REVIEW

Immunotherapy of COVID-19 with poly (ADP-ribose) polymerase inhibitors: starting with nicotinamide?

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Albumin, aryl hydrocarbon receptor, COVID-19, dexamethasone, glucocorticoids, indoleamine 2,3-dioxygenase, kynurenine pathway, nicotinamide, non-esterified fatty acids, poly (ADP-ribose) polymerase 1, plasma free tryptophan, proinflammatory cytokines, tryptophan 2,3dioxygenase

What is already known?

- A proinflammatory environment exists in COVID-19 patients that is stronger in those requiring intensive care.
- COVID-19 over-expresses the immune modulator the aromatic hydrocarbon receptor (AhR) and by inference the nuclear NAD^+-consuming enzyme poly (ADP-ribose) polymerase (PARP), which causes cell death by depleting NAD^+ and ATP.

What this study adds?

- Reviews evidence for the above immune changes
- Proposes PARP inhibition as immunotherapy of COVID-19
- Proposes the use of nicotinamide as a PARP inhibitor initially along with standard clinical care or in combination with other agents and subsequently as an adjunct to stronger PARP inhibitors

What is the clinical significance?

- Preventing death from COVID-19 infection with a widely available vitamin-like substance with a unique biochemical and activity profile can present a great clinical advance worldwide.

Abstract

COVID-19 patients in China exhibit a proinflammatory environment that is stronger in severe cases requiring intensive care. The immunity modulators, the aryl hydrocarbon receptor (AhR) and the nuclear NAD^+-consuming enzyme poly (ADP-ribose) polymerase 1 (PARP 1) may play a critical role in COVID-19 pathophysiology. The AhR is over-expressed in a variety of coronaviruses, including COVID-19 and, as it regulates PARP gene expression, the latter is likely to be activated in COVID-19. PARP expression is enhanced in other lung conditions: the pneumovirus respiratory syncytial virus (RSV) and chronic obstructive pulmonary disease (COPD). I propose that PARP 1 activation, which leads to cell death mainly by depleting NAD^+ and ATP, is the terminal point in a sequence of events culminating in patient mortality and should be the focus of COVID-19 immunotherapy. Potent PARP 1 inhibitors are undergoing trials in cancer, but a readily available inhibitor, nicotinamide, which possesses a highly desirable biochemical and activity profile, merits exploration. It conserves NAD^+ and prevents ATP depletion by PARP inhibition, enhances NAD^+ synthesis, and hence that of $NADP^+$ which is a stronger PARP inhibitor, reverses lung injury caused by ischaemia/reperfusion, inhibits proinflammatory cytokines, and is effective against HIV infection. Its unique biochemical properties qualify nicotinamide for therapeutic use initially in conjunction with standard clinical care or combined with other agents, and subsequently as an adjunct to stronger PARP 1 inhibitors.

INTRODUCTION

Understanding the immune effects of COVID-19 can pave the way for a rational choice of the appropriate immunotherapy. Huang et al reported recently[1] that COVID-19 patients from Wuhan, China exhibit a proinflammatory environment characterised by elevated levels of plasma cytokines and chemokines, which is stronger in those requiring intensive care. A proinflammatory environment can lead to activation of the immune modulator the aryl hydrocarbon receptor (AhR) which in turn can activate the nuclear NAD^+-consuming enzyme poly (ADP-ribose) polymerase 1 (PARP 1). PARP 1 activation leads to cell death by a mechanism involving in part depletion of NAD^+ and ATP. Accordingly, targeting PARP 1 with inhibitors has been proposed[2-4] as a potential therapy of COVID-19. The latter three studies[2-4] did not review evidence for modulation of the AhR by COVID-19, mainly because of the rapid advance in COVID-19 research currently taking place at an unprecedented pace.

In the present review, a detailed analysis of changes in cytokines and chemokines induced by COVID-19, other coronaviruses and the other conditions affecting lung function, the respiratory syncytial virus (RSV) and chronic obstructive pulmonary disease (COPD), will be made, evidence for upregulation of the AhR in COVID-19 and other coronaviruses and of that of PARP in RSV and COPD will be described, and a sequence of events culminating in cell death following COVID-19 infection will be proposed. Experimental and clinical studies addressing potential changes in the proposed sequence will be suggested. Finally, a review of some currently tested medications and of potential pharmacotherapies based on PARP 1 inhibition with particular emphasis on nicotinamide will be made. This review is not intended to be exhaustive, but will be limited mainly to a discussion of issues related to the above aspects.

