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Jack of All Trades: Pleiotropy and the application of Chemically Modified Tetracycline -3 in Sepsis and the Acute Respiratory Distress Syndrome (ARDS)

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Abstract

Sepsis is a disease process that has humbled the medical profession for centuries with its resistance to therapy, relentless mortality, and pathophysiologic complexity. Despite 30 years of aggressive, concerted, well-resourced efforts the biomedical community has been unable to reduce the mortality of sepsis from 30%, nor the mortality of septic shock from greater than 50%. In the last decade only one new drug for sepsis has been brought to the market, drotrecogin alfa-activated (XigrisTM), and the success of this drug has been limited by patient safety issues. Clearly a new agent is desperately needed. The advent of recombinant human immune modulators held promise but the outcomes of clinical trials using biologics that target single immune mediators have been disappointing. The complex pathophysiology of the systemic inflammatory response syndrome (SIRS) is self-amplifying and redundant at multiple levels. In this review we argue that perhaps pharmacologic therapy for sepsis will only be successful if it addresses this pathophysiologic complexity; the drug would have to be pleiotropic, working on many components of the inflammatory cascade at once. In this context, therapy that targets any single inflammatory mediator will not adequately address the complexity of SIRS. We propose that Chemically Modified Tetracycline-3, CMT-3 (or COL-3), a non-antimicrobial modified tetracycline with pleiotropic anti-inflammatory properties, is an excellent agent for the management of sepsis and its associated complication of the Acute Respiratory Distress Syndrome (ARDS). The purpose of this review is threefold: 1) to examine the shortcomings of current approaches to treatment of sepsis and ARDS in light of their pathophysiology, 2) to explore the application of COL-3 in ARDS and sepsis, and finally 3) to elucidate the mechanisms of COL-3 that may have potential therapeutic benefit in ARDS and sepsis.

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Keywords

Sepsis; Shock; Acute Respiratory Distress Syndrome; ARDS; MODS

1.1- Introduction- An Old Nemesis

Sepsis is a disease process that has humbled the medical profession for centuries with its resistance to therapy, relentless mortality, and pathophysiologic complexity. Medical practitioners and the public at large have recognized the key features of sepsis and septic shock for centuries. A casual observation by Macchiavelli reflects the understanding of the time course and severity of the disease that was already in common parlance 600 years ago, “Hectic fever (sepsis) at its inception is difficult to recognize but easy to treat. Left untended, it becomes easy to recognize but difficult to treat.”[1] This observation very accurately characterizes the treatment challenge presented by sepsis and shock: that once the disease has manifested it is often so severe that therapy is doomed to failure. The 21st century is an era in which many of our therapies are successful at managing, if not necessarily curing the diseases that afflict our patients. In this era of successes outnumbering failures, sepsis and its complications retain disturbingly high mortalities and morbidities. In the US there are 750,000 cases per year with an estimated cost of \$17 billion, this year 934,000 cases are anticipated [2]. Despite 30 years of aggressive, concerted, well-resourced efforts the biomedical community has been unable to reduce the mortality of sepsis from 30%, nor the mortality of septic shock from greater than 50% [3]. Indeed, in the last decade only one new drug for sepsis has been brought to the market, drotrecogin alfa-activated (Xigris™), and the success of this drug has been limited by patient safety issues.[4, 5]

One of the most common and feared complications of sepsis is the Acute Respiratory Distress Syndrome (ARDS). ARDS, occurring in nearly 200,000 patients yearly, claims the lives of a third of the patients that it afflicts [6, 7]. The dismal prognosis of ARDS has been similarly resistant to therapeutic research over the last 30 years [8]. Although intensive clinical outcomes research using protocol-driven supportive modalities such as the *Surviving Sepsis Campaign* and the *ARDS Network (ARDSnet)* have helped establish standards of care and have improved outcomes, these treatment strategies are not based on targeting the specific deranged physiology of either sepsis or ARDS [9]. It can be argued that there is a theoretical limit to the success of supportive measure-based treatment; more precisely if we can target what is *wrong*, we can offer a *cure*. Recent basic science research has significantly improved our understanding of the mechanisms of these related diseases and a holistic view of this pathophysiology is required in order to direct our attempts at managing sepsis and ARDS.

A century ago Sir William Osler commented on the topic of sepsis that, “Except on few occasions, the patient appears to die from the body’s response to infection rather than from it.” In this rather prescient deduction, based purely on observing his patients, Osler correctly identified the overly prolific immune response as the key pathophysiologic step in the development of sepsis [10]. Any approach to the treatment of sepsis and ARDS requires a clear and comprehensive understanding of the mechanisms of this hyper-prolific immune response.

