Literature search summary :
an approach to H2S protective and therapeutic in COVID-19 disease ,
in which endogenous H2S formation from NAC is essential and beneficial .

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Introduction to COVID-19

Acute febrile respiratory illness (FRI) leading to respiratory failure is a common reason for admission to the ICU. Viral pneumonia constitutes a portion of these cases, and a small number of viral agents may lead to acute FRI, respiratory failure, and ARDS: seasonal influenza, avian influenza, coronavirus associated with severe ARDS, respiratory syncytial virus, adenovirus, varicella, human metapneumovirus, and hantavirus.

In SARS-CoV (2002) combination of lopinavir and ritonavir was associated with substantial clinical benefit,but in 2020 again is negative for current COVID-19 infection. So no antiviral treatment has been proven to be effective.
Only supportive and symptomatic therapies were used for most COVID-19 cured patients : Fluids for hydration, antiphlogistics, antibiotics (preventive) for secondary bacterial infection, supplemental oxygen, ultimately mechanical ventilation in most severe cases.

Predictors of fatality from a recent retrospective, multicentre study of 150 confirmed COVID-19 cases in Wuhan, China, included elevated ferritin (mean 1297·6 ng/ml in non-survivors *vs* 614·0 ng/ml in survivors; p<0·001) and IL-6 (p<0·0001).Also D-dimere is a discerning factor.This suggests that mortality might be due to virally driven hyperinflammation and that those laboratory results can be used to scale up treatment.
Conclusion :New treatment options with antiviral and antiinflammatory effect are desperately needed.

Antiviral and Antiinflammatory model in the airways:

The following animal study identifies H2S as a novel molecule that can modulate viral replication and airway inflammatory responses.

Hydrogen Sulfide Is an Antiviral and Antiinflammatory Endogenous Gasotransmitter in the Airways ;Role in Respiratory Syncytial Virus Infection
Ivanciuc et al.: *Am J Respir Cell Mol Biol 55,684–696,*
https://*doi*.org/*10.1165/rcmb.2015-0385OC*

This study identifies H2S as a novel molecule that can **modulate viral replication and airway inflammatory responses**, both important determinants of lung injury in respiratory syncytial virus infection, with the potential for rapid translation of such findings into novel therapeutic approaches for viral bronchiolitis and pneumonia.

The authors have shown previously **) that levels of intracellular H2S modulates cellular responses and viral replication in an *in vitro* model of paramyxovirus infection,including RSV.

In this study, they provide evidence that H2S has a protective role in RSV infection *in vivo* as well, by modulating **both** inflammatory responses and viral replication.Overall, their results support the notion that the antiviral activity of H2S appears to be largely **independent** of its antiinflammatory properties.

**) see additional references at the end

Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation Zanardo 2006 *FASEB J.*20,E1411 DOI: 10.1096/fj.06-6270fje

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This animal study demonstrates suppression by H2S of leukocyte adhesion ,infiltration and edema formation in a vascular inflammatory response
[? reminiscent of the pathogenesis in ARDS syndrome ?.
Would too low or a shortage of H2S play a role in the pathogenesis of ARDS?]
Also a warning to investigate the interaction NSAID vs H2S genesis.

In this animal study, the authors have demonstrated that
a) several H2S donors can suppress leukocyte adherence to the vascular endothelium and can reduce leukocyte infiltration and edema formation.
These effects of H2S were seen irrespective of the inflammatory stimulus used (carrageenan, aspirin, fMLP).

b) Suppression of endogenous H2S synthesis, through blockade of CSE, resulted in enhanced leukocyte adhesion, leukocyte infiltration, and edema formation.

These findings therefore suggest an important role for endogenous H2S as a modulator of some of the key components of acute inflammatory responses, particularly those occurring at the leukocyte-endothelial interface.

Of the four H2S donors used in this study, only N-acetylcysteine requires metabolism in order for H2S to be released.The observation that the antiinflammatory actions of N-acetylcysteine were reversed by an inhibitor of CSE (-cyanoalanine) is consistent with the effects being mediated by H2S.
Also the ability of N-acetylcysteine to reduce carrageenan-induced leukocyte infiltration was dependent on CSE activity.

