



Endotoxin and Vitamin C

Part 1 – Sepsis, Endotoxin and Vitamin C

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Introduction

Endotoxin has been on the clinical research 'map' since 1892 when Richard Pfeiffer published a paper demonstrating that the severe toxicity of cholera (*Vibrio cholerae*) was caused by a "poison" released by the cholera organism on its death. He called this poison "endotoxin", i.e. "inside toxin" because he thought that the poison was inside the cholera organism and was released upon its death. Pfeiffer showed that cholera organisms, even if mixed with antiserum or if all viable organisms were killed, could still produce the same severe toxic effects as the living cholera. This discovery threw the fledgling world of bacteriology on its head — it had been assumed as axiomatic that it was the multiplication of the living bacteria (i.e. the "infection") that caused *all* the toxic effects of various infectious diseases. This confusion was compounded by the almost simultaneous discovery that living cholera organisms also secrete a poison, albeit we now know a different one, which is termed an *exotoxin*. Exotoxins also produce pathological effects in infections, e.g. botulism toxin (Botox) is an exotoxin.

The scientific and medical worlds could barely believe the idea that the *death* of the infected animal (sepsis) was due to bacteriolysis and release of "endotoxins", and that the multiplication of the bacteria themselves was in the most part benign. The idea seemed patently ridiculous, however, the enormous amount of clinical and laboratory research that this debate sparked over the next 110 years has proved Pfeiffer to be correct. The basic structure of endotoxin was characterized in 1933 but the real progress in understanding of endotoxin variability and host interaction has occurred in the last decades.

Many of the key clinical discoveries in endotoxin research after the Second World War are due to the work of Abraham I. Braude. Braude discovered that blood that contained endotoxin from Gram negative bacteria could cause vascular collapse and death in people who received transfusions of this blood. Critically, these people had no bacterial multiplication in their blood (no bacteraemia). Braude had demonstrated that endotoxin was responsible for the pathogenesis of shock in these patients, and that it was the endotoxin, not the infection as such, that caused the shock.

Braude spent the rest of his life studying the pathogenesis and treatment of septic shock and produced numerous landmark papers in these areas, including research into the structure of endotoxin from various bacterial species, the pathogenesis of the Schwartzman reaction to endotoxin (a haemorrhagic reaction following massive vascular damage – it can rapidly lead to intravascular coagulation and multiple organ failure) and critical papers on the distribution of endotoxin in various organs after intravenous administration.

Braude died in 1984, but his insight into endotoxin paved the way for deep research into the role of endotoxin in normal and pathological physiology. The scope of the research into endotoxin now is astounding: from mapping of the genes in bacteria responsible for its manufacture; identification of multiple classes of endotoxin; identification and purification of the toxic part of endotoxin – the "Lipid-A" portion; identification of receptor proteins for endotoxin in various mammalian cells; identification of endotoxin transport mechanisms; extensive understanding of the effects of endotoxin on the immune system (including an expanding knowledge of how endotoxin turns on the genes that code for various cytokines); an understanding of the "coalface" of endotoxin toxicity – free radical damage; and understanding of various control elements for endotoxin toxicity – including antioxidant control and defence. The list goes on.

It has been known for some time now that several antioxidants, including vitamin C, in sufficient concentration, can effectively neutralize some of the toxic effects of endotoxin. Exactly how this occurs is not fully understood. However it is clear from animal research that vitamin C does indeed interfere with the pathogenesis of endotoxic shock. This paper will cover some basic aspects of endotoxin and give a brief on the state of research, as well as begin to unwind the interaction of vitamin C with endotoxin.

1. Endotoxin - What is it?

This is one of those simple questions with a complex answer. In its simplest distillation, endotoxin is a component of the cell walls of Gram negative bacteria. Strictly, the term endotoxin refers to the lipopolysaccharide complex associated with the outer membrane of *all* Gram negative bacteria. It is the

lipopolysaccharide (LPS) that gives Gram negative bacteria their characteristic pink counterstain. These bacteria include commensal and/or opportunistic pathogenic species, such as *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, *Helicobacter* and *Haemophilus*, as well as many other common species.

