Consensus Meeting on "Relevance of Parenteral Vitamin C in Acute Endothelial Dependent Pathophysiological Conditions (EDPC)"

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Abstract

The 22nd Hohenheim Consensus Workshop took place in at the University of Stuttgart-Hohenheim. The subject of this conference was vitamin C and its role in the treatment of endothelial dysfunction. Scientists, who had published and reviewed scientific and regulatory papers on that topic were invited, among them basic researchers, toxicologists, clinicians and nutritionists. The participants were presented with eleven questions (bold letters), which were discussed and answered (italic letters) at the workshop, with the aim of summarising the current state of knowledge. The explicatory text accompanying the short answers was produced and agreed on after the conference and was backed up by corresponding references.

The therapeutic relevance of administration of the physiological antioxidant vitamin C in high parenteral doses in Endothelial Dependent Pathophysiological Conditions (EDPC) was discussed. Endothelial dysfunction is defined as including disturbed endothelial dependant relaxation of resistance vessels, breakdown of the microvascular endothelial barrier and/or loss of anti-adhesive function. It occurs in severe burn injury, intoxications, acute hyperglycemia, sepsis, trauma, and ischemic-reperfusion tissue injury and is induced by oxidative stress. Reduced plasma ascorbate levels are a hallmark of oxidative stress and occur in severe burns, sepsis, severe trauma, intoxication, chemotherapy/radiotherapy and organ transplantation. Vitamin C directly enhances the activity of nitric oxide synthase, the acyl CoA oxidase system and inhibits the actions of proinflammatory lipids. There is experimental evidence that parenteral high-dose vitamin C restores endothelial function in sepsis. In vitro, supraphysiological concentrations (> 1mM) of ascorbate restore nitric oxide bioavailability and endothelial function. Only parenterally, can enough vitamin C be administered to combat oxidative stress. There is no evidence that parenteral vitamin C exerts prooxidant effects in humans. Theoretical concerns in relation to competitive interactions between vitamin C and glucose cellular uptake are probably only relevant for oxidised vitamin C (dehydroascorbate).

Key words: endothelial dysfunction, vitamin C, parenteral, shock, trauma, oxidative stress

1. Definition of Endothelial Dysfunction

Consensus: **Depending on vessel type: resistance vessels: disturbance of endothelial relaxation as a result of a stimulus (either physiological or pharmacological). Microcirculatory dysfunction of the endothelial barrier. Loss of anti-adhesive function.**

With a weight of 1.5 kg and surface area of 700 m^2, the endothelium can be seen to represent a major organ of the body. The main function of an intact endothelium is to maintain blood flow in order to supply tissues and organs with oxygen and nutrients and to remove metabolites. In addition, it serves an endocrine function. It generates a number of extracellular messenger molecules that mediate a variety of vital functions [1]. The physiological function of the endothelium varies depending on the type of vessel. As the vital regulator of arterial vascular tone, it controls local blood flow in response to changes in the metabolic demands of the surrounding tissue but in certain tissues this function is subordinate to its role in contributing to the maintenance of the organism's blood pressure. The endothelium is also vital for maintaining

blood fluidity by preventing inappropriate coagulation while being actively involved in initiating coagulation in response to damage of the endothelial barrier. The permeability of the postcapillary venule endothelial barrier is regulated in order to allow the passage of immune and inflammatory cells from the blood into tissue in response to tissue damage or infection. The endothelium plays a part in regulating these critical steps in immune and inflammatory processes. The growth of new blood vessels is also a vital physiological process that is regulated in part by the endothelium. It is a major source of angiogenic factors and exerts trophic effects on vascular smooth muscle cells.

Any dysfunction of the endothelium may be a primary contributor to the development of various diseases including hypertension, atherosclerosis and chronic inflammation as well as a secondary factor in the pathophysiology of other disease such as diabetes. The typical consequences of endothelial dysfunction are disturbed vasodilatory function, anticoagulative function, increased adhesivity of the vessel wall for platelets and leucocytes, reduced fibrinolytic activity and breakdown of barrier function causing leakage and oedema formation [2]. Which aspect of endothelial dysfunction predominates depends on the type of the vessel involved.

A key mediator of endothelial function is nitric oxide which is generated by the enzyme, nitric oxide synthase, the activity of which is under physiological control. Nitric oxide is a major mediator of endothelial induced vasodilatation, it inhibits endothelial cell adhesion, is antithrombotic, and inhibits smooth muscle cell proliferation. It is regarded as serving a vital vascular protective and antiatherosclerotic function. It appears that the characteristic feature of endothelial dysfunction relates to the bioavailability of nitric oxide, which has been shown to be reduced in disorders, such as hypertension, dyslipidemia, diabetes, and heavy smoking, which are associated with endothelial dysfunction.

