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ORIGINAL ARTICLE

The Role of Neutrophils in ARDS and Drugs that Modify their Formyl Peptide Receptor Activity

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Abstract

Adult Respiratory Distress Syndrome (ARDS) represents a sustained neutrophil driven attack on the lung, where raised alveolar neutrophil numbers are known to predict severity and mortality. Neutrophil infiltration to the lung is in response to released Formyl Peptides from infected and damaged tissue which can drive oxidant and protease release with toxic effects. Formyl Peptides can stimulate neutrophils directly via their surface Formyl Peptide Receptors (FPR'S), escalating their activation and cytokine release. Highly stimulated and stressed neutrophils can be driven to release their nuclear material as Neutrophil Extracellular Traps, producing additional damage and arterial obstruction with increased hypoxaemia and mortality in ARDS. The sulphonamide Dapsone can block these FPR'S in a dose dependant manner reducing the inflammatory response and the risk of acute lung injury in animal models which is also seen for Cyclosporine. Trimethoprim and cotrimoxazole also reduce neutrophil FPR'S stimulation including data presented from our medical staff following oral dosing. In this manuscript, we examine the immune effects and role of neutrophils and their FPR's in acute lung injury and ARDS along with human and animal data. The global Covid-19 pandemic has brought ARDS into sharp focus including the limited knowledge of effective drug treatments in severely affected patients. We present our own and other published data showing the ability of cotrimoxazole and trimethoprim to reduce Covid-19 induced ARDS mortality and to speed recovery. These drugs clearly offer a way of calming an "out of control host response" that drives ARDS and they are inexpensive antibiotics already licensed for respiratory infections with Immunological benefit that may finally change the course of this disease and thereby warrant inclusion in future trials.

Keywords

Cotrimoxazole, Formyl peptide receptors, ARDS (adult respiratory distress syndrome), Neutrophils, Immune Responses, Covid-19

Abbreviations

ARDS: Adult Respiratory Distress Syndrome; FP: Formyl Peptide FPR-1: Formyl Peptide Receptor-1; NET's: Neutrophil Extracellular Traps; DNA: Deoxyribonucleic Acid; NE: Neutrophil Elastase; PKC: Protein Kinase-C; PMA: Ligand-Phorbol 12-Myristate 13-Acetate; CTX: Cotrimoxazole; TMP: Trimethoprim; ST: Standard Therapy

Introduction

Neutrophils and tissue damage

Neutrophils are critical in responses to infection; however, their infiltration into the lung en masse with intense cytokine activation leads to neutrophil generated reactive oxidants and released proteases that are toxic to tissue. These collectively damage the capillary endothelium, causing alveolar oedema and reduced gas exchange that encourages fibroblast activation and collagen deposition that forms the basis of ARDS [1-3].

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There is now ample evidence that ARDS is a picture of a self-perpetuating and sustained neutrophil attack, with large number of neutrophils seen in early broncho-alveolar lavage samples in patients developing the condition. Their numbers independently predict severity and mortality [4]. In animal models of ARDS, the depletion of neutrophils attenuates immune responses and lung injury confirming their key role [4,5]. As inflammation promotes neutrophil activation and priming it produces a more protracted transit time through the pulmonary vasculature bed relative to unprimed cells [4-6]. In ARDS, the neutrophil de-priming activity of the pulmonary vascular endothelium is disrupted thus allowing highly activated neutrophils to produce greater endothelial injury. These hyper-reactive neutrophils can also enter the systemic circulation and mount attacks upon other organs, offering an explanation for the multi-organ failure observed in ARDS [7,8]. Likewise serious abdominal inflammation or trauma can also generate local hyper-reactive neutrophils that may reach the lung in large numbers via the mesenteric 'gut-lymphatics' driving ARDS from events "outside the lung".

Neutrophil function

Neutrophils are short lived phagocytic cells involved in acute inflammation, phagocytosis and host defense. On average they spend 36 hrs in the circulation before migrating into tissue where they offer defense against invading pathogens [9-11]. They are equipped with a full arsenal of anti-microbial proteins and cytoplasmic secretory granules to effect phagocytosis and intracellular killing [11,12]. Neutrophils can also mediate cytotoxicity against virally infected cells through antibody dependent mechanisms, free radicals and complement activation. They can migrate out of the circulation in response to chemotactic stimuli and may activate macrophages, platelets and clotting factors to escalate inflammation [11].

