Chapter 5

The Role of Vitamin C in the Protection and Modulation of Genotoxic Damage Induced by Metals Associated with Oxidative Stress

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Abstract

This chapter reviews the effects of vitamin C on metal-induced genotoxicity. By focusing on cutting-edge studies, including our own results in experiments with vanadium(V) and chromium(VI), the suggestion that vitamin C can be used effectively to protect against or reduce the genotoxic effects induced by metal exposure by suppressing oxidative stress is particularly explored. After explaining the chemical mechanisms involved in oxidative stress associated with heavy metals, this chapter discusses the various proposals regarding the physiological processes of vitamin C at the molecular level, its relationship with oxidative stress, levels of 8-hydroxydeoxyguanosine (8-OH-dG, 7,8-dihy-dro-8-oxodeoxyguanosine) and apoptosis, and its role in the protection and modulation of DNA damage, as well as how they fit with our own results that showed an increase in apoptosis and 8-OH-dG when vitamin C was administered in addition to the metallic compounds. The relevant gaps in our understanding of the role of vitamin C with regard to these issues are highlighted, as well as the key importance of its clinical use, and ultimately, human health.

Keywords: vitamin C, antigenotoxic, genotoxic damage, antioxidant, heavy metals, oxidative stress

1. Introduction

Several studies have suggested that diets rich in fresh fruits and vegetables are associated with a lower risk of cardiovascular diseases and cancer because of the high levels of antioxidants

such as vitamin C and polyphenols present in these foods [1]. The antioxidant effects of vitamin C have been observed both *in vitro* and *in vivo*. Ascorbic acid, which is a water-soluble bioactive form of vitamin C (**Figure 1**), can be found in all body fluids. At physiological pH, the 99% of vitamin C is present as $AscH^-$ (**Figure 1b**), indicating that its chemical form confers its main antioxidant effects. The remaining percentage is covered by 0.05% of AscH2 (**Figure 1a**) and 0.004% of $Asc2^-$ (**Figure 1c**). The antioxidant activity of vitamin C develops in two ways: (a) directly, by scavenging oxygen free-radicals, more generally known as reactive oxygen species (ROS) and (b) indirectly, by regenerating other antioxidant systems [2, 3].

A significant number of studies have focused on metal-induced toxicity and carcinogenicity by emphasizing their role in the generation of ROS. Metal-mediated formation of free radicals may cause modifications to DNA bases, lipid peroxidation, and changes in calcium and sulfhydryl homeostasis [4, 5]. However, these effects can be influenced by the action of low molecular weight antioxidants such as vitamin C, which is capable of chelating metal ions, reducing their catalytic activity, and resulting ROS formation. Since the genotoxicity of heavy metals associated with oxidative stress is based on the oxidative mechanism during reduction [5], vitamin C can be used effectively to protect or reduce the induced genotoxic effects by suppressing oxidative stress caused by these metallic compounds [6–8]. However, paradoxically under certain conditions (i.e., low concentration *in vitro* and the presence of metal ions), vitamin C can exert a pro-oxidant effect, increasing oxidative damage to lipids, DNA and protein, besides being a potential direct or indirect modulator of gene expression [9]. In fact, our understanding of the physiological processes of vitamin C at the molecular level and its relationship with oxidative stress, as well as its role in the protection and modulation of DNA damage is still incomplete. As a consequence, the evidence indicating the potential of vitamin C in counteracting oxidative stress, a key component in various pathological conditions including cardiovascular disease, neurological disorders, diabetes, and cancer [3, 10, 11], has not been translated, at least conclusively, in many randomized controlled trials.