Please Table 1 near here

THE CYTOKINE STATUS OF COVID-19 PATIENTS

Preliminary results by Huang et al[1] in 13 patients requiring and 28 not requiring intensive care and 4 healthy controls demonstrate a proinflammatory environment in COVID-19 patients. Whereas plasma levels of most cytokines and chemokines analysed were higher in patients than in controls (see Supplementary Figure 1 in Huang et al[1]), a largely and stronger proinflammatory profile was observed in those requiring, compared with those not-requiring, intensive care (ICU) (Table 1 here). It would therefore be prudent not to aggravate this environment with use of immunosuppressants (see below). A second paper from China[5] examined cytokine and chemokine levels in a smaller number of COVID-19 patients: 8 with severe and 4 with mild symptoms and 8 control subjects. The authors[5] confirmed the higher levels in severe-, compared with mild-symptom patients of IL-2, IL-7, IL-12 and IL-17. Additionally, they reported higher levels of IL-1ra, IL-4, IL-10 and IFN-γ, whereas this group of cytokines were reported in the Wuhan study[1] to be comparable between ICU and non-ICU patients. Clearly, more detailed studies are required in larger patient and control samples. In the second study,[5] IFN-α was measured and found to be elevated in all patients and higher in severe, than in mild, patients. The potential significance of this latter finding in relation to the efficacy of dexamethasone in COVID-19 therapy will be discussed below.

Please insert Table 2 near here

PREVIOUS FINDINGS WITH CORONAVIRUSES AND A PNEUMOVIRUS

Plasma and tissue cytokine and chemokine concentrations were determined in patients with the coronaviruses SARS,[6-8] MERS,[9,10] the pneumovirus RSV[11] and in COPD patients.[12] The changes depicted in Table 2 show clearly that a proinflammatory environment also characterises these conditions. The increases in proinflammatory cytokines in plasma or serum of SARS and COPD patients occurred in subjects not requiring hospitalisation or intensive care, thus suggesting that, as with COVID-19, a proinflammatory environment exists in patients with these viral infections irrespective of severity. In one study in SARS patients,[6] time-dependent differences in plasma cytokine changes following the onset of symptoms were observed, hence the need to perform time-course studies of plasma cytokine changes in COVID-19 patients as soon as possible after diagnosis. In the COPD study,[12] the authors noted that the plasma changes in cytokines and chemokines are not seen in lung (broncho-alveolar lavage) material and suggested that the lung pathology originates elsewhere.

Other aspects of immune function emerge from studies with a range of coronaviruses, the RSV pneumovirus and from those in COPD patients. Thus, activation of the AhR was demonstrated with SARS, MERS, COVID-19,[13] and the mouse hepatitis virus (MHV) model,[14] and the with pneumovirus RSV.[15] MHV activates the AhR independently of indoleamine 2,3-dioxygenase (IDO),[14] causing cytokine modulation and inducing expression of PARP, and RSV-infected mesenchymal stem cells regulate immunity via interferon-β (IFN-β) and IDO.[15] PARP 1 is activated in COPD patients.[16] These findings, most of which are recent, coupled with the important observation that the AhR regulates the PARP 1 gene[17] provide a justification for targeting PARP 1 (and possibly also the AhR) for COVID-19 therapy.

THE AhR

The ligand-activated transcription factor the AhR can elicit both protective and destructive influences on immune responses. In general, endogenous ligands activate the AhR in a manner that prevents excessive inflammation and autoimmunity, whereas exogenous ligands enhance inflammatory responses to infection, resulting in a state of pathological immunosuppression.[18] Endogenous AhR ligands include Trp metabolites formed in the kynurenine (Kyn) pathway (KP) (Figure 1). Since demonstration of Kyn as an endogenous Trp metabolite AhR ligand,[19] it has generally been assumed that Kyn is the sole ligand. However, subsequent studies with other Kyn metabolites established that kynurenic acid (KA) has the greatest affinity for the AhR, followed by xanthurenic acid (XA), with Kyn itself being a much weaker ligand.[20] The latter authors[20] suggested that KA can activate the AhR at physiologically relevant concentrations. They showed that KA enhances the expression of CYP1A1 mRNA in HEP-G cells significantly at a 100 nM concentration that is ~3-fold higher than the normal plasma level of 31 nM quoted. A 100 nM concentration (representing the KA IC_{25} or the concentration that can achieve 25% of AhR activation by 10 nM TCDD)[20] can be reached easily if [Kyn] is increased moderately. For example, a ~50% increase in human plasma [Kyn] following a small dose oral Trp load (1.15 g to a ~ 70 kg adult) results in a 3-fold increase in [KA].[21]

