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A paradigm shift in the role of NSAIDs in COVID-19: new pathological mechanisms and potential treatment targets

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ABSTRACT

Objectives: The pathogeny of SARS-COV2 infection is currently not well defined. In this paper, we present a new perspective of how the SARS-CoV2 infection can lead to severe cases and potential pathways of preventing such cases.

Methods: In the first part, we describe the role of cyclooxigenase 2 and in the second part, we describe the role of hypoxia inducible factor (HIF).

Findings: We hypothesize that cyclooxigenase 2 and hypoxia inducible factor with subsequent inflammation and hypoxia can up-regulate each other in a vicious circle of feed-forward that can ultimately lead to "cytokine storm".

Conclusions: Breaking this cycle early potentially will have beneficial effects either by improving oxygenation (oxygen being given earlier in the course of the treatment) or by inhibiting COX-2. We also believe, that the use of COX-2 inhibitors early in the course of the disease can improve the outcome and clinical trial are urgently needed.

KEYWORDS

Paradigm Shift; NSAIDs; Covid-19; COX-2 inhibitors.

1. INTRODUCTION

The pathogeny of SARS-COV2 infection is currently not well defined. Viral infections can often lead to inflammatory responses mediated by many soluble factors and cellular effectors. The balance between the protective and deleterious effects of these can determine the course after the initial viral infection. In this paper we present a new perspective of how the SARS-CoV2 infection can lead to severe cases and potential pathways of preventing such cases.

We hypothesize the important role played by cyclooxigenases (COX)-2 and hypoxia inducible factor(HIF), the interaction between these two (feed-forward) and the inflammatory and coagulation factors to explain progression of the disease to severe cases; and also how patients are tolerating high levels of hypoxia rarely seen before, as described by many clinicians. Below we explore the role of COX in coronavirus infections (Figure 1).