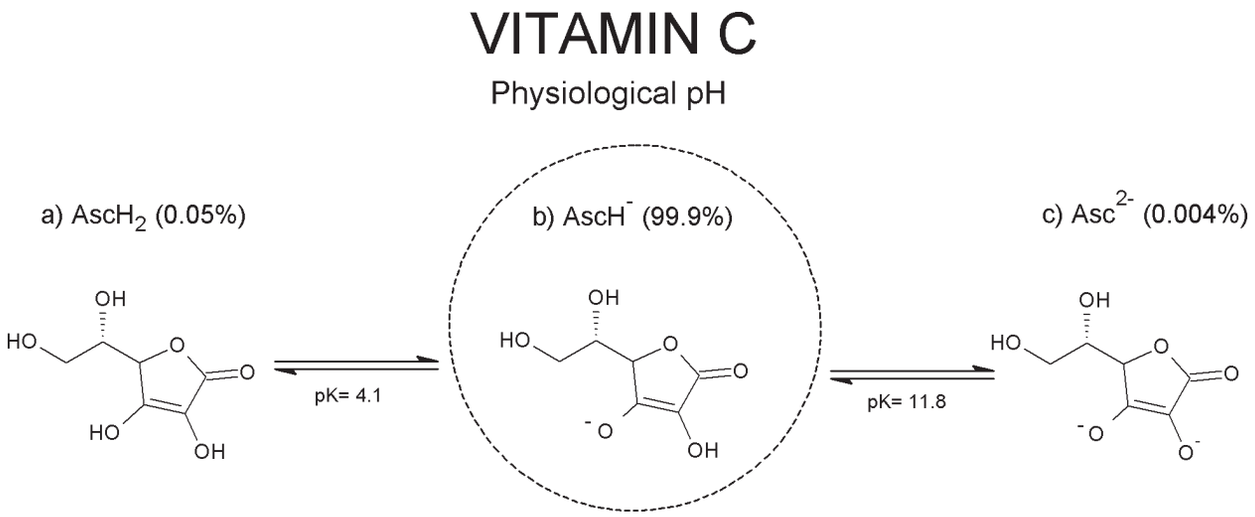

Figure 1. Bioactive forms of vitamin C at physiological pH. a) Ascorbic acid with two ionisable hydroxyl groups, AscH2; b) Ascorbate anion, $AscH^-$; and c) Ascorbate dianion, $Asc2^-$.

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2. Heavy metals and oxidative stress: the case of vanadium and chromium

It is well established that redox-active metals participate closely in the generation of different free radicals [6]. Exposure to transition metal ions$^{(n+)}$ such as chromium (Cr) and vanadium (V) hence represent a realistic *in vivo* production of ROS and free radicals due to intra-cellular reduction. The majority of the hydroxyl radicals (•OH) generated *in vivo* come from the metal-catalyzed breakdown of hydrogen peroxide (H_2O_2) through the Fenton and Haber-Weiss reactions [4, 12]:

$$\text{Transition metal ion}^{(n+)} + H_2O_2 \rightarrow \text{Transition metal ion}^{(n+1)} + {}^{\cdot}OH + OH^-$$

The •OH is the most reactive of all the ROS (half-life <1 ns) and interacts with all components of the DNA molecule. The initial stage of mutagenesis, carcinogenesis, and aging involves the permanent modification of genetic material. In fact, it has been well documented that in various cancer tissues, free radical-mediated DNA damage has occurred. ROS-induced DNA damage involves single- or double-stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, and DNA cross-links [5, 13, 14].

As mentioned above, the main genotoxic mechanism of V(V) and Cr(VI) compounds has been linked to reduction and generation of •OH [15, 16]. Reduction of V(V) to V(IV) takes place outside the cell (**Figure 2**). In plasma, V(V) is rapidly reduced to V(IV) by nicotinamide adenine