AhR activation in viral infection must involve participation of an endogenous ligand(s). As stated above, MHV activates the AhR by an IDO-independent mechanism.[14] As KA appears to be the major endogenous AhR ligand among Kyn metabolites, it is possible that this mechanism

can still be mediated by KA produced from Kyn by other mechanisms, such as upregulation of liver TDO and/or increased flux of plasma free Trp down the KP (see below). The possibility of increased flux of plasma free Trp in viral infections will be considered below.

A KA-mediated activation of the AhR is not a simple process. While details of the complex interactions underlying this process are outside the scope of this article, it is important to emphasize here that IDO expression is controlled by the AhR through an autocrine loop involving AhR-IL-6-STAT3 signaling[22,23] and that, while KA can induce IL-6 production, IL-6 generated by inflammation can induce IDO to produce sufficient amounts of KA to activate the AhR.[20]

Please insert Figure 1 near here

THE KYNURENINE PATHWAY OF TRYPTOPHAN METABOLISM

The KP is the major Trp-degradative pathway, accounting for ~ 95% of dietary Trp metabolism.[24] The liver is the major site of the KP, being responsible for ~ 90% of Trp degradation, with the remaining 5% occurring elsewhere, including immune cells. The KP is controlled mainly by TDO in liver and IDO extrahepatically. As shown in Figure 1, Kyn undergoes mainly oxidative metabolism, to 3-hydroxykynurenine (3-HK) by kynurenine monooxygenase and then to 3-hydroxyanthranilic acid (3-HAA) by kynureninase (Kynase). This latter enzyme can also hydrolyse Kyn to anthranilic acid. 3-HAA is further oxidised by its dioxygenase (3-HAAO) to an unstable intermediate: 2-amino-3-carboxymuconic acid-6-semialdehyde (ACMS), which occupies a central position at 2 junctions of the pathway. The pathway favours the non-enzymic cyclisation of ACMS to quinolinic acid (QA) and subsequent synthesis of NAD^+. The minor junction involves decarboxylation of ACMS by ACMS decarboxylase (picolinate carboxylase) to form 2-amino-3-muconic acid-6-semialdehyde (AMS), which can undergo either non-enzymic cyclisation to picolinic acid (PA) or further metabolism to acetyl-CoA. Kyn and 3-HK can also be transaminated to KA and XA respectively in a minor reaction catalysed by Kyn aminotransferase (KAT). KA and XA formation, however, is limited by availability of the respective substrates in view of the high K_m of the enzyme.[24] Thus, formation of the AhR ligands KA and XA is dependent on [Kyn]. Details of NAD^+ synthesis from QA in the main (*de novo*) pathway and the "salvage" pathway from nicotinic acid and nicotinamide are illustrated in ref.[24] Trp is a more effective source of NAD^+ synthesis via QA in the *de novo* pathway than is nicotinamide or nicotinic acid in the salvage pathway (for references, see[24]).

The KP produces a range of metabolites that are biologically active at various physiological levels and body systems.[24-26] Of particular relevance to COVID-19 are the immunomodulatory properties of some KP metabolites, namely the proinflammatory 3-HK, 3-HAA and QA, the antiinflammatory PA and the dually acting KA. Increased production of these metabolites can be achieved by a number of mechanisms: TDO induction by glucocorticoids, IDO induction by interferon-γ (IFN-γ), flux of plasma free (non-albumin-bound) Trp down the pathway and upregulation of KMO and subsequent enzymes. Raising levels of proinflammatory Kyn metabolites by whatever intervention or mechanism may aggravate the immunosuppressive environment of COVID-19 to a greater pathological level.