Clinical ARDS

In 1967, an association between respiratory failure and non-cardiac pulmonary oedema was first described. Lung biopsies showed similarity to infantile respiratory distress syndrome that led to the name "adult respiratory distress syndrome or ARDS". The clinical picture is that of refractory hypoxaemia unresponsive to an increasing inspired oxygen fraction in association with reduced lung compliance and bilateral pulmonary infiltrates without cardiac impairment. Here acute inflammation affects the alveolar-capillary membrane, increasing permeability and producing oedema in association with neutrophil recruitment and mediator release. This inflammatory exudate inactivates surfactant leading to collapse and consolidation of the distal airspaces with loss of gas exchange. The "hypoxic pulmonary vasoconstriction" required to correct this V/Q mismatch, becomes paralysed by the inflammatory milieu resulting in profound hypoxaemia [13]. The mortality is high at 50-60%, and survivors demonstrate long-term morbidity with a reduced quality of life [13]. There is a wide variety of recognised causes with 75% occurring from direct insults to the lung (infections, toxic fumes or aspiration of gastric contents) and 25% from indirect involvement (abdominal and systemic sepsis, massive haemorrhage, blood transfusions, major trauma, obstetric crisis) [13,14].

Despite intense research elucidating the complex pathophysiology of ARDS and the use of lung protective ventilation, antibiotics and oxygen, there remains an urgent and unmet need for effective therapeutic agents to reduce the unacceptably high mortality in the most severely affected patients [13,14]. In life-threatening ARDS unresponsive to conventional therapies, **E**xtra **C**orporal **M**embrane **O**xygenation technology can be used as a bridge for lung recovery in suitable patients. The cost of hospital treatment in ARDS is extremely high with 10% of intensive care patients affected. All this has been brought into sharp focus following the global Covid-19 pandemic with over 200 million estimated cases of ARDS occurring in high-risk patients [9,15-17].

The initial stage of ARDS is exudative with diffuse alveolar damage and alveolar leak. In the second stage, resolution of the pulmonary oedema is associated with proliferation of alveolar type II cells and myofibroblasts. Some patients progress to the third stage of fibrosis, where the normal lung architecture is replaced by diffuse fibrosis and cyst formation affecting lung function and survival [18-20].

To date, broad spectrum antibiotic cover forms part of the current guidance with neutral fluid balance [13]. Steroids were traditionally used for their anti-inflammatory effects, but past studies showed mixed results without clear improvements in survival, leading to their discontinuation unless required for refractory hypotension unresponsive to vasopressors [13,18,21]. Steroids have a wide range of biological effects, from anti-inflammatory properties to suppression of antigen presentation and mediator release. They also reduce cell traffic, lymphocyte numbers and cause neutrophil demargination. Many of these effects are non-specific but Immune suppression increases concerns over the risk of super-infection in ARDS [13,21].

The National UK Recovery Study for Covid-19 did show steroid benefit in oxygen dependant patients with ARDS, where dexamethasone 6 mg/day for 8 days reduced mortality by 11% [22]. This benefit was not seen in SARS-Covid-1 (severe acute respiratory syndrome) **n**or MERS (middle east respiratory syndrome), where data gave mixed results with delayed viral clearance [23,24]. Since the UK Recovery findings, small trials of low dose methylprednisolone have been assessed in Covid-19 with a meta-analysis confirming benefit in reducing mortality and the need for mechanical ventilation. The latest Japanese guidelines for ARDS

have now added low dose methylprednisolone to their treatment regimen [25,26].