In summary, the results of this study have demonstrated a role for endogenous H2S as a modulator of key inflammatory events occurring at the interface of leukocytes and the vascular endothelium. H2S functions as a tonic regulator of leukocyte adherence to the endothelium and of endothelial permeability.
The anti-inflammatory effects of H2S appear to be mediated by activated K_{ATP} channels.

Pro memori :Authors reported that NSAIDs suppress H2S synthesis by reducing expression of CSE. The accompanying reduction of H2S synthesis may contribute to the increase in leukocyte adherence that is seen after NSAID administration. DOI: 10.1053/j.gastro.2005.07.060

N-Acetyl Cysteine Functions as a Fast-Acting Antioxidant by Triggering Intracellular H2S and Sulfane Sulfur Production

Ezerina et al, Cell Chemical Biology 2018;25,447 doi.org/10.1016/j.chembiol.2018.01.011

This preclinical study presents a completely new view on the processes of H2S generation and resultant antioxidant effects ;
The old stories about NAC now unmasked:

Overall, a consensus explanation for the cytoprotective and antioxidative agency of NAC does not seem to exist, as noted previously (DOI: 10.1016/j.bbagen.2013.04.016 and 10.1100/tsw.2010.104).
Hence,it remains unclear how the large number and wide-ranging variety of studies reporting the ability of NAC to rescue cells from pro-oxidative and electrophilic insults can be explained.

In this paper, authors show now that NAC-derived cysteine is desulfurated to generate hydrogen sulfide,which in turn is oxidized to sulfane sulfur species, predominantly within mitochondria.

They offer a new perspective on the antioxidative prowess of NAC, and demonstrate that NAC is first desulfurated to H2S, which is subsequently oxidized to sulfane sulfur species in mitochondria.

They also provide evidence suggesting that sulfane sulfur is a key mediator of the anti-oxidative and cytoprotective effects of NAC, and that its generation is dependent on the activities of sulfane sulfur-producing enzymes 3-mercaptopyruvate sulfurtransferase (MST) and/or sulfide:quinone oxidoreductase (SQR).

Enzymatic synthesis of H2S

DiNicolantonio JJ, *et al. Open Heart* 2017 DOI: 10.1136/openhrt-2017-000600

With regard to the former study, now introduction of nutraceuticals for enzymatic synthesis of H2S can be proposed in this animal study.

At least three enzymes generate endogenously H2S in the human body: [DOI:10.1155/2015/925167]

cystathionine γ-lyase (CSE),
cystathionineß-synthase (CBS), and
3-mercaptopyruvate sulfurtransferase

CSE appears to be of primary importance. It is notable that CSE's Km for cysteine has been found Km to be around 3.5 mM—a concentration far higher than 70 ambient levels of free cysteine in cells. It should follow that supplementation with nutraceuticals that can boost cellular levels of cysteine will boost CSE-mediated H2S production to a commensurate degree.

Hence, there is reason to suspect that increasing cellular cysteine levels should proportionately increase H2S generation by all three enzymatic sources of this gas.

N-acetylcysteine (NAC),a well-tolerated and well-absorbed nutraceutical that is rapidly cleaved in vivo to yield cysteine, has long been employed clinically to enhance cellular levels of glutathione. The rate-limiting enzyme for glutathione synthesis, γ-glutamylcysteine synthetase, also has a rather high Km for cysteine, which is why NAC supplementation is effective for boosting glutathione levels.
The clinical efficacy of NAC in this regard demonstrates that feasible NAC intakes do indeed meaningfully enhance the cysteine content of cells.

There is no evident reason why supplementary NAC should not in a comparable manner stimulate H2S production by CSE.

Taurine Supplementation Lowers Blood Pressure and Improves Vascular Function in Prehypertension

Sun *Hypertension* 2016;67 ; https://doi.org/10.1161/HYPERTENSIONAHA.115.06624).

This first human randomized, double-blind, placebo-controlled clinical trial investigated taurine's ability to lower modestly elevated BP but also the amazing effects of taurine supplements on H2S generation :
"The TURBO goes on"

Supplemental taurine increases (vascular) CSE expression.An exciting recent research discovery may provide an additional complementary strategy for boosting CSE-mediated H2S production.
In relatively high dietary doses,the physiologically essential amino acid osmolyte taurine has long been known to exert important protective effects in vascular rodent models.Taurine is essentially free of toxicity (except in severe kidney failure), well absorbed, quite inexpensive in multigram doses, highly soluble,devoid of flavour can be added in high amounts to any food/beverage.

