Micro-immunotherapy
International Medical Experience

Benefits of using vitamin C and micro-immunotherapy in the management of infections

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Introduction

The immune system is among those that are fatally damaged by the deficiency of vitamin C. Recent research[1] has specified a number of components of immunity the function of which is vitally dependent on vitamin C and has defined the mechanisms that impair immunity when this vitamin is deficient in the body. These findings are of crucial importance because, as it has been shown[2], deficiency of vitamin C is much more prevalent in the civilized world than previously expected. For these reasons, the incidence of anti-infective immune disorders as well as other chronic diseases is increasing. In this article, we present an overview of the mechanisms by which vitamin C affects various components of the immune system, impacting on anti-infective immunity. At the same time, we outline the importance of combining vitamin C supplementation with specific immunomodulatory treatments such as micro-immunotherapy and we discuss on their synergy in the management of infections. Finally, we propose some guidelines for clinical practice.

Vitamin C and immune function

Vitamin C (ascorbic acid) has essential significance in both innate (non–specific) and adaptive (specific) immune function.

First of all, vitamin C plays a crucial role in maintaining the barrier function provided by the skin and mucous membranes[1]. The skin is one of the first lines of defense in the body, protecting it against the penetration of pathogenic microorganisms. The main cellular components of the epidermis are keratinocytes, whereas dermis is composed mainly by fibroblasts, which secrete collagen, essential constituent of the extracellular matrix. Both types of cell need vitamin C to fulfill their barrier function. For example, vitamin C is used as a cofactor by the enzymes involved in the synthesis of collagen. Deficiency of this essential nutrient results in impaired production of collagen, impacting negatively on the healing process[3]. Leukocyte function, especially neutrophils and macrophages, is as well dependent on vitamin C. This nutrient contributes to their antibacterial properties, which are essential for the healing process. In addition, vitamin C may improve anti-infective characteristics of the mucous membranes, such as the epithelium from the respiratory and gastrointestinal tract, by increasing the expression of proteins that ensure the maintenance of the so-called tight-junctions between epithelial cells[1].

On another hand, vitamin C contributes to the expression of multiple immune mediators, including interferon, which has a crucial role in antiviral defense[1,4]. It is also required for the migration and function of leukocytes such as neutrophils, during an acute infection[5]. Neutrophils infiltrate the infected site, remove pathogenic microorganisms through phagocytosis, then die out by apoptosis and are removed from the infected site. These processes require vitamin C, which leukocytes of all types actively accumulate[6]. The level of vitamin C in these cells is up to 100 times higher than in blood plasma. People with severe infections often suffer from impaired chemotaxis of neutrophils linked to a deficiency of ascorbate[7]. In fact, clinical trials in patients with recurrent infections have shown that deficient chemotaxis of neutrophils can be restored by administration of high doses of vitamin C[8]. Furthermore, the efficacy of phagocytosis depends as well on a sufficient high level of vitamin C in the cells, as confirmed by several studies[9]. After supplementation of high doses of this vitamin, the phagocytic function of neutrophils is enhanced significantly, and is followed by improvement of the patients' clinical condition[1].

After neutrophils in the infected tissues complete the elimination of the pathogenic microorganisms by phagocytosis, another step follows, which represents their destruction by apoptosis and removal by macrophages from the affected tissue. This is the final step of the physiological acute inflammatory process. With this step, macrophages prevent the development of pathological, chronic inflammation. If the process of neutrophils' apoptosis is disrupted, they necrotize and release substances that lead to chronic inflammation and damage to the affected tissue. In cardiovascular and respiratory disease, which are linked to chronic inflammation and oxidative stress, leukocytes further increase their need for vitamin C: for example through increased intake of dehydroascorbate, which is consecutively reduced to ascorbate in cells. Sufficient level of ascorbate reduces the activation of pro-inflammatory cytokines in immune cells and acts on its signaling pathways (e.g nuclear factor kappa B (NF-kappa B)), thus protecting tissues from chronic inflammation. Another variant of the pathological process in infected tissue is the so-called NETosis, which is the process related to the formation of so-called neutrophil extracellular traps (NET). NET is a network of DNA strands, histones and enzymes that normally capture pathogens, but with severe infection and concomitant deficiency of ascorbate, this network can lead to the damage of tissues and organ failure. Studies have shown that increased supplementation of vitamin C can prevent NETosis[1].