Formyl peptides and neutrophil activation

Mechanistically, neutrophils migrate to inflamed tissue in response to Formyl Peptide signals even at very low concentrations of 1 nmol. Formyl peptides (FP's) are inflammatory 'Danger Signals' released by bacteria and damaged or dying cells including mitochondrial DNA derived FP's due to their known bacterial ancestry [27,28]. FP's give a specific chemotaxic signals that marks the presence of invading organisms or tissue damage. FP's can stimulate the abundantly expressed surface Formyl Peptide Receptors (FPR) on neutrophils, increasing their activation and driving a myriad of different processes including superoxide generation, chemotaxis and the secretion of oxidants and proteolytic enzymes including tumour necrosis factor-α, interleukin-8, neutrophil elastase, myeloperoxidase and matrix metalloprotease 8 + 9 [4]. Clinical studies in ARDS confirm high levels of FP's in the serum and alveolar lavage fluid within 6-24 hours following acute lung injury [29,30]. FP driven mediator release causes alveolar injury and apoptosis especially to alveolar type-1 cells due to their limited concentrations of protective catalase [27,31]. Neutrophil Elastase (NE) is a protease that allows neutrophils to digest and migrate through dense extracellular matrix and NE release is increased 6-fold in hypoxic conditions like ARDS. NE also drives alveolar type-2 cells and myofibroblast to proliferate via NE-induced activation of the fibrotic cytokine transforming growth factor-beta. This may produce interstitial fibrosis in severe cases of ARDS [32].

Formyl peptide receptors

Formyl peptide receptors are transmembrane G-protein coupled receptors critical in myeloid trafficking, inflammation and the immune response. Three different FPR receptors are recognised in man. FPR-1 is the most studied and abundantly expressed on neutrophils and monocytes and activated by released FP's from infection or tissue damage [27,28,33-35]. FPR-2 is present on neutrophils, monocytes, epithelial and endothelial cells where it binds lipid mediators such as lipoxin-A4, amyloid protein-A, annexin-A1 and resolvin-D1 [20,29,36,37]. FPR-3 is largely insensitive to FP's and present on eosinophils, monocytes and dendritic cells with likely involvement in allergic responses [38]. The physiological responses to FP's are determined by the number of surface FPR's which can range between 10,000-100,000 receptors per neutrophil [39].

The functional importance of FPR-1 is demonstrated in animal models of acute lung injury following intra-tracheal hydrochloric acid, where blockade of the FPR-1 by an antagonist such as cyclosporine H reduces injury whether administered before or after the insult. Mice genetically deficient in FPR-1 show a marked reduction in alveolar neutrophils relative to wild type mice. Pulmonary vascular leak in early ARDS, is also reduced in FPR-1 deficient mice and neutrophil depletion in animal models also ameliorates protein leak [4,29].

Immunologically, FPR-1 has also been shown to be a tissue specific driver of pulmonary fibrosis, with FPR-deficient mice showing reduced neutrophil recruitment and fibrotic responses following bleomycin compared with wild-type mice. This was shown to be intrinsic to the FPR-1 receptor [40,41]. Further studies have shown that lung fibroblasts migrate via FPR-1 mediated calcium influx and protein kinase C pathway signalling with detrimental effects on the fibrotic process [39].

FPR-2 has a lower affinity for bacterial peptides with a protective role in respiratory diseases via their FPR-2 agonists of lipoxin A4, annexin-A1 and resolvin-D1 that reduce neutrophil migration and drive monocytes to increase their phagocytosis of neutrophils [39]. In acute lung injury, pre-treatment with resolvin-D1 attenuates injury through suppression of nuclear factor-kappa-β generated cytokines [31,35,39]. Inflammatory effects via the FPR-2 are however particularly produced by mitochondrial FP's, which induce interleukin-8 and CXCL-2 chemokine production leading to increased neutrophilic inflammation and lung fibroblast activation [39].

A significant deficiency of FPR's can increase the risk of infections such a listeria monocytogenes with neutrophils also required to repair the lung epithelium following ARDS through their secretion of matrix metalloprotease-9. Therefore, excessive loss of FPR function may also have detrimental effects on the immune system [42-44].

Monocytes and macrophages FPR's

The FRP-1 receptors on human macrophages are less studied but all monocytes and macrophages express FPR-1 receptors including resident alveolar macrophages. In some this surface receptor can be rendered non-responsive by the cytokine milieu [38]. The classically stimulated pro-inflammatory M1 macrophages are activated by lipopolysaccharides, interferon-γ and tumour necrosis factor-α to produce heightened immune surveillance to infection via their FPR-1 receptors. In contrast, the alternatively activated M2 macrophages are largely anti-inflammatory with reduced FPR-1 expression which is unresponsive to bacterial peptides but activated by interleukin-4, 10 and 13 and transforming growth factor-β [38]. Monocytes and macrophages are critical for appropriate protective responses in bacterial, viral and fungal infections, with animal studies demonstrating increased lung pathology and neutrophil recruitment when monocytes are depleted [3,45]. Bronchial lavage from ARDS patients show alveolar macrophages to be enriched with M1-like genes on day 1 and M2-like genes by day 4-8, with

this change predicting survival at 28 days. In contrast, a maintained M1-gene enrichment predicts death [45].