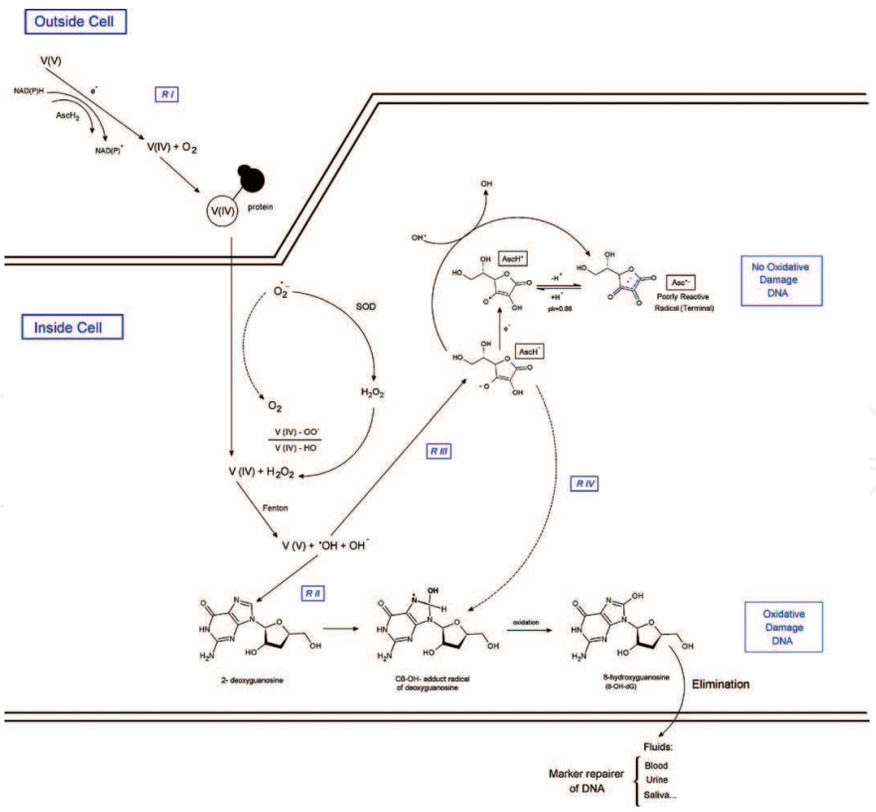

Figure 2. Routes of V(V) involved in the induction, protection and modulation of DNA oxidative damage (outside and inside cell).

dinucleotide phosphate (NADPH) and ascorbic acid. Once reduced, it is bonded with plasma proteins that carry it into the cell, where peroxovanadyl radicals [V(IV)–OO•] and vanadyl hydroperoxide [V(IV)–HO•] are formed. The generated superoxide is further converted into H_2O_2 by the dismutation reaction with superoxide dismutase (SOD). V(IV) can react through the Fenton reaction with H_2O_2 forming a •OH (**Figure 2**, *RI*) [5, 17, 18]. Nevertheless, Cr(VI) can actively enter the cells through channels for the transfer of isoelectric and isostructural anions, such as those for SO_4^{2-} and HPO_4^{2-} [19]. Once inside the cell, Cr(VI) quickly forms a complex with glutathione, reducing to Cr(V) (**Figure 3**, *RII*). Additionally, NAD(P)H can also reduce Cr(VI) to Cr(V), mediated by ascorbate (**Figure 3**, *RI*). Cr(V) can react through the Fenton reaction with H_2O_2 forming •OH [15].

The genetic damage by the production of C8-OH-adduct radical of deoxyguanosine is generated by the interaction between •OH and 2-deoxyguanosine. Therefore, there are two ways in which the protection and modulation of DNA oxidative damage could be caused. First, $AscH^-$ could react with •OH, quenching and converting it into a poorly reactive semi-hydroascorbate radical, which do not cause DNA damage (**Figures 2** and **3**, *RIII*). Second, $AscH^-$ can activate the repair mechanisms to eliminate C8-OH-adduct radical of deoxyguanosine. During catalysis of •OH in the reaction with 2-deoxyguanosine with molecular oxygen, C8-OH-adduct radical of deoxyguanosine is formed (**Figures 2** and **3**, *RII* and *RIV* respectively), which is a

