

Review Article

An update in the utilization of N-acetyl cysteine & vitamin c for tackling the oxidative stress in acute kidney injury secondary to robust sepsis - A systematic review

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Abstract

The commonest etiology of acute kidney injury (AKI) is Sepsis that results in an escalation of morbidity and mortality in the hospital intensive care units. Existentially, the therapy of septic AKI rather than being definitive or curative is just supportive, without tackling the pathophysiology. Usually, Sepsis gets correlated with systemic inflammation, along with the escalated generation of Reactive oxygen species (ROS), in particular superoxide. Simultaneously liberation of nitric oxide (NO) subsequently reacts with the superoxide, thus, resulting in the generation of reactive nitrogen species (RNS), that is mostly peroxynitrite. This sepsis stimulated generation of ROS in addition to RNS might cause a reduction in the bioavailability of NO that modulates microcirculation aberrations, localized tissue hypoxia as well as mitochondrial impairment, thus starting a vicious cycle of cellular damage which results in AKI. Here we conducted a systematic review utilizing search engine PubMed, Google Scholar; Web of Science; Embase; Cochrane review library utilizing the MeSH terms like septic AKI; ROS; inducible nitric oxide synthase (iNOS); nicotinamide adenine nucleotide phosphate (NADPH) oxidase complex; Oxidative stress; Renal medullary hypoxia; Hypoxia inducible factor 1; hypoxia responsive enhancer A; mitochondrial impairment; Intrarenal oxygenation; urinary oxygenation; erythropoietin gene; RRT; NAC; Vitamin C from 1950 to 2021 till date. We found a total of 6500 articles out of which we selected 110 articles for this review. No meta-analysis was done. Thus here we detail the different sources of ROS, at the time of sepsis, besides their pathophysiological crosstalk with the immune system, microcirculation as well as mitochondria that can result in the generation of AKI. Furthermore, we detail the therapeutic utility of N-acetylcysteine (NAC), besides the reasons for its success in ovine as well as porcine models of AKI. Moreover, we discuss preclinical along with clinical for evaluation of Vitamin C's antioxidant effects as well as pleiotropic effects as a stress hormone that might aid in abrogation of septic AKI.

Introduction

Sepsis is the commonest etiology of acute kidney injury (AKI) that attributes to about 50% of patients with renal impairment in intensive care units [1]. Generation of AKI at the time of sepsis is important in addition to an independent factor with regards to the prognosis for continuous hospital admission apart from mortality in the hospital [2]. Furthermore, there is escalated epidemiological proof

existent in the context of those who survive mild or short periods of AKI possessing greater propensity of generation of Chronic Kidney Disease (CKD) in addition to end stage renal disease (ESRD) in the latter stage of life [3]. Antibiotics along with rejuvenation with fluids besides vasopressors are the existent, recommendations as far as treatment of human sepsis is concerned. Replacement therapy (RRT) is advocated for patients who generate robust septic AKI [4]. Nevertheless, these can maximally aid in alleviation

More Information

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Keywords: Sepsis; Acute kidney injury; Oxidative stress; Renal medullary hypoxia; NAC; Vitamin C





as an intervention measure hoping that patient's kidney capacity recovers. Considering this getting a good insight into the pathophysiology of septic AKI is the requirement for the generation of efficacious mode-based interventional approaches.

In spite of global renal ischemia having been posited as the etiology of septic AKI [5], both experimental along with clinical proof questions this posit. Histopathological evaluation conducted on post mortem Kidney tissue from patients dying from septic AKI illustrates heterogeneous focal patchy tubular damage with least tubule-epithelial demise (< 5%), apical vacuolization in addition to the focal mesangial expansion, that are not the properties of robust renal ischaemic damage [6]. Additionally, robust proof from clinically significant ovine along with porcine models is existent concerning sepsis generated AKI occurs despite the lack of global renal ischemia [7]. Akin to the histopathological observation in human sepsis, acute tubular necrosis in addition to tubular cell apoptosis were not the properties of AKI in these large mammalian models of hemodynamic sepsis [7,8]. At the time of inflammatory disorders, like sepsis, there is apparent uncoupling of the renal microcirculation from the macrocirculation [9]. In case of ovine sepsis, particular renal medullary tissue ischemia besides hypoxia antecede the generation of AKI by 12-24 h in spite of the escalation of renal blood flow in addition to renal cortical medullary tissue perfusion oxygenation [10,11]. Oxidative stress (OS) possesses a key part in the facilitation of adaptive reaction towards local tissue hypoxia via stabilization of Hypoxia inducible factor 1 (HIF 1) that facilitates the transcription of a lot of genes [12]. Nevertheless, in sepsis, an imbalance is existent amongst Reactive oxygen species (ROS) along with Reactive nitrogen species (RNS) as well as the host's antioxidant defense mode.

Here the sources in addition to part of Oxidative along with nitrosative stress with regards to the pathophysiology of septic AKI highlighting on its cross talk with inflammation, microcirculation anomalies, tissue hypoxia as well as mitochondrial impairment is discussed. More recently, pre-clinical along with clinical studies have evaluated the utilization of antioxidants, mainly N-acetylcysteine (NAC) in addition to Vitamin C in the form of a probable treatment option with regards to septic AKI. Subsequent to conducting a review on the role of Vitamin C for the treatment besides prevention of acute respiratory failure that was inclusive of pneumonia common cold, or any other viral infections like influenza & more specifically in the COVID-19 epidemic we faced all through the world in addition to sepsis [111], we decided to review the role of this antioxidant in therapy of septic AKI.

Methods

Here we conducted a systematic review utilizing search engine PubMed, Google Scholar; Web of Science; Embase;

Cochrane review library utilizing the MeSH terms like septic AKI; ROS; inducible nitric oxide synthase (iNOS); nicotinamide adenine nucleotide phosphate (NADPH) oxidase complex; Oxidativstress; Renal medullary hypoxia; Hypoxia inducible factor 1; hypoxia responsive enhancer A; mitochondrial impairment; Intrarenal oxygenation; urinary oxygenation; erythropoietin gene; RRT; NAC; Vitamin C from 1950 to 2021 till date.

Results

We found a total of 6500 articles out of which we selected 110 articles for this review. No meta-analysis was done.

Crosstalk amongst the septic inflammatory step way as well as oxidative stress

The inflammatory reaction represents the body's firstline defense from the invader pathogen's, however, this might work in the form of a key triggering parameter for renal damage. In case of sepsis inflammatory modulators that is inclusive of the damage or pathogen-associated molecular patterns (DAMP/PAMP) get liberated into the intravascular regions, besides getting estimated by toll like receptors (TLR) that are existent on tubular along with endothelial cells [13]. Stimulation of these receptors results in the progression of numerous downstream events that aid in tubular repair, vascular nonrecruitment or temporary perfusion blockade, besides acceleration of proinflammatory immunomodulation at the region of damage that result in vascular congestion along with endothelial impairment [14]. These events seem to come together for the stimulation of superoxide stimulated tissue hypoxia in addition to cellular damage (Figure 1).