Neutrophil NETosis

Excessive stimulation of the neutrophil surface FPR-1 receptor stresses the neutrophil and increases intracellular oxidants production with activation of intracellular protein kinase C (PKC). Highly activated and stressed neutrophils can undergo neutrophil NETosis [11,38,46,47]. In this process, first identified in 1996, the neutrophil extrudes its DNA and chromatin as a NET (**N**eutrophil **E**xtracellular **T**rap) to catch and kill infectious agents, reduce bacterial virulence and prevent micro-organism spread. These NET's can produce additional tissue damage by activating coagulation with thrombosis within the scaffold of the NET that effectively blocks the circulation [48]. Activation of intracellular PKC is a potent inducer of neutrophil NETosis with calcium influx required to triggers the entry of activated neutrophil elastase (NE) and myeloperoxidase into the nucleus to drive the NETosis cascade [48,49]. This triggers nuclear disintegration and chromosomal DNA release with gasdermin-D generating pores in the neutrophil membrane for its expulsion [48]. NETosis is also seen in viral illnesses like influenza and was demonstrated in the plasma of patients with H1N1 and H7N9 viral infections. These viral NET's were shown to be ineffective against bacterial infection suggesting a functional difference [46]. NETS produce injury to the host and may contribute to the development of autoimmune diseases via released nuclear material as seen in DNase-deficient mice, who develop a lupus-like syndrome with anti-nuclear antibodies due to their inability to digest released nucleic acids [11,28,36,47,49,50,51].

A high level of NETosis within the pulmonary circulation predicts ARDS and increases mortality as shown in Covid-19 postmortems findings [43]. Generally neutrophil migration and phagocytosis is reduced in those > 60 yrs, but spontaneous free radical IS increased by age and also by hyperglycaemia. This produces higher bystander tissue damage with infections that increases susceptibility to NETosis and ARDS in older patients with higher mortality [43]. Platelets and neutrophils cooperate in sepsis with platelet migration following the neutrophil, a process that ends if neutrophils are depleted or migration reduced [11]. Plasma from septic patients can induce platelet-neutrophil interactions and NETosis within 5-10 minutes. Both gram positive and gram negative bacteria are susceptible to NET-mediated killing, but some bacteria express DNase which degrades the NET scaffold of DNA allowing bacterial escape. This is seen particularly in Group A streptococcus and Strep. Pneumoniae allowing spread through the airway [11].

Dapsone

Many antibiotics have effects on immune function. Studies of the sulphonamide Dapsone (4, 4-diaminodiphenol sulphone) confirmed its ability to reduce the generation of both intracellular and extracellular oxygen free radicals generation by neutrophils, in a dose dependant manner via blockade of their FPR-1 [37,52-56]. This blockade also reduces NETosis as Dapsone is able to reduce intracellular Protein Kinase C (PKC) stimulation by its ligand PMA (Phorbol 12-myristate 13-acetate). This effect would discourage neutrophil NETosis and reduce NE release into the extracellular space thereby offering protection against an evolving ARDS [32,57].

Cyclosporine

Cyclosporine H and A, have a high affinity for FPR-1 and lock the receptor into an inactive state thereby reducing acute lung injury in animal models [29,31,44,48]. This protection by cyclosporine is seen both before and after a lung insult. The release of oxygen free radicals and the production of NETosis are both reduced in a dose dependant manner by cyclosporine. In addition, both drugs reduce alveolar neutrophil numbers and even alveolar protein leak following lung injury. Since protein leak is related to the severity of hypoxaemia, these drugs could offer considerable additional benefit in ARDS but like Dapsone they have not been investigated in man [31]. Cyclosporine A + H, also reduces Protein Kinase C activation and calcium influx thereby blocking the key steps in the NETosis cascade [47-49,]. Cyclosporine A is shown to reduce NETosis induced by the calcium ionophore (ionomycin), indicating that its action is downstream from the calcium activating affects [48,49]. Following cyclosporine A treatment, isolated neutrophils show reduced motility and degranulation [48]. Mice with genetically absent FPR's show protection from acute lung injury indicating a potential therapeutic target in ARDS to down regulate the excessive neutrophil activation [44].