Conversely, an enhanced release of nitric oxide may also contribute to endothelial dysfunction. During inflammation, the inducible form of nitric oxide synthase is expressed and nitric oxide serves as an important proinflammatory regulator. During chronic inflammation or acute systemic inflammation (e. g. sepsis) there is an overproduction of nitric oxide. Potentially toxic micromolar concentrations of nitric oxide may be attained that cause a loss of endothelial barrier function, release of cytokines and adhesion molecules which are typical features of the systemic inflammatory response that occurs in conditions of acute inflammatory shock [3].

In clinical settings there is no parameter of endothelial dysfunction that is generally accepted. Most often an impairment of endothelial dependent vasodilatation is taken as an indicator of endothelial dysfunction. A reduced response to stimulation of endothelial dependent vasodilatation has been shown to be a prognostic marker of cardiovascular risk [4]. Acute endothelial dysfunction occurs in a number of acute conditions in which there is local or systemic disturbance or breakdown in endothelial function that may lead to organ failure and a state of shock [5, 6].

2. WHAT CLINICAL STATES INVOLVE ACUTE ENDOTHELIAL DEPENDENT PATHOPHYSIOLOGICAL CONDITIONS (EDPC)?

Consensus: **Burn injury, intoxications, acute hyperglycemia, sepsis, situations involving ischemia/ reperfusion e. g., heart failure, shock, trauma, compromised circulation.**

There are conditions of chronic and progressive damage to the endothelium and there are conditions such as those listed above, in which there is acute endothelial damage and dysfunction. Although it is clear that oxidative stress and a reduction in nitric oxide underlies each of these pathological conditions, the details of the molecular events that lead to endothelial dysfunction remain largely unclear. In particular, which specific species of oxygen derived radicals are involved in these different conditions have yet to be identified. The recent finding of the presence of nitrosylated proteins in the damaged endothelium associated with severe burns provides some initial insight into the molecular processes involved, at least in this condition [7].

ISCHEMIA/REPERFUSION TISSUE INJURY

Various conditions of tissue ischemia with subsequent tissue reperfusion generate local oxidative stress that leads to endothelial dysfunction [8]. These include various conditions of acute disturbance of local blood flow such as apoplexia, myocardial infarct, embolisms or acute suboptimal perfusion in the extremities or any surgical procedure that involves short-term blockade of blood supply.

MYOCARDIAL INSUFFICIENCY

In the special case of myocardial insufficiency, there is, in addition to local endothelial dysfunction within the myocardium, a resultant condition of cardiac shock that involves ischemia within systemic organs and results in oxidative stress and consequent endothelial dysfunction [9].

ACUTE TISSUE TRAUMA

Various factors released from cells within severely damage tissue evoke local oxidative stress as part of a local inflammatory reaction that can be severe enough to cause endothelial dysfunction which is partly manifest by oedema within the surrounding tissue [10].

HYPOVOLEMIC SHOCK

Hypovolemic shock involves primarily a suboptimal perfusion of all target organs which alone will cause endothelial dysfunction and which is further enhanced by the general compensatory response of vasoconstriction. In the case of extreme and irreversible hypovolemic shock a maximal oxidative stress associated with a general state of vasodilatation causes a breakdown of vascular regulatory mechanisms and circulatory collapse in vital organs [11].

Sepsis

Acute systemic infection or toxemia can lead to severe shock and the complete breakdown of vascular function. Endotoxins and endotoxin induced proinflammatory factors evoke a systemic inflammatory response by acting on the systemic microcirculation. There is a breakdown of local microvascular control that involves oxidative stress and endothelial dysfunction. The precise molecular events involved remain unclear but it is likely that proinflammatory cytokines and toxins are able to generate oxygen derived radicals in endothelial cells [12].

Acute Hyperglycemia

Acute hyperglycemia causes endothelial dysfunction by inducing oxygen derived radicals generation by the endothelium [13, 14].

Burns

Severe burn refers to burn injury of a large surface area of the body. In such conditions, endothelial dysfunction plays a special role as the severe burn syndrome develops as essentially a summation of a number of those factors that are discussed above as causes of endothelial dysfunction. Within the burned tissue there is local hyperemia, inflammation and breakdown of the endothelial function. During the ensuing inflammation activation of macrophages and the nonspecific immune system occurs. This in turn leads to the generation of humoral proinflammatory cytokines that evoke a systemic inflammatory response. This can cause endothelial dysfunction but not the massive barrier breakdown that is characteristic within 24 hours of severe untreated burn. The consequent leakage through the vascular wall of proteins of size greater than 80.000 Dalton leads to increased oncotic pressure in the interstitium and to massive oedema. The oedema in the non-damaged tissue adjacent to the burned tissue causes hypoperfusion and ischemia. There is also a general hypoperfusion of systemic tissue that results from the accompanying hypovolemic shock. Even before the oedema, there is a reduced cardiac output, probably in response to a neural reflex that is evoked by stimulation of sensory receptors within the burned tissue. Hence, it is assumed that the massive breakdown of the microvascular barrier following severe burns results from endothelial dysfunction following a combination of ischemia/reperfusion, hypovolemic shock, cardiac depression, and trauma.