Sepsis stimulated tubular along with vascular in association with Oxidative stress result in the enrolling of polymorphonuclear neutrophils that results in the stimulation of a stepwise immunomodulation process that results in the downstream generation of ROS as well as, RNS that results in the further progression of damage [16]. Neutrophils possess the capacity of developing superoxide via the development of an event that is known as "Oxidative burst" [17]. Expression of numerous receptors by neutrophils that are inclusive of β 1, β 2, as well as β 3 integrins. These on activation possess the capacity of binding fibronectin, fibrinogen, besides collagen that causes the modulation of them translocating to extracellular matrix (ECM), by P-selectins as well as E selectins [18]. A number of signaling pathways finally result in the downstream liberation of intracellular calcium along with the generation of nicotinamide adenine nucleotide phosphate (NADPH) oxidase complex that causes the generation of ROS [17]. Moreover, the generation of inducible nitric oxide synthase (iNOS) action gets up regulated in immune cells (Figure 2). The escalation of generation of NO might crosstalk with the ROS that gets generated by NADPH oxidase for the generation of RNS peroxynitrite (Figure 2). Peroxynitrite aids in nitrosative

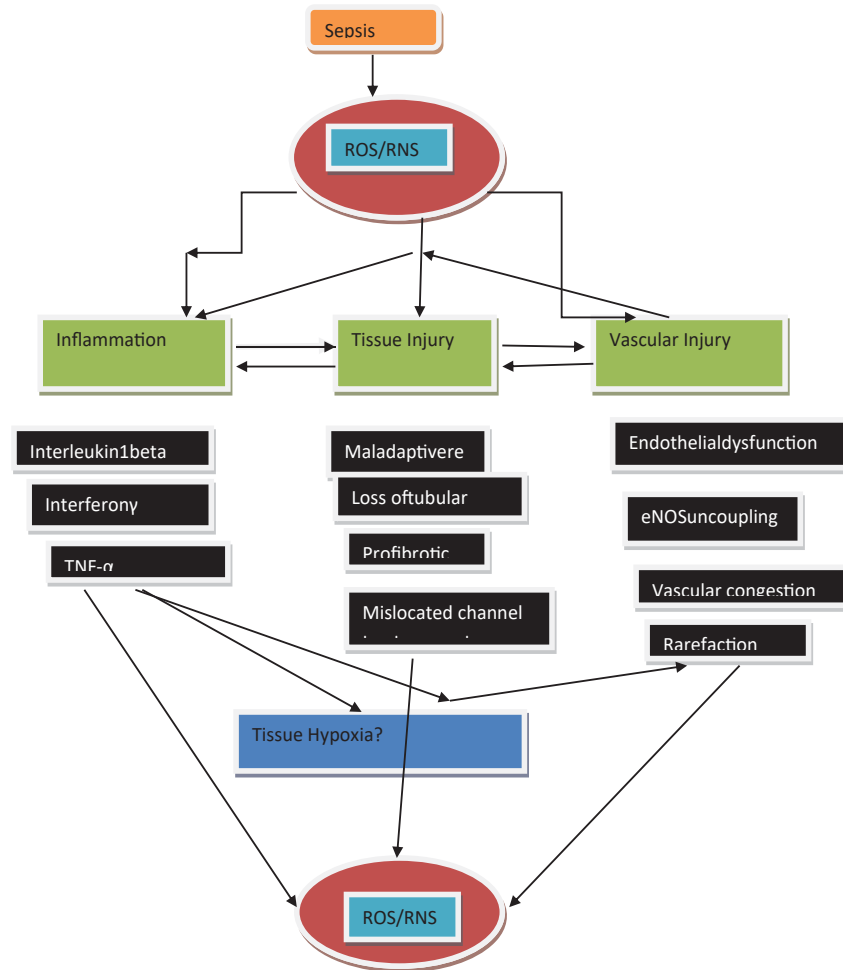


Figure 1: Courtesy ref no-15-Scheme of the posited association amongst sepsis- stimulated generation of reactive oxygen species (ROS) along with reactive nitrogen species (RNS) besides inflammation, tissue, in addition to vascular damage. ROS and RNS result in escalated generation of immunomodulatory cells at regions of damage in vessels as well as tubules, thus starting a complex cascade of inflammation along with damage. Significantly, tissue along with vascular damages in robust sepsis can have harmful, actions as they can aid in an uncoupling of endothelial nitric oxide synthase (eNOS), modulated endothelial impairment as well as tissue hypoxia, thereby aid in escalating the accrual of ROS and RNS.

injury like S-nitrosylation of proteins thus implicating the normal working of proteins. The thought of ROS stimulated vascular damage is validated by the finding in patients of sepsis of exhaustive generation of superoxide from leukocyte microparticles that further result in an escalation of adhesion molecules action, as well as, endothelial activation [19]. Furthermore, escalated plasma amounts with regards to lipid peroxidation markers (F2-isoprostanes along with isofurans have been robustly correlated with septic AKI patients [20]. The development of oxidative stimuli via neutrophils leads to the luring of proinflammatory chemokine ligand 5 along with intracellular cell adhesion molecule [ICAM]-1, that are significant parameters which promote the enrolment of leukocytes to regions of tissue damage[21]. Leukocytes that have got mobilized besides activated are responsible for stimulation of cytokine storm that implicates the enrolment of proinflammatory cytokines that are inclusive of interleukin-1 β (IL-1 β), interferon-gamma (IFN γ) as well as (TNF α) [22], that can further cause generation of ROS (Figure 1). Actually, inflammatory, as well as, Oxidative stress biomarkers, like TNF α , IL-1 β along with myeloperoxidase

action, malondialdehyde (MDA) in addition to hydrogen peroxide (H₂O₂) have all been documented to get important escalation in patients of septic AKI [23]. Collectively, the original inflammatory step pathway in sepsis seems to be a key parameter responsible for the progression of Oxidative stress that can possess harmful actions concerning renal microcirculation.

Oxidative stress exaggerates microcirculation aberrations besides vascular apparent compressive/blockade action

Endothelial impairment in addition to microcirculation apparent compressive/blockade action alias rarefaction have got documented as the usual pathophysiological characteristics of septic AKI along with are posited to be the key parameters responsible for the modulation of propagation of CKD subsequent to the recovery from AKI [24]. The NO system is a significant controller of the vascular tone amongst renal microcirculation, however, it might be harmfully influenced in sepsis (Figure 2). Amongst the healthy situation, the bio generation of NO by vascular