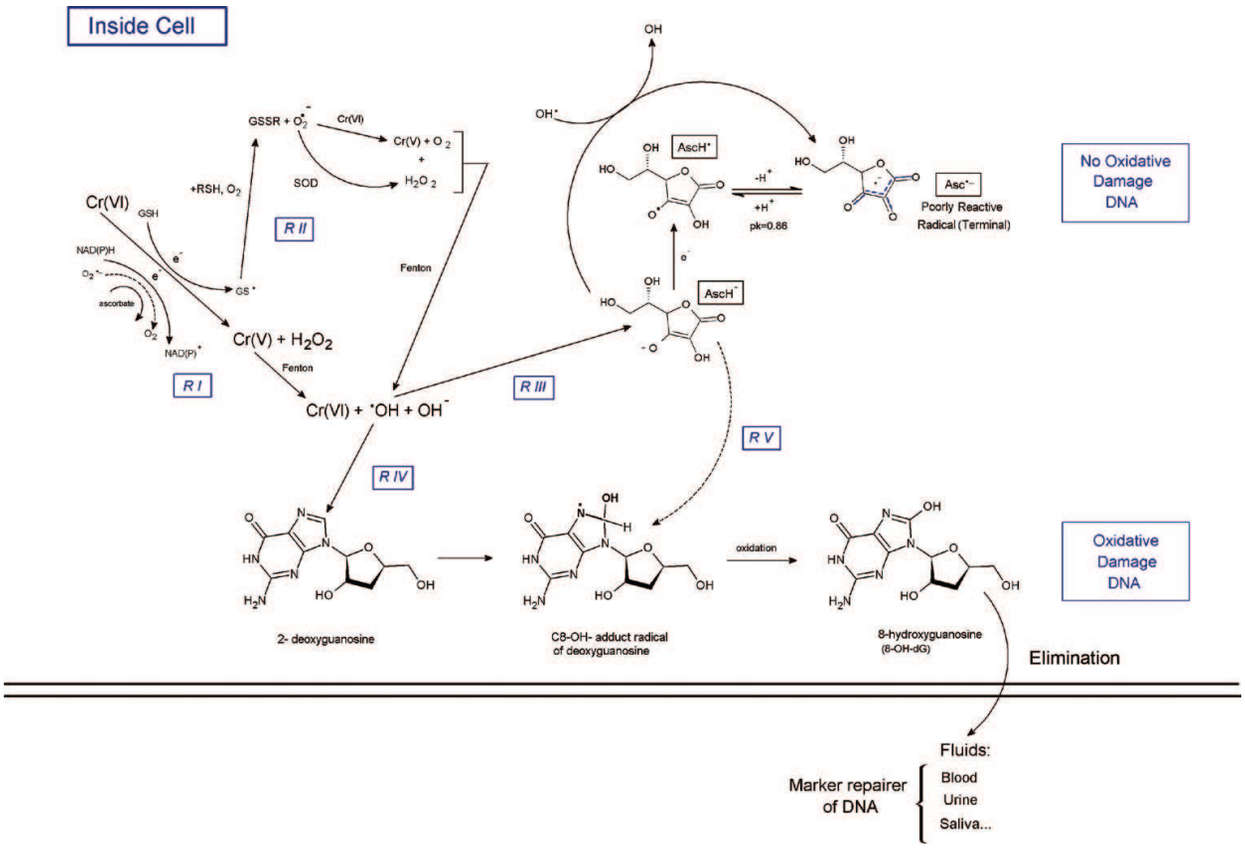

Figure 3. Routes of Cr(VI) involved in the induction, protection and modulation of DNA oxidative damage (all occurring inside the cell).

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form of oxidative DNA damage because it induces DNA strand breaks [20, 21]. Thus, $AscH^-$ could activate repair mechanisms and eliminate this radical through 8-hydroxydeoxyguanosine (8-OH-dG, 7,8-dihy-dro-8-oxodeoxyguanosine), which is a marker repairer of oxidative stress in biological systems that can be measured in fluids such as blood, urine, and saliva (**Figures 2** and **3**, *RIV* and *RV*, respectively).

Although the direct relationship between DNA damage and $^{\bullet}OH$ is not completely clear, Patlolla et al. [22] have suggested a role for ROS in Cr(VI)-induced genotoxicity and cytotoxicity. They showed that Cr(VI) induced genomic DNA damage through the formation of 8-OH-dG. Nevertheless, Rudolf and Cérvinka [23] observed that Cr(VI) induced time- and concentration-dependent cytotoxicity, resulting in oxidative stress, but through suppression of antioxidant systems and by activation of p53-dependent apoptosis. Other studies have questioned the genotoxic/mutagenic effect of $^{\bullet}OH$ in the context of Cr exposure, suggesting that reduction of Cr(VI) by physiological concentrations of vitamin C generates ascorbate-Cr(III)-DNA crosslinks and binary Cr(III)-DNA adducts. Therefore, Cr-DNA adducts are responsible for both the mutagenicity and genotoxicity of Cr(VI) [24].