Factors arising from the burned tissue, including postulated "burn toxins", are thought to be the trigger for the systemic breakdown of the microvascular barrier but these remain to be identified. The recent discovery of nitrosylated proteins in endothelial cells of both the burned and non-burned tissue is direct evidence of widespread oxidative stress. The oxidative damage of cellular proteins and lipids within the endothelium by oxygen derived radicals is likely to be a major factor in causing endothelial dysfunction. Although the interaction of the processes of intoxication, ischemia/reperfusion and hypovolemic shock activate the endothelium, this cannot be solely responsible for the systemic endothelial dysfunction that increases the risk of organ failure and that occurs within 24 hours of severe burn. It is more likely that the normal inflammatory reaction to tissue injury within the burned tissue spills over to induce inflammatory processes within the adjacent tissue and eventually other tissues of the body. Thus, a "systemic inflammatory reaction syndrome" (SIRS) occurs that is life threatening since it causes widespread endothelial dysfunction and the consequent risk of organ failure with reduced immune competence and consequent risk of bacterial infection. The "systemic inflammatory reaction syndrome" (SIRS) is driven by widespread oxidative stress [15].

3. What Are the Underlying Mechanisms of Acute Endothelial Dependent Pathophysiological Conditions (EDPC)?

Consensus: **Basic mechanism is oxidative stress.**

Oxygen derived radicals are generated during normal oxidative metabolism and by leukocytes (monocytes, granulocytes) during inflammatory reactions. They are also important intracellular mediators of proinflammatory responses. Since oxygen derived radicals are highly reactive and can damage cell structure and function, the organism requires antioxidant mechanisms to keep these molecules in check. When the production of oxygen derived radicals exceeds the antioxidant capacity of the organism, oxidative stress occurs and excessive amplification of inflammatory processes occurs. The endothelium is especially susceptible to oxidative stress [16]. Increased oxidative stress is associated with cardiovascular risk factors (hypertension, smoking, dyslipidemia, diabetes, ischemia/reperfusion events) and the activation of leukocytes (monocytes, granulocytes) in inflammatory states. Stress exerted on the vessel wall or on leukocytes generally causes an increased generation of reactive oxygen species. The mechanisms underlying this oxidative stress appear to involve a variety of processes (uncoupling of the electron flow in mitochondria, disturbance of the nitric oxide synthase complex, activation of oxygen derived radicals generating enzymes including nicotinamide adenin dinucleotidephosphat oxidase, xanthine oxidase, myeloperoxidase, lipoxygenases) that lead to generation of oxygen derived radicals which is always accompanied by a significant reduction of antioxidant capacity.

The endothelium is an important site of regulation of normal inflammatory reactions. During inflammation oxygen derived radicals activate redox sensitive proinflammatory transcription factors and genes in the endothelium (e. g. the transcription factor Nuclear Factor-κB), cytokines (Interleukin-6, Monocyte Chemotactic Protein-1) and adhesion molecules (Vascular Cell Adhesion Molecule-1, Intercellular Cell Adhesion Molecule-1). These processes are inappropriately activated during oxidative stress and, in addition, the vasodilatory, antithrombotic and anti cell adhesion properties of the endothelium are disturbed [5, 17, 18]. These additional disturbances are very likely to be a di-

rect consequence of the reduced bioavailability of nitric oxide caused by oxidative stress [19]. There is a decrease of nitric oxide due to its interaction with oxygen derived radicals and the generation the peroxynitrite radical which further increases the oxidative stress. Furthermore oxygen derived radicals are considered to uncouple the cofactor biopterin from nitric oxide synthase and so inhibit the enzyme's nitric oxide generating activity [20].

Oxidative stress is associated with a number of important conditions in which endothelial dysfunction is considered a common pathophysiological factor and these include hypertension, atherosclerosis, diabetes, and ischemia/reperfusion tissue damage [21]. Acute endothelial dysfunction occurs when a localised inflammatory reaction develops into a systemic inflammatory response. In such circumstances there is an overspill from the original site of inflammation of proinflammatory cytokines and a dramatic reduction of the organism's antioxidant capacity. The vascular endothelium becomes exposed to an acute oxidative stress and the processes outlined above are induced and there is a subsequent increased risk of organ failure.

4. What Are the Therapeutic Approaches Based on the Mechanisms of Acute Endothelial Dependent Pathophysiological Conditions (EDPC)?