Cotrimoxazole

Early studies by Anderson showed cotrimoxazole (sulphamethoxazole + trimethoprim) to also reduce neutrophil-derived oxidative stress and intracellular protein kinase C activation similar to that seen for Dapsone [57,58]. These findings would be consistent with the shared sulphonamide ring and antibiotic sensitivities of sulphamethoxazole and Dapsone [59]. Previously published data from our lung fibrosis study using cotrimoxazole, examined cotrimoxazole (CTX) and trimethoprim (TMP) effects upon neutrophils and monocytes (National Research Ethics Committee for London-South East: REC reference 2006-004927-12) [60]. It confirmed the ability of cotrimoxazole to significantly block both the neutrophil and monocyte FPR-1 stimulation by the bacterial peptide f-Met-Leu-Phe (an FPR-1 ligand) in patients taking cotrimoxazole. Eight healthy medical staff involved in the study took a 7-day course of cotrimoxazole (960 mg 12 hrly) to

establish if the immune effects differed in lung fibrosis patients relative to healthy controls. After a 2 months washout, they also took trimethoprim (200 mg 12 hrly) for 7 days as described in the published manuscript [60]. Further expansion of this to a total of 12 healthy medical staff (unpublished data) is shown here in Figure 1 + Figure 2 and demonstrates the effects upon neutrophil and monocyte FPR-1 activation as assessed by flow cytometric measurement of 10,000 cells following 2 stimulants [60]. Figure 1 shows neutrophil stimulation of the FPR-1 by the bacterial peptide f-MLP (N-formyl-Met-Leu-Phe) to be reduced 70% by oral cotrimoxazole ($p = 0.005$) and trimethoprim ($p = 0.0001$) at 7 days. For monocytes, FPR stimulation was also reduced by 60% by cotrimoxazole ($p = 0.0007$) and 75% by trimethoprim ($p = 0.0001$). These results confirmed Anderson's original neutrophil findings now with the additional monocyte findings indicating their ability to reduce cellular activation in both cell types.

Figure 2 shows that neutrophil stimulation of Protein Kinase C (PKC) by its ligand PMA (phorbol 12-myristate 13-acetate) to be reduced by 30% following both trimethoprim and cotrimoxazole. While in monocytes, stimulation by PMA was reduced by 68% for cotrimoxazole alone ($p = 0.003$), suggesting that the sulphamethoxazole component produced this effect. Since PMA stimulates intracellular protein kinase C activity producing oxidant release, this reduction could reduce neutrophil NETosis in ARDS with CTX effects upon monocytes offering an additional clinical benefit [41,47,57].

In the absence of formal clinical trials of cotrimoxazole and trimethoprim, there is circumstantial data and case reports suggesting that these FPR-blocking drugs have likely benefit in ARDS [59,60]. Certainly, the literature contains dramatic case reports of rapid recovery from severe ARDS following the addition of intravenous cotrimoxazole in Middle Eastern Respiratory Syndrome and other cases of ARDS [61-63]. A Nationwide analysis of Japanese patients ventilated for rapidly progressive respiratory failure in Idiopathic Pulmonary Fibrosis, showed a significant survival benefit with cotrimoxazole and rescue steroids (odds ratio 0.2); indicating an 80% reduction in the risk of death compared to a range of other drugs. The benefit was presumed to be immunological or related to effects upon pneumocystis infection [64]. Cotrimoxazole has also been shown to reduce interleukin-1, 2, 6 and 8 and tumour necrosis factor-α (TNFα) production by neutrophils and macrophages and these cytokines are raised in ARDS [65,66].

Translational studies

Animal models of ARDS have led to human clinical trials, but these have not generated positive findings to date, and include studies of aspirin, statins, neuromuscular blockers; intranasal nitric oxide, intravenous beta-2 agonists, antagonists of CXCR2, DNase enzyme treatments and 2 Neutrophil Elastase inhibitors (Sivelestat & AZD9668) [18,46,67]. Colchicine trialled in the UK Covid-19 recovery trial to reduce neutrophil recruitment, did not show survival benefit at 28 days ($p = 0.063$) relative to standard therapy [68].