1.2- Complex Pathophysiology of Systemic Inflammation

The overly prolific inflammatory response to severe injury or infection, known as the Systemic Inflammatory Response Syndrome (SIRS), is centrally implicated in the pathophysiology of sepsis, septic shock, and their feared potential outcome, multiple organ

dysfunction syndrome (MODS). Recent data support the longstanding clinical observation that more people die from MODS and septic shock than from sepsis alone [11]. SIRS causes a massive release of a wide repertoire of inflammatory mediators from immune cells resulting in endothelial dysfunction, profound vasodilation, and subsequent increases in vascular permeability [12]. The inflammatory mediators released number in the hundreds, indeed over 600 unique genes are upregulated in human SIRS patients [12, 13]. Each mediator is responsible for cross-regulatory, overlapping actions on immune cells, endothelium, neurohormonal function, metabolic function, and coagulation as shown by Figure 1 [14–53]. These actions include multiple components including the release of proteolytic enzymes such as matrix metalloproteinases, induction of apoptosis of endothelial cells, and both pro- and anti- thrombotic effects on the coagulation cascade [54]. Furthermore each mediator has the capacity to trigger the release of more inflammatory mediators leading to a self-augmenting, overwhelmingly proinflammatory state [12, 54–56].

As Figure 1 illustrates, the inflammatory response is extremely complex, multifaceted, and redundant at multiple regulatory levels. This complexity has been indicted as the reason why targeted pharmacotherapies against sepsis have proven so disappointing in clinical trials. Many drugs that have shown promise in small animal studies work against single mediators in this dense web of intertwined cross-regulatory pathways to cell damage, death, and further propagation of inflammation. Unfortunately, these ‘single-mediator’ therapies’ results were not reproduced in the clinical arena. Among the reasons cited for this failure are the inability of the small animal “knockout mouse” model to adequately replicate the complexity of the human disease state; and the multifactorial complexity of the pathology itself [57]. For pharmacotherapy to be successful in the management of sepsis and its complications the drug would clearly have to address the complex, redundant pathophysiology. In order for a single drug to do this successfully it would have to work on multiple targets at once, in other words this drug would have to be pleiotropic.

Chemically Modified Tetracycline -3 (CMT-3, or COL-3) is a drug that has distinct pleiotropic actions. This review will focus on the unique pleiotropic actions of Chemically Modified Tetracycline-3 and its potential applications as pharmacotherapy against SIRS-sepsis as well as SIRS-induced ARDS. CMT-3 is the first chemically modified non-antimicrobial tetracycline to be administered to humans in clinical trials [58]. CMT-3 was first described as a host-modulating drug by Golub et al [59] and was produced by removing the dimethylamino group (needed for antimicrobial efficacy) from carbon-4 of the “A” ring of the tetracycline molecule, as well as the methyl & hydroxyl groups from the carbon-6 position of the “C” ring; i.e 6-demethyl 6-deoxy 4-dedimethylamino tetracycline, or CMT-3/ COL-3. This compound was found to be a more potent MMP inhibitor than tetracycline and doxycycline [60]. Rudek et al described some of the pleiotropic benefits of this drug in the first human clinical trial of COL-3 in 2001 [58]. In order to fully elucidate the therapeutic potential of the pleiotropy of COL-3 it is essential to understand the complexity of the pathologic process. The purpose of this review is threefold: 1) to examine the shortcomings of current approaches to treatment of sepsis and ARDS in light of their pathophysiology, 2) to explore the application of COL-3 in ARDS and sepsis, and finally 3) to elucidate the mechanisms of COL-3 that may have potential therapeutic benefit in ARDS and sepsis.

The pathophysiology of organ failure from SIRS can be conceptually divided into an initiating *inflammatory* phase followed by secondary *tissue damage* phase [61]. In the inflammatory phase trauma, hemorrhage, and severe sepsis cause a massive release of multiple inflammatory mediators that result in the clinical manifestations of acute shock. In the tissue damage phase, these inflammatory mediators trigger organ damage by promoting a variety of proteolytic and oxidative effectors including matrix metalloproteinases (MMPs) and reactive oxygen species [62]. As these effectors exert their damaging actions on tissue,

various organs lose function and eventually fail altogether leading to the feared entity of Multisystem Organ Failure or Multiple Organ Dysfunction Syndrome (MOSF or MODS). Compounding this process is the fact that tissue damage itself triggers the further release of inflammatory mediators thus creating a “vicious cycle” of amplification of the proinflammatory state, tissue destruction, further inflammation, eventual organ failure and death. Often the lung is among the first organs to fail as SIRS is transduced into MODS [63]. ‘Lung failure’ or ARDS is a progression of the injurious mechanisms that characterize SIRS.