THE PARPs

The PARPs [poly (ADP-ribose) polymerases] family of enzymes catalyse the transfer of adenosine diphosphate-ribose to target proteins, thereby influencing many important processes, including DNA repair.[27] PARP activation results in a multifaceted programmed cell death pathway involving in part depletion of NAD^+ and ATP[27,28] and preclinical data suggest that PARP inhibitors may be effective therapies for inflammatory, metabolic and neurological disorders.[29] The deleterious effects of PARP activation are further emphasised by observations in patients with lung diseases. Thus, COPD is associated with activation of systemic PARP in peripheral blood lymphocytes[16] and the Ala allele polymorphism in the PARP1 gene is associated with a decreased risk of asthma.[30]

ADDITIONAL FINDINGS OF RELEVANCE TO THE IMMUNE EFFECTS AND THERAPY OF COVID-19

AhR activation and enhanced PARP1 expression by MHV

In the study with the coronavirus MHV mouse model,[14] the AhR is activated by a mechanism(s) independent of IDO1 induction. The question arises if IDO induction by administration of inducers such as IFN-β, which is being trialled in COVID-19 patients, will additionally activate the AhR, thus further enhancing PARP expression (see below). A potential AhR activation by IFN-β is likely to be additive to that by COVID-19, if the two act by different mechanisms. Embarking on IFN-β therapy should therefore be approached with caution. If AhR activation by COVID-19 is also IDO-independent, it is possible that it could still involve production of Kyn metabolites by other mechanisms, such as glucocorticoid induction of liver TDO or increased flux of plasma free Trp through TDO and down the KP (see below).

Expressions of interferon-β and IDO in RSV infection

RSV infected mesenchymal stem cells exhibit a 100-fold increase in IFN-β expression and a 70-fold increase in that of IDO, and neutralising IFN-β reverses the increased IDO expression and activity.[15] Upregulation of these two expressions was suggested[15] to modulate the immune regulatory function, causing impairment of immune cell proliferation, which may account for the lack of protective RSV immunity and for chronicity of RSV-associated lung diseases such as asthma and COPD. The authors[15] however did not observe a difference in viral load or increased IFN-β expression between wild-type and IDO KO mice and suggested that this mouse model does not reflect the human disease. Other studies with RSV patients did not report on IFN-β (which may not have been overexpressed), but observed enhanced expression of a wide array of cytokines and chemokines in the lower and upper respiratory tracts (reviewed in[31]). Levels of IFN-β and IDO expression therefore need to be measured in COVID-19 patients. A significant increase in IFN-β in COVID-19 would not justify the use of this cytokine as a therapy.

Please insert Figure 2 near here

HYPOTHESIS: SEQUENCE OF EVENTS WITH COVID-19 INFECTION

A hypothesis is graphically illustrated in Figure 2. A sequence of events is proposed of the likely effects of COVID-19 culminating in cell death. Activation of PARP 1 is the final step preceding cell death as a result of depletion of both NAD^+ and ATP. Lung function is especially vulnerable to impaired mitochondrial oxidative energy metabolism and it is therefore

very likely that PARP 1 may play a central role in COVID-19-induced mortality. So far, the increase in plasma proinflammatory cytokines, the over-expression of the AhR and the decrease in albumin (see below) are the only known changes induced by this coronavirus. All other changes listed in Figure 2 are therefore hypothesis-driven, but based on observations in other viral infections, and can be assessed experimentally.

WHAT NEEDS TO BE MEASURED IN COVID-19 PATIENTS?

Immune modulators and the tryptophan-metabolic status: In addition to assessing the expressions of the AhR, PARP1, IFN-β, IDO and pro-and anti-inflammatory cytokines in larger patient and control samples, the Trp-metabolic status of COVID-19 patients should be established through measurements of fasting plasma Trp, Kyn and the Kyn metabolites 3-HK, 3-HAA, KA and QA.

Cortisol: The other Trp-degrading enzyme, liver TDO, may also be involved through glucocorticoid induction if cortisol levels are elevated in COVID-19. Activation of the hypothalamic-pituitary-adrenal (HPA) axis resulting in increased release of cortisol is a feature of the stress response to viral (and bacterial) infections.[32]The cortisol status in COVID-19 infection is unclear and a prospective study is currently underway.[33] Raised cortisol levels are observed in RSV and Ebola patients and in SARS patients with lymphopenia.[34] In the Wuhan study,[1] lymphopenia was significantly more predominant (85%) in patients requiring intensive care, compared with those not requiring it (54%), though numbers were small. It is therefore possible that cortisol will be raised in a proportion of COVID-19 patients irrespective of severity. In such cases, TDO induction by cortisol is likely to elevate the availability of Kyn to subsequent enzymes of the KP and thus promote the formation of proinflammatory Trp metabolites.[24]