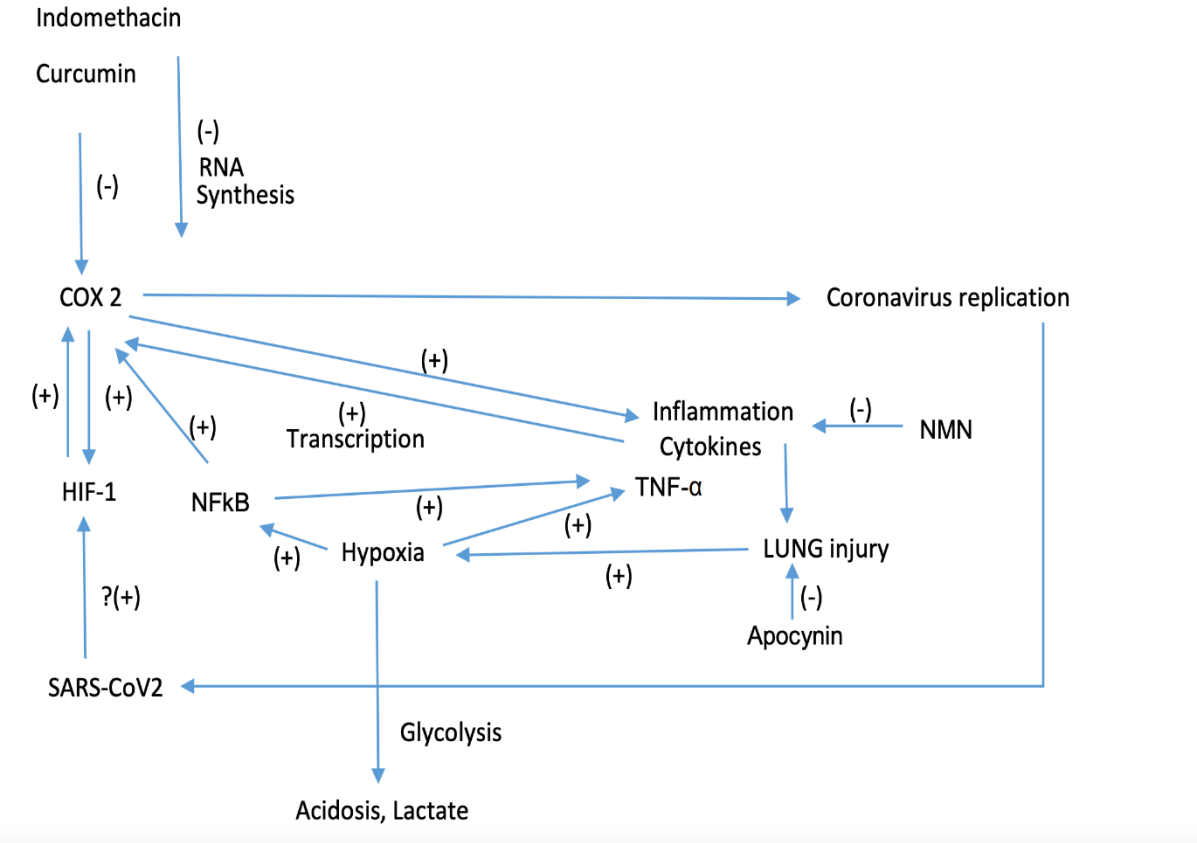

Figure 1. The role of COX in Covid-19 infections

2. THE ROLE OF CYCLOOXIGENASE 2

Raben et al have clearly shown that for an efficient replication in vitro of a coronavirus strain (Mouse hepatitis virus - MHV3), COX-2 activity is required [1]. Moreover, COX inhibitors (Indomethacin and curcumin), interfere with viral RNA, protein synthesis and consequently with production of infectious particles in a dose-dependent manner. A significant decrease in the yield of infectious viral pathogeny by more than 95% and 85%, respectively was observed after inhibition of COX activity by curcumine and Indomethacin. When Indomethacin was added immediately after the cells were placed at 37°C, the maximum inhibitory effect was obtained. No significant inhibition of the infection was observed if Indomethacin was added 2 h later which demonstrates that COX activity plays an important role early in the virus infection cycle. The authors concluded that: COX activity appears to be required for efficient MHV replication, providing a potential target for anti-coronaviral therapy. For a wide range of viruses, an important role for COXs and PGs has been described during infection. Two structural proteins from SARS-CoV able to induce the expression of COX-2 in vitro were described.

Indomethacin has a potent direct antiviral activity against the coronaviruses SARS-CoV and CCoV, as described by Amici et al [2]. This activity was not induced by affecting coronavirus entry into the host cell or binding but by blocking viral RNA synthesis at cytoprotective doses, independent of cyclooxigenase [2]. This in vitro finding was subsequently confirmed in vivo in CCoV-infected dogs (>1000 fold reduction in virus yield).

COXs convert arachidonic acid into PGH2, which can be isomerized to generate different biologically active forms of (prostaglandins) PGs. PGs from the E series (PGE2), could exhibit immunomodulatory effects, preventing activation of the innate cellular immunity. This potential role of PGE2 was explored by Abecassis et al in an experiment of fully susceptible Balb/cJ mice infected with MHV3, a coronavirus strain that caused fulminant hepatitis. They have described that animals treated with PGE2 prior or following infection demonstrated little of no biochemical and histologic evidence of disease, in-spite of high titres of infectious virus recovered from both PGE2 treated and non-treated animals [3]. They have also noticed that the animals treated with PGE2 had no increase in the splenic macrophage, which was detected in non-treated animals. As nicotine and nicotinic acid, seem to correct PGE2 deficit it might explain the better results in terms of infection and outcomes observed in COVID-19 for smokers. In the blood of SARS-CoV-infected individuals, were found elevated levels of PGE2.