Consensus: **Stimulation of anti-inflammatory response, improvement of antioxidant defences, reduction of toxins including oxidants, improvement of circulation, preconditioning of ischemia/reperfusion situations are the therapeutic approaches based on the mechanisms of acute Endothelial Dependent Pathophysiological Conditions (EDPC). In cases of elective surgery: improvement of nutritional status, especially of antioxidants.**

The pivotal observation that guides the therapeutic approaches derived herein from the knowledge of Endothelial Dependent Pathophysiological Conditions (EDPC) pathophysiology is the pathological reduction of antioxidant defence mechanisms observed in critically ill patients including patients with adult respiratory distress syndrome or septic shock [22, 23, 24]. Also, antioxidant defence mechanisms are severly compromised in patients with diabetes, other antioxidant consuming diseases, and patients awaiting organ transplantation [25]. This latter situation is particularly problematic since these patients not only suffer from the burden of a defective organ system (i. e. renal failure), the challenges of ongoing medical interventions (i. e. dialysis, drugs, etc.), but are also faced with the unavoidable trauma related to the surgical intervention, multiple transfusions, and in particular the ischemia/reperfusion injury of the transplanted organ. Recently, a group of surgeons from Seattle have reported on their observations in almost 600 critically ill surgical patients where antioxidant prophylaxis with vitamins C and E significantly lowered the duration of mechanical ventilation and the incidence of multiple organ failure [26]. These data confirm the previous report by Porter and coworkers, in which administration of an antioxidant cocktail including vitamin C and E as well as selenium and N-acetylcysteine at the time of resuscitations significantly reduced the incidence of later infectious complications and multiple organ failure [27]. The putative beneficial action of antioxidants, in particular of vitamin C will be discussed later in this manuscript. In the search for a link between antioxidant plasma and tissue levels and the consecutive protection from organ injury, several authors have documented reduced levels of inflammatory mediators, in particularity tumor necrosis factors and interleukins, in patients with antioxidant prophylaxis [26]. This brings us to a complementary, albeit significantly more frustrating approach to the management of critically ill patients: the elemination of inflammatory mediators. The basic rationale is to restore the balance between aggressive, pro-inflammatory mechanisms and the body`s intrinsic defence mechanisms. In the context of Endothelial Dependent Pathophysiological Conditions (EDPC), these include the antiadhesive and antiaggregatory properties of the endothelium, its control of transendothelial fluid exchange, and the orchestration of vasodilatory and vasoconstrictive functions. Since virtually all of these mechanisms are defective in septic shock and since animal models have documented a dramatic rise in inflammatory mediators, including tumor necrosis factors and Interleukin-I proceeding the microvascular collapse, a robust effort was undertaken to eliminate inflammatory mediators from the blood stream [28]. As much as these efforts using pharmacological agents, plasmaphoresis and eventually antibodies and receptor antagonists resulted in impressive beneficial effects in animal models of septic shock, the translation into the clinical situation in critically ill patients was and still is until today a frustrating chapter of medical history.

A much more effective approach has been derived from the observation that a high consumtion of n-3 fatty acids from maritime sources (fish oil) appears to protect from cardiovascular diseases. It has been proposed that n-3 fatty acids replace n-6 fatty acids, including arachidonic acid and that the generation of proinflammatory Leukotriene-B4 and proaggregatory leukotriene is shifted to a generation of biologically ineffective lipoxygenase products. Indeed, feeding animals for 4 - 6 weeks with fish oil supplemented diets resulted in a significant improvement of ischemia/reperfusion injury in striate muscle [29]. Another important aspect to keep in mind trying to keep the microcirculation of a critically ill patient is to prevent microvascular thrombosis and fibrin deposition, as a consequence of a pathological activation of the coagulation cascade. In this respect, particular attention has focused on Protein C, especially when converted to its active form [30].

As much as we can – and now start to actually do – improve the balance between oxidants and antioxidants and as effectively as we succeed in lowering the burden of inflammatory mediators in the systemic circulation, there still remains the microcirculation per se as a target for protective interventions. Particularly ischemia/reperfusion injury is a pathophysiological condition in which tissue injury stands at the end of

an impressive effort of the microcirculation to remain patent. A time honored, albeit currently challenged concept of microcirculatory perfusion salvage is extreme hemodilution. Even though the reduction of the red cell mass theoretically reduces the oxidant transport to tissue, the benefit of improved tissue perfusion clearly outweighs this problem. Other interventions to improve tissue perfusion have included the exogenous administration of nitric oxide donors – with enormous clinical benefit in primary pulmonary hypertension, or nitric oxide synthase inhibitors – to treat endotoxin associated hypotension [31]. Whether or not hyperbaric oxygen has the potential to improve postischemic microcirculatory blood flow as suggested from animal experiments remains to be shown [32]. Finally, small volume resusitation aims at rapid restoration of critical nutritional blood flow following ischemia, especially in rapid – and effective reperfusion [33]. In summary, therapeutic and / or prophylactic targets to improve acute Endothelial Dependent Pathophysiological Conditions (EDPC) include rapid and effective restoration of nutritional microcirculatory blood flow at the same time correcting the balance of inflammatory/anti-inflammatory and oxidant/antioxidant mechanisms.