The truly intriguing finding was **a virtual doubling of plasma H2S.**
Plasma H2S levels in the taurine group rose from 43.8 µmol/L at baseline to 87.0 µmol/L after 12 weeks ($p<0.001$)
They also found that expressions of both CSE and CBS rose markedly and **dose dependently**; the increase in **CSE expression was over fivefold** at 40 mM taurine for 24 hours.

PM: *Indeed, taurine is currently a standard constituent of so-called 'energy drinks.' (Unjustly, the dangerous side effects of the hypercaffeination which overconsumption of these drinks can induce have led some to question taurine's safety; ironically, the taurine may make these drinks safer.)*

A nutraceutical regimen for boosting H2S synthesis

Assuming that the recent research linking taurine with H2S can be replicated (one must bear in mind that, to date, only one clinical study has reported the impact of supplemental taurine on plasma H2S levels),
it is logical to propose that a supplementation regimen featuring clinically meaningful doses of both NAC and taurine should boost endogenous production of CSE.Clinical studies evaluating the impact of various dose regimens of taurine and NAC on plasma H2S levels appear warranted.

Dose range

The dose range in which NAC has shown clinical benefits—and hence presumably achieves a meaningful increase in tissue cysteine levels—is 1200–1800 mg daily, in divided doses.
Taurine has been used in daily doses as high as 6 g without any evident adverse effects; 1.6 g daily was sufficient to elevate H2S in a trial in patients with prehypertension (Azuma 1985,1994) https://doi.org/10.1002/clc.4960080507

PM:
In regard to NAC, it has been suggested that the elderly have an increased requirement for cysteine owing to the fact that the efficiency of glutathione synthesis and glutathione tissue levels decline with age. This age-related deficit in glutathione can be corrected with supplemental NAC. This observation may help rationalise epidemiology which concludes that, whereas relatively low dietary protein intakes are associated with lower mortality risk in people under 65 (possibly by downregulating growth factor activities which drive the ageing process), low protein intakes (as a fraction of total calories) predict higher mortality in those over 65. 10.1136/openhrt-2017-000599

Supplementation with NAC in the elderly may provide health protection by boosting the production of both glutathione and H2S, each of which is crucial for optimal physiological function and health promotion.
NAC may be of particular merit for 'rejuvenating' immune function in the elderly, and alleviating the symptoms of influenza.
Eur Respir J 1997;10:1535 NAC & Flu . PMID: 9230243 DOI: 10.1183/09031936.97.10071535

N-ACETYLCYSTEINE AND INFLUENZA

This is the "retrospective" clinical proof of the concept .

Human-RCT--Doubleblind-Multicenter

Attenuation of influenza-like symptomatology and improvementofcellmediated immunity with long-term N-acetylcysteine treatment

S De Flora et al -European Respiratory Journal 1997 10: 1535 DOI: 10.1183/09031936.97.10071535

Authors studied the effect of long-term treatment with NAC on influenza and influenza-like episodes in a randomized, doubleblind multicenter trial. In total 262 subjects were randomized to receive either placebo or NAC tablets (600 mg) twice daily for 6 months. Patients suffering from chronic respiratory diseases were not eligible, to avoid possible confounding by an effect of NAC on respiratory symptoms.

NAC treatment was well tolerated and resulted in a significant decrease in the frequency of influenza-like episodes, severity, and length of time confined to bed.
Both local and systemic symptoms were sharply and significantly reduced in the NAC group. Frequency of seroconversion towards A/H1N1 Singapore 6/86 influenza virus was similar in the two groups, but only 25% of virus-infected subjects under NAC treatment developed a symptomatic form, *versus* 79% in the placebo group.

Administration of N-acetylcysteine during the winter, thus, appears to provide a significant attenuation of influenza and influenza-like episodes, especially in elderly high-risk individuals. N-acetylcysteine did not prevent A/H1N1 virus influenza infection but significantly reduced the incidence of clinically apparent disease.

The authors speculated that their findings appear to confirm, in a clinical study, the *in vitro* experimental evidence concerning the immunomodulant properties of NAC.
In addition, NAC is known to play a regulatory role on the immune system by modulating production and release of cytokines.

The ACE2 receptor and effect of H2S

Only a few preclinical studies can be found on this subject.