1.3- Acute Respiratory Distress Syndrome Pathogenesis

Vasodilation and endothelial dysfunction resulting from massive inflammation cause an increase in endothelial permeability, sometimes termed ‘capillary leak’ (Figure 2). The effect of capillary leak in the lung is a heterogenous exudative edema that coats the gas-exchanging surface of the alveolus. The inflammatory exudates filling the alveolar surface cause surfactant dysfunction resulting in dependent atelectasis, and heterogenous alveolar expansion and collapse with each breath. Figure 2 demonstrates these effects at the histologic level: the lung alveolar surfaces are coated with fibrinous exudates, intra-alveolar hemorrhage is present, and abundant sequestration of neutrophils is seen. As a result of these processes the clinically observable features of ARDS manifest as hypoxemia, hypercarbia and changes in pulmonary mechanics that reflect the ‘stiffening’ of the lung i.e. reductions in compliance, increase in mean airway pressures, peak airway pressures, and plateau pressures. [6, 64]

Exacerbating the processes described above is the active proteolytic destruction of lung architecture by immune cell associated proteases. The respiratory units of the lung are mechanically integrated and therefore interdependent on one another via a network of ‘tethering’ connective tissue composed largely of elastin and collagen. This architectural integration results in coordinated movement of multiple respiratory units (i.e. Air Sacs) together with respiratory motion (i.e. changes in lung volume). Proteases including matrix metalloproteinases (MMPs) released by a variety of immune cells, particularly polymorphonuclear leukocytes, monocytes and mast cells, are known to be upregulated in terms of both production and activation in ARDS [65–68] (Figure 2). These enzymes actively destroy parenchymal architecture thereby worsening the lung’s ability to move efficiently and exchange gas.

Alveoli are normally stable in size and shape throughout the respiratory cycle; however subsequent to the onset of inflammation the alveoli become unstable- collapsing and reopening with each breath (Figure 3) [69]. This alteration in alveolar mechanics (i.e. the dynamic change in alveolar size and shape during tidal ventilation) is caused by the combination of edema-induced surfactant dysfunction and destruction of architectural integrity [70, 71]. Figure 3 illustrates this process clearly showing an alveolus that is inflated at inspiration (Panel F) but completely collapsed at expiration (panel E). Unstable alveoli like those seen in Figure 3 experience mechanical and biological trauma with each cycle of expansion and collapse [69]. Lung tissue destruction results in further localized inflammation, further release of inflammatory mediators and activation of proteolytic enzymes [71–74]. Thus it can be seen that the pathophysiology of ARDS (Figure 2) is an extension of the pathophysiology of SIRS (Figure 1) in that it is complex, multifaceted, self-augmenting, and redundant at multiple levels (Figure 2). ARDS is lung dysfunction/failure and is therefore a subset of the organs that fail in MODS. In this sense ARDS is situated along the SIRS - MODS continuum of disease and can be considered a consequence of SIRS. This similarity in pathophysiologic mechanisms between SIRS and ARDS could prove to be of therapeutic advantage to patients at risk of developing organ failure from

SIRS if the mechanisms could be targeted by pharmacotherapy. In effect treating SIRS would treat ARDS.

2.1- Trail of Tears: The Track Record of Biomedical Research in ARDS

In a recent review of ARDS clinical trials by McIntyre et al [8], 15 different therapeutic modalities are analyzed with respect to evidence-based support of therapeutic efficacy. The review utilized the standard validated rating system for medical evidence and best-practice recommendations [See Table 1]. Of the interventions examined in the review, 6 were not recommended, 7 received recommendations at either the “C” or “D” levels, and one (protective ventilation) received a recommendation at the “B” level. None received a recommendation at the “A” level. These findings are tabulated in Table 2. [8]

Cepkova et al found similarly disappointing results in their review of non-ventilatory (i.e. pharmacological) clinical trials for ARDS. Among the pharmacological interventions reviewed were surfactant therapy, vasodilators, anti-inflammatory agents, and anti-oxidants. In their Summary they state “There is no pharmacological therapy that can be recommended in standard therapy of acute lung injury (ALI).”[7]