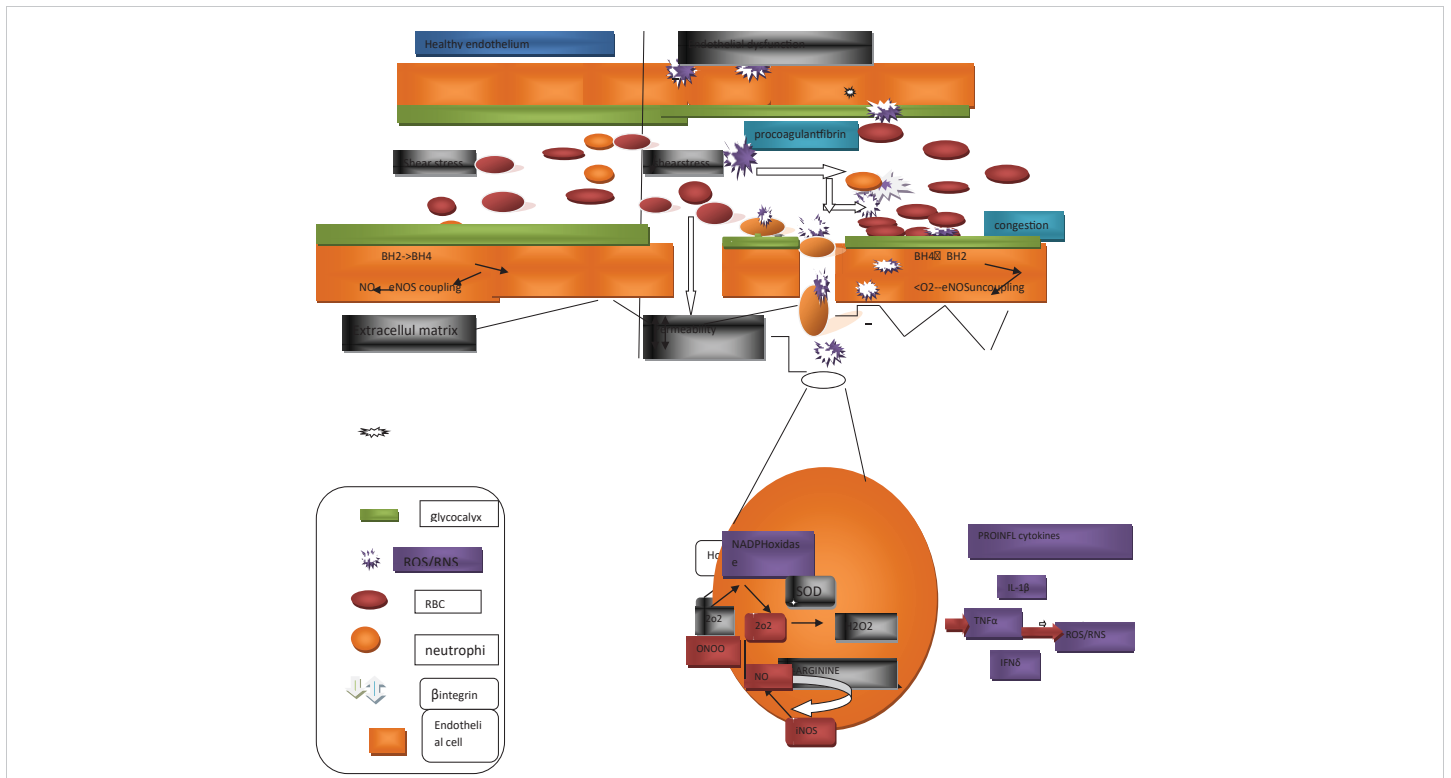


Figure 2: Courtesy ref no-15-Schemedetailing the starting of oxidative injury to the endothelium, the downstream consequences for endothelial impairment as well as the progression of oxidative stress. The layer of glycocalyx lining the apical surface of endothelial cells is significant for the sustenance of shear-stress and flow- modulated nitric oxide (NO) liberation and vasodilation of the endothelium. Sepsis- stimulated oxidative stress resulted in glycocalyx thinning and shedding, leads to the depletion of gap junctions, along with subsequently escalated permeability impairment as well as vascular leakage. Depletions of gap junctions further greatly promotes extravasation of neutrophils in the circulation, modulated by β -Integrins, via to the extracellular matrix. The resultant superoxide (O_2^-) that is generated can crosstalk with nitrosative species produced by inducible nitric oxide synthase (iNOS), generating the greatly reactive peroxynitrite ($ONOO^-$) in addition to then hydrogen peroxide (H_2O_2) subsequently. Oxidative bursts from neutrophilsaid in escalating oxidative injury along with the downstream generation of pro-inflammatory cytokines which have the intrinsic capability of generating reactive oxygen species (ROS) along with reactive nitrogen species (RNS). Oxidative stress can further induce the recruitment of pro-coagulants to the site of injury, causing vascular congestion, ultimately impeding blood flow. Increased oxidative stress at the endothelium depletes resulting in oxidation of the pool of tetrahydrobiopterin (BH4), resulting in the uncoupling of endothelial nitric oxide synthase (eNOS) in endothelial cells, thereby enhancing the generation of ROS. Interleukin-1beta (IL- β); tumor necrosis factor-alpha (TNF- α), interferon-gamma (IFN- δ).

endothelial cells is based on the coupling status of endothelial nitric oxide synthase (eNOS) along with the bioavailability of the cofactor tetrahydrobiopterin (BH4) [25]. Once the endogenous amounts of BH4 are enough, L-arginine coupling occurs with a decrease of oxygen amounts that results in the generation of the robust vasodilator NO [26]. Nevertheless, with low amounts of BH4 there is uncoupling of eNOS with generation of superoxide taking place rather. Moreover, BH4 possess greater proneness towards oxidation to BH2 once greater amounts of superoxide are existent further results in deletion of the pool of the rate limiting cofactor BH4 [27]. Uncoupling of eNOS has been documented to aid in the pathophysiology of several Kidney Diseases that occur secondary to type 2 diabetes mellitus (T2DM), hypertension along with ischemia-reperfusion damage [28]. Intravenous supplementation of BH4 in case of an ovine model of sepsis aids in the rectification of microvascular impairment through the escalation of the amounts of vessels getting perfusion, the percentage of small vessels getting perfusion in addition to the microvascular index amongst the sublingual circulation [29]. In case of an ovine model of robust septic AKI, that got stimulated by the live intravenous infusion of *Escherichia*

Coli, for 48 h eNOS gene expression got selectively downregulated in the renal medulla, however, not in the renal cortex [8]. Nevertheless, if an uncoupling of eNOS aids in the early initiation of microcirculation aberration in the renal medulla in an ovine septic AKI [10] it asks for a greater evaluation.

In case of sepsis, escalation of superoxide formation as well as collection in parallel with inflammation further caused a direct structural injury to the vasculature that caused leaking from the vasculature besides tissue edema (Figure 2) [30]. The endothelial glycocalyx is a key controller of endothelial working along with vascular tone. The endothelial glycocalyx that lines the apical surface of the endothelium, is made up of hyaluronic acid, heparin sulfate, glycoproteins along with proteoglycans, that makes sure of the existence of the intactness of vascular permeability, thus it confers protection from the vascular leaking, coagulation, in addition to continuous inflammation (Figure 2) [30]. Further, it is a significant, mechanotransducer in view of its capacity of detection of shear stress, thus aid in shear stress modulated NO generation along with vasodilation.



During pathological situations that possess the properties of Oxidative stress, the generation of hyaluronidases which catalyze the depolymerization along with degranulation of the hyaluronic acid of the glycocalyx gets up regulated. The shedding that results, besides the thinning of the endothelial glycocalyx can result in the reduction of the capacity of the endothelium to respond to the shear stress modulated liberation of NO (Figure 2) [31]. Plasma amounts of endothelial glycosaminoglycans in the patients who were the survivors of sepsis were twice in contrast to the healthy volunteers [32]. Furthermore, this casting action of the vascular endothelial cadherin, a protein necessary for the sustenance of gap junctions amongst endothelial cells was importantly higher in case of septic patients with AKI which needed RRT in contrast to the ones without AKI [33]. These clinical findings validate the belief that deletion of endothelial intactness might possess a key part with regards to pushing aberrations in the microcirculation in septic AKI.

The injured endothelium further lures leukocytes towards the region of damage, as an integral part of the, innate immune response that gets promoted by the exposed intercellular cell along with vascular adhesion molecules. This homing of the proinflammatory cells along with the gap junctions that have got negotiated, results in extravasation of the proinflammatory cells from the endothelium within the tissue that is surrounding, aids in the generation of inflammation [34]. Noticeably inflammatory cells possess the capacity of generation of ROS by themselves in addition to the bioavailability of NO [34], thus aiding the exhaustive pool of superoxide, that necessarily results in the generation of a vicious cycle of Oxidative stress, inflammation along with vascular damage [16] (Figure 1). Microvascular damage that occurs secondary to sepsis stimulation can further cause the liberation of microparticles into the systemic circulation. Microparticles represent cell membrane obtained particles that are comprised of vasoconstrictive agents like prostaglandin E2, thromboxane A2, inflammatory modulators, procoagulants as well as fibrin. Hence microparticles might work in the form of localized generators of Oxidative stress, inflammation along with disseminated intravascular coagulation that causes vascular congestion [35]. It can be considered that these microcirculatory disconcertion might get exaggerated in particular, amongst the smaller arteries of the renal medullary vasa recta that might reason out why the renal medulla is prone with regards to the generation of hypoxia at the time of sepsis.

Hypoxia of the renal medullary tissue: a key process in acute Kidney injury?

Hypoxia of the renal medullary tissue is being probed in the form of a usual pathophysiological characteristic of AKI that arises from sepsis [10,36], cardiopulmonary bypass [37], along with radiocontrast induced Nephropathy [38]. Moreover, hypoxia of the renal medullary tissue has

been believed to be a significant stimulator with regards to transition, as well as, chances for propagation from AKI to CKD [39]. The proportionally high metabolic needs of the tubular elements that are existent in the renal medulla, that are coupled with the terrain of vascular along with tubular engineering amongst the medulla cause a steep oxygen gradient amongst the capillaries (vasa recta) in addition to both the thick as well as thin ascending loop of Henle along with the collecting ducts [40]. Furthermore, the probability for diffusive oxygen shunting amongst the renal medullary microcirculation (from descending to ascending vasa recta) that might further negotiate the renal medullary oxygen supply [41]. Graded clamping of the renal arteries in case of healthy sheep, thus result in a continuous drop in renal blood flow caused a greater amount of renal medullary ischemia, as well as, hypoxia as per percentage in contrast to the renal cortex that pointed to an internal deficiency with regards to the autocontrolling ability of the renal medullary microcirculation [42]. Thus at the time of pathophysiological situations like sepsis, renal microcirculation disturbances that result in just modest decreases in medullary oxygen supply or escalation in renal oxygen utilization might have had sequelae for the medullary tissue oxygenation.

