Vitamin C and Neutrophil Function

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Vitamin C is known to support immune function and is accumulated by neutrophils to millimolar intracellular concentrations suggesting an important role for the vitamin in these cells.

Keywords: vitamin C ; ascorbic acid ; neutrophils ; polymorphonuclear leukocytes ; migration ; chemotaxis ; apoptosis ; phagocytosis ; oxidative burst

1. Introduction

Neutrophils are a vital component of the innate immune system, providing a first line of defense against invading pathogens [1]. Following microbial invasion, neutrophils migrate to the site of infection in response to pathogen- and host-derived pro-inflammatory mediators, known as chemotaxis [1]. The neutrophils then proceed to phagocytose, kill and digest the invading pathogens via both oxidative and enzymatic mechanisms [2]. Spent neutrophils subsequently undergo a process of programmed cell death which results in recognition and clearance of the cells by macrophages [3]. Effective clearance of neutrophils from inflammatory loci is vital for resolution of the pro-inflammatory response as release of necrotic cell contents results in tissue damage [4]. Chromatin released from neutrophils, known as neutrophil extracellular traps, comprises both oxidative and proteolytic enzymes, and has been implicated in host tissue damage and various pathologies [5].

Defective neutrophil function is observed in a number of conditions, such as chronic granulomatous disease and Chédiak-Higashi syndrome, which result in recurrent infections [6][7]. Patients with recurrent infections and sepsis can also present with dysfunctional neutrophils, sometimes referred to as immune paralysis due to the inability of the cells to migrate appropriately [8]. It is noteworthy that patients with severe infections and sepsis present with depleted vitamin C status [9][10]. Vitamin C is known to have pleiotropic roles in the immune system, through its antioxidant and enzyme cofactor activities, including potentially supporting neutrophil function [11]. Preclinical studies indicate that neutrophils isolated from scorbutic guinea pigs exhibit attenuated chemotaxis, phagocytosis, oxidant production and microbial killing compared with control animals, and supplementation with vitamin C reversed the dysfunctional activities [12][13][14]. Vitamin C-deficient gulonolactone oxidase (Gulo) knockout mice exhibit dysfunctional neutrophil cell death and diminished uptake by macrophages [15], and vitamin C supplementation can decrease neutrophil extracellular traps formation in septic Gulo knockout mice [16].

Although mean plasma vitamin C concentrations are typically around 50 µmol/L, neutrophils accumulate millimolar intracellular vitamin C concentrations against a concentration gradient which is thought to indicate an important role for the vitamin in these cells [17]. Thus, the depleted vitamin C status of neutrophils observed during infectious episodes could potentially compromise their function [18]. Numerous non-controlled studies have investigated the effects of vitamin C supplementation on the neutrophil functions of chemotaxis, phagocytosis, oxidant generation and microbial killing and predominantly showed positive effects (reviewed in [11]). A number of these studies included patients with known neutrophil dysfunction e.g., those with chronic granulomatous disease or Chédiak-Higashi syndrome, or individuals with allergic or infectious conditions. Exercise, both single bouts and prolonged training over several weeks, can produce changes in the distribution and function of various cellular and humoral components of the immune system [19]. Studies have reported high susceptibility of athletes to infections, especially upper respiratory tract infections, following heavy and intensive training as well as after marathon and ultramarathon running [20][21]. Thus, the effect of vitamin C supplementation on neutrophil function in athletes is also of interest.

2. Current Insights on Vitamin C and Neutrophil Function

Overall, nine of the 16 RCTs included in this review reported no effect of supplementation with vitamin C alone, or in combination with other micronutrients or antioxidants, on various neutrophil functions [19][22][23][24][25][26][27][28][29]. The seven studies which did show effects of supplementation on the neutrophil functions assessed (i.e., chemotaxis, oxidative

burst activity, antioxidant enzyme activity and apoptosis) were in hospitalized patients or outpatients [30][31][32][33] or athletes [34][35][36]. None of the other studies carried out with healthy volunteers showed any effects of additional supplementation.

Eight of 10 RCTs showed no effect of supplementation on neutrophil phagocytosis and/or oxidative burst activity [19][23][24][25][26][27][28][29]. The two studies that showed an effect on oxidative burst activity used combination supplements and they showed opposite effects [33][35]. Herbaczynska-Cedro et al. [33] showed decreased oxidant burst activity in acute myocardial infarction patients treated with a combination of vitamins C and E. It is well known that vitamin C can recycle vitamin E and vitamin C has been proposed to interact with vitamin E in vivo, thus they appear to work synergistically [37]. Although another study also tested a combination of vitamins C and E, they saw no effect on oxidative burst activity [25]. However, this study was carried out in smokers and it is known that smoking causes enhanced oxidative stress and smokers require significantly higher intakes of vitamin C to reach the same circulating concentrations as non-smokers [38]. In contrast, Robson et al. [35] showed an increase in oxidative burst activity in trained runners supplemented with an antioxidant combination following prolonged exercise. Since the baseline vitamin C status of the participants was already at saturating levels (i.e., 70 µmol/L), this suggests that the observed effects may have been due to other components of the antioxidant mixture, although it does not rule out vitamin C acting synergistically with these components.