The reasons for the failure of these initially promising interventions are multifactorial. Both authors suggest that novel therapies based on highly simplified small-animal models, such as knockout mice, fail in human clinical trials due to their inadequate modeling of the complexity of the human disease state. Both authors cite that mortality may be a poor outcome measure in clinical studies evaluating ARDS since most patients do not die of hypoxia but rather succumb to other complications of sepsis or shock. As Figure 1 illustrates, no individual component of SIRS pathologic process occurs independently. The complement system, arachidonic acid metabolites, cytokines, nitric oxide, reactive oxygen species (ROS), proteases including MMPs and the coagulation cascade are intimately interconnected (Fig 1) and if uncontrolled can lead to serious tissue damage ultimately causing organ failure. The changes induced by these pathways include epithelial & endothelial permeability changes, surfactant dysfunction, neutrophil sequestration and activation (with the accompanying proteolytic and oxidative tissue damage), cell death in the form of necrosis and apoptosis and abnormalities in vascular reactivity; all of which occur simultaneously (Figs 1&2). Furthermore tissue damage itself reinitiates the inflammatory process resulting in a self-amplifying, self-exacerbating cycle. Therefore it is not mechanistically possible to prevent SIRS-induced ARDS by targeting a single endogenous mediator, as the clinical research in this field has repeatedly shown.

The unique therapeutic opportunity offered by COL-3 for both sepsis and ARDS lies in the pleiotropy of the drug. COL-3 inhibits both the mediators that *initiate* and perpetuate systemic inflammation and the mediators that *effect* tissue injury. COL-3 acts at multiple sites in the inflammatory process that are key in ARDS pathogenesis (Figs 1 & 2). Among the inflammatory initiators this modified tetracycline is active against include: tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), interleukin 6, and other interleukins via NF κ B, platelet activating factor (PAF), Thromboxanes, and Prostaglandins [75–77]. Among the effectors the drug has activity against are matrix metalloproteinases (MMPs), reactive oxygen species (ROS) and nitric oxide (NO) [78–80]. Furthermore COL-3 is able to inhibit apoptosis via its actions against caspase pathways [81, 82]. Our group hypothesized that COL-3 would be effective at inhibiting the systemic inflammation that causes ARDS by blocking a critical combination of mediators. This pleiotropic blockade could override the cross-regulatory redundancy of SIRS, shut down the amplification of inflammation, and ameliorate the damage to tissue and organ function.

3.1- Application of Chemically Modified Tetracycline- 3 in ARDS and Sepsis

Although there are only a few studies investigating the effect of COL-3 on sepsis and ARDS the studies that have been conducted have shown that COL-3 is a powerful drug for ARDS prevention and treatment. COL-3 is known to be a potent inhibitor of the gelatinase family of matrix metalloproteinases, MMP-2 and MMP-9, by at least two mechanisms. First, COL-3 chelates the zinc in the active sites of the enzymes resulting in both reversible and irreversible competitive inactivation in the extracellular matrix. Second, COL-3 reduces MMP mRNA transcription at the nuclear level resulting in reduced production of MMP-2 and MMP-9. Therefore the drug has a twofold effect reducing both MMP activity and MMP quantity [80]. As described earlier, and illustrated in Figure 1; MMPs are important *effectors* of organ injury in the SIRS-MODS cascade. The proteolytic destruction of tissue that eventually leads to organ failure in general and ARDS in particular is mediated by MMPs. Moreover COL-3 is the only tetracycline (TC) to date that has demonstrated the ability to *directly* inhibit neutrophil elastase (a serine protease) although various TCs can indirectly inhibit neutrophil elastase by protecting serum α -1 antitrypsin from MMP attack and can inhibit macrophage metalloelastase, which is a matrix metalloproteinase (MMP-12). Since neutrophils are key effectors of injury (Fig 1) the direct action of COL-3 against neutrophil elastase is another potentially beneficial mechanism. The therapeutic implications of COL-3 as an MMP inhibitor in these inflammatory disease states has been investigated only in small and large animal models but shows tremendous promise.