Renal medullary hypoxia might be a robust stimulator of stepwise processes that result in cellular damage, vascular damage along with, tubular impairment [43]. Any acute insults that is inclusive of endotoxemia possess the capacity of escalation of renal tissue oxygen utilization. Like these alterations possess the capacity of causing tubular damage besides compression, along with inappropriate localization of Na/K ATPase as well as transportation proteins amongst renal tubular epithelial cells thus result in a reduction of the effectiveness of the oxygen consumption with regards to sodium reabsorption [44]. Furthermore, it is of significance that Oxidative stress can cause escalation of utilization of tubular oxygen by a reduction in effectiveness of the ATP generation amongst the mitochondria, as well as/or by escalation of oxygen consumption with regards to tubular sodium reabsorption [45]. Furthermore, renal insults that acutely arbitrate the vascular intactness or continuously facilitate vascular compressive/blockade action alias rarefaction might result in reduction of renal medullary oxygen supply that might result in deterioration of the tissue hypoxia. In a clinically appropriate ovine model of gram-negative sepsis the generation of renal medullary hypoxia anteceded AKI, as estimated by the escalation of plasma creatinine along with oliguria by 12-24 h [10,11]. Bladder urinary oxygenation got corroborated in the exact model that was utilized earlier. For the provision of a dependable evaluation of renal medullary tissue oxygenation at the time of generation of septic AKI [46] along with in reaction to clinical management, that is inclusive of building up the patients, with fluids [47], vasopressin [11], as well as, diuretics [48]. Subsequently, in case of human studies, the



existence of renal tissue medullary hypoxia, as indirectly evaluated by estimation of bladder urinary hypoxia was illustrated in patients with septic AKI [49]. Cellular modes that confer protection, stimulated by HIFs possess the capacity of protection of the Kidneys once there is mild tissue hypoxia/or temporary, however, they might crash if tissue hypoxia becomes robust/or continued as might take place at the time of stress [50].

HIFs represent cellular oxygen detectors. Stabilization of the α subunit at low oxygen amounts causes the generation of a dimer with the β subunit. Subsequently, the dimer gets translocated towards the nucleus, binding to the hypoxia response element that causes changed transcription of numerous proteins that are necessary for numerous crucial signaling pathways [51]. This upregulation in addition to stabilization of the HIFs is considered to be adaptive, as it causes stimulation along with a generation of erythropoietin that facilitates the generation of red blood cells [52]. The escalation of the blood oxygen carrying ability thus, it possesses the capacity of abrogation of tissue hypoxia. ii) The other significant protein which gets upregulated secondary to the stabilization of the HIFs is eNOS that has the probability of causing an escalation of the NO bioavailability, thus mitigating vasoconstriction, which restricts greater progression of hypoxia along with ROS/RNS [51]. Nevertheless, HIFs might work in the form of a double-edged weapon in view of accelerated generation of HIFs in reaction to continued hypoxia at the time of robust sepsis might cause exhaustive generation of vasoconstrictive in addition to ROS stimulated proteins, like Inos [53], besides proteins that result in fibrogenesis [54]. Relevant to these findings hypoxia at the time of early initiation of renal tissue medullary hypoxia during sepsis causes prolongation of phases of tissue hypoxia which can result in destabilization of the HIFs, which result in prolongation of Oxidative as well as nitrosative damage, that ends in AKI. Validating this belief HIF-1 α gene got down regulated amongst the renal medulla in case of septic sheep with robust AKI subsequent to 48 h of sepsis [8].

It is the knowledge we possess that the mode amongst renal medullary hypoxia along with a reduction in glomerular filtration rate (GFR) has not got found in AKI as well as might vary based on the etiology of AKI. Nevertheless, it might be thought that continued tissue hypoxia specifically in case of metabolically active areas of the Kidney, like renal medulla, result in mitochondrial impairment, that causes escalation of ROS generation which further results in augmentation of renal cellular damage.

Mitochondrial impairment secondary to sepsis stimulates generation of ROS

It has been posited that mitochondrial impairment is the etiology apart from the pathogenesis of septic AKI [55].

Mitochondria remain the ones that cause the utilization of oxygen maximum in the kidney. Hence, the generation of physiological amounts of mitochondrial ROS in the mitochondrial matrix is significant as ROS work as signals in addition to controllers of numerous biological events. These are inclusive of getting adapted to hypoxia via the control of the stabilization of the HIFs [56], ii) promote the generation of autophagosomes via the Oxidation of the cysteine protease gene4 by H₂O₂ [57], along with iii) ROS based stimulation of phosphatidyl inositide 3-kinase (PI3K) that results in the downstream generation of proinflammatory cytokines that are inclusive of caspase 1, IL-1 β , as well as, IL-18 [58]. Nevertheless, with continuous exposure of the cells to hypoxia, alterations in metabolism, apart from bad oxygen consumption with regards to ATP generation in the mitochondrial electron transport chain (ETC), that causes an escalation of leakage of electrons along with escalation of generation of free radicals/ROS [59]. Oxygen consumption by mitochondria occurs in the form of the ultimate acceptor of the respiratory chain, however, its incomplete reduction can further generate ROS, in particular superoxide [60]. Complex III of the electron transport chain (ETC), represent an inherent oxygen detector at the time of acute hypoxia, besides its control of superoxide generation inversely as per oxygen being available [61]. The conversion of Complex I from its active towards inactive form was further documented for possessing the capacity of generation of ROS outbursts at the time of acute hypoxia [81]. Septic AKI patients possess escalation of receptor interacting protein kinase (RIPK3) in urine along with plasma [62]. RIPK3 facilitates Oxidative stress in addition to mitochondrial impairment in Kidney tubular epithelial cells by an escalation of the expression along with mitochondrial transportation of NADPH oxidase as well as hampering of mitochondrial complexes I as well as III [63]. Thus it is not astonishing that mitochondrial damage has been usually associated with multiorgan impairment in case of patients with sepsis [64].

Preclinical along with clinical studies illustrate that the adaptive events of mitochondrial fission get downregulated in sepsis, that probably aids in the deletion of mitochondrial mass, thus aid in progression of ROS stimulated damage at the time of septic AKI. Sepsis gets correlated with marked morphological alterations in mitochondria. These alterations are inclusive of reduction in numbers of cristae that is secondary to the swelling of intercrystal space in addition to the mitochondrial matrix along with vacuolation amongst the mitochondrial space [63,65]. On the basis of the robustness of mitochondrial injury, the depletion of mitochondria might be brought about by 2 pathways namely mitophagy, as well as, apoptosis (Figure 3). Localized in addition to exhaustive morphological abnormalities amongst the mitochondria can result in the collection of superoxide ultimately resulting in the opening of the mitochondrial membrane channels. This results in reduction of the mitochondrial membrane