As the name suggests, lipopolysaccharide contains a lipid portion and a polysaccharide portion. The lipid portion, called lipid A is highly conserved amongst Gram negative species. This means that despite considerable genetic differences between the various Gram negative species, the lipid A portion of LPS in all of these species is *almost* identical. The polysaccharide portion however differs *widely* amongst the various Gram negative species and different strains of the same species.

The lipid A portion of LPS is not generally antigenic, i.e. it does not usually provoke the production of antibodies and immune defences *specific* to it. Having said this, antibodies which have been developed to the core components of LPS are prime candidates for anti-endotoxin drug therapy. Despite its low antigenicity, it is the lipid A portion which is essentially responsible for the toxicity of LPS. Lipid A, if stripped from LPS and injected intravenously, will produce all the consequences of intact LPS injection and almost all the consequences of injecting the live bacteria themselves. Because the Lipid A portion is highly conserved across species of bacteria, the reaction to different varieties of LPS from different bacteria is roughly similar (in the same host), however the Lipid A of some species is considerably more toxic than that of others.

The polysaccharide portion of LPS is quite antigenic, i.e. it provokes a specific immune response. The polysaccharide portion contains a core and an "O specific chain". The O specific chain is the portion of LPS *most* responsible for its immune *recognition*. Minor variations in the structure of the O polysaccharide make enormous differences to the virulence of bacterial infections, i.e. the capacity of the bacteria to multiply, infect and ultimately cause harm. While it is the function of the immune system to recognize the O polysaccharide and mount defences against the invading bacteria, it is the Lipid A fragments that produce the noxious harm caused by the infection. Paradoxically, destroying the pathogen increases the harm it does because the LPS is released in large quantities from the dead cell (this is what Pfeiffer guessed in 1982). Lipid A released into phagocytic lysosomes gets into the bloodstream, cells and tissue spaces and invokes a powerful non-specific immune reaction. It is this reaction, if on a large enough scale, that causes shock and death in so many patients.

This reaction to LPS occurs even with *minute* doses entering the bloodstream – picograms – in whatever way the LPS got there in the first

place. LPS can of course readily cross the gut barrier into the blood directly – no systemic infection is required. Endotoxin released from massive kill rates of Gram negative bacteria in the gut can occur in many clinical situations, such as in severe burns, antibiotic use/abuse,^{1,2,3,4,5,6,7} severe trauma, intense or endurance exercise, ischaemia and reperfusion of the gut (and other organs) and several common gastrointestinal pathologies. If LPS is released into the gut in large amounts, it is more or less certain that *significant* amounts of it will find their way into the bloodstream⁸. This in itself can cause severe complications in critically ill and surgical patients, including shock and death.

2. Our cells have receptors for LPS

The coexistence of the cells of eukaryotic organisms with bacterial cells goes back as far of the original emergence of eukaryotes (cell or organism with membrane-bound, structurally discrete nucleus and other well developed subcellular compartments eukaryotes include all organisms except viruses, bacteria, and blue green algae). Conservatively, it is estimated that 50% of the cells in a human body are bacterial. This situation is of course normal, and accordingly essential for normal health. Since our cells have been exposed to bacteria for a long time and bacterial cells have been exposed to us for just as long, it follows that we have developed defences and checks and balances to each other.

Because of this co-evolutionary exposure, it is really no surprise at all that we have systems for recognition and transport of LPS. The large amount of LPS present in the gut makes it inevitable that a continuous low-dose stream of LPS enters the circulation. The presence of LPS in the circulation turns on host defence and stimulates resistance to infection and malignancy⁹. Exposure to continuous low doses of LPS leads to tolerance to LPS (endotoxin tolerance) and is a normal and desirable situation.

For LPS to have any effects at all in higher organisms it is necessary for the LPS to interact in some way with various groups of transporters and receptors. Knockout mice bred without major classes of endotoxin receptors (e.g. CD-137, CD-14 receptors) display little or no reaction to LPS even when they are injected with quite large amounts¹⁰. Given the extremely long association in evolution between bacteria and eukaryotes, it is also not surprising that there are many different classes and distributions of endotoxin receptors in humans. How LPS binds to and activates these classes of receptors largely determines the outcome of the LPS exposure⁹. The list of known transporters and receptors in various species is currently enormous, and this list is growing in complexity.