3.2- COL-3 In ARDS

Studies by the Nieman group have clearly shown the effects off COL-3 mediated MMP blockade in the context of ARDS. Carney et al showed that COL-3 caused a decrease in MMP-2 and MMP-9 concentrations in the bronchoalveolar lavage fluid of pigs injured by cardiopulmonary bypass (CPB) combined with a very small dose of LPS infusion and pretreated with COL-3 [61] (Figure 4 & 5). Note how COL-3 treatment reduces gelatinase and elastase activity to nearly that of naive controls in Figure 5. Steinberg et al demonstrated a 64% reduction in MMP-2 activity, a 34% reduction in MMP-9 activity, and a reduction in neutrophil elastase concentration in the bronchoalveolar lavage fluid of a chronic, clinically relevant porcine shock-ARDS model [83]. Inhibition of these proteases was correlated with a significant reduction of lung injury (Figure 6). Particularly dramatic among these results is the histologic data from the Steinberg study demonstrating that treatment with COL-3 almost completely eliminates the stigmata of ARDS exhibited by the untreated group, resulting in alveoli that are as healthy and well inflated as the naive controls (Figure 6). The Zwischenberger group demonstrated significant improvements in hemodynamics, pulmonary gas exchange, lung histology and survival when sheep injured with smoke inhalation and cutaneous burns were treated with COL-3. In their chronic, clinically applicable, translational model they were able to show marked improvements in outcomes, Figure 7 shows survival data from this elegant study [84].

3.3- COL-3 In Sepsis

Ramamurthy et al have shown COL-3 induced improvements in both survival and liver injury in rats subjected to polymicrobial sepsis by cecal ligation and puncture (CLP). Their data demonstrated strong evidence that reductions in MMP-9 and Nitric Oxide were the mechanism by which COL-3 reduced organ injury and improved survival [85]. Maitra et al reproduced similar effects in a rat CLP model and further characterized the hepatoprotective effect of COL-3 [81]. They demonstrated that Tissue Inhibitor of Metalloproteinase - 1 (TIMP-1) was increased in the livers of animals treated with COL-3. Figure 8 shows that treatment of septic rats with COL-3 not only reduced expression of MMP-2 and MMP-9 but

it also increased the expression of TIMP-1 in liver specimens from these animals. They further showed that transforming growth factor beta-1 (TGF- β 1) and caspase-3 were reduced (secondary to TIMP-1 induced reduction in MMP-9 & -2 activity) thereby improving survival in their CLP model [81, 82].

4.1 Pleiotropy and the potential of Chemically Modified Tetracycline 3

Two recent studies have demonstrated that MMPs are present in the 3rd space fluids of human patients; peritoneal ascites fluid [86] and interstitial fluid collected from cutaneous blisters [87]. Both studies demonstrated that concentration and activity of MMPs in third space fluids correlate directly with the severity of sepsis as well as patient survival. A recent study from Italy identified MMP 9, MMP 10 and TIMP levels in the serum of human sepsis patients as biomarkers for both severity of sepsis and mortality [88]. The implications of these studies are that COL-3 with its pronounced activity against MMPs might reduce sepsis severity and improve survival. Preliminary translational data also support these implications. Preliminary data from the Nieman group showed improvement in survival with COL-3 treatment in a porcine model of shock induced by ischemia/reperfusion and peritoneal sepsis.

Although several studies have shown that COL-3 is a powerful drug in the treatment of SIRS and ARDS the exact mechanism of protection is not known since COL-3 inhibits multiple inflammatory mediators. COL-3 has been shown to be active against an array of endogenous mediators in the inflammation-tissue injury axis. It is possible that inhibition of multiple pathways simultaneously may be much more effective than is blocking single mediators in stopping SIRS and preventing ARDS. The exact combination of mediators that COL-3 inhibits is unknown but regardless of the exact mechanism the fact remains that COL-3 has been shown to prevent ARDS in very sophisticated translational animals models.

Since the bulk of clinical research on COL-3 has investigated the antiprotease activity of the drug largely in the context of cancer [80] such as Kaposi's sarcoma [89], there is a dearth of research examining other mechanisms of COL-3 in the context of sepsis and ARDS. There is however a body of bench science work that confirms that COL-3 is active against an array of other components of the inflammation-tissue injury axis, suggesting that these pathways may also be targeted by COL-3 in the context of SIRS. This literature provides an intriguing window into the potential mechanisms of COL-3 that are not well characterized and that may prove advantageous if articulated in studies specifically designed to address the efficacy of the drug in SIRS and its related complications.