3. POTENTIAL PATHOGENY MECHANISMS

An excellent animal model for the study of immunological disorders is a coronavirus, the mouse hepatitis virus type 3 (MHV3). We believe the changes described in this animal model to be very similar with the changes described during the COVID-19 disease. Macphee et al have studied the acute and chronic effects of MHV3 on the microcirculation [4]. They observed that the induction of a specific macrophage protease (procoagulant activity (PCA)) correlated directly with susceptibility to disease. They have also considered that it was responsible for disturbances in the microcirculation characterized by microthrombi, vasculitis, thrombosis and cellular necrosis. During the acute phase, at day 10, two patterns of chronic disease were observed. 80% of mice developed chronic granulomatous hepatitis whereas 20% had a more severe aggressive hepatitis. It was characterised by ongoing hepatocellular necrosis and a marked mononuclear infiltrate. The onset of microcirculatory abnormalities was described to be concomitant with a rise in monocyte related PCA.

They have noticed that during the early stage of infection, splenic macrophage PCA did not discriminate between mice that did or didn't develop the aggressive forms of disease. Only by day ten, the authors could identify distinctive patterns of PCA response, which were correlated with the severity of the histologic lesions. The abnormalities in the microcirculation were detected following infection with MHV3 but prior to viral replication and the authors postulated as potential cause the viral-induced generation of monocyte/macrophage PCA resulting in activation of the coagulation cascade. This can trigger the compliment and kallikrein systems resulting in bradykinin - mediated vasodilataton, increased vascular permeability and eventually granular blood flow. This can explain the clinical findings in the lungs of patients with COVID-19 who exhibit symptoms of pulmonary edema with no other clear cause. Circulating leukocytes would be recruited to the area of inflammation by potent chemotactic factors (C3a, C5a, C567 and thrombin). Moreover, continued elaboration of PCA by monocytes would further amplify the original response leading to sinusoidal blockage by microthromi, with viral replication within sinusoidal lining cells and hepatocytes (with disruption of mithocondria and endoplasmic reticulum) making the cells susceptible to ischemic damage and injury by inflammatory mediators. Hepatocelular necrosis, leukocyte infiltration and edema may result in further sinusoidal blockage and parenchymal damage. This can correspond with pneumonia seen in some of the severe cases of COVID-19. PCA can act by influencing both humeral and cellular immune mechanism by recruitment of activate T cells macrophages and NK cells into the chronically acted area.

The activity of PCA was also describe as being responsible for inducing lupus nephritis in the BXSB murine model, an autoimmune mouse strain [5]. In this recombinant inbread mouse strain, almost 100% of mice develop disease, that affects males much earlier than females. The authors have described striking changes in PCA expressed by viable splenic macrophages that have varied with age (6 months for these animals). Values varied from 37 mU/10^6 splenic macrophages - 1 mo; 800mU/10^6 -3 mo; 29000mU/10^6 - mo 4 - 6). The authors have shown that PCA activity was dependent of coagulation Factor II, calcium and fibrinogen. Interestingly, Jerrard-Dunne has described lower levels of antithrombin III as well as lower protein S and protein C that can lead to a hypercoagulable state in black Caribbeans and black Africans. By neutralizing the enzymatic activity of thrombin, anthithrombin III inhibits coagulation (coagulation factors IIa, IXa, Xa). It is a protease, non-vitamin K-dependent. These variations can explain more severe cases of COVID-19 in the black population. Some have described PCA as a prothrombinase. The increase of PCA seems related to interaction of lymphocytes with factors from plasma such as Immune Complex (IC), as suggested by their experiment. Impaired immunoregulation results in development of IC in the plasma with age. IC might trigger PCA-inducing lymphokine with a major increase in the expression of monocyte/machrophages PCA.

Analyzing the above evidence, we believe that further studies should urgently look at the value of COX2 inhibitors used in the early stages of the disease as well as to explore the potential role of PGE2 and curcumin.