It is evident that LPS does not occur free in biological fluids. LPS is always associated with protein binders, transporters or receptors in bacteria and hosts. Furthermore, there are specific binders and receptors for Lipid A and polysaccharide portions of LPS distributed in different tissues. There are binders to carry LPS fragments across membranes; there are nuclear receptors for Lipid A and LPS polysaccharide in multiple cell types; there are binders and receptors on many classes of immune cells; there are binders produced by immune cells that have blocking or activating activity on LPS; and there are binders involved in immune signalling and immune cascades.

Receptors and transporters for LPS are coded by genes, which of course, are inherited. Enormous variation exists between individuals as to the extent of expression of these genes, and the ultimate frequency and distribution of the various LPS signalling components. The major transporter is LPS-binding protein (LBP) and the major receptor is membrane CD-14 (on various immune cells), the final endotoxin signal is mediated by Toll-like receptors (TLR-4). *Endotoxin has no effect without transporters and receptors*, so ultimately the expression and regulation of these receptors and signalling components determines the effect on the host. There is reasonable evidence to support the observation that sepsis is more severe in certain receptor genotypes, however the regulation of LPS recognition and signalling is far more complex than basic genetics¹¹. Multiple factors interact, such as immune surveillance, antioxidant status, increased nitric oxide signalling and increased expression of nitric oxide synthase genes.

There is little point detailing the great lists of known receptors and binders, since this adds little clarity to the *clinical* consequences of LPS exposure. Suffice to say that much R&D has gone into developing drugs that can block either the LPS itself or inactivate receptors. Because of the variability in the O region, research effort has gone into developing drugs and antibodies that target the more conserved Lipid A and core polysaccharide regions. Of great promise have been human antiserum^{12,13} and M monoclonal antibodies¹⁴ against the LPS core components and antibodies against Macrophage Inflammatory Factor (MIF)¹⁵. Antioxidants can block various aspects of LPS signalling, and can also, if in high enough concentration, diminish the effects of the cytokines that LPS signalling releases.

3. The effects of LPS

This again is a simply stated concept which is increasingly complex in detail. The effects of LPS on macrophages, neutrophils, B and T cells and the cascades of cytokines and inflammatory mediators that can be released has been studied in enormous detail. An excellent review of the core of this material has been written by Fujihara et al.¹⁵

LPS exposure leads to an increase in circulating levels of nitrate and nitrite, which are stable by-products of nitric oxide. In sepsis, LPS induces inducible nitric oxide synthase (iNOS) expression leading to an increased synthesis of nitric oxide (NO)¹⁹. NO is a potent vasodilator and its continuous overproduction during inflammation, along with the production of various cytokines, leads to vasodilation.

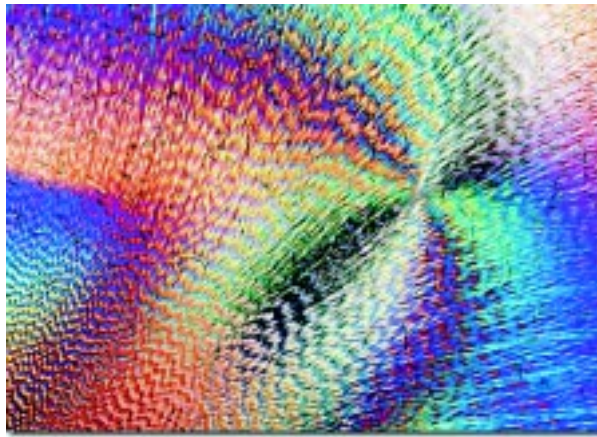
Almost regardless of the endotoxin exposure, a similar set of consequences ensues. Inhaled endotoxin inevitably affects the lungs first, and gut-derived endotoxin inevitably affects the liver first. However and wherever it is presented, once bound, LPS is a potent initiator of immune responses. Phagocytes ingest LPS and the LPS signalling induces gene transcription for cytokines and iNOS. Phagocytes produce large amounts of cytokines in response, typically TNF- α and NF- κ B. These cytokines leave the phagocytes and enter the general circulation and tissue spaces. Control of the production, release and response to these and other cytokines determines the clinical course of the LPS exposure. The production of NO, TNF- α and NF- κ B after exposure to even picogram amounts of LPS can literally cause the vascular collapse, inflammation, coagulation and multiple organ failure characteristic of severe sepsis.