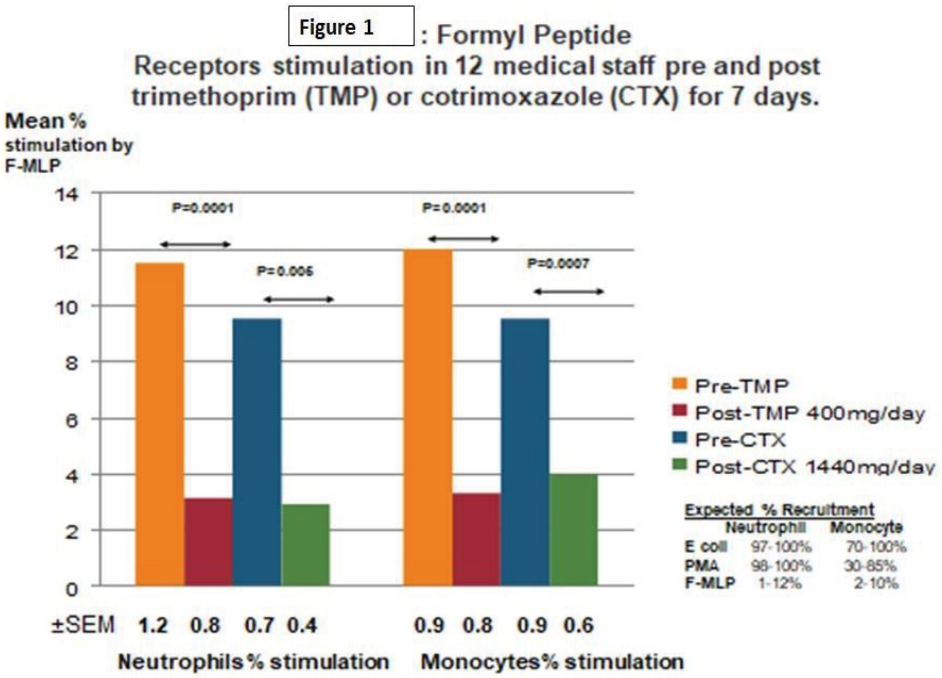

Figure 1: Shows the results of neutrophil and monocyte FPR stimulation by the bacterial peptide f-MLP as percent of positively stimulated Cells. It shows group means ± SEM (standard error of mean) for the 12 healthy medical staff, and the change from baseline following 7 days of cotrimoxazole (CTX) 1440 mg/day or trimethoprim (TMP) 400 mg/day.