Plasma free Trp and determinants of its flux: Another determinant of Kyn formation is the flux of plasma free (non-albumin-bound) Trp through TDO and down the pathway, without the need for TDO/IDO induction.[24] The bulk of plasma [Trp] is albumin-bound, with only 5-10% freely circulating. Free [Trp] is therefore controlled by concentrations of the binder albumin and the physiological displacers nonesterified fatty acids (NEFA).[24] In the Wuhan study,[1] [albumin] (median and range in g/L) was 27·9 (26·3–30·9) in intensive care patients and 34·7 (30·2–36·5) in non-intensive care patients. No control values were given. However, as the normal range is 35-50 g/L, the value for non-intensive care patients approaches the normal range. Consequently, intensive care patients can be assumed to have at least 20% less albumin. A decrease in [albumin] of ≥19% can significantly cause the release of bound Trp.[26] Serum [NEFA] has not been measured in COVID-19 patients, but is known to be elevated in infections with other viral classes, e.g., the flavivirus Dengue[35], the lentivirus HIV[36] and the asfivirus, the swine (fever) influenza.[37] Albumin depletion coupled with NEFA elevation provide the maximum effect on Trp binding and the resultant increase in free Trp availability can enhance the production of Kyn through IDO/TDO and thus provide the substrate for proinflammatory KP metabolite formation.[24] Measurements of plasma free [Trp] along with albumin and NEFA in COVID-19 patients can be informative. The mouse MHV model is the first coronavirus to induce simultaneous increases in liver [Kyn] and [Trp] [38] that are consistent with increased flux of circulating free Trp through liver TDO and down the KP.[39]

POTENTIAL THERAPIES OF COVID-19

Existing drugs in current trials

Until vaccine therapy becomes available, clinicians are currently trialling various therapies previously used in viral and other diseases. These include the antiviral agent Remdesivir, the antimalarial drugs chloroquine and hydroxychloroquine, glucocorticoids, IFN-β, IL-6 antagonists and angiotensin-converting enzyme 2 (ACE 2) inhibitors.

Remdesivir and antimalarials: With Remdesivir, efficacy is partial, though valuable and much needed: it does not lower death rates, but reduces the time to recovery by ~ 27%.[40,41] With the antimalarials chloroquine and hydroxychloroquine, efficacy is unproven, results of clinical trials are equivocal and the drugs possess serious potential side effects, especially in severely ill patients.[42]

Glucocorticoids: With corticosteroids (glucocorticoids), current evidence does not justify their use and the WHO does not recommend them for COVID-19 therapy.[43] Glucocorticoids can compromise anti-viral defences and enhance respiratory viral and other infections, especially when given in high doses and/or for long durations.[44-46] Additionally, glucocorticoids could increase production of proinflammatory Kyn metabolites by TDO induction and thus aggravate the proinflammatory environment of COVID-19 patients. One glucocorticoid, dexamethasone (DEX), however, has been shown to decrease the death rate of COVID-19 patients in a recent trial.[47] The rate reduction in ventilated patients was ~ 30%, whereas that in patients on O_2 only was ~ 20%. No benefit was accrued by patients not requiring respiratory support.

How does DEX differ from other glucocorticoids, apart from being the strongest antiinflammatory[48] and a much stronger TDO inducer than cortisol,[49,50] based on dose requirements. Whereas these differences may not explain the efficacy of DEX in COVID-19, other differences provide potential explanations, in particular, effects on IDO induction by interferons and inhibition of prostaglandin biosynthesis. Thus, interferon induction of IDO is inhibited much more strongly by DEX than by cortisone (IC_{50} = 1 nM for DEX and 20 nM for cortisone).[51] This strongly suggests that IDO induction may play an important role in COVID-19 patients, as suggested in Figure 2 here, especially in those with severe symptoms requiring intensive care, and, by inference, IDO-dependent production of proinflammatory Kyn metabolites may be a vital component of the pathophysiology of this viral infection. In the above study,[51] the type of interferon was not defined. DEX at 10 nM inhibits IDO induction by IFN-α by 71%, but potentiates that by IFN-γ by 2-fold in human peripheral blood monocytes.[52] IFN-γ-induced IDO activity potentiated by DEX is 28-fold greater than the IFN-α-induced IDO activity inhibited by DEX. Does this suggest that DEX could be effective in COVID-19 patients with raised IFN-α, but without benefit or even harmful in those with raised IFN-γ? Perhaps the balance between the α- and γ-IFNs determines DEX efficacy and could be assessed as a possible predictor of response to therapy with DEX. IFN-γ is the strongest IDO inducer among interferons, followed by IFN-β, with IFN-α being the weakest inducer.[53] Though the weakest, IFN-α is strong enough to induce Trp degradation along the KP.[54,55]