4. HYPOXIA-INDUCIBLE FACTORS (HIFS)

Hypoxia has been described as a key symptom for COVID-19. The hypoxia-inducible factors (HIFs) allows the adaptation to hypoxia, by regulating hypoxia activated gene expression [6]. HIF is a heterodimer complex composed of two subunits, HIF-α (three major isoforms) and HIF-ß. Under normoxia, the hydroxylation of proline and asparagine residues suppresses HIF activity. Under low O2 concentration α-subunit, is stabilized and transactivates gene expression that regulates adaptive response to hypoxia. Moreover, hypoxia, HIF stabilization can be regulated by reactive oxygen species, nitric oxide, metabolic intermediates of tricarboxylic acid cycle, pro-inflammatory mediators (TNF-α, interleukins 1ß and hormonal factors) [6].

Under normoxic conditions, mitochondria respiration consumes above 90% of oxygen. The remaining 10% is utilized for HIF-1α degradation. During sustained hypoxia, mitochondria consume all the oxygen causing rapid stabilization of HIF-1α. Activated HIF-1α leads to increased transcription

of a number of genes (VEGF, EPO, inducible nitric oxide synthase (iNO). These factors participate in the response to hypoxia and lead to increased tissue perfusion and oxygenation. In intermittent hypoxia, activation of NFkB, possible through mitochondrial stress, triggers production of inflammatory mediators such as TNF-α [7]. NFκB and HIF display synergistic behaviour during hypoxic inflammation and crosstalk at different levels (monocyte, T-cell, B-cell, neutrophil) [8]. NFkB can also induce upregulation of COX 2. This can be an aggravating mechanism in patients with COVID-19 with low levels of SO2 (oxygen saturation) and addressing it earlier might reduce the risk of releasing further inflammatory mediators that can be responsible for the cytokine storm. The cytokine storm was described more often during the night, probably related to lower SO2 during sleep. Also, an interesting clinical finding was the observed drop in oxygen saturation immediately after intubation. We believe that a contributing factor can be the use of Propranolol which was shown to inhibit HIF 1α. Adaptation to hypoxia, promotes a metabolic switch to glycolysis, and lactate production to provide ATP and this can explain some of the metabolic changes (acidosis, high lactate, etc).

A variety of viral pathogens are able to activate the HIF-1 pathway, inducing different downstream effects such as altering host cellular metabolism, promoting inflammation, and facilitating viral replication [9]. Spike protein-displayed recombinant baculovirus (SSDRB) has increased expression of HIF-1α.

HIF-1 up-regulates direct transcription of COX-2 [10] and HIF-1 is involved in hypoxia-induced COX-2 up-regulation in cancerous cells. Moreover, Csiki et al. reported that COX-2 is up-regulated in hypoxic lung cancer cells in an HIF-1-dependent manner [11]. Other studies indicate that COX-2 is important for IL-1β, but not hypoxia, driven HIF-1α activation.

We hypothesize that COX 2 and HIF with subsequent inflammation and hypoxia can up-regulate each other in a vicious circle of feed-forward that can ultimately lead to "cytokine storm." Indeed, breaking this cycle early potentially will have beneficial effects either by improving oxygenation (oxygen being given earlier in the course of the treatment) or by inhibiting COX-2.

It was seen that, the suppression of PGE2, via genetic ablation of mPGES-1 or by the pharmacological inhibition, improved survival after Influenza A viral infection, while the addition of PGE2 reversed this effect [12]. It was also observed that the inhibition of COX-2 with celecoxib had a suppressive effect on lung inflammation explained by a COX-2-dependent mechanism involved in the inhibition of HIF-1α signaling, that can contribute to restoring the structure of bronchial epithelium [13].

Inhibition of COX activity has been reported to be associated with decreased expression of pro-inflammatory cytokines and chemokines including tumour necrosis factor α (TNF-α) and monocyte

chemotactic protein-1. Moreover, numerous factors, including cytokines, growth factors, tumour promoters, and oncogenes stimulate COX-2 transcription via transcription factors, such as NFAT, NF-κB, NF-IL6, NF-κB, activator protein, and PEA3 in cancer.