3. Protective effects of vitamin C against genotoxic damage from vanadium(V) and chromium(VI)

For humans, vitamin C is an essential micronutrient that plays multiple biological roles. It must be obtained from the ingestion of particular foods, mainly fresh fruits and vegetables, since our body is incapable of synthesizing it. The consequences of the intake of very high doses of vitamin C (>2 g/day) remain a subject of intense debate. However, it has been observed that supplementation of vitamin C reduces the incidence of stomach, lung and colorectal cancer; likewise, low serum levels of vitamin C in high-risk populations may contribute to increased risk of gastric metaplasia or chronic gastritis, which are both precancerous lesions [5, 25]. Nevertheless, analyses of the effects of vitamin C are rather complicated because diet and vitamin supplementation determine the levels of vitamin C in plasma.

Cameron and Pauling highlighted the beneficial properties of vitamin C in the 1970s. They suggested that high doses of vitamin C (>10 g/day) cure and prevent cancer by promoting collagen synthesis [26]. However, researchers now suggest that vitamin C prevents cancer by neutralizing ROS before they can damage DNA and initiate tumor growth. Furthermore, it has been proposed that vitamin C may also act as a pro-oxidant, helping the body's own ROS destroy early-stage tumors [27, 28]. Currently, the recommended dietary allowance (RDA) in many countries ranges from 40–90 mg/day, although the results of various studies suggesting that the protective vitamin C concentrated in plasma for the minimum risk of free radical diseases corresponds to an intake of 124.2 mg/day (in the range of 92–181 mg) [10, 29].

Vitamin C possesses double bonds with an associated electron deficiency, making it highly reactive to free radicals from molecular oxygen. It donates two electrons from C-2 and C-3 double-bonded carbons, resulting in the formation of tricarbonyl ascorbate radical ($AscH^{\bullet}$), which is present in the nonprotonated form, a semidehydroascorbate radical ($Asc^{\bullet-}$). The resulting

ascorbate free radicals reduce to a neutral ascorbate molecule (**Figures 2** and **3**, *RIII*). Thus, the oxidation of ascorbate by many ROS is $Asc^{\bullet -}$, a poorly reactive radical that is considered terminal [30–32], and the level of $Asc^{\bullet -}$ radical function is an effective measurement of the degree of oxidative stress in biological systems [33].

In a previous study, we observed that the frequency of micronuclei in polychromatic erythrocytes (MN-PCE) increased with the administration of 40 mg/kg of V_2O_5 through ip [8], consistent with other studies testing soluble vanadium compounds (Na_3VO_4, SVO_5, and NH_4VO_3) [34–36]. However, the *in vivo* administration of vitamin C prior to the V_2O_5 injection decreased MN-PCE formation compared to administering V_2O_5 alone, reducing basal MN-PCE, and presenting the strongest protection against genotoxic damage induced by V_2O_5. This was probably because the vitamin C acted as a potent antioxidant (reducing agent) that scavenged free radicals of reactive oxygen and nitrogen species and prevented them from damaging nucleic acids [27, 37]. Interestingly, the MN-PCE decrease we observed with vitamin C was more effective than with the administration of beverages with high levels of antioxidants such as green tea [38], red wine [39] and their antioxidant components such as polyphenols [40–42].

Despite the important studies on the cytotoxic and anticarcinogenic effects of antioxidants in tumor model systems, it is clear that the molecular mechanisms underlying the benefits of antioxidants in cancer prevention are not yet well understood. Some ascorbyl forms of stearate inhibited cell proliferation by interfering with the cell cycle, reversing the phenotype and inducing apoptosis in human brain tumor glioblastoma (T98G) cells. Therefore, it has been proposed that the chemopreventive properties of antioxidants are related to their ability to target specific cellular signaling pathways that regulate cellular proliferation and apoptosis [43]. This proposal is consistent with our results since the frequencies of apoptotic cells (particularly, late apoptotic cells) indeed increased significantly with the administration of vitamin C, and their administration prior to treatment of V_2O_5 increased them even further [8]. Additionally, other studies have reported that the apoptosis-inducing activity of antioxidants might be synergistically enhanced by a combined treatment with chemopreventive [44] or genotoxic agents [40]. Therefore, it is plausible that enhanced induction of apoptosis following a combined treatment may positively contribute to the elimination of the cells with V_2O_5-induced DNA damage (MN-PCE).