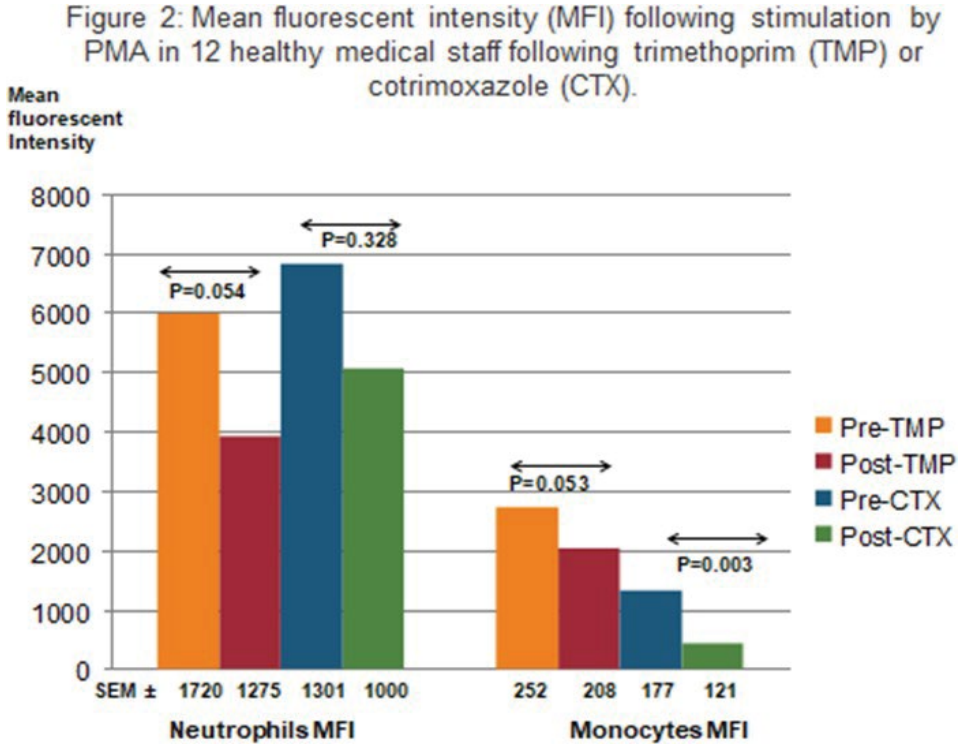

Figure 2: Shows the results of neutrophil and monocyte stimulation by PMA-ligand (phobol 12-myristate 13-acetate) upon intracellular protein kinase C activation as measured by flow cytometric mean fluorescent intensity (MFI) following PMA stimulation. The graph shows group means ± SEM for the 12 healthy medical staff and the change from baseline following 7 days of cotrimoxazole (CTX) 1440 mg/day or trimethoprim (TMP) 400 mg/day.

Table 1: Shows the summarised findings of 3 different patient cohorts with severe Covid-19 induced ARDS who received added cotrimoxazole (CTX) or trimethoprim (TMP) added to standard care (ST) versus ST-alone. The table shows the mortality and mechanical ventilation rate relative to standard care with the 4th cohort of 15 home treated Covid-19 ARDS patients supplied with oxygen and CTX and their outcome.

Author (reference No.)	Randomised	No. ST*	No. ST* + CTX or TMP	Added Steroids	Mortality ST	Mortality CTX (p value vs. ST)	Mechanical Ventilation ST	Mechanical Ventilation (p value vs. ST)
Quadery [75]	Yes	55	58 Δ	100%	29%	11% (p = 0.020)	13%	9% (p = 0.90)
Singh [76]	No	50	151 Δ	100%	40%	13% (p = 0.001)	40%	15% (p = 0.001)
Siddiqui [77]	No	0	15 Δ	Nil	-	0%	-	0%
Varney [67]	No	84	46 Θ	ST = 73% TMP = 32%	36%	17% (p = 0.014)	7%	4% (p = 0.9)

*ST: Standard Therapy of broad spectrum antibiotics, heparin, oxygen: ΔCTX (cotrimoxazole 960 mg) 8 hrly: ΘTMP (trimethoprim) 400 mg/day in place of CTX

A recent study of beta-interferon significantly reduced mortality in phase I + II trials, encouraging optimism from its ability to generate extracellular adenosine to improve barrier function and reduce alveolar leak in ARDS. Sadly the phase III study did not reduce mortality nor ventilator free days [46,47,69-73]. Drugs that inhibit molecules involved in NETosis (PAD4 and gasdermin-D) are in development, with Disulfiram already known to inhibit gasdermin-D and shown to reduce lung injury in animal models [47,74]. Most of these translational studies to reduce NETosis in ARDS, will be acting late in the pathological process and this may explain the failure seen with these studies. Since ARDS can result from different insults, some researchers consider this relevant to the lack of therapeutic progress, suggesting that future studies should focus upon the initial underlying insult.

Cotrimoxazole Data from Covid-19 ARDS

A 2017 review of neutrophils in ARDS concluded that: -" The holy grail of ARDS therapy will be to limit neutrophil recruitment, priming and activation while preserving host defense" [5]. This statement and the reported effects of these drugs upon the FPR's, led to a decision

to try these in Covid-19 induced ARDS. Cotrimoxazole (CTX) was given orally to 4 severely hypoxic Covid-19 patients within the first week of the UK pandemic and was observed to reduce their oxygen requirements from 15 L/min to 2 L/min within 12 hrs followed by rapid recovery and discharge. This observation led on to the following data using cotrimoxazole or trimethoprim (TMP) in critical Covid-19 patients with radiological ARDS (Table 1).