4.2 Non-antiproteinase Mechanisms of COL-3 with Potential Benefit

4.2.1- Cytokines

The anti-cytokine activity of COL-3 is well described and has been shown to be directly active against TNF, IL1 β , and IL6 [75, 90, 91]. COL-3 also inhibits IL-12 synthesis and secretion via post-transcriptional inhibition [92]. Preliminary data from the Nieman group showed that COL-3 was able to prevent lung injury in a porcine model of shock-induced ARDS and that lung protection correlated with reduced plasma cytokine concentration [93]. The inhibition of multiple proinflammatory cytokines by COL-3 may produce an overall "de-escalation" of the Systemic Inflammatory Response Syndrome thereby mitigating the progression from SIRS to tissue damage.

4.2.2- Mast Cells

Mast cells are implicated in the progression of ARDS and sepsis, specifically the histamine release associated with the inflammation induced degranulation of these cells. Histamine

locally promotes vasodilation, an increase in vascular permeability and subsequent 3rd spacing of fluid. In a recent study Sandler et al demonstrated that COL-3 and other chemically modified tetracyclines (CMTs) promoted apoptosis of mast cells through inhibition of caspase pathways [94, 95]. This anti-mast cell activity of COL-3 suggests that this may also be a mechanism of lung protection as well as the mitigation of sepsis in the acute SIRS response.

4.2.3- Apoptotic Pathways

Caspase and its associated pathways are particularly important in the development of organ dysfunction. When activated, caspase pathways can lead to cell death through apoptosis. A number of studies have found chemically modified tetracyclines to inhibit caspase [81, 82, 95, 96]. This anti-apoptotic activity of COL-3 mediated via its inhibition of caspases adds to the potential of the drug to exert cytoprotective and organ preserving effects during sepsis and septic shock. This cytoprotective potential of COL-3 is particularly relevant since caspase mediated cell-death is a known feature of ARDS, and other forms of organ failure in sepsis-induced-MODS.

4.2.4- Free Radical Scavenging

The cells of the immune system charged with the task of killing cells and propagating inflammation are Polymorphonuclear Cells (PMNs) (see Figure 1). These cells exert their lethal effects by producing an “Oxidative Burst” in which an array of reactive oxygen species (ROS) are released into tissue killing cells and damaging tissue [62]. The four phenol rings that are the foundational structure of all tetracyclines allow COL-3 to be an effective scavenger of oxygen radicals [96]. As ROS are centrally implicated in the destruction of tissue architecture during shock and MODS [62], particularly in the lung, this ROS scavenging capacity of COL-3 makes the drug a promising potential anti-MODS agent.

4.2.5- Arachidonic Acid Metabolites

Metabolites of arachidonic acid along with nitric oxide are known to play a role in both acute and chronic inflammation as well as playing a role in platelet function. Patel et al showed that COL-3 inhibits the production of cyclooxygenase derived prostaglandin-E2 (PGE-2) *in vitro* [77]. Pruzanski et al have shown that COL-3 also inhibits production of secretory non-pancreatic phospholipase A2 (PLA-2) [97]. Furthermore COL-3 has been shown to regulate inducible Nitric Oxide Synthase (iNOS) at the post-transcriptional level in murine macrophages thereby reducing the production of NO [78]. These *in vitro* studies suggest that the mechanism of COL-3 efficacy in sepsis and ARDS treatment may be the combined effect of blocking multiple inflammatory mediators involved in SIRS. To put it another way we can present once again the complexity of the inflammatory response in Figure 1 and add the action of COL-3 (Figure 9). Note that some of these drug actions have been demonstrated *in vivo* in large or small animal studies and some of these actions have so far only been shown *in vitro*. Nevertheless this pleiotropic blockade of multiple injurious pathways in the inflammatory response cascade represents a powerful new approach in the pharmacologic management of injury, infection, and inflammation: rather than targeting a specific pathway in this complex web, we can target the entire cascade itself. This pleiotropy could result in a substantial therapeutic advantage for the septic or traumatically injured patient. Further research is required at both the translational and clinical level in order to actualize the potential that COL-3 has in this field.

5.1 Conclusions

In summary, COL-3 has been shown to be a powerful drug in the treatment of SIRS-induced ARDS in both small and large animal models. In a small animal CLP model COL-3 was

shown to improve both survival and liver function [82, 85]. Rigorous, clinically applicable large animal models have shown COL-3 efficacious at preventing lung injury and improving survival in a 2-hit model of sepsis and ischemia-reperfusion and in a burn plus smoke inhalation model [83, 84]. Although these large animal studies clearly showed the beneficial effects of COL-3 they did not measure all of the mediators that COL-3 is known to inhibit. COL-3 did reduce both MMP and cytokine concentrations but it is not known if these are the only mediators essential to the efficacy of COL-3. Moreover, COL-3 is the only chemically modified tetracycline that has ever been administered to humans in clinical trials such as the work of Dezube in which Kaposi Sarcoma patients were orally administered COL-3 with beneficial results [89]. Further research is necessary to identify the precise protective mechanisms of COL-3 and to determine whether COL-3 is clinically effective in human SIRS caused by sepsis, trauma or hemorrhage.