Minute quantities of LPS open the blood brain barrier and expose the CNS to abnormal blood components, leading to catastrophic meningeal and/or brain inflammation. This is the essential mechanism of the pathology seen in meningitis. LPS depletes membrane glutathione (GSH) and severely depletes ascorbate, as well as other key antioxidants. Drastic reductions in antioxidant concentrations in multiple tissues are characteristic of sepsis.

4. LPS Translocation and Response in the Liver

Despite advances in sepsis care, still today approximately 400,000-500,000 people each year develop sepsis in Europe and the USA, half of these people show signs of shock. Over half of the people who develop shock will die despite all treatment efforts – that is approximately 170,000-180,000 people *per year* in intensive care¹⁶. LPS is an incredibly potent initiator of immune cascades. Because of this it is extremely difficult to detect it accurately and it is extremely difficult to interrupt the LPS signalling process to a clinically useful extent. Anti-LPS drugs have not shown as much clinical efficacy as has been hoped. In severely ill people, it is virtually impossible to stop significant LPS translocation into the blood stream from the gut – you really don't need much to cause a lot of harm.

In most cases sepsis patients are exposed to LPS primarily from the gut. Gut-derived LPS is a major route of exposure for surgery patients, burns patients, trauma patients and in general critically ill patients. This of course is the route of exposure for people with gastrointestinal pathologies. LPS may also be derived from blood-borne bacteria or from some other tissue infection. Efforts to control infection with antibiotics are documented to increase LPS loads, leading to the paradoxical tension between controlling the infection and limiting the damage it does from LPS release.



LPS which has crossed into the blood from the gut is taken to the liver first; however any LPS carried in the blood will eventually end up in the liver. The liver throughout our evolution has been the primary detoxifying interface between the “outside” world and us. The liver is the prime organ for mounting an effective immune response and detoxifying response to gut-derived bacteria and toxins. Kupffer cells and hepatocytes are able to recognize LPS via Toll-like receptors and respond to it. Because of the evolutionary association of LPS with the liver, the liver has developed mechanisms to prevent catastrophic overreaction to normal doses of LPS, i.e. the response to LPS is tightly regulated. Larger doses of LPS however can overwhelm liver defence, can cause diffuse hepatitis and lead to a significant increase in circulating toxins.

Considerable evidence has emerged recently that alcohol induces liver damage through an LPS mechanism. Alcohol significantly increases gut permeability to LPS, which in turn reaches the liver and has the potential to overwhelm defences. Once significant LPS signalling ensues in Kupffer cells, large amounts of inflammatory cytokines are released leading to various extents of organ damage. The evidence for this is strong — alcohol does not cause liver damage in knockout mice which do not express various LPS receptors, or in mice with suppressed Kupffer cell function¹⁷.

The precise mechanisms for LPS detoxification by the liver are unclear; however endotoxin is normally processed by

hepatocytes and excreted back into the digestive system in bile¹⁸. Most of the “normal dose” endotoxin encountered by the liver appears to be handled this way. In liver pathology, however, there can be a significant retention of endotoxin in hepatocytes. Increases in endotoxin concentration are associated with increased rates of hepatocyte apoptosis, increased permeability of tight junctions and the pathogenesis of cirrhosis.

Transgenic mice which over-express various glutathione peroxidase (GPx) enzymes show considerable resistance and increased survival to large doses of LPS¹⁹. The GPx enzymes are largely involved in the detoxification of hydrogen peroxide and hydroperoxides – molecules which can lead to the generation of extremely damaging free radicals. GPx enzymes are structurally selenium-dependent and their maintained concentration depends on selenium availability. In sepsis, glutathione metabolism is significantly altered – GSH is rapidly consumed, requiring replacement of GSH. The GSH can be replaced by reducing oxidized glutathione (GSSG) and/or by synthesising more GSH from cysteine. In sepsis the GSH/GSSG ratio drops and blood concentrations of GSH are significantly lowered²⁰, leading to increased oxidative stress.