Please insert Figure 3 near here

Inhibition of phospholipase A_2 may provide another explanation of efficacy of DEX in COVID-19. IDO induction by interferons involves participation of prostaglandin biosynthesis and the above enzyme involved in this process exhibits different sensitivities to inhibition by glucocorticoids, with DEX being a much stronger inhibitor than cortisone (IC_{50} = 3.6 μM for

DEX and 120.7 μM for cortisone).[51] A detailed discussion of the relationships between and interactions of cytokines and prostaglandins is outside the scope of this article, but a few relevant features are noteworthy. Thus: (1) interferons enhance the synthesis and activation of phospholipase A_2.[56,57] (2) As well as by nonsteroidal antiinflammatory drugs (NSAID), COX2 is also inhibited by adrenal glucocorticoids[58,59] and by DEX.[60] A simple diagram summarising differences between DEX and cortisone that may explain the efficacy of DEX in COVID-19 patients is presented in Figure 3.

A study of 3 severe cases of COVID-19 who did not respond to therapy with the IL-6 inhibitor tocilizumab showed a rapid improvement with pulse administration of a high daily intravenous dose of methylprednisolone of 1g for 3 days and intravenous immunoglobulin.[61] These findings with another potent antiinflammatory synthetic glucocorticoid underscore the need to perform detailed investigations into differences between synthetic and natural glucocorticoids on aspects of tryptophan and prostaglandin metabolism that impact immune function as illustrated by the above findings.

Interferon-β, IL-6 antagonists and ACE 2 antibodies: IFN-β is currently being trialled and results are awaited. Little is known about the effects of this interferon on prostaglandin biosynthesis or any likely modulation thereof by glucocorticoids. As far as I could ascertain, the status of IFN-β in COVID-19 is unknown. A number of IL-6 antagonists are also being trialled, including tucilizumab[61]. Inhibition of angiotensin-converting enzyme ACE 2 has also been suggested. Whereas recent discussions have centred on potential sensitivity of ACE 2 inhibitor-treated patients to COVID-19,[62] clinical trials of ACE 2 antibodies have been suggested.[63]

Therapies countering proposed effects

Researchers can devote effort to assessing the changes proposed in Figure 2 and, if demonstrated, counter measures could be suggested as follows: TDO and IDO induction can be blocked with glucocorticoid antagonists and IDO inhibitors respectively. Increased flux of Trp down the KP can be minimised by albumin infusions, antilipolytic agents or both. These measures can minimise excessive production of proinflammatory Kyn metabolites and potentially also activation of the AhR and consequently that of PARP1.

PARP 1 Inhibitors

In the short term, efforts should focus on the distal changes in the proposed sequence of events in Figure 2, in particular PARP 1. PARP 1 inhibition will most likely prevent cell death due to NAD^+ and ATP depletion. PARP 1 inhibition has been suggested using nicotinamide[2] or CVL218 (Mefuparib).[3] This latter compound inhibits SARS viral replication with an EC_{50} of 5.12 μM, and IL-6 production by activated peripheral blood mononuclear cells.[3] As will be described below, nicotinamide exerts similar effects, but at higher concentrations.

Nicotinamide: an ideal profile for PARP 1 inhibition

While various PARP inhibitors are currently being trialled in other conditions[4] a more readily available inhibitor is the NAD^+ precursor nicotinamide[64,65] (NAM) (and not nicotinic acid). By inhibiting PARP and restoring ATP levels as well as acting as NAD^+ precursor, NAM possesses the desired profile for a potential effective therapy of COVID-19. Examples of its favourable

profile include abrogation of acute lung injury caused by ischaemia/reperfusion,[66] inhibition of proinflammatory cytokines[64] and efficacy against HIV infection (see[67] for review).