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List of Abbreviations

ARDS	Acute Respiratory Distress Syndrome
SIRS	Systemic Inflammatory Response Syndrome
MODS	Multiple Organ Dysfunction Syndrome
MMP	Matrix Metalloproteinase
CMT-3	Chemically Modified Tetracycline-3
COL-3	Chemically Modified Tetracycline-3
ROS	Reactive Oxygen Species
TNFα	Tumor Necrosis Factor- α
NFκB	Nuclear Factor- κ B
PAF	Platelet Activating Factor
NO	Nitric Oxide
RNA	Ribonucleic Acid
CPB	Cardiopulmonary Bypass
CLP	Cecal Ligation and Puncture
TIMP	Tissue Inhibitor of Metalloproteinase
PGE-2	Prostaglandin E-2
PLA-2	Phospholipase A-2
IL-1β, 2, 6, 8, 10, 12	Interleukin 1 β , 2, 6, 8, 10, 12

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Figure 1. The Complexity of Inflammation

This diagram depicts the web of interactions that unfold as the result of a provoking inflammatory stimulus. There is tremendous redundancy and cross-talk between different arenas as inflammatory mediators are stimulated and perpetuated by multiple sources, making control of this cascade once all aspects are activated, very difficult. Each physiological component of this process has been color-coded; (Cytokines-pink; Coagulation cascade-red, Complement pathways-blue, Arachidonic acid metabolites-green, Matrix metalloproteinases – orange, Reactive oxygen species-purple) AA-arachidonic acid; aPC-activated Protein C; CRP-C-reactive protein; EC-endothelial cell; IL-interleukin; iNOS-inducible nitric oxide synthase; LTB4-leukotriene B4; MMP-matrix metalloproteinase; Mono-monocyte; NF-κB-nuclear factor Kappa light chain enhancer of activated B cells; PGE2-prostaglandin E2 or dinoprostone; PMN-polymorphonuclear cell; ROS-reactive oxygen species; TF-tissue factor; TNF-tumor necrosis factor; TXA2-thromboxane.

Figure 2. The Pathophysiology of ARDS

[6]The Normal Alveolus (Left-Hand Side) and the Injured Alveolus in the Acute Phase of Acute Lung Injury and the Acute Respiratory Distress Syndrome (Right-Hand Side). In the acute phase of the syndrome (right hand side) there is sloughing of both the bronchial and alveolar epithelial cells, with the formation of protein-rich hyaline membranes on the denuded basement membrane. Neutrophils are shown adhering to the injured capillary endothelium and marginating through the interstitium into the air space, which is filled with protein-rich edema fluid. In the air space, an alveolar capillary macrophage is secreting cytokines, interleukin-1, 6, 8 and 10, (IL-1, 6, 8, and 10) and tumor necrosis factor- α , which act locally to stimulate chemotaxis and activate neutrophils. Interleukin-1 can also stimulate the production of extracellular matrix by fibroblasts. Neutrophils can release oxidants, proteases, leukotrienes, and other proinflammatory molecules, such as platelet activating factor (PAF). A number of antiinflammatory mediators are also present in the alveolar milieu, including interleukin-1-receptor antagonist, soluble tumor necrosis factor receptor, autoantibodies against interleukin-8, and cytokines such as interleukin-10 and 11 (not shown). The influx of protein rich edema fluid into the alveolus led to the inactivation of surfactant. MIF denotes macrophage inhibitory factor.

Figure 3. Alveolar Collapse and Expansion

[69]Photomicrographs depicting individual alveoli as they are inflated from end expiration (Expiration) to peak inspiration (Inspiration) during tidal ventilation in the acutely injured lung (Tween lavage). Alveoli of interest have been highlighted with white dots and represent the same alveolus at expiration and inspiration. Alveolar inflation patterns were separated into three types depending on the appearance of alveolar area changes with tidal ventilation. Type I alveoli change volume imperceptibly from end expiration (A) to peak inspiration (B). Type II alveoli change volume from end expiration (C) to peak inspiration (D) but stay inflated at end expiration. Type III alveoli collapse totally at end expiration (E) and re-inflate with inspiration (F). In the normal lung all alveoli exhibit type 1 inflation patterns.