On the other hand, some compounds including vanadium-oxide(V) have been proposed for clinical use as therapeutic drugs for cancer because the intracellular cascade mechanisms may be involved in causing apoptotic cell death. For many decades, vanadium was considered a low-toxicity essential trace element with anticarcinogenic properties [45]. However, important events have taken place since then. In 2006, the International Association for Research on Cancer (IARC) classified vanadium pentoxide (V_2O_5) as a Group 2B substance (possibly carcinogenic to humans) based on results in experimental animals [46]. In 2009, the American Council of Government and Industrial Hygienists (ACGIH) placed V_2O_5 in category A3 (confirmed animal carcinogen with unknown relevance to humans) [47].

The low levels of ROS promoting mRNA formation and encoding proteins known to be regulated by vanadium can induce the activation of transcription factors. In contrast, high levels

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of ROS are cytotoxic to the cells and trigger apoptotic mechanisms. Therefore, it has been proposed that the cytotoxic effects of vanadium compounds should be used to generate ROS and reactive nitrogen species to combat cancer cell lines [48, 49]. Of all the proposed mechanisms of V(V) toxicity, the induction of oxidative stress is of particular importance for biological systems [50, 51]. As explained above, antioxidants can deactivate highly reactive molecules such as ROS that are generated during various biochemical processes in the cells [3]. As a consequence, substances with antioxidant properties emerge as putative preventatives and co-adjuvants in the treatment of chronic degenerative diseases related to oxidative stress and DNA damage [41]. Additionally, the promising low costs of vanadium-based drugs make it particularly attractive, and the ability to overcome the adverse effects of vanadium compounds during therapeutic action is an urgent and crucial issue for its future use in medicine [49]. Our findings strongly suggest that vitamin C can be used effectively in therapy either alone (antioxidant) or in combination with other agents such as V_2O_5 to reduce their genotoxicity [8].

With regard to Cr(VI) compounds, they have been of particular interest and broadly studied because of their importance in different industrial applications including chrome plating, metallurgy, pigment manufacturing, leather tanning, and wood preservation and, most relevant to this chapter, because they are associated with the induction of cancer [52, 53]. Cr usually exists in various oxidation states, primarily Cr(III) and Cr(VI). The former is an essential micronutrient that plays a key role in protein, sugar, and fat metabolism. The latter is particularly effective in inducing genotoxicity by producing several types of DNA lesions and gene mutations. Some of the major factors that may play a significant role in determining cellular genotoxicity are Cr(VI)-induced DNA-DNA interstrand crosslinks, oxidative DNA damage, and mutations in the tumor suppressor gene p53 [19, 54]. It has been observed that Cr(VI) induces DNA damage through changes in the 8-OH-dG levels in DNA in rats. Furthermore, both endogenic (enzyme system) and exogenic (antioxidant consumption) antioxidant systems might counteract ROS and free radicals. In a recent study, we observed that administration of Cr(VI) increased MN-PCE (genotoxic damage), nonviable cells (cytotoxic damage), and glutathione (GSH) levels (a molecule that intervenes in its reduction to Cr(V), (**Figure 3**, *RII*) and decreased the total levels of antioxidants. Treatments with vitamin C prior to administration of CrO_3 decreased MN frequencies (protection or modulation of genotoxic damage) and nonviable cells (decreased cytotoxic damage). A decrease in the levels of 8-OHdG in CrO_3 group was observed, which could be related to the inhibition of repair mechanisms. However, when the organism was treated with vitamin C, a significant increase in the levels of 8-OHdG was observed, suggesting that it increases DNA repair. Our findings showed a protective effect of vitamin C on genotoxic damage induced by Cr(VI), possibly related to its ROS-suppression properties before the oxidative stress generated by the reduction of Cr(VI) to Cr(III) [55–57]. **Figure 4** summarizes the proposal of the interaction between vitamin C and heavy metals, that is, (1) the free radicals generated by heavy metals can be scavenged by vitamin C inhibiting their genotoxic effects; (2) the repair mechanisms inactivated by heavy metals can be reactivated by vitamin C; and (3) heavy metals induce apoptosis by damaging DNA and vitamin C contributes to this process.