Because LPS is generally excreted in bile it is a reasonable assumption that LPS is at least in some way handled by liver detoxifying enzymes. The major classes of enzymes likely to be involved are phase I cytochrome P450s (CYP) and phase II glutathione S-transferases, since it has been shown that LPS can significantly decrease the activity of these enzymes²¹. GSH acts as the ‘power supply’ for GST enzymes, so a *decreased* activity of GST enzymes implies that *less* GSH will be used up during GST detoxification reactions. It is not clear at this stage whether the lowered GST activity is due to a lack of GSH, or due to some direct or indirect interaction with endotoxin. It has been reported however, at least in rats, that LPS reduces the expression of GST genes²². The drop in GSH concentration during sepsis is apparently due to GSH consumption by oxidising molecules.

LPS is known to affect the expression of P450 genes and the activity of the enzymes. Numerous studies have demonstrated that the cytokines released in LPS signalling can reduce the expression of genes coding for various CYP enzymes²³.

Regardless of the order of events, if LPS reduces GST and/or P450 activity this will have consequences for the patient. GSTs are heavily involved with the detoxification and removal of toxic xenobiotics and various endogenously derived toxins, P450s are heavily involved in metabolism of multiple classes of drugs. Critical care patients are likely to be on high doses of multiple pharmaceuticals. A

diminution in GST and P450 activity in sepsis will in general raise the toxic profile of the patient and complicate the clinical picture.

5. LPS and Vitamin C Studies – a Selection

Antioxidants theoretically should be able to attenuate the effects of LPS exposure. Antioxidants in general if in sufficient concentration can block the effects of multiple steps in LPS signalling. Multiple antioxidants have been demonstrated to block the production of NF- κ B and also block the effects of the reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are produced by phagocytes in response to LPS¹⁹. Vitamin C in particular has been shown to reduce the expression of iNOS²⁴ in sepsis.

In sepsis patients, plasma and cerebro-spinal fluid ascorbate levels are decreased significantly²⁵. Several animal studies have also shown that sepsis depletes ascorbate levels in various tissues²⁶ and that LPS decreases ascorbate uptake into cells. Because the blood-brain barrier is compromised by LPS, sepsis patients commonly show neurological symptoms resulting from inflammation in the Central Nervous System (CNS), a 'septic encephalopathy'. Decreasing ascorbate concentrations in the CSF are directly correlated with the severity of neurological symptoms²⁵.

There are many studies which demonstrate the beneficial effect of ascorbate loading in LPS injury. Pleiner et al.²⁷ studied the effect of LPS on forearm blood flow in humans and looked at the effects of vitamin C on this. LPS administration caused systemic vasodilation, increased white blood cell count, elevated body temperature and reduced plasma vitamin C concentrations. In this study, vitamin C completely prevented the endothelial dysfunction caused by LPS without altering the responsiveness of the vascular smooth muscle. Also, vitamin C had no effect on the reactivity of subjects not exposed to LPS. Vitamin C was given intravenously in this study at a rate of 24 mg/min for 4 hours (approx 6 grams over 4 hours), and reached blood concentrations of approx 375 micromoles per litre (2-3 times higher than can be achieved with massive oral doses).

In an in vitro study on mouse macrophages by Victor et al.²⁸, macrophages were challenged with *E. coli* endotoxin and then treated with various concentrations of vitamin C. "The increased adherence, ingestion and superoxide anion production by macrophages from animals with endotoxic shock were lower in the presence of AA (ascorbic acid), reaching similar values to those of the control animals. The most effective AA concentration in cells from mice with endotoxic shock was 0.01 mM." This concentration is significantly lower than in the Pleiner et al. study; however, these are cell concentrations, not blood concentrations.

Another study by Armour et al.²⁹ looked at the effects of vitamin C on microvascular dysfunction in the skeletal muscle of the septic rat. Rats were given a caecal ligation and perforation (CLP) to introduce bacteria into their general circulation. 24 hours after the surgery, plasma ascorbate levels dropped by 50% and urinary ascorbate concentrations increased by 1000% in rats that were not given vitamin C. Arterial pressure dropped by 20% and there was a 30% decrease in the density of perfused capillaries in these rats. Intravenous ascorbate (7.6 mg/100 g body weight – approx 5-6 grams for a 70 kg human) given, as a single bolus after surgery, restored all of these parameters to near control levels.