Figure 4. COL-3 Reduced Gelatinase concentration in Bronchoalveolar Lavage Fluid (BAL) in porcine ARDS

[61]Gelatin zymography on BAL specimens from pigs randomized to LPS, COL-31LPS, or control. Numbers (0, 120, 240, 360) are time in minutes following LPS or sham LPS at which BAL was sampled [61]

Figure 5. Effect of COL-3 on Gelatinase & Elastase Activity in Bronchoalveolar Lavage Fluid in ARDS

[68]Matrix Metalloproteinase activity. Groups: Control = Naive Controls CPB = animals injured by Cardiopulmonary Bypass, LPS = animals injured by Lipopolysaccharide Endotoxin, CPB+LPS = Animals injured by both CPB and LPS. CPB+LPS+CMT= Animals subjected to CPB+LPS that were treated with COL-3. [68]

Figure 6. COL-3 and Lung Histology in porcine ARDS

[83] A) Control group showing fully inflated alveoli. B) 2-hit injury by clamping of superior mesenteric artery and fecal peritonitis (SMA+FC) showing alveolar collapse, leukocyte infiltration, edema, and fibrin deposition consistent with ARDS. C) FC+SMA injury treated with COL-3 showing dramatically healthy alveoli.

Figure 7. COL-3 Improves survival from smoke inhalation in ovine ARDS [98]. Kaplan-Meier curve for 96-hour survival after smoke-inhalational, burn, and barotrauma injuries. Data are mean $\pm$ standard deviation; $n = 5$ each group.

Figure 8. Effect of COL-3 on Hepatic MMP and TIMP in Sepsis

[82] Hepatic matrixmetalloproteinase (*MMP*)-9, MMP-2, and tissue inhibitor of metalloproteinase-1 (*TIMP*)-1 activity after 0, 24, and 48 hrs of cecal ligation and puncture (*CLP*) in untreated and chemically modified tetracycline (*CMT-3*)-treated rats. *A*) Representative Western blot. *B*) densitometric analysis of five blots. $N = 5$ rats per group. * $p < .05$ compared with CLP 0 hrs.

Figure 9. Pleiotropy

The Drug Actions of COL-3 on the Inflammatory Cascade. This is a pictorial representation of all literature supported actions of COL-3 in the inflammatory response.

Table 1**Hierarchy of Evidence****[8] A Validated Rating System for Scientific Evidence and Recommendations**

Rating system for quality of evidence	
Level 1.	Large, randomized, prospective, controlled investigations
Level 2.	Small, randomized, prospective, controlled trials with uncertain results
Level 3.	Nonrandomized, concurrent, or historical cohort investigations
Level 4.	Peer-reviewed state-of-the-art articles, review articles, editorials, or substantial case series
Level 5.	Nonpeer-reviewed published opinions, such as textbook statements or official organizational publications
Rating system for recommendations	
A.	Convincingly justifiable on scientific evidence alone (two level 1 investigations)
B.	Justifiable by available scientific evidence (one level 1 study)
C.	Reasonably justifiable by available scientific evidence and strongly supported by expert critical care opinion (level 2 investigations only)
D.	Adequate scientific evidence is lacking but widely supported by available data and expert critical care opinion (level 3 data)
E.	No scientific data exists to justify support (level 4, 5 publications)

Table 2

ARDS Treatments, Evidence & Recommendations Systematically Reviewed (Adapted from Ware et. al [6] and Cepovka et al [7])

Treatment Modality	Evidence Level	Recommendation Grade
Ventilatory		
-Low Tidal Volume	1 & 2	B
-Open Lung	2	B
-Inverse Ratio	3 & 4	D
-Liquid Ventilation	4	E
Extracorporeal Life Support	Level 1 & 2 <i>Against</i>	Not Recommended
Prone Positioning	3	D
Restrictive Fluid Management	2	C
Surfactant	3	C
Pulmonary Vasodilators		
-Inhaled Nitric Oxide	Level 1 <i>Against</i>	Not Recommended
-Almitrine	3	C
-Prostacyclin	3	C
Immune Modulators		
-PGE ₁	Level 1 <i>Against</i>	Not Recommended
-Ketoconazole	Level 1 <i>Against</i>	Not Recommended
-Ibuprofen	Level 2 Mixed Results	Not Recommended At Any Level
-Corticosteroid	2	C
N-Acetylcysteine	Level 2 Mixed Results	Not Recommended At Any Level