b.

- The possibility that NAC could act as an H_2S donor was initially in 2016 demonstrated by *R.C.Zanardo* [17] and in 2018 by *D.Ezerina* [18]; then endorsed by others in 2019 and 2020. This finding has since been somewhat overlooked or ignored, with more emphasis on the often cited "antioxidant" activity (which is actually only related to the sulfane-sulfur component) and less emphasis on the associated H_2S release by NAC.
- COVID-19 can be viewed from two treatment sides: *X.Yang* [14] looked at it from a "H_2S point of view", while *F.Poe*, *J.Corn* [65] and *S. de Flora* [43] from a "NAC as an anti-oxidant" position. In fact, we may conclude that they are all looking at the same process and we are not surprised that the results are the same or match flawlessly. Both pathways (antioxidant and H_2S) signal via oxidation reactions with protein cysteine sulfur and both produce identical effector responses [66].
- Multiple targets can be used by H_2S against the coronavirus, such as cell entry, the virus replication and the escalation of inflammation to a cytokine storm, which were also targets explored in the recent drug trials, as mentioned in the introduction.

The attack of the virus suddenly collapses both the cellular innate immunity as well the (supply of) sulfur amino acids. The latter ensures that H_2S decreases quickly.

On the other hand, to correct this acutely, the generation of endogenous H_2S also appears to be a very dynamic, fast-acting process and this suggests that, from a safety standpoint, H_2S will immediately degrade once it has fulfilled its function.

But even in severe cases of COVID-19, a fast and high dose of NAC appears to have sufficient effect for supplementation, according to the clinical success in the patient cases [54] This safety is also evidenced by the relatively low serum and tissue concentrations measured *in vivo* versus the high and fast concentrations by the artificial H_2S donors in the previous *in vitro* studies. Endogenous H_2S generation is apparently safe, but further intensive dose finding studies are warranted, especially when administered in the high dose range.

The rationale for using H_2S in COVID-19 is supported in 4 other studies, like *X.Yang* [14]; *V.Citi* [16]; *M.B.Evgen'ev* [15]; *M.Datillo* [53], but in those studies the use of H_2S has not been linked to NAC as a H_2S donor.

On the other side *F.Poe*, *J.Corn* [65] and *S. de Flora* [43] investigate a rationale for using NAC in COVID-19, but then again without regarding the H_2S generation power of NAC.

Also, the timing of combination in treatment is very important and is determined by the ratio of the viral load to inflammation. By starting antiviral therapy in a timely manner and then containing the inflammatory process at an early stage, it is possible to prevent the disease from developing and causing severe damage during the inflammation phase [46]. NAC only, and started too late was without clinical benefit [48].

Preferentially the viral killing therapy (NAC, orally or by nebulizer) is introduced in an early phase. Steroids [e.g. dexametason] in the second phase, will follow the evidence of the Recovery trial [1].

The large safety margin of NAC and the various options in its administration make it possible to scale up with the time course and severity of the SARS-CoV-2 infection, starting with prevention in case of (suspected) virus contact, via home medication [home confinement for isolation or for quarantaine] to hospital or ICU application.

A proposal for such an incremental therapy is on a Poster "Adapted Incremental Treatment Plan in COVID-19" [67].

The repurposed drug NAC used here, is over-the-counter, very safe, without side effects, and cheap, making it very feasible for conducting randomized controlled clinical trials [6] especially in low income countries. DNDi [68] works closely with 26 prominent African and global research and development (R&D) organizations to coordinate the rapid acceleration of development for promising medical countermeasures at 19 sites in 13 countries by the ANTICOV consortium. It would be worthwhile to consider NAC for inclusion in one of the ANTICOV master protocols.

NAC as a endogenous H_2S generator reactivates the collapsed innate immunity in COVID-19 but off course it is also clear that not one drug alone will give a sufficient effect.

An intelligent combination of different drugs may prove necessary, but even if it is not possible to fully suppress the virus, also a mild course with less hospitalizations or ICU admissions, will suffice.

Conclusion

The described process of endogenous H_2S generation provides us with a multi-targeted antiviral Host Factor in COVID-19 infection by reactivating the collapsed innate immunity. The use of this endogenously generated H_2S as a pharmacological antiviral agent is already supported by the successful results in now three dozen case studies in very severe COVID pneumonia.

We may assume that the milder phases of COVID-19 may also be treatable with this antiviral host factor, perhaps even preventively, avoiding unwanted socio-economic measures and healthcare overload.

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