"At autopsy, CLP rats were found to have an accumulation of purulent peritoneal fluid and inflamed intestine, marked by swelling of the intestinal wall. In contrast, a normal peritoneal cavity was found in control rats and those CLP rats that had been infused with ascorbate."

The authors also did some in vitro work on the effect of ascorbate on bacterial replication. Ascorbate at 100 micromolar concentration inhibited bacterial replication significantly in faecal samples taken from the rats (approx 65% reduction); increased concentrations had little additional effect. Note however that higher concentrations of vitamin C in vivo have other effects apart from bacteriostatic properties.

Intense or endurance exercise produces a concentration of endotoxin in the blood similar to that found in patients with sepsis. Oral ascorbate pretreatment of as little as 1 gram has been demonstrated to completely block the increase in circulating endotoxin and nitrite typically found during and after intense exercise³⁰. It appears from this study that oral

vitamin C prevents endotoxin translocation from the gut.

Amazingly, there are very few human clinical studies on the effect of ascorbate in sepsis. The studies that do exist use very small doses of ascorbate, doses that could not possibly produce the effective blood and tissue concentrations seen in animal studies.

How does vitamin C work?

This is a good question. It has variously been reported that vitamin C "neutralizes" or "detoxifies" endotoxin. It is probably more accurate to say that vitamin C protects from the deleterious effects of endotoxin if it is in a sufficient and sustained concentration.

Stress (including exercise) in general causes a mild ischaemia to the gut and LPS translocation increases dramatically. We know from exercise studies that oral ascorbate decreases or completely blocks LPS translocation from the gut³⁰. The most likely explanation here is that ascorbate attenuates the mild inflammatory response in the gut epithelium which is a response to decreased perfusion. Preventing mild inflammation prevents the increased vascular leaking associated with it.

Lipid A is a huge molecule and only picograms are required to initiate an uncontrollable inflammatory response. Ascorbate is a tiny molecule and many grams are required to overwhelm or control this response. Because of this it is most likely that rather than ascorbate deactivating endotoxin by binding it (or some such), ascorbate works by controlling the flow-on effects of endotoxin signalling. Further evidence for this idea comes from normal animal physiology. Most animals manufacture significant amounts of ascorbate in their livers (in some cases tens of grams a day) and this ascorbate enters the circulation. The very same animals are also exposed to continuous minute doses of endotoxin from their gut, as also occurs in humans. We know that this endotoxin exposure is symbiotic and is necessary to maintain normal immune function. This normal endotoxin exposure does not appear to be 'detoxified' by the ascorbate circulating in these animals.

We know that once the endotoxin signalling process has begun, the damage done by endotoxin is actually damage done by the immune system. Cytokines released in high concentrations produce overwhelming amounts of ROS and RNS, leading to free radical production and catastrophic tissue damage. Equally overwhelming amounts of antioxidants are required in *sustained* concentrations to both prevent and combat this.

By far the safest, cheapest, easiest to sustain and easiest to administer effective antioxidant is ascorbate. Ascorbate can be safely given intravenously in tens of grams over multiple doses. To achieve the sorts of plasma concentrations seen in the Pleiner et al. study, *intravenous* ascorbate administration is necessary. The maximum transient plasma

concentration achievable with oral administration is approximately 200 micromoles per litre, more typically 100 micromoles per litre. Note that this is a plasma concentration, not an end tissue concentration. Plasma ascorbate concentrations are fairly tightly controlled after oral administration, mostly due to saturation of absorption versus rate of excretion. On the other hand, an intravenous dose of 50 grams of ascorbate can achieve a plasma concentration of approx 13,000-14,000 micromoles per litre. 50 grams is a perfectly safe dose to give and these sorts of doses are common and are well represented in the clinical and research literature with no side effects. These very high concentrations are more likely to increase tissue ascorbate concentrations than are achievable with acute oral doses.

The high concentrations achievable with large doses of intravenous ascorbate will also maintain the plasma concentration of ascorbate at an effective level for a longer period. Ascorbate is rapidly oxidized/metabolized/excreted in sepsis; therefore, as a therapeutic strategy, it makes sense to dose often to maintain effective plasma concentrations. In a clinical setting of sepsis, the concentrations of ascorbate theoretically necessary to combat the acute phase of sepsis can only be maintained by regular high doses. A single 'megadose' could not be expected to be as effective, especially in severe cases.