5. WHAT IS THE POTENTIAL THERAPEUTIC VALUE OF PARENTERAL VITAMIN C ON ACUTE ENDOTHELIAL DEPENDENT PATHOPHYSIOLOGICAL CONDITIONS (EDPC)?

Consensus: **Sealing the endothelium. Reduce adhesiveness. Improvement of tissue perfusion. Breaking the viscous circle of inflammation.**

A principal mode of action of vitamin C in restoring endothelial dysfunction seems to be in increasing the bioavailability of nitric oxide by regeneration of the nitric oxide synthase cofactor, tetrahydrobiopterin, and preventing the oxidative conversion of nitric oxide to peroxynitrite [34, 35]. Through this mechanism the anti-adhesive, antithrombotic, vasodilatatory and anti-atherosclerotic properties of nitric oxide are ensured and proper tissue perfusion is re-established.

Consistent with this notion are the reported observations that intraarterial infusion of vitamin C acutely restores endothelium dependent vasodilatation in heavy smokers, diabetic, hypercholesterolemic and essential hypertensive patients [36, 37, 38, 39, 40 41]. These groups of patients have obvious endothelial dysfunction which underlies their hypertensive state and lower than normal plasma ascorbate levels [42, 43]. Restoration of normal endothelial function is now recognised as a key target in reducing the risk of cardiovascular disease. Physiological concentrations of vitamin C can inhibit the oxidative modification of low density lipoprotein, a critical event in initiation of endothelial dysfunction and atherosclerosis [44]. In addition, there is evidence that vitamin C directly inhibits inflammation by suppressing activation of Nuclear Factor-κB, which is a key transcription factor that triggers the endothelium to express pro-inflammatory molecules [45, 46].

6. IS THERE EVIDENCE FOR A DOSE-RESPONSE RELATIONSHIP?

Consensus: **There is no evidence.**

There has been so far no systematic testing of the dose-efficacy relationship of parenteral vitamin C in treating acute Endothelial Dependent Pathophysiological Conditions (EDPC), and hence no evidence for a clear dose-response relationship. Since critically ill patients experience different degrees of oxidative stress and due to the difficulty of quantifying therapeutic outcomes, it is probably not easy to obtain evidence in a clinical setting of such a dose-response relationship. However, both clinical and pharmacological studies indicate that supraphysiological doses in the gram range are necessary in order to achieve therapeutic benefit.

The oxidative stress that causes the acute Endothelial Dependent Pathophysiological Conditions (EDPC) reduces the ascorbate levels and antioxidant capacity of the body [47]. It was actually reported many years ago by different research groups that trauma patients as well as surgical patients have reduced plasma levels of ascorbate and that supplementation with high doses of vitamin C is required in order to restore ascorbate levels to normal [48, 49, 50, 51]. These findings have been confirmed more recently. Schorah and coworkers have reported abnormally low plasma ascorbate concentrations in critically ill patients [22]. Long and coworkers tested various doses of parenteral vitamin C and demonstrated that supraphysiological doses are required to rapidly restore ascorbate levels [52]. They showed that two days of 1000 mg/day restored plasma levels to the low normal range and that a more significant increase was achieved with 2 days of 3000 mg/day. These authors recommended an early and rapid repletion of the body's ascorbate pool in order to allow the critically ill patient to effectively combat the oxidative stress. This was emphasized by the findings of a randomised prospective study of critically ill surgical patients demonstrating that 3000 mg vitamin C/day (given in combination with 2000 IU α-tocopherol/day) leads to a reduced risk of pulmonary morbidity, of multiple organ failure and a shorter duration of mechanical ventilation and of stay in the intensive care unit [26].

Recent pharmacological studies further emphasize the need for supraphysiological doses of ascorbate for restoring endothelial function. Furthermore, they suggest that doses must be sufficient to achieve mM-concentrations of plasma ascorbate and that simply restoring physiological ascorbate levels (70-100 μM) is inadequate. Endothelial Dependent Pathophysiological Conditions (EDPC) are due to oxidative stress causing a reduction of the bioavailability of nitric oxide and the generation of peroxynitrite. Abnormally low levels of nitric oxide cause a breakdown of endothelial homeostasis whereas the generation of peroxynitrite adds to the oxidative stress that directly damages the endothelium. Jackson and coworkers have used an in vitro model in which isolated arterial vessels were exposed to an oxidative stress that was

generated chemically [53]. They measured the plasma levels of superoxide and peroxynitrite as well as arterial relaxation in response to acetylcholine, which is an endothelial and nitric oxide dependant process. Ascorbate was found to inhibit the generation of peroxynitrite by the endothelium and to restore oxidative stress-impaired arterial relaxation which is an indicator of a recovered nitric oxide bioavailability. These effects were found to be dose-dependent but required pharmacological doses (millimolar) well above normal physiological levels (70-100 µM). A virtually complete attenuation of oxidative stress induced peroxynitrite levels was achieved with 10 mM ascorbate although 100 µM was sufficient to completely quench superoxide levels. The recovery of impaired arterial relaxation was not complete at 10 mM ascorbate but this probably reflects the brief time course of the experiments which was 20 minutes. These results are consistent with studies performed in hypertensive patients [37, 54, 55]. In these patients, an impaired arterial endothelial function can be demonstrated pharmacologically and this could be restored by acute intra-venous infusion of vitamin C. Each of these studies demonstrated that local levels of ascorbate must attain supraphysiological levels (> 1 mM) to affect any improvement while having no effect in healthy subjects with normal endothelial function [37, 54, 55].