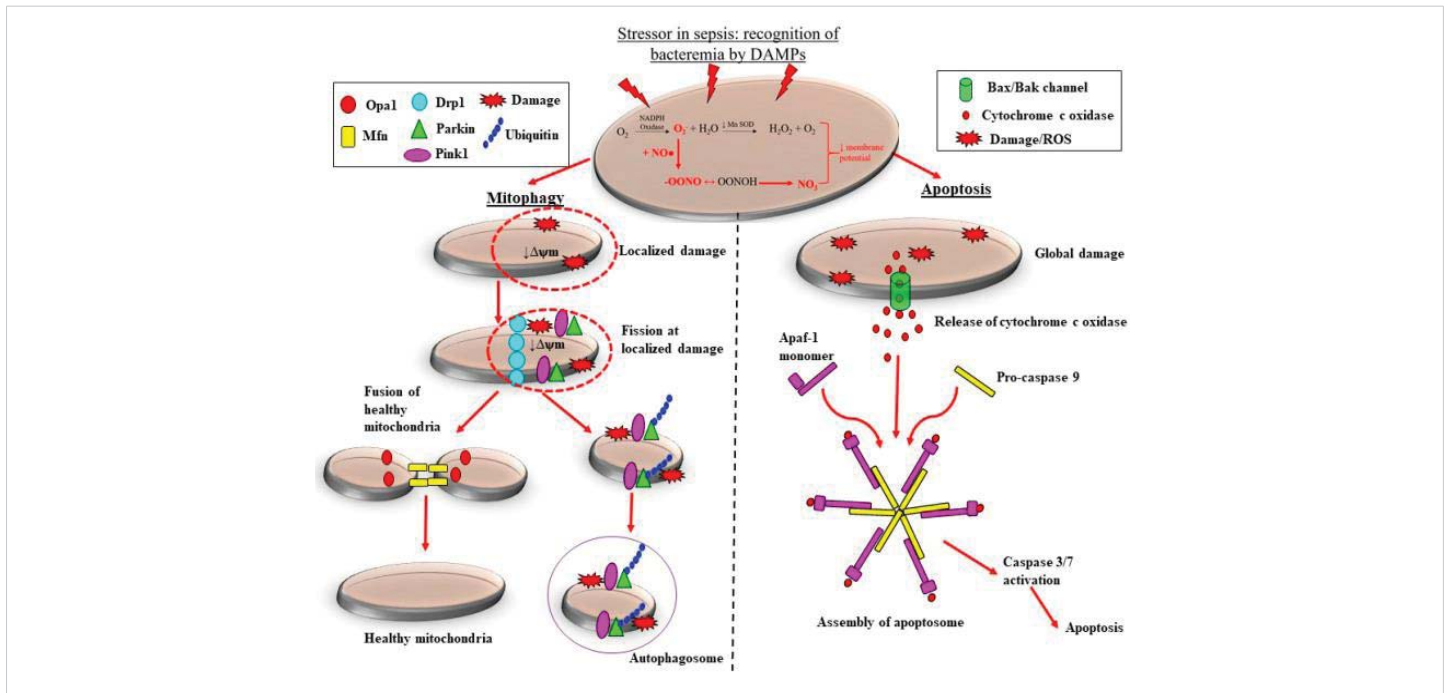


Figure 3: Courtesy ref no-15-Adaptive and maladaptive reactions of the mitochondria to sepsis-stimulated oxidative stress. Escalated generation of superoxide (O_2^-) by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase coupled with the reduction in action of manganese superoxide dismutase (MnSOD) causes accrual of O_2^- . Cytosolic nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS) reacts with O_2^- , forming the highly reactive peroxynitrite ($ONOO^-$). The accrual of O_2^- and $ONOO^-$ causing continued oxidative stress along with a decrease in mitochondrial membrane potential (ψ_m) and so mitochondrial impairment. In the case of localized mitochondrial injury, mitochondrial quality control modes are activated which arrest mitochondrial impairment. In addition to restrict escalated mitochondrial depletion. Recruitment of mitochondrial fission proteins to sites of damage targets the injured regions of the mitochondrion for fission. Subsequently, ubiquitin, PTEN-induced kinase (PINK1) and E3 ubiquitin-protein ligase (Parkin) proteins are recruited to the injured mitochondrion, removed by mitochondrial fission and crosstalk with phagosome, consequently generating an autophagosome. The healthy regions of the mitochondrion undergoes mitochondrial fusion, that adds to the existing mitochondrial pool and restricting escalated mitochondrial depletion. Conversely, Comprehensive injury to mitochondria in robust, sepsis causes the liberation of cytochrome c oxidase, along with the generation of an apoptosome when it crosstalks with apoptotic protease activating factor-1 (Apaf-1) monomers as well as pro-caspase 9. This results in downstream activation of caspase 3/7, ultimately causing the containment of sepsis-induced injury via apoptosis. Mitochondrial dynamin-like GTPase (OPA-1); dynamin related protein-1 (DRP-1); damage-associated molecular patterns (DAMPs).

potential, that is restricted towards the area of damage, thus further result in escalation of generation of ROS [66]. Moreover, escalation of amounts along with/or collection of up regulation of the uncoupling protein-1 [67], which as a consequence cause an escalation of proton leakage, as well as, inhibiting the generation of ATP [68]. Here in view of the injury having a tendency of being localized, the mitochondrion getting targeted for mitochondrial fission that is subsequently followed by the deletion of the injured part of the mitochondrion by mitophagy (Figure 3). At the time of mitochondrial fission, the mitochondrial membrane gets pinched at the damaged region that gets promoted by the assembling of dynamin associated protein1 which promotes the deletion of the injured area of the mitochondria from the noninjured area (Figure 3) [69]. Subsequently PTEN stimulated kinase-1 along with ubiquitin-protein ligase (Parkin) proteins recruitment takes place towards the injured mitochondria that finally result in generation of the autophagosomes, thereby getting depleted by mitophagy [69]. Mitochondrial fusion proteins mitofusin in addition to mitochondrial dynamin like GTPase, get recruitment towards the left healthy mitochondrion (Figure 3). Hence, mitochondrial fusion that is followed by fission, is apparently

a significant adaptive healing event, as besides putting a halt to subsequent mitochondrial injury in addition to avoidance of escalation of depletion of the mitochondrial mass, thus restricting ROS stimulated damage. Validating this belief, reduction in expression of mitochondrial PINK1 along with Parkin mRNA has been documented in biopsies from patients having mortality secondary to septic AKI [70]. Exhaustive injury to the mitochondria causes global fall of mitochondrial permeability that might result in superoxide collection, that finally result in opening of the mitochondrial membrane channels. These damages stimulate a cascade of downstream processes that is initiated by the liberation of pro-apoptotic factors like Bcl2 correlated X protein BAX as well as B cell lymphoma protein. This causes modulation of the liberation of cytochrome c, that in turn crosstalks with the apoptotic proteaseactivating factor1 proteins, that finally causes generation of an apoptosome which stimulate a procaspase 9-modulated downstream inherent apoptotic cascade [71] (Figure 3). This event promotes the depletion of the whole mitochondria in robust sepsis, as well as, causes decrease in mitochondrial mass [70] that might further arbitrate the generation of host antioxidants along with escalate the generation of superoxide (Figure 3). Hence



ROS stimulated damage of the mitochondria in sepsis can cause escalation of ROS generation as well as collection that might aid in a vicious progressing cycle of Oxidative stress, macrovascular damage along with cellular damage that ends in AKI. Thus atleast in theoretical manner the generation of Pharmacologic treatment with the objective of halting as well as /or restriction of generation of along with the collection of ROS/ as well as, or RNS at the time of sepsis might ameliorate the progression of vascular, mitochondrial, as well as, cellular damages, as well as, restrict the robustness of septic AKI. Here details of preclinical along with clinical studies which have evaluated the utilization of NAC or Vitamin C in the form of a therapeutic approach for mitigation of Oxidative stress stimulated multiorgan impairment in sepsis are discussed.

N-acetyl cysteine

NAC represents a robust synthetic antioxidant that forages superoxide, H_2O_2 , besides hydroxyl radicals in addition to the restoration of glutathione amounts, thus escalating the antioxidant reactions of the host [72]. NAC has been demonstrated to cause repression of production of oxygen radicals by the human neutrophils as well as macrophages *in vitro* [73], cause reduction in leukocyte to endothelial cells adhesion, as well as, vascular leakage in case of rodent endotoxemia [74] besides ameliorate the activation of nuclear factor κB (NF κB) along with IL-8 amounts in sepsis [75]. These observations yielded the stimulus for evaluation of NAC for reversal of the harmful actions of Oxidative, as well as, nitrosative stress in sepsis.

Evaluation of NAC has numerous experimental studies in the form of precautionary treatment or rescue treatment at the time of early stages of sepsis. In case of a rodent model of sepsis that has been stimulated by cecal ligation as well as puncture (CLP), subcutaneous NAC in a dosage of 20 mg/kg therapy started 3 h subsequent to CLP caused a reduction in systemic inflammation, Oxidative stress along with mitochondrial impairment that results in a significant escalation of survival (10% - 40%) [76]. In case of rodent endotoxemia intravenous NAC treatment) started 20' subsequent to delivery of lipopolysaccharides (LPS) caused a reduction in serum creatinine along with blood urea nitrogen amounts, an action correlated with a decrease in inflammatory cytokines (TNF α as well as IL-6) [77]. Nevertheless, treatment started at a markedly early stage of sepsis are of restricted value with regards to a clinical scenario. This is in view of patients with sepsis get usually diagnosis made with, the recipient of management, once there is the existence of a proven multiorgan impairment, as estimated by sequential organ failure assessment (SOFA) scores [78]. In this context intravenous treatment with NAC (150 mg/kg bolus+20 mg/kg/h) in pigs 12 hrs subsequent to development of endotoxemia could not respond with no enhancement in systemic, pulmonary, as well as, hepatospanchnic hemodynamics or be correlated with the

reduction in a biomarker of Oxidative stress (8-isoprostane), inspite of escalation of glutathione amounts [79].