NAM possesses the following unique advantages regarding ATP, NAD^+ and PARP. (*i*) It inhibits PARP activity by competing with NAD^+ for the enzyme active site. (*ii*) As a result, it conserves NAD^+ and (*iii*) restores ATP levels. (*iv*) As the NAD^+ precursor, it further increases its levels. (*v*) The increased concentration of NAD^+ provides the substrate for NAD^+ kinase, leading to production of $NADP^+$, which is a stronger PARP inhibitor,[68] thereby strengthening the PARP inhibitory capacity. (*vi*)$NADP^+$ which acts by competing with NAD^+ thus conserving NAD^+ levels, is able to inhibit various classes of PARP other than PARP1.[68]

PARP 1 inhibition by nicotinamide appears to be a weak one: a 50% inhibition at 0.5 mM,[65] in contrast with the strong one by CVL218 (IC_{50} = 3.2 nM).[69] However, $NADP^+$, which has been proposed to be the endogenous PARP 1 inhibitor, is a stronger inhibitor than nicotinamide, demonstrating inhibition at 10 µM.[68] Perhaps the weak inhibition by nicotinamide in cell systems *in vitro* may be due to its slow conversion to $NADP^+$. The study by Bian et al[68] showed that: (1) PARP 1 recognises $NADP^+$, but does not use it as substrate; (2) the ratio of $[NADP^+]/[NAD^+]$ is an important determinant of PARP 1 inhibition, with a modest increase producing a significant inhibition; (3) NAD(P)H do not inhibit PARP activity. That nicotinamide administration can increase $[NADP^+]$ has been demonstrated in rat liver.[70] When given in drinking water at a concentration of 100 mg/L, resulting in a daily dose of 10-15 mg/kg body weight, nicotinamide increases hepatic $[NADP^+]$ by 42-60% and [NADPH] by 26-30% respectively.[70] With a normal rat liver $[NADP^+]$ of ~ 54 µM,[71] the above increase by nicotinamide raises it to ~ 80 µM. As $NADP^+$ is formed from NAD^+, it is almost certain that the $[NADP^+]/[NAD^+]$ ratio will have been increased by nicotinamide. Given that, compared to humans, rodents generally require doses of chemicals a few orders of magnitude higher, the nicotinamide dosage needed to inhibit PARP 1 in COVID-19 patients is unlikely to be excessive. In humans, doses of nicotinamide of up to ~ 3 g daily (equivalent to ~ 40 mg/kg for a 70 kg adult) are generally acceptable, with higher doses needing supervision.[72] As a nutritional "vitamin-like" substance, NAM should, if required, receive rapid approval from Ethics Committees and other regulatory authorities.

A comparison of the effects of nicotinamide and Mefuparib may be useful. The only clear advantage of Mefuparib over NAM is its strong PARP 1 inhibitory potency. Both decrease IL-6 production and conserve NAD^+ and ATP. However, only NAM will cause an additional increase in NAD^+ and increased synthesis of $NADP^+$. Finally, whereas NAM has been shown to possess a favourable profile in a number of inflammatory states, Mefuparib has not as yet undergone corresponding studies other than inhibition of viral replication.[3] Thus, given the urgent need to address the COVID-19 pandemic, the use of nicotinamide can be initiated before current and future clinical trials and experimental studies with the stronger PARP 1 inhibitor Mefuparib and other strong inhibitors establish their safety and other requirements and efficacy in COVID-19 patients. Even if stronger PARP 1 inhibitors are proven to be effective in COVID-19 patients, nicotinamide, by virtue of its additional effects, can be used as an adjunctive therapy.

A note of caution is appropriate here. In a mouse model of acute lung injury induced by mechanical ventilation, Jones et al[73] reported that nicotinamide causes hypoxemia, yet no such effect of nicotinamide has been reported in other studies referenced by these authors[73] with

other models of inflammatory diseases, including acute lung injury, in mice, rats and hamsters. As stated above, NAM abrogates lung injury caused by ischaemia/reperfusion.[66] Jones et al[73] did not specify the mouse strain(s), which included the C57, used in their experiments. Mouse strains are known to differ in their response to immune insults, with the C57BL being the most vulnerable.[74] However, clinicians should nevertheless consider this possibility by monitoring blood O_2 levels during clinical management when using nicotinamide.