Additionally, sufficient levels of vitamin C may influence multiple pathways in adaptive immunity, supporting for example lymphocyte and Natural Killer (NK) cells function. Under physiological conditions NK cells, B-lymphocytes and T-lymphocytes actively accumulate high concentrations of vitamin C. This vitamin plays an important role in these cells not only as an antioxidant, thus protecting them from oxidative stress, but as well supporting their proliferation[10,11]. It is as well necessary for NK and T-cells cytotoxic function and contributes to the adequate production of antibodies by B-lymphocytes (or more accurately, by their final differentiation stage - plasma cells)[1,12,13].

Vitamin C deficiency and infectious disease

Due to the significant effect of vitamin C on the immune system, its deficiency leads to a weakening of the immune system and an increased susceptibility to infectious diseases[14]. Studies have shown that patients with acute respiratory diseases, such as bronchopneumonia, often suffer from vitamin C deficiency[15]. Supplementation of this vitamin to patients with respiratory infections improves the clinical outcome[16,17]. In fact, antiviral activity of ascorbate has been demonstrated in a variety of viruses, such as influenza, herpes, poliovirus, parvovirus, rabies virus, HIV and others[18-29].

As vitamin C contributes to the adequate production of interferon and to the reduction of pro-inflammatory cytokines in the lungs, vitamin C deficiency during viral infection is linked to higher titers of the virus in the lungs and a decrease in antiviral cytokines, especially interferon alpha and beta (IFN-α/β)[15]. In addition, several studies have shown that vitamin C deficiency is related to inflammatory changes in the lungs when exposed to viral infection (e.g. influenza)[30], and point out its beneficial effect when administered in individuals with viral pneumonia[31]. For example, vitamin C deficiency leads to increased production of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1 in the lungs[15].

Deficiency of vitamin C, which often precedes the onset of infectious diseases, is itself exacerbated by the course of the disease due to increased consumption of vitamin accompanied by more intensive metabolism during inflammation. This is also the reason why the requirements for its supplementation as part of the treatment of infectious diseases are significantly higher than preventive doses. For example, it has been proven that vitamin C reduce viral load in cells infected with Epstein-Barr virus (EBV)[32] or cytomegalovirus (CMV)[33]. A study has shown that increasing levels of vitamin C in endothelial cells before exposure to viruses reduce viral load in cells[33].

Modern medical research has shown that one of the pathophysiological consequences of deficiency of vitamin C is the development of oxidative stress, caused by insufficient neutralization of reactive oxygen species[34]. Under physiological circumstances, exposure to a viral infection leads to the activation of phagocytes, with increasing intracellular production of reactive oxygen species (ROS). ROS play a role in deactivation of viruses. However, the predominance of ROS can be detrimental to the host's cells themselves and thus it can exacerbate tissue damage caused by viral infection. Oxidative stress results in a pro-inflammatory condition with harmful consequences in all systems of the body. One example may be provided by the respiratory syncytial virus (RSV), one of the most common causes of infectious diseases of the upper and lower respiratory tract, especially in children. Infection of respiratory epithelial cells by RSV leads to increased production of ROS and to the inhibition of antioxidant enzymes. This imbalance increases the danger of RSV to the respiratory tract and reduces defensive capabilities of the respiratory epithelium[35]. A sufficient supplementation of vitamin C may play a positive role reducing inflammatory damage by removing excess ROS[36]. Vitamin C not only acts directly as an antioxidant, but it also helps to activate other intracellular antioxidants such as tetrahydrobiopterin and alpha-tocopherol[37,38].