Clinical studies with the utilization of NAC as ancillary treatment for stress have mostly resulted in poor results specifically when NAC was delivered to patients with robust sepsis for longer periods. In maximum patients, the utilization of NAC was done in the dosage of (150 mg/kg) that was dependent on the regimen that was used in patients who got treatment for acetaminophen poisoning [80]. In case of patients with sepsis, intravenous NAC delivered over a short duration relatively (150 mg/kg bolus + 12.5 mg/kg/h for 90') resulted in significant escalation of cardiac index (5.7 (treatment) vs. 5.0 (usual care) L/min/m², $p = 0.01$). Nevertheless, NAC delivery for longer periods of time (50 mg/kg at 4 h that was followed by 100 mg/kg/24 h for 44 h) resulted in deterioration of organ failure (average SOFA scores at 48 h: 7.7 \pm 3.8 (treatment) vs. 5.1 \pm 2.1 (usual care) L/min/m², $p < 0.05$) without any enhancement of microalbuminuria/ creatinine ratio in patients with sepsis [81]. As far as NAC delivered to patients with the diagnosis of septic shock made for time periods greater than 24 h resulted in cardiac depression that had the properties of reduction in cardiac output along with enhancement of hypotension [82]. Akin to that greater needs with regards to inotropic support got illustrated in critically ill patients who received treatment with NAC (150 mg/kg bolus + 12 mg/kg/h) for periods over 24 h in contrast to that with usual care [83]. The maximum dosage of NAC delivered to patients with the diagnosis of sepsis (3 g 6 hrly x 3 days) did not result in significant enhancement with regards to patient mortality, however, possessed harmful actions that was inclusive of escalation of the risk of the inflammation that was correlated with deteriorated AKI [84]. Nevertheless, at present there is a deficiency of pharmacokinetics along with pharmacodynamics results with regards to NAC as far as initiation, disease propagation, as well as, the robustness of sepsis is not clear. Hence further clinical in addition to experimental studies are needed prior to abandonment of the advantageous actions of NAC is considered (Table 1).

Vitamin C

Vitamin C represents a necessary antioxidant, that hunts ROS in addition to RNS [85], besides resulting in restoration of the endogenous glutathione amounts, thus causing restoration of host's antioxidant defenses [86]. Akin to NAC, Vitamin C possesses antiinflammatory actions by hampering the actions of nuclear factor κB (NF κB) along with generation of proinflammatory modulators [87]. Nevertheless, not like NAC, there is an escalation of proof that Vitamin C, that possesses actions other than its effect as a Vitamin, in the form of a stress hormone, might be playing a key part in the modulation of the adrenocortical stress response, specifically in sepsis, that is it possesses pleiotropic characteristics. Vitamin C, acts in the form of



Table 1: On actions of N-Acetyl Cysteine(NAC)in case of oxidative stress pertaining to sepsis in both models.

Reference no	Author name	Effects of NAC	Mode of actions (Antioxidants influenced)	Radicals influenced	Restoration of physiological substances	Efficacy
72.	Arouma, et al. 1989	NAC(synthetic)	Robust Antioxidants	superoxide, H ₂ O ₂ , hypochlorous acid,	glutathione amounts	
73.	Kharazmi, et al. 1988	ditto		cause repression of production of oxygen radicals by the human neutrophils as well as macrophages in vitro hampering of neutrophils as well as chemotaxis		
74.	Schmidt, et al. 1997			cause reduction in leukocyte to endothelial cells adhesion as well as vascular leakage in case of rodent endotoxemia		
75.	Patterson, et al. 2003			ameliorate the activation of nuclear factor κB (NFκB) along with IL-8 amounts in sepsis		observations yielded the stimulus for evaluation of NAC for reversal of the harmful actions of observations yielded the stimulus for evaluation of NAC for reversal of the harmful actions of Oxidative as well as nitrosative stress in sepsis .
76.	Ritter, et al. 2004	NAC + desferoxamine	a rodent model of sepsis that has been stimulated by cecal ligation as well as puncture (CLP)			subcutaneous NAC in a dosage of 20 mg/kg therapy started 3 h subsequent to CLP caused a reduction in systemic inflammation, Oxidative stress along with mitochondrial impairment that result in significant escalation of survival (10% - 40%)
77.	HsuBG et al.2006		rodent endotoxemia			intravenous NAC treatment) started 20' subsequent to delivery of lipopolysaccharides (LPS) caused reduction in serum creatinine along with blood urea nitrogen amounts, an action correlated with decrease in inflammatory cytokines (TNFα as well as IL-6).
78.	Singer, et al. 2016					Nevertheless, treatment started at markedly early stage of sepsis are of restricted value with regards to a clinical scenario. This is in view of patients with sepsis get usually diagnosis made with, recipient of management, once there is existence of proven multiorgan impairment, as estimated by sequential organ failure assessment (SOFA) scores
79.	Vassilev, et al. 2004					context intravenous treatment with NAC (150 mg/kg bolus+20 mg/kg/h) in pigs 12 hrs subsequent to development of endotoxemia could not respond with no enhancement in systemic, pulmonary, as well as hepatospanchnic haemodynamics or be correlated with reduction in a biomarker of Oxidative stress (8-isoprostane), inspite of escalation of glutathione amounts
80.	Szakmany, et al. 2007					Clinical studies with utilization of NAC as ancillary treatment for stress have mostly resulted in poor results specifically when NAC was delivered to patients with robust sepsis for longer periods. In maximum patients the utilization of NAC was done in the dosage of (150 mg/kg) that was dependent on the regimen that was used in patients who got treatment for acetaminophen poisoning



81.	Spapen, et al. 2005					In case of patients with sepsis, intravenous NAC delivered over a short duration relatively (150 mg/kg bolus+12.5 mg/kg/h for 90') resulted in significant escalation of cardiac index (5.7 (treatment) vs. 5.0 (usual care) L/min/m ² , <i>p</i> = 0.01). Nevertheless, NAC delivery for longer periods of time (50 mg/kg at 4 h that was followed by 100 mg/kg/24 h for 44 h) resulted in deterioration of organ failure (average SOFA scores at 48 h: 7.7 ± 3.8 (treatment) vs. 5.1 ± 2.1 (usual care) L/min/m ² , <i>p</i> < 0.05) without any enhancement of microalbuminuria/creatinine ratio in patients with sepsis
82.	Peake, et al. 1997					NAC delivered to patients with the diagnosis of septic shock made for time periods greater than 24 h resulted in cardiac depression, that had the properties of reduction in cardiac output along with enhancement of hypotension
83.	Molnar, et al. 1999					Akin to that greater needs with regards to inotropic support got illustrated in critically ill patients who received treatment with NAC (150 mg/kg bolus+12 mg/kg/h) for periods over 24 h in contrast to that with usual care
84.	Najafi, et al. 2014					The maximum dosage of NAC delivered to patients with the diagnosis of sepsis (3 g 6 hrly x 3 days) did not result in significant enhancement with regards to patient mortality, however, possessed harmful actions that was inclusive of escalation of the risk of inflammation that was correlated with deteriorated AKI. Nevertheless, at present there is deficiency of pharmacokinetics along with pharmacodynamics results with regards to NAC as far as initiation, disease propagation as well as robustness of sepsis is not clear. Hence further clinical in addition to experimental studies are needed prior to abandonment of the advantageous actions of NAC is considered.

an immune stimulator by which it causes enhancement of macrophages [88], besides might possess the capacity of direct hampering of bacterial proliferation [89]. In cultured human endothelial cells, Vitamin C in a dose-based manner caused hampering of the TNF α stimulated expression of intracellular adhesion molecule [ICAM]-1, thus it possesses the capacity of reduction of microvascular leukocyte that plug in addition to microcirculatory impairment [90]. Vitamin C further has the capacity of reversing BH₄ oxidation along with the escalation of BH₄ based generation of nitric oxide (NO) from eNOS [91], as well as/or escalate eNOS bioavailability through fast phosphorylation of eNOS-Ser1177 [92], actions not observed with antioxidants like NAC [93]. Furthermore, Vitamin C, acts as a significant cofactor for the generation of endogenous vasoconstrictors, like noradrenaline, as well as, vasopressin [94], that might further help. In the circulatory

treatment of patients with sepsis, that commonly develop nonresponsiveness towards vasopressor treatment. Critically ill patients possess aberrantly low amounts of ascorbate [95] that gets further worsened in humans not possessing the capacity of generation of Vitamin C, that gives one extra reason for Vitamin C supplementation, in patients with sepsis [96]. In view of enteral uptake being not enough for normalization of amounts of ascorbate in view of saturable intestinal Sodium dependent Vitamin C cotransporters (SVCTs), the need for intravenous therapy exists [97].