Eight of the 10 RCTs investigating oxidative burst activity and/or phagocytosis were carried out in healthy participants or athletes [19][23][24][26][35][27][28][29]. However, only one of these studies assessed baseline vitamin C status, which was already saturating [35]. It should also be noted that neutrophils saturate at lower vitamin C levels than plasma [39]. Therefore, it is unlikely that supplementing healthy volunteers or athletes with additional vitamin C over and above their normal baseline levels would have an effect on neutrophil function [40]. Exercise trials can also be complicated in that, depending on the exercise intensity, the effects on the immune system can vary such that moderate exercises may have immunopotentiating effects while intense exercise is potentially immunosuppressive [41]. Although two combination trials showed positive effects of supplementation on neutrophil function in athletes [35][36], with combination studies, it is not always possible to determine which of the component(s) is having the effects, or if there are synergistic interactions occurring between the components.

Four of the RCTs administered intravenous vitamin C in hospital or outpatient settings, three of which saw an effect of the intervention on neutrophil chemotaxis and apoptosis [30][22][31][32]. Intravenous vitamin C is known to provide significantly higher plasma concentrations of vitamin C than oral administration [42]. Anderson et al. showed that administration of intravenous vitamin C (1000 mg once daily) was associated with improved neutrophil chemotaxis in asthmatic patients. Maderazo et al. [31] reported an increase in plasma vitamin C status and enhanced neutrophil chemotaxis in trauma patients administrated the combination of vitamins C and E. Ferron-Celma et al. [32] observed a decrease in pro-apoptotic enzymes and an increase in anti-apoptotic proteins in septic patients administered vitamin C alone. The forth study was carried out in healthy children undergoing elective surgery and although their baseline vitamin C levels were not assessed, these may have already been adequate [22]. Furthermore, the vitamin C was administered as a single dose only (10 mg/kg bodyweight), which is less likely to have an effect than repeated dosing [43].

There are a number of limitations of using the RCT paradigm to assess the effectiveness of nutrients such as vitamin C [44]. RCTs were specifically developed and designed to test the safety and efficacy of pharmaceutical drugs, not nutrients. For example, it is not possible to have a true placebo group in RCTs of nutrients as all of the participants will be consuming variable amounts of the nutrient of interest. Of note, a number of the assessed studies did not control for dietary intake or the participants were asked to maintain their normal diet. More than half of the RCTs included in this review did not assess baseline plasma vitamin C status, and a majority of the studies were carried out in healthy individuals, who likely already had adequate plasma vitamin C status prior to beginning the supplementation, thus negating any effect of supplementation. Only one study measured the vitamin C content of the neutrophils pre- and post-supplementation [34]. There was also variability in the analysis of the neutrophil functions, with the chemotaxis, enzyme activity, and apoptosis assays being carried out with isolated cells in buffer or culture media, and the phagocytosis and/or oxidative burst assays being carried out with either whole blood or isolated cells. Furthermore, two of the studies used only a single dose of the vitamin [22][23]. Because vitamin C is water soluble, it is cleared rapidly from circulation by the kidneys, with a half-life of approximately two hours, therefore a regular intake is required to maintain adequate levels [42]. Finally, smoking status is well known to impact on vitamin C status and requirements, due to enhanced oxidative stress [45][38], however, this was not taken into account in a majority of the studies.

3. Conclusions

Overall, 44% of the RCTs assessed in this review showed effects of vitamin C supplementation on various neutrophil functions. The studies were very heterogeneous, comprising different participant cohorts (athletes, hospitalized patents or healthy volunteers) and different dosing regimens (oral or intravenous, monotherapy or multi-supplements, synthetic or food-derived, and from one-off to many months in duration). There were also a number of limitations inherent in the design of many of these RCTs. Unlike drug trials, evidence indicates that RCTs of vitamin C supplementation will be more likely to have a positive effect in participants who are suboptimal or deficient in the vitamin at baseline [44]. Therefore, future RCTs should incorporate prescreening of potential participants for low vitamin C status or utilize cohorts known to have low vitamin status, such as hospitalized patients. The effects of vitamin C administration on more recently discovered functions of neutrophils, such as the formation of neutrophil extracellular traps, should also be explored in future studies based on promising in vitro and preclinical data [16]. Meta-analyses have indicated that vitamin C intakes of at least 200 mg/day can decrease the risk of acquiring respiratory infections [46][47], however, gram doses of vitamin C are required once an infection has taken hold, due to increased requirements for the vitamin [10][48]. Therefore, future RCTs should also comprise appropriate vitamin C dosing for the specific cohort under investigation.

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