The role of micro-immunotherapy in infectious disease

Micro-immunotherapy (i.e low dose immunotherapy) is a therapeutic approach that aims to restore immune balance by targeting the host's immune system. It is a type of immunotherapy that uses immunomodulatory mediators (such as cytokines, chemokines, growth factors, neuropeptides…) in low doses, in order to respect the natural functioning of the body and improve treatment tolerability.

Current micro-immunotherapy treatments use low doses that are similar or below the physiological concentrations at which these substances normally circulate in the body (ranging from micrograms [10^{-6}g], picograms [10^{-12}g] to fentograms [10^{-15}g]). They follow sequential sequences in accordance with the steps of the immune response (chronobiology) and are administered via the sublingual route, taking advantage of the histological and immunological features of sublingual mucosa.

Micro-immunotherapy formulas can be used to regulate the immune response in multiple therapeutic areas, from infections to inflammatory or autoimmune diseases, in allergy[39,40], as well as

a complementary treatment in oncology. In infectious diseases, micro-immunotherapy may be used with different objectives, depending on the treatment:

- As a preventive strategy in infectious diseases (e.g. common cold, flu, influenza, rhinitis, pharyngitis…)
- To support the immune system en case of immunodeficiency (e.g. frequent & recurrent infections, induced by chronic stress…)
- In specific viral infections, such as those caused by herpes simplex virus, Epstein-Barr virus, Cytomegalovirus, papillomavirus or varicella-zoster virus: to prevent viral replication, promote an effective immune response and control the development of diseases associated with viral latency[41].

Due to its characteristic features, micro-immunotherapy formulas can be used alone or synergistically combined with other therapies, and they can be administered to all types of patients, as they have a good safety profile. It is a treatment designed for daily clinical practice.

To name a few examples, here we describe two micro-immunotherapy formulas widely used in clinical practice by health professionals internationally: EID and PAPI.

- EID micro-immunotherapy formula is a complex formula that combines multiple active substances, such as interleukins IL-1, IL-2, IL-6, TNF-α and interferon-gamma (IFN-γ), in low (LD) and ultra-low doses (ULD) that aim to enhance primary immune response against infections. IL-1, IL-6 and TNF-α are pro-inflammatory cytokines that both participate in innate and adaptive immunity by stimulating, for example, macrophages and neutrophils mobilization to the site of infection. They are as well responsible for promoting phagocytosis and antigen presentation to lymphocytes, in order to activate adaptive immune response. These cytokines can as well activate lymphocytes T, B and NK cells, and stimulate antibody production by B cells or cytotoxic activity from T-lymphocytes. IL-2 is strongly implicated in T-lymphocytes proliferation; it is an "activator" of immune response. IFN-γ, on the other hand, is an important cytokine in antiviral defense. Formula EID can be used in clinical practice to support immunity in cases of acute, chronic and recurrent infections (e.g winter infections), both in adults and in children[42].

- PAPI micro-immunotherapy formula is a complex formula that combines multiple active substances, such as interleukins IL-1, IL-2, interferon-alpha (IFN-α) and specific nucleic acids (SNA®) targeting viral proteins, in order to prevent viral replication and modulate immune response in HPV infections. The efficacy of the medicine 2LPAPI® has been proven in a clinical study published in the *Advances in Infectious Diseases*[41]. 2LPAPI® was administered in a daily dose of 1 capsule sublingually for 6 months to women with cytological confirmed HR-HPV infection. The presence of HR-HPV was then tested in a follow-up at 6 and 12 months. The results highlighted the effectiveness of the formula in high-risk papillomavirus (HR-HPV) clearance in patients aged over 25 years, which is the population with the higher risk of subsequently developing cervical cancer. A higher clearance in this group compared to the control group was observed at 12 months showing a long-term therapeutic action of the medicine on the immune system[41].