CONCLUSIONS

It is almost certain that PARP 1 is activated by COVID-19 and is responsible for death of patients. The partial success of some current therapies may be explained by the agents used not targeting PARP 1 directly, but are able to modulate steps in the proposed sequence of events presented in this paper with varying degrees of success. It is hoped that the above account will stimulate research efforts aimed at understanding the pathophysiology of COVID-19 viral infection and encourage clinicians to explore the potential therapeutic use of nicotinamide as a PARP 1 inhibitor along standard clinical care in the first instance and subsequently as an adjunct to stronger PARP 1 inhibitors and other drugs.

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CONFLICT OF INTEREST

The author does not have any conflict of interest regarding this article.

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Table 1. Pro- and anti-inflammatory cytokines in COVID-19 patients from Wuhan, China according to intensive care needs

ICU vs Non-ICU-patients			
Higher Proinflammatory	Similar Proinflammatory	Higher Antiinflammatory	Similar antiinflammatory
IL-2	IL-1β	IL-9	IL-1ra
IL-7	IL-6		IL-4
IL-8	IL-13		IL-5
IL-12	IFN-γ		IL-6
IL-17			IL-10
IP-10			
MCP-1			
MIP-1α			
MIP-1β			
TNF-α			
RANTES			

This Table is derived from data in Supplementary Figure 1 of the article by Huang et al[1] (Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395: 497-506). Abbreviations used: ICU, intensive care use; IFN-γ, interferon gamma; IL, interleukin; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; TNF, tumor necrosis factor; RANTES, regulated on activation and normal T-cell expressed.

Table 2. Previous studies of cytokines and chemokines in SARS, MERS, HBV, RSV and COPD

	SARS	MERS	RSV	COPD
Reference No	(6)(7)(8)	(9)(10)	(11)	(12)
Proinflammatory				
IL-1β	↑ ↑		↑	
IL-2	↑	-	↑	↑
IL-6	↑ ↑ ↑	↑	↑	
IL-7				↑
IL-8	↑ ↑	↑		↑
IL-12	↑	↑	↑	↑
IL-13		-	↑	
IL-17		↑	↑	↑
IFN-γ	↑ ↑ ↑	↑ ↑	↑	↑
IP-10	↑ ↑	↑		
MCP-1	↑ ↑ ↑	↑		
MIP-1α	↑	↑		
MIP-1β				
TNF-α	↑	↑	↑	↑
RANTES	↑	↑	↑	
Antiinflammatory				
IL-1ra				
IL-4				
IL-5				
IL-6	↑ ↑ ↑	↑		
IL-9			↑	
IL-10			↑	↑

The symbols ↑ and – indicate respectively an increase above, or no change from, controls. Absence of a symbol indicates that a parameter has not been measured. Numbers next one another indicate different studies referenced as follows: SARS (6-8), MERS (9,10), RSV (11), COPD (12). See the text for abbreviations and further comments.

Legends to Figures

Figure 1. The kynurenine pathway of tryptophan degradation up to the quinolinic acid and picolinic acid steps. Adapted from Figure 1 in ref.[24] Abbreviations used: 3-HAAO (3-hydroxyanthranilic acid 3,4-dioxygenase); IDO (indoleamine 2,3-dioxygenase); KAT (kynurenine aminotransferase); KMO (kynurenine monooxygenase); Kynase (kynureninase); N′FK (N′-formylkynurenine); TDO (tryptophan 2,3-dioxygenase). Colours indicate a proinflammatory (red), antiinflammatory (green), a mixed or partially proinflammatory (amber) or a normal metabolite (yellow).

Figure 2. Diagrammatic representation of known and likely changes in immune function and tryptophan-metabolic parameters in COVID-19. For details and comments, see the text. Abbreviations used: AhR, aromatic hydrocarbon receptor; Kyn, kynurenine; IDO, indoleamine 2,3-dioxygenase; NEFA, non-esterified fatty acids; PARP, poly (adenosine-diphosphate-ribose) polymerase; PI, proinflammatory; Trp, tryptophan; TDO, tryptophan 2,3-dioxygenase. Colours indicate a serious change (red), an intermediate change (amber) and a primarily normal physiological change (green).

Figure 3. Differential effects of dexamethasone and cortisone on inflammatory mediators explaining their likely pharmacotherapeutic differences in COVID-19. Abbreviations used: DEX, dexamethasone, IDO1, indoleamine 2,3-dioxygenase 1; IFN, interferon; NSAID, nonsteroidal antiinflammatory drugs. Colours indicate a normal metabolic pathway (yellow), glucocorticoids and NSAID (blue) and agents causing harm (red),