Experimental studies have given sufficient proof with regards to the possible advantages of intravenous Vitamin C in sepsis. Murine sepsis that gets stimulated by CLP, pre-treatment with Sodium ascorbate (200 mg/kg), avoided vascular leaking by hampering the induction of iNOS



superoxide generation along with peroxynitrite generation in skeletal muscle tissue [98]. Sodium ascorbate resulted in a reduction of CLP stimulated superoxide development by avoidance of NADPH oxidase activation in addition to uncoupling eNOS in the same study [98]. Ascorbate treatment in mice with fecal induction of peritonitis (FIP), 6 h post FIP resulted in reversing of platelets adhesion, enhancement of muscle capillary blood distribution along with the escalation of survival, however, these advantages were not visualized in eNOS knockout mice [99]. Pre-treatment with sodium ascorbate (76 - 200 mg/kg), has been documented to ameliorate the reduction in pressor reactions to vasopressors that is inclusive of noradrenaline, as well as, angiotensin II that have been visualized in CLP mice through hampering of iNOS action [100]. These experimental findings have yielded the scientific basis for clinical trials that evaluated the provisional advantages of Vitamin C in human sepsis.

In spite of the posited advantages of Vitamin C that have been evaluated in animal models of early stage sepsis, its effectiveness in human sepsis is contradictory. 2 small, single-center randomized clinical trials (RCT; $n = 24$ & $n = 28$), demonstrated, that intravenous infusion of ascorbic acid, at dosages varying from 50 - 200 mg/kg/day, caused a reduction in inflammatory markers, as well as, sequential organ failure assessment (SOFA) scores [101] along with enhancement of vasopressor sensitivity (25 mg/kg 6 hrly x 3 days) [102]. One more single centre retrospective prior, as well as, subsequent study ($n = 47$) with the utilization of a combination treatment of ascorbic acid (1.5 g 6 hrly) along with hydrocortisone as well as thiamine isolated significant drop with regards to hospitalized mortality (8.5% vs. 40.4%, $p < 0.001$), time period of vasopressor utilization (18.3 ± 9.8 h, $s, 54.9 \pm 28.4$ h%, $p < 0.001$), with the need for RRT (10% vs. 37.4%, $p < 0.05$) [103]. Nevertheless, further multicentre RCT (10% vs. 37.4%, $p < 0.05$) at 72 h following the; [103]. Nevertheless, further multicentre RCT, VITAMINS [104], ACTS [105] as well as ATESS [106] in which trials of ascorbic acid 6 g daily with thiamine, with or without corticosteroid for upto 10 days, did not possess the capacity of estimation the variation in mortality or need for RRT in cases of sepsis. The CITRIS ALI trial where utilization of 16 g daily of intravenous ascorbic acid for 4 days in cases of sepsis, further were unable to find enhancement of average modified SOFA score, despite a decrease in 28 days mortality from 46% - 30% was seen [107]. Sepsis possesses the properties of a significant systemic inflammation status that has been demonstrated to cause downregulation of cellular SVCTs [108]. As per this, greater dosages in contrast to those previously trialed in clinical sepsis might be needed to tide over the reduction in SVCTs, besides pushing Vitamin C into cells for attaining its intracellular advantages.

As significantly greater dosages of Vitamin C have been documented to possess safety in cancer patients or those with burns [109], the preclinical safety along with the effectiveness of a mega dosage of Sodium ascorbate (150 g

for a 40 kg sheep) was more recently, evaluated in an ovine model of sepsis with proved AKI [110]. Subsequent to 24 h of proved sepsis, mega dosage of Sodium ascorbate therapy reversed renal medullary ischemia or hypoxia in septic sheep that was associated with a total reversion of AKI, as estimated by marked escalation of urine flow, as well as, creatinine clearance besides the return of plasma creatinine to normal values [110]. Furthermore, Sodium ascorbate significantly resulted in a reduction in the needs for noradrenaline required, for the restoration of target blood pressure (BP), cause a reduction of arterial blood lactate amount besides enhancement of lung function in septic sheep [137]. Additionally, a critically ill septic patient with COVID-19 stimulated hypotension, as well as, AKI got treated with intravenous Sodium ascorbate (60 g) used on compassionate grounds. Akin to septic sheep Sodium ascorbate resulted in a reduction in plasma creatinine (118 to 84 μ mol/l) besides escalation of urine flow (10 - 400 ml/h, besides average arterial pressure restoration inspite of total removal of noradrenaline, for a minimum period of 7 h intervention duration [110]. In total these preclinical as well as, clinical studies pointed that the ideal dosage, timing in addition to time period of Vitamin C treatment are probably the key factors which estimate its treatment effectiveness, in case of sepsis, however, these factor at present require further evaluation, in details [96]. Thus, 2-pilot placebo controlled RCT are being evaluated at present for the actions of intravenous mega dosage of Sodium ascorbate (60 g as well as 120 g) treatment (on renal outcomes besides vasopressor needs in patients with sepsis (ACTRN1262000651987p; NCT04796636) (Table 2).

Conclusion

Proof is existent with regards to early stage sepsis, with renal microcirculatory impairment, due to inflammation as well as Oxidative stress, causes localized tissue hypoxia, along with mitochondrial impairment, thus starting a vicious cycle of cellular damage besides propagative AKI. Studies with regards to experimental sepsis suggested that anti-inflammatory besides antioxidant effects of NAC along with Vitamin C result in multi-organ protection from failure specifically on administration as a prophylactic agent or at the time of early stage. Nevertheless, these presumed advantages of NAC along with Vitamin C as visualized in animal studies have not got translated all the times in heterogeneous populations of patients with sepsis that have full development of multi-organ impairment on delivery at different time points of disease robustness over continued periods of time of treatment. As per preclinical studies in clinically applicable models of sepsis are the need of the hour for escalation of our insight of the modes of effects of antioxidants in case of sepsis, besides the determination of ideal dosages at a particular correct time period of sepsis (as in comparison in Tables 1 & 2), Vitamin C appears to have greater efficacy in contrast to NAC. These types of studies are necessary for a justified scientific basis for fashioning a larger RCT.



Table 2: On actions of Vitamin C on Oxidative stress in Sepsis (in human & animal models).