7. Is there any Evidence that Clinical Conditions Cause a Decrease of Plasma Vitamin C?

Consensus: **Yes, there is evidence in cases of: burn, sepsis, severe trauma, intoxication, chemotherapy/ radiotherapy, organ transplantation.**

There is sufficient evidence that surgical stress increases ascorbic acid requirements and that plasma levels are drastically reduced [52]. Long and coworkers showed that plasma ascorbic acid levels following trauma and infection are extremely low and are not normalized with 300 or even 1000 mg of parenteral ascorbic acid supplementation. From their results the authors conclude that there is an increased turnover and catabolism of ascorbic acid in the presence of trauma and infection, ascorbic acid at 3000 mg should be given for at least the first three days following severe stress from trauma and infection [52]. Schorah and coworkers found median ascorbic acid concentrations in critically ill reduced to below one forth of the values found in healthy control subjects as well as in two other clinical conditions (diabetes, gastritis) in which reactive oxygen species are reported to be increased [22]. In patients suffering from burn injury, the initial plasma vitamin C levels are very low and tend to increase over time [56, 57]. Intensive care patients with sepsis have been shown to have extremely low concentrations of antioxidants including vitamin C [24]. During radiotherapy and chemotherapy of tumour patients, an oxidative stress is generated that increases the demand for antioxidants such as vitamin C.

8. Which States of Acute Endothelial Dependent Pathophysiological Conditions (EDPC) Require Intravenous Vitamin C therapy?

Consensus: **Clinical conditions leading to low plasma vitamin C require intravenous supply to overcome vitamin C deficiency.**

For diseases involving acute endothelial dysfunction – severe burns, critical illness and trauma – there is evidence of drastic decline in blood levels of ascorbate. However, so far only preliminary evidence exists to indicate that parenterally administration of vitamin C in order to achieve supraphysiological levels is of benefit. In a number of chronic diseases – diabetes, heavy smoking, hypertension – reduced blood levels of ascorbate have been reported as well as endothelial dysfunction. In such conditions, human pharmacological studies have demonstrated that high parenteral doses of vitamin C acutely restore normal endothelial vasodilator function without causing inappropriate vasodilatation [58, 59, 60].

An oral supply of vitamin C or low parenteral doses in critically ill patients to overcome the decrease of ascorbic acid plasma concentration is ineffective regarding improvement of low plasma levels and effects of vitamin C on endothelial dysfunction. According to Crandon and coworkers, the low levels of vitamin C in surgical patients were not corretced by parentral administration of 100 mg day [61]. Shorah and coworkers reported that the decreased plasma vitamin C levels in critically ill patients cannot be prevented by the use of parenteral nutrion containing ascorbic acid (200 mg/day for 13 days) [22]. Parenteral administration of 3.5 g ascorbic acid per week or more than 5 g/week did not increase the low plasma levels above the mid-normal range [62, 63].

A number of clinical studies have investigated parenteral vitamin C administration in critically ill patients. Several clinical status has been postulated using vitamin C to prevent oxidant mediated tissue injury. Nathens and coworkers have reported on a randomised prospective study which demonstrated that 3000 IU tocopherol plus 3000 mg intravenous ascorbic acid during 28 days in patients in an intensive care unit was more effective than routine treatment in attenuating the alveolar inflammatory response, in reducing the incidence of less multiple organ failure score, and in reducing the period of stay in the intensive care unit [26].

Also, in animal models, ascorbic acid prevents lung oxidant injury and inhibits polymorphonuclear leukocytes influx into the pulmonary tissue [64]. Similar findings have been obtained in asbestos induced lung disease [65]. Ascorbic acid has also been shown to reduce liver damage caused by oxidative stress following paraquat intoxication [66].

Vitamin C could attenuate the neurological symptoms observed in a mouse model of amyotrophic lateral sclerosis which is a disease in which oxidative stress is a major pathophysiological factor [67].

The results of investigations by Tanaka and coworkers indicate that high-dose parenteral vitamin C (1 g/

hour), administered in third degree burns affecting 70% of body surface area in guinea pigs is able to maintain adequate hemodynamic stability, even in the presence of a reduced resuscitation fluid volume [68]. The fluid volume could be reduced by up to 75% of the Parkland Volume without depression in cardiac output. Tanaka and coworkers also reported that high-dose vitamin C counteracts the negative interstitial fluid hydrostatic pressure and early oedema development in thermally injured rats [69].