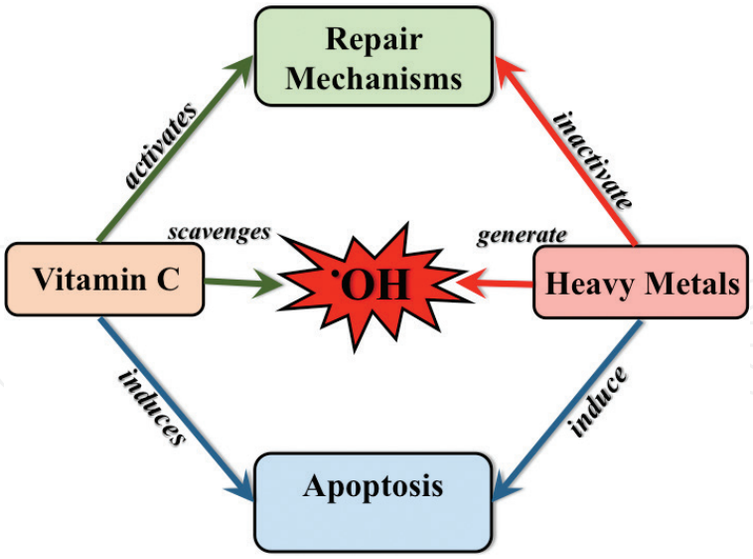

Figure 4. Summary of the interactions between vitamin C and heavy metals.

4. Conclusions

Vitamin C is a potent antioxidant found mainly in fresh fruits and vegetables. It can be readily absorbed and concentrated in tissues and biofluids at a physiologically relevant level, presenting effects in both the aqueous and membrane domains. Furthermore, it plays an essential role in the organism since it scavenges free radicals, chelates redox metals, and regenerates other antioxidants within the "antioxidant network." All these characteristics make the study of the effects of the *in vivo* administration of vitamin C on the genotoxic effects induced by agents associated with oxidative stress particularly important. In this sense, heavy metals such as V(V) and Cr(VI) are of particular relevance since they generate a realistic *in vivo* production of ROS and free radicals due to intracellular reduction. ROS-induced DNA damage involves single- or double-stranded DNA breaks, purine, pyrimidine or deoxyribose modifications, and DNA cross-links. Several studies, including our own results, have solidly concluded that vitamin C does play a significant role in the protection against the genotoxicity caused by metal compounds such as V(V) and Cr(VI). Although the main described mechanism of antioxidants is the scavenging of free radicals, our studies suggest that DNA repair and apoptosis are possible pathways involved in the protection and modulation of DNA. However, it has to be taken into account that under certain conditions (i.e., low concentration *in vitro* of vitamin C and the presence of metal ions), vitamin C can exert a pro-oxidant effect, increasing oxidative damage to DNA. More studies are necessary to fully understand the mechanisms involved in the modulation of and protection against metal-induced genotoxic damage and the adequate doses of vitamin C to stimulate these properties. In addition, it is possible that the impact of vitamin C on DNA damage depends also on both background values of vitamin C within the organism and the level of exposure to xenobiotics or oxidative stress.

Conflict of interests

The authors declare that they do not have any competing interests.

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Abbreviations

$AscH_2$, $AscH^-$, Asc^{2-}	Forms of ascorbic acid (vitamin C)
$AscH^{\bullet}$	Tricarbonyl ascorbate radical
$Asc^{\bullet-}$	Semidehydroascorbate radical
Cr	Chromium
Cr(VI)	Chromium hexavalent
CrO_3	Chromium trioxide
GSH	Glutathione
H_2O_2	Hydrogen peroxide
ip	Intraperitoneal
MN	Micronucleus
MN-PCE	Micronucleated polychromatic erythrocytes
NADPH	Nicotinamide adenine dinucleotide phosphate
$NAD(P)^+$	Oxidated form of nicotinamide adenine dinucleotide phosphate
Na_3VO_4	Sodium orthovanadate
NH_4VO_3	Ammonium metavanadate
$^{\bullet}OH$	Hydroxyl radical
PCE	Polychromatic erythrocytes
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SVO_5	Vanadyl sulfate
V	Vanadium
$V(IV)–OO^{\bullet}$	Peroxovanadyl radicals
$V(IV)–HO^{\bullet}$	Vanadyl hydroperoxide
V(V)	Vanadium pentavalent
V_2O_5	Vanadium pentoxide
8-OH-dG	8-hydroxydeoxyguanosine

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