Since LPS detoxification occurs primarily in the liver, the liver must be protected in sepsis patients. Circulating LPS can simply sustain or start the sepsis process again if it is not physically blocked or removed. In sepsis, ascorbate and GSH levels in the liver are critically low, having been "used up" in defense of oxidant damage. We know from guinea pig research that ascorbate influences P450, expression and synthesis in the liver. Animals with deficient ascorbate make a *lot* less P450 which means of course that they will have trouble removing endotoxin. Also, high concentrations of ascorbate in the liver will protect cells from oxidant damage, overall leading to less liver damage in the face of sepsis. Ascorbate dosing has also been shown to significantly spare GSH, since GSH is normally used to reduce dehydroascorbate back to ascorbate. In short, high concentrations of ascorbate would be expected to increase liver GSH concentrations, increase P450 synthesis and activity, and directly block the tissue-damaging effects of free radical production. The combined effect means that the liver can continue to do its job at reducing the blood burden of endotoxin.

During this process, the ascorbate concentration must be maintained until at least the endotoxin exposure is reduced to an acceptable level. Since ascorbate is nontoxic in very high and regular doses, there is no pharmacological reason *not* to maintain ascorbate concentrations with regular

intravenous infusion during sepsis. It also makes sense to give ascorbate orally, since we know from evidence that ascorbate can decrease further LPS translocation from the gut.

Gut disturbances, such as parasitic infestation, ulcerative colitis, dysbiosis etc. will result in increased oxidative stress, thereby decreasing the levels of vitamin C in the gut and in the gut endothelium. Insufficient levels of vitamin C may result in an increased translocation of endotoxin, potentially producing multiple organ damage in lungs, liver and brain.

Summary

Endotoxin is a component of the cell walls of Gram negative bacteria. In various physiological or clinical conditions, significant amounts of endotoxin can cross the gut barrier into the blood. Endotoxin may also be derived from blood-borne bacteria or tissue infections.

Endotoxin is a potent stimulator of immune response. Picograms of endotoxin can produce an immune reaction that is overwhelming and is responsible for the majority of problems encountered in sepsis. Endotoxin signalling is mediated by a host of specific transporters and receptors. Once activated, endotoxin signalling produces enormous amounts of inflammatory cytokines which in turn produce ROS, RNS and free radicals. The cytokine response leads to the overproduction of NO, which in part is responsible for the vasodilation seen in septic shock.

Endotoxin translocated from the gut is delivered to the liver. The liver is the principal organ for endotoxin detoxification; however if the liver is not protected by antioxidants or the endotoxin load is too high, severe liver inflammation can result.

Endotoxin severely depletes antioxidants, in particular GSH and ascorbate. A depletion of antioxidants is directly associated with the severity of the reaction to endotoxin exposure. The deleterious effects of endotoxin are mediated by the immune system; the damage done to tissues is due to direct attack by radicals released from phagocytes.

Ascorbate can overcome the radicals produced by the immune system if it is in sufficient and sustained concentration. Ascorbate also decreases or prevents endotoxin translocation from the gut, is directly bactericidal, and increases circulation and liver GSH concentrations. Ascorbate also prevents a decline in the hepatic detoxifying enzymes responsible for endotoxin clearance. Ascorbate blocks the increase in iNOS expression responsible for increased NO production in sepsis.

In sepsis, it is reasonable to suggest that patients should be supplemented continuously with oral and intravenous ascorbate. Oral ascorbate decreases endotoxin translocation

from the gut to the blood, and intravenous ascorbate achieves clinically effective plasma concentrations. Since sepsis is in essence endotoxin signalling, the signal will not be removed unless the endotoxin is removed. Endotoxin is removed through the liver and excreted in bile. The liver must be protected by continuous ascorbate administration. Ascorbate concentration must be maintained in order to effectively neutralize endotoxin signalling. Effective concentrations can be achieved by the regular administration of several grams of ascorbate throughout the entire presentation of sepsis. Ascorbate can be given regularly in doses of tens of grams without toxicity.

The potential association of various bacterial toxins with SIDS and Shaken Baby Syndrome will be discussed in Part 2.

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“Men stumble over the truth from time to time, but most pick themselves up and hurry on as if nothing happened.”

Winston Churchill