In another controlled clinical study, Tanaka and coworkers investigated patients with more than 30% burns and treated with a dose of 66 mg/kg/hour vitamin C for 24 hours. The aim of this prospective, randomized study was to assess whether high-dose vitamin C treatment attenuates post-burn lipid peroxidation, resuscitation fluid volume requirements, and oedema generation in severely burned patients [57]. Thirtyseven patients with burns over more than 30% of their total body surface area (TBSA), and hospitalized within 2 hours of the burn injury, were randomly divided into ascorbic acid and control groups. In the ascorbic acid group, ascorbic acid was infused during the initial 24-hour study period. In the control group, no ascorbic acid was infused. Hemodynamic parameters, respiratory function, lipid peroxidation, and fluid balance were assessed for 96 hours after burn injury. Heart rate, mean arterial pressure, central venous pressure, arterial pH, base deficit, and urine outputs were equivalent in both groups. The 24-hour total fluid infusion volumes in the control and ascorbic acid groups were 5.5 ± 3.1 and 3.0 ± 1.7 mL/kg per percentage of burn area, respectively ($p < 0.01$). In the first 24 hours, the ascorbic acid group gained $9.2\% \pm 8.2\%$ of pre-treatment weight; controls, $17.8\% \pm 6.9\%$. Burned tissue water content was 6.1 ± 1.8 vs. 2.6 ± 1.7 mL/g of dry weight in the control and ascorbic acid groups, respectively ($p < 0.01$). Fluid retention in the second 24 hours was also significantly reduced in the ascorbic acid group. In the control group, the ratio of partial oxygen pressure to fraction of inspired oxygen at 18, 24, 36, 48, and 72 hours after injury was less than that of the ascorbic acid group ($p < 0.01$). The length of mechanical ventilation in the control and ascorbic acid groups was 21.3 ± 15.6 and 12.1 ± 8.8 days, respectively ($p < 0.05$). Serum malondialdehyde levels were lower in the ascorbic acid group at 18, 24, and 36 hours after injury ($p < 0.05$). Administration of high-dose vitamin C during the first 24 hours after thermal injury also significantly reduced resuscitation fluid volume requirements, body weight gain, and wound oedema. A reduction in the severity of respiratory dysfunction was also apparent in these patients [57].

Reperfusion-associated tissue injury is thought to be caused by oxygen radicals. Rhee and coworkers reported that vitamin C combined with vitamin E reduced the oxidative stress in a rat experimental model of hepatic ischemia-reperfusion injury [70]. Also high-dose parenteral vitamin C significantly reduces myocardial damage in a dog model of myocardial infarction [71].

9. WHAT ARE THE MECHANISMS OF VITAMIN C WITH RESPECT TO ACUTE ENDOTHELIAL DEPENDENT PATHOPHYSIOLOGICAL CONDITIONS (EDPC)?

Consensus: **From animal experiment there is evidence that vitamin C interacts with nitric oxide synthase, the acyl CoA oxidase system and with proinflammatory lipid mediated effects.**

On the basis of experimental and clinical studies, vitamin C has been proposed to act via different mechanisms to restore endothelial dysfunction. Ascorbate activates endothelial nitric oxides synthase probably by stabilising tetrahydrobiopterin which is a necessary cofactor in the conversion of arginine to nitric oxide[34, 35]. This is supported by the pharmacological observations of the effects of infusion of vitamin C in improving endothelium-dependent vasodilatation in smokers, diabetics, hypercholesterolemic and hypertensive patients [36, 37, 38, 39, 40, 41]. Ascorbate acts to prevention oxidative stress-induced lipid peroxidation, which potently impairs endothelial function. In addition, they are part of the system maintaining the generation of oxidative stress. Lipid peroxidation can be prevented by vitamin C [44, 72, 73]. Endothelial dysfunction is associated with the activation of the Nuclear Factor-κB by reactive oxygen species which redox-sensitive proinflammatory genes (cytokines, adhesion molecules) and inflammatory breakdown the endothelial barrier. Reactive oxygen species activated Nuclear Factor-κB can be blocked by vitamin C [45, 46]. In an animal model of sepsis ascorbate was shown to be excreted in the urine during sepsis and parenteral ascorbate was shown to prevent microvascular dysfunction in the skeletal muscle of septic animals [74]. In healthy human subjects, Pleiner and coworkers demonstrated that high doses of parenteral vitamin C reverses endotoxin induced endothelial dysfunction [75].

10. CAN VITAMIN C IN PARENTERAL EXERT PROOXIDATIVE EFFECTS?

Consensus: **There is no firm evidence in humans.**

The continual generation of oxygen derived free radicals is a key pathophysiological factor in inflammatory diseases and leads to the reduction of the protective antioxidant reserves of the body. This is reflected in the reduced blood levels of ascorbate in patients with such diseases. In other words, chronic and acute inflammation involves oxidative stress which causes a reduction in the body's ascorbate levels. This general reduction in the body's antioxidant capacity we believe to be a major pathophysiological factor in diseases such as rheumatoid arthritis, gastritis, diabetes, pancreatitis, and critical illness [76, 77, 78, 79, 80].