Reference no	Author	Effects of Vitamin C	Mode of action	Radicals foraged	Restoration of physiological substances	
85.	Padayatty, 2016	antioxidant		hunts ROS & RNS	restoration of the endogenous glutathione amounts	Thus antioxidant Status of host
86.	Buetner, et al. 1996	Besides antioxidant	It can act as a prooxidant as a reducing agent to reduce redox-activemetal. s such as copper and iron,			
87.	Carcamo, et al. 2002		Akin to NAC, Vitamin C possesses antiinflammatory actions by hampering the actions of nuclear factor κB(NFκB) along with generation of proinflammatory modulators	Vitamin Causes repression of TNF-α stimulated NFκB activation, by hampering I kappa B phosphorylation		
88.	Victor, et al. 2000		peritoneal macrophages from BALB/c mice suffering lethal endotoxic shock caused by intraperitoneal (i.p.) injection of Escherichia coli lipopolysaccharide (LPS; 100 mg/kg) a high production of superoxide anion		The increased adherence, ingestion and superoxide anion production by macrophages from animals with endotoxic shock were lower in the presence of AA, reaching similar values to those of the control animals.	Vitamin C in various doses showed 0.01 nm most efficacious
89.	Armour, et al. 2001		Nevertheless, not like NAC, there is escalation of proof that Vitamin C, that possesses actions other than its effect as a Vitamin, in the form of a stress hormone, might be playing key part in the modulation of adrenocortical stress response, specifically in sepsis, that is it possesses pleiotropic characteristics. Vitamin C, acts in the form of an immune stimulator by which it causes enhancement of	In case of CLP animals iv ascorbate restored Vitamin C, amounts hampered replication of bacteria & avoided H ₂ O ₂ induced cultured microvascular endothelial cells injury	Thus vit c lost in urine & iv vit c can restore skeletal. muscle function	
90.	Mo, et al. 2003		In cultured human endothelial cells, Vitamin C in a dose based manner caused hampering of the TNFα stimulated expression of intracellular cell endothelial cells adhesion molecule [ICAM]-1, thus it possess the capacity of reduction of microvascular leukocyte that plug in addition to microcirculatory impairment			
91.	Kim, et al. 2006			In cultured human endothelial cells, Vitamin C in a dose based manner caused hampering of the TNFα stimulated expression of intracellular cell endothelial cells adhesion molecule [ICAM]-1, thus it possess the capacity of reduction of microvascular leukocyte that plug in addition to microcirculatory impairment		
92.	Ladurner, et al. 2003	as /or escalate eNOS bioavailability through a fast phosphorylation of eNOS-Ser1177i i.				
93.	Schneider, et al. 2005	actions not observed with antioxidants like NAC				
94.	Carr, et al. 2005	Acts as a cofactor for the generation of endogenous vasoconstrictors, like noradrenaline as well as vasopressin				Thus helpful in sepsis & septic shock for sustenance of BP.



95.	Carr, et al. 2018	that might further help In the circulatory treatment of patients with sepsis,that commonly develop nonresponsiveness towards vasopressor treatment. Critically ill patients possess aberrantly low amounts of ascorbate [Hypo Vitaminosis C generation & deficiency	Despite advocated togienteral & parenteral intakes in these critically ill patients			
96.	May, et al. 2021	Megadose of Vitamin C	Conscious sheep received an infusion of live Escherichia coli for 31 hours. At 23.5 hours of sepsis,	in all animals, sodium ascorbate (0.5 gm/kg over 0.5 hr or 0.5 g/kg/hr x 6.5 hrs dramatically improved the	Fever, HR, creatinine all reduccding in renal medullary pO2.decin noradr vasopressordose	
97.	Wilson, et al. 2013		In view of enteral uptake being not enough for normalization of amounts of ascorbate in view of saturable intestinal Sodium dependent Vitamin C cotransporters (SVCTs),with need for intravenous therapy			
98.	Zhou, et al. 2012	Murine sepsis that gets stimulated by CLP (cecal ligation and puncture	Pretreatment with Sodium ascorbate (200 mg/kg), avoided vascular leaking by hampering the induction hampering of iNOS superoxide generation along with peroxy nitrite generation in skeletal. muscle tissue			Sodium ascorbate resulted in reduction of CLP stimulated superoxide development by avoidance of NADPH oxidase activation in addition to uncoupling eNOS in the same study
99.	Tym, et al. 2008		Ascorbate treatment in mice with faecal induction of peritonitis (FIP), 6 h post FIP resulted in reversing of platelets adhesion, enhancement of muscle capillary blood distribution along with escalation of survival, however these advantages were not visualized in eNOS knockout mice			
100.	Wu, et al. 2004		Pre treatment with sodium ascorbate (76-200 mg/kg), has been documented to ameliorate the reduction in pressor reactions to vasopressors that is inclusive of noradrenaline as well as angiotensin II that have been visualized in CLP mice through hampering of iNOS action.			These experimental findings have yielded the scientific basis for clinical trials that evaluated the provisional advantages of Vitamin C in human sepsis.
101.	Fowler, et al. 2014		Inspite of the posited advantages of Vitamin C that have been evaluated in animal models of early stage sepsis, its effectiveness in human sepsis is contradictory. 2 small, single center randomized clinical trial (RCT; n = 24 & n = 28), demonstrated, that intravenous infusion of ascorbic acid ,at dosages varying from 50-200 mg/kg/day ,caused reduction in inflammatory markers as well as sequential organ failure assessment (SOFA) scores			
102.	Khalili, et al. 2016		along with enhancement of vasopressor sensitivity (25 mg/kg 6 hrly x 3 days)			



103.	Marik, et al. 2017		One more single centre retrospective prior as well as subsequent study ($n = 47$) with the utilization of a combination treatment of ascorbic acid (1.5 g 6 hrly) along with hydrocortisone as well as thiamine isolated significant drop with regards to hospitalized mortality (8.5% vs. 40.4%, $p < 0.001$), time period of vasopressor utilization (18.3 ± 9.8 h,s, 54.9 ± 28.4 h%, $p < 0.001$), with the need for RRT (10% vs. 37.4%, $p < 0.05$)	Nevertheless, further multicentre RCT (10% vs. 37.4%, $p < 0.05$) at 72 h following the Intervention did not corroborate		
104.	Fuji, et al. 2020		further mult centre RCT, VITAMINS	In which trials of ascorbic acid 6 g daily with thiamine, with or without corticosteroid for upto 10 days	Did not possess the capacity of estimation the variation in mortality or need for RRT in cases of sepsis	
105.	Moscowitz, et al. 2020		Effects of ascorbic acid, corticosteroids and thiamine on organ injury in septic shock (ACTS RCT)		,	
106.	Hwang, et al. 2020		Combination treatment of Vitamin C and thiamine for septic shock; a multicentre, double blinded randomized controlled study ATESS RCT		DITTO	
107.	Fowler, et al. 2019		CITRIS ALI trial where utilization of 16 g daily of intravenous ascorbic acid for 4 days in cases of sepsis, further were unable to find enhancement of average modified SOFA score, despite a decrease in 28 days mortality from 46% - 30% was seen			
108	Subramaniam, et al. 2019		Sepsis possesses the properties of a significant systemic inflammation status, that has been demonstrated to cause downregulation of cellular SVCTs	TNF- α reduces intestinal Vitamin C absorption		
109	Yahase, et al. 2021		As significantly greater dosages of Vitamin C have been documented to possess safety in cancer patients or those with burns			
110	Lankadeva, et al. 2021		the pre-clinical safety along with effectiveness of a mega dosage of Sodium ascorbate (150 g for a 40 kg sheep) was more recently, evaluated in an ovine model of sepsis with proved AKI	Subsequent to 24 h of proved sepsis, mega dosage of Sodium ascorbate therapy reversed renal medullary ischemia or hypoxia in septic sheep, that was associated with a total reversion of AKI [as estimated by marked escalation of urine flow as well as creatinine clearance besides return of plasma creatinine to normal values]	Furthermore, Sodium ascorbate significantly resulted in reduction in the needs for noradrenaline required, for the restoration of target blood pressure (BP), cause reduction of arterial blood lactate amount besides enhancement of lung function in septic sheep	Additionally, a critically ill septic patient with COVID-19 stimulated hypotension as well as AKI got treated with intravenous Sodium ascorbate (60 g) used on compassionate grounds. Akin to septic sheep Sodium ascorbate resulted in reduction in plasma creatinine (118 to 84 μ mol/l) besides escalation of urine flow (10-400 ml/h, besides average arterial pressure restoration inspite of total removal of noradrenaline, for a minimum period of 7 h intervention duration



Summing up	In total these preclinical as well as clinical studies pointed that the ideal dosage, timing in addition to time period of Vitamin C treatment are probably the key factors which estimate its treatment effectiveness, in case of sepsis, however these factor at present require further evaluation, in details				Thus2 pilot placebo controlled RCT are being evaluated at present for the actions of intravenous mega dosage of Sodium ascorbate (60 g as well as 120 g) treatment (on renal outcomes besides vasopressor needs in patients with sepsis (ACTRN1262000651987 p; NCT04796636).	
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