Other micro-immunotherapy formulas that may be of interest in the field of infections may be: Formula EBV and CMV (used in clinical practice in cases of Epstein-Barr or Cytomegalovirus infections, respectively) and Formula HERP and ZONA (used in clinical practice in cases of herpes simplex or varicella-zoster virus, correspondingly).

The joint effect of vitamin C and micro-immunotherapy in the management of viral infections

As vitamin C plays an important role in the functioning of the immune system, being its deficiency one of the causes of immune disorders and other diseases[43,44], supplementation of this vitamin in sufficient doses and in a suitable form may be of considerable importance in infectious diseases. Simultaneously, micro-immunotherapy is a safe therapeutic strategy that can be used in clinical practice to regulate the immune system in the management of infections. It uses low doses of its own mediators, ensuring therefore a good tolerability of the treatment.

Here we suggest that a combination of both strategies may benefit from a joint effect that can impact synergistically on immune function. The objectives of combining micro-immunotherapy and vitamin C supplementation are the following:

- To correct immune alterations linked to a vitamin C deficiency, that is supporting the barrier function, the expression of inflammatory mediators, the leukocyte function (e.g. migration and phagocytosis), etc.
- To enhance primary immune response against infection, that is stimulate antiviral defense by influencing the cytokine microenvironment.
- To obtain a long-term therapeutic action, by providing the immune system the tools it needs to recover its own balance.

Guidelines for clinical practice

In cases of vitamin C deficiency, sufficient supplementation is important to achieve saturation of the organism with this vitamin. In such cases, the usual oral form of vitamin C, whose absorption is limited by intestinal transporters, is not sufficient. Therefore, it is advisable to use a form that ensures achieving of sufficiently high plasma level.

One possibility may be to administer high doses of the vitamin by infusion. In these cases vitamin C should be measured in urine and the dosage recommendation based on the corresponding results. In our InPHARM Clinic, we usually monitor the levels of vitamin C using UroC Kontrol strips developed internally and that are part of our vitamin C LipoC Askor package.

Another form of administration of vitamin C is the oral form of vitamin C with liposomal delivery (liposomal vitamin C), which is an advantageous form that provides substantially higher bioavailability of ascorbate than conventional oral forms thanks to its specific pharmacokinetics. Prophylactic use requires dosing in the mg range, while treatment of current infections requires dosing in the gr range. As a general recommendation, we usually prescribe 2grams of liposomal vitamin C LipoC Askor Forte daily (2x 2cps, 1 cps = 500mg of liposomal vitamin C). This recommendation is based on epidemiologic data from our Clinic, because the most common urine level of vitamin C is 20mg/dl and this dosage is necessary for effective saturation. If the deficit is deeper, we recommend 4 or 6grams daily. If the deficit is mild, then we prescribe just 1gram daily,and if the patient is well saturated by vitamin C in urine, only 500 mg daily. The intake of liposomal vitamin C should always be at least 3 months.

The combination of micro-immunotherapy with vitamin C supplementation may depend on the nature of the infection:

▶ For prevention of coronavirus infection, we recommend micro-immunotherapy formula EID 1x daily + LipoC Askor Forte 2x2 cps (=2g daily) for 3 months.
▶ In the case of infection by herpes simplex virus, we recommend micro-immunotherapy formula HERP 1caps x daily + LipoC Askor Forte 2x2 cps (=2g daily) for 3 months.
▶ In the case of papillomavirus infection, we recommend micro-immunotherapy formula PAPI 1caps x daily + LipoC Askor Forte 2x2 cps (=2g daily) for 3 months.

Conclusion

The immune system daily defends our body from external and internal aggressors and prevents it from disease. It is the very pillar of our health. Most diseases, including infections, are therefore linked to an immune dysfunction. Regulation of the immune response should therefore be considered in any therapeutic strategy and integrated in any preventive treatment plan. The combination of vitamin C supplementation and micro-immunotherapy is of interest as an immunomodulatory strategy due to its multiple advantages.

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