Hydrogen Sulfide Attenuates Atherosclerosis in a Partially Ligated Carotid Artery Mouse model via Regulating Angiotensin Converting Enzyme 2 Expression

Yanjun Lin; Front Physiol. 2017; 8: 782.doi:10.3389/fphys.2017.00782

Hydrogen sulfide has been suggested to play an essential role in atherogenesis.
There is a paucity of information about the association between H2S and angiotensin converting enzyme 2 (ACE2), a novel homolog of ACE, which is also the receptor for the SARS coronavirus. Authors found that carotid partial ligation in high-fat fed apoE−/− mice significantly inhibited endogenous H2S synthesis in the ligated carotide artery. Application of NaHS, an H2S donor considerably attenuated the severity of atherosclerosis with upregulating carotid expression of ACE2, thus converting pro-atherosclerotic angiotensin II (Ang II) to anti-atherosclerotic angiotensin 1-7 (Ang-(1-7)).

Hiding in plain sight:Fedson 2020.mBio doi.org/10.1128/mBio.00398-20.

The host response is a major determinant of the pathogenesis of infectious diseases. Another approach to treating patients with severe COVID-19 infection might be hiding in plain sight. The tissue receptor for the virus is ACE2, which is also the receptor for the SARS coronavirus. ARBs and statins upregulate the activity of ACE2, and higher levels of ACE2 are associated with a reduced severity of ARDS. Both statins and ARBs target the host response to infection, not the virus Both drugs counter endothelial dysfunction by affecting the ACE2/angiotensin-(1–7)/Mas and angiopoietin/Tie-2 signaling axes (9). Combination treatment with these two drugs appears to accelerate a return to homeostasis, allowing patients to recover on their own.

Note:*Whether the upregulation of the activity of ACE2, and higher levels of ACE2 give rise to possibly more corona COVID-19 virus particles to employ angiotensin-converting enzyme 2 (ACE2) for the cell entry,remains to be seen.*

ACE and ACE2 in Inflammation: A Tale of Two Enzymes

Gaddam Inflamm Allergy Drug Targets. 2014;13(4):224.
DOI: 10.2174/1871528113666140713164506
chapter:"THE RAS AND HYDROGEN SULFIDE IN INFLAMMATION"

In the past few years, hydrogen sulfide (H2S) has been identified as an endogenous gas mediator of inflammation.
ACE and ACE2 are metalloprotease enzymes containing zinc (Zn+2) in their structure. Zinc acts as a co-factor for ACE and ACE2. H2S is a gas mediator having potential to react with metal cations in metalloproteins.

Laggner *et al*.reported the direct interaction between H2S and ACE in human endothelial cells (HUVECs). They studied the effect of H2S on activity and mRNA expression of ACE in HUVEC homogenates and reported dose dependent inhibition of H2S on ACE activity but no effect on mRNA expression.
They also studied the effect of H2S on ACE activity in the presence of Zn+2 and reported the counteracted effect of Zn+2 on H2S mediated inhibition of ACE .
These studies together suggest that direct inhibitory effect of H2S on ACE activity results from an interaction with internal Zn+2. However, no interaction between H2S and ACE2 has been shown so far. In addition, H2S and ACE are proinflammatory in most of the inflammatory diseases, but there are no reports on the interaction between these systems.

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Postscript:

(** from page 2): **Further reading: similar studies in viral respiratory diseases :**

2: Bazhanov N, Ansar M, Ivanciuc T, Garofalo RP, Casola A.
Hydrogen Sulfide: A Novel Player in Airway Development, Pathophysiology of Respiratory Diseases, and Antiviral Defenses.
Am J Respir Cell Mol Biol. 2017;57(4):403-410.

3: Ivanciuc T, Sbrana E, Ansar M, Bazhanov N, Szabo C, Casola A, Garofalo RP.
Hydrogen Sulfide Is an Antiviral and Antiinflammatory Endogenous Gasotransmitter in the Airways. Role in Respiratory Syncytial Virus Infection.
Am J Respir Cell Mol Biol. 2016;55(5):684-696.

4: Li H, Ma Y, Escaffre O, Ivanciuc T, Komaravelli N, Kelley JP, Coletta C, Szabo
C, Rockx B, Garofalo RP, Casola A.
Role of hydrogen sulfide in paramyxovirus infections.
J Virol. 2015 May;89(10):5557-68.

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