Quadery and colleagues openly randomised 111 patients with severe Covid-19 to standard therapy (ST) of antibiotics (benzyl penicillin and clarithromycin), dexamethasone and heparin OR oral cotrimoxazole (960 mg 8 hrly) with ST [75]. Entry criteria included an oxygen requirement of 10-15 litres/min with randomisation 1:1. Both groups well matched demographically. The results showed a significant 18% reduction in mortality with added CTX (11% versus 29% for ST, p = 0.020). A further case series from West Bengal also showed benefit [76]. Here, outcomes from 50 critical Covid-19 patients given standard therapy (ST) were compared to 151 patients treated with added CTX (960 mg 8 hrly) + ST. The ST consisted of piperacillin/tazobactam with clarithromycin or azithromycin, dexamethasone, heparin, remdesivir and favipiravir. These patients had severe disease with initial saturations < 90% on air and chest-X-rays and CT chest scans showing lung infiltrates. The mortality was 40% in the ST-only group and 13% in the CTX + ST group. Differences in the requirement for mechanical ventilation were seen with CTX at 15% versus 40% for ST only. ITU admissions were 6 days for CTX versus 11 days with ST (p = 0.001); with a reduced total mean hospital stay (11 days versus 15 days for ST: p = 0.001).

An interesting case series from Bengal India, examined domiciliary managed acute Covid-19 infection (n = 15) with mean oxygen saturations of 87.6% on air and radiological changes seen in 9 patients who were offered a chest-X-ray [77]. This cohort had home treatment of oxygen and oral CTX 960 mg/day added to ST of azithromycin 500 mg/day. Oxygen saturations were significantly improved by 48-72 hrs with radiological improvement seen in all 9 chest-X-rays at 7 days. This approach prevented hospital admission in 14 out of 15 cases, with the single admitted case due to coexistent dysentery requiring treatment. All patients made a full recovery**.** The authors concluded that it was the CTX added to azithromycin gave the improvement and suggested formal trials of CTX. The low cost of TMP or CTX makes it an affordable drug for many poorer countries to reduce admissions and mortality as suggested by this various data [77].

Our published case series of non-randomised covid-19 positive patients admitted in 2020-2021 showed benefit to the 46 cases who received oral trimethoprim (TMP) 200 mg 12 hrly + ST compared with 84 patients who received ST (benzyl penicillin and clarithromycin, dexamethasone, heparin, remdesivir) along with entry to the UK recovery trial. This case series showed reduced mortality in the TMP-treated group at 17% versus 36% taking ST + Recovery trial entry. There was a significantly faster reduction in oxygen requirement (p = 0.03), fever (p = 0.0001) and inflammatory markers (0.0001) at 48 hrs with TMP that was not seen for ST + recovery trial. The Hospital length of stay was reduced to a mean 10.6 days for TMP versus 17.1 days for ST (p = 0.003) [77]. The overall clinical findings are summarized in Table 1.

ARDS clearly requires effective treatments that have not yet been identified. Since drugs such as Dapsone, CTX, TMP and cyclosporine have the ability to reduce early activation of neutrophils and monocytes, they could significantly reduce the escalating host responses and prevent acute lung injury from progressing to ARDS. These inexpensive drugs have shown benefit in Covid-19 ARDS, but have never been assessed for non-Covid related ARDS in man, although the shared mechanisms would predict likely benefit. Since broad spectrum antibiotics are already part of the standard treatment protocols in ARDS, the unique Immunological properties of CTX and TMP to calm the host's immune response warrants investigation. Since their effects are immunological, antibiotic sensitivities are irrelevant to the therapeutic effect in this situation compared to standard antibiotics. Their absorption is rapid with their use in a timely manner important in early ARDS, to inhibit the evolving neutrophil activation and migration before the disease process becomes established. Since ARDS represents a sustained neutrophil attack upon the host, then calming this early component of the Immune system may be all that is required to maintain oxygenation and protect against multi-organ failure. The targeting of individual mediators much later in the disease process, when injury is already established may offer little benefit.

Conclusion

ARDS can be life threatening and has no effective drug therapies to date. Since it represents a sustained neutrophil attack driven by ongoing activation of the neutrophil Formyl Peptide Receptors, blockade of these receptors as demonstrated in our medical staff, could calm the "out of control host" offering an effective drug treatment. Our data shows that like Dapsone and cyclosporine, both cotrimoxazole and trimethoprim reduce neutrophil activation with evidence of benefit through reduced mortality seen in Covid-19 ARDS suggesting human studies are justified. Early recognition of a deteriorating patient is essential, as delay may reduce the ability of these inexpensive drugs to act before irreversible alveolar damage and NETosis occurs.

Declarations

Conflict of interest

The authors declare no conflict of interest with data available upon request from the corresponding author upon request.

The medical staffs are co-authors and have approved this publication which was part of a previously published study with Ethical approval as cited with this manuscript.

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Author contributions

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