Fisher and Naughton have recently claimed that supplementation with vitamin C only serves to add to the burden of oxidative stress in chronic inflammatory diseases such as rheumatoid arthritis and Crohn's Disease [81]. There is considerable published evidence that contradicts this hypothesis. Fisher and Naughton

base their claim on the premise that ascorbate acts as a prooxidant in the presence of free ions of transition metals such as those of iron and copper [81]. Levels of such free ions are thought to increase to abnormal levels in inflamed tissues. Several in vitro studies support the notion that ascorbate can exert oxidative effects and is thus potentially damaging to the organism such as by causing damage to desoxyribonucleic acid [82, 83]. Fisher and Naughton cite Rehmann and coworkers as providing evidence that vitamin C supplementation in healthy people can cause oxidative damage [81, 84]. However, in their studies Rehmann and coworkers showed only a transient increase in oxidised desoxyribonucleic acid when vitamin C was combined with iron. These authors point out that it is not possible to assess whether this response is due to iron alone or in combination with ascorbate. Also they speculate that this transient increase in oxidised desoxyribonucleic acid may reflect a potential harmful effect or a protective response [84]. With respect to protein and lipid oxidation due to iron overload, ascorbate has been shown in vitro to be antioxidant [85, 86]. Antioxidant effects appear to predominate at high levels of vitamin C supplementation [87, 88]. In in vivo genotoxicity experiments, high concentrations of vitamin C have been tested without any toxicity being observed [89, 90, 91].

11. IS THERE ANY REASON FOR CONCERN WITH REGARD TO INTERACTIONS AND SIDE EFFECTS WITH HIGH-DOSE VITAMIN C?

Consensus: **From in vitro data there is evidence for a competitive interaction of cellular glucose and vitamin C uptake.**

Vitamin C in its reduced form, ascorbate, is transported ino cells via a sodium-dependent transporter which is present in variable amounts in different cell types [92]. In contrast, oxidised vitamin C, dehydroascorbate, enters the cell via certain glucose transporters (GLUT-1 and GLUT-3) [93, 94]. This is explained by the similar molecular structure of dehydroascorbate and glucose and means that all cells are able to take up dehydroascorbate. The rate of transport of dehydroascorbate into cells is much more rapid than the energy- and sodium-dependent uptake of ascorbate [93]. Dehydroascorbate is normally present in low extracellular concentrations [95]. This is probably explained by its rapid uptake into cells following its formation due to oxidation of ascorbate. The uptake of dehydroascorbate into cells is thought to be followed by reduction to ascorbate which can then be released from the cell. This is considered to be an important mechanism for recycling ascorbate following oxidation [96]. Also it has been recently shown that the rapid uptake of dehydroascorbate and its reduction intracellularly allows the accumulation of ascorbate intracellularly and serves to protect cells during oxidative stress [96].

Can glucose interfere with DHA uptake into cells and / or vice versa? Since ascorbate is not transported by glucose transporters, the possible interaction of vitamin C with glucose relates to possible competition between glucose and dehydroascorbate for specific glucose transporters. A large number of glucose transporters have now been identified. These include sodium dependent transporters (SGLT-1 – SGLT-6) as well as many transporters (a total of 12 have so far been identified) that facilitate cellular glucose uptake independently of sodium. Thus the cellular uptake of glucose is complex and varies between tissues and under different physiological conditions. So far the transport of dehydroascorbate via glucose transporters has been tested for the glucose transporters GLUT-1, GLUT-2, GLUT-3, GLUT-4, GLUT-5 and the sodium dependent transporter SGLT-1. Only GLUT-1 and GLUT-3 which are both present in all tissues, exhibited high affinity for dehydroascorbate (apparent values of the Michaelis Menten Constant are 1.1 and 1.7 mM respectively) while GLUT-2, GLUT-5 and SGLT-1 did not transport dehydroascorbate. GLUT-4 exhibits very low affinity for dehydroascorbate which is physiologically insignificant. The high affinities of GLUT-1 and GLUT-3 for dehydroascorbate suggest possible competition between dehydroascorbate and glucose (glucose affinities of GLUT-1 and GLUT-3 are both about 1 mM). Since systemic levels of dehydroascorbate rarely attain levels greater than $10 \mu M$ and never more than $100 \ \mu M$, a significant interference of dehydroascorbate with glucose uptake is unlikely [95]. However, it is theoretically possible that glucose can interfere with the recycling of ascorbate in conditions of oxidative stress. It is speculated that the hyperglycemia of Type-II-Diabetes causes such an impairment of ascorbate recycling and that the resultant reduced antioxidant capacity is of pathophysiological significance.

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