The ethnic variability in COVID-19 may be explained by variability of immunological and biological factors. In one study, obese black women were found to have higher expression of HIF-1 when compared to obese caucasian women [14]. Ribeoro et al [15] have shown that AA genotype of HIF1α 1790G/A was positively associated with renal cell carcinoma risk in overall populations and furthermore, the G allele was negatively associated with susceptibility for prostate cancer in overall populations. Also in Asians, GG genotype was negatively associated with prostate cancer susceptibility. In the same time, gene HIF polymorphism was associated with increased risk of digestive cancers in Asians.

Braksy et al. identified 8 single nucleotide polymorphisms (SNPs) for COX-2. In a population-based case–control study in Western New York, they have examined their association with risk of breast cancer [16]. Only one SNP, rs2745559, was associated with an increased risk of breast cancer (OR 1.23, 95% CI 1.03–1.46).

5. COVID-19 AND OBESITY

Under morbid obesity conditions, immunoreactive proteins and the COX-2 gene have been documented to be elevated and highly expressed in adipose tissue [17]. High-fat diet (HFD) feeding of rats has been reported to increase expression of COX-1 and COX-2 [18].

6. COVID-19 AND SEX

Sex differences were observed by Xu et al [19] who have concluded that the COX-2 signaling pathway appears to be required for acclimatization in oxygen-limiting environments only in males, whereas female COX-2-deficient mice may be able to achieve hypoxic acclimatization by accessing COX-2-independent mechanisms.

7. COVID-19 AND DIABETES

Via protein kinase C signalling, high glucose induces oxidative stress and up-regulation of COX-2. In this way, resulting in reduced nitric oxide availability and altered prostanoid profile [20].

8. COVID-19 AND AGEING

One of the best-characterized feature of immunosenescence and the most significant is declining T cell function. This age-associated decline in T cell function is caused by intrinsic changes within T cells and extrinsic factors. The most recognized extrinsic factor is the increased production of T cell-suppressive factor PGE2 by macrophages. A result of increased COX-2 expression is increased PGE2 production in old macrophages. This leads to higher COX enzyme activity, which is subsequently associated with the ceramide-induced up-regulation of NF-κB. Treatment with nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor reduced reactive oxygen species production in adipose tissue, attenuated the dysregulation of adipokines, hyperlipidemia, and hepatic steatosis [21]. The potential value of NADPH oxidase inhibitors in sepsis was also described before. Studies have found that mice with NOX2 subunit knockout exhibited attenuated production of the cytokines IL-1β, TNFα, IL-6, and CCL2, than wild-type mice following LPS-induced inflammation [22].

NADPH oxidase inhibitor apocynin was found to attenuate sepsis-induced lung injury in guinea pigs, neutrophil ROS generation, and neutrophil-mediated human umbilical vein endothelial cell injury in another study [23]. By providing reducing equivalents to antioxidants such as glutathione and thioredoxin, the reduced form of NADPH protects against redox stress. Ageing is linked with a decline of nicotinamide adeninedinucleotide (NAD+). Cytoplasmic and mitochondrial NAD+ kinases synthesises NADP+ exclusively from NAD+. So, a decline in the cytoplasmic or mitochondrial NADPH pool may also contribute to the ageing process [24]. NAD^+ precursors can have anti-inflammatory effects according to existing growing body of evidence. One study found that treatment with NMN for one week of 24-month-old mice reduced the expression of inflammation markers in skeletal muscle, such as TNFα and IL-6 [25].

9. CONCLUSIONS

To conclude, from the above described findings and the proposed mechanism of infection and interaction between the virus and host, we believe that early inhibition of COX-2 as well as supportive treatment for hypoxia early in the course of the disease have the potential to improve the outcome. It can also be the base for further studies and treatments options.

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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