

# Catalytic Therapy of Cancer by Ascorbic Acid Involves Redox Cycling of Exogenous/Endogenous Copper Ions and Generation of Reactive Oxygen Species

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## Key Words

Catalytic therapy · Cancer · Prooxidants · Ascorbate · Polyphenol antioxidants · Copper · Oxidative DNA breakage · Apoptosis · Comet assay

## Abstract

Catalytic therapy is a cancer treatment modality based on the generation of reactive oxygen species (ROS) through administration of ascorbate/medicinal herbal extracts and copper. It is known that antioxidants such as ascorbate also exhibit prooxidant activity in the presence of transition metals such as copper. Based on our work and that in the literature, in this review we propose a mechanism for the cytotoxic action of ascorbate against cancer cells. It involves redox cycling of exogenous/endogenous copper ions and the consequent generation of ROS leading to oxidative DNA breakage. Using human peripheral lymphocytes and the Comet assay, we have shown that ascorbic acid is able to cause oxidative breakage in cellular DNA. Such DNA degradation is inhibited by neocuproine (a Cu(I) sequestering agent) and scavengers of ROS indicating that the cellular DNA breakage involves the generation of Cu(I) and formation of ROS. Similar results are also obtained with plant polyphenol antioxidants that are important constituents of medicinal herbal extracts. Copper is an essential component of chromatin and can take part in

redox reactions. It is well established that tissue, cellular and serum copper levels are considerably elevated in various malignancies. Therefore, cancer cells may be more subject to electron transfer between copper ions and ascorbate/plant polyphenols to generate ROS. In this review we cite evidence to indicate that in catalytic therapy cytotoxic action against cancer cells involves redox cycling of exogenous/endogenous copper ions.

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## Introduction

There are currently a number of treatment strategies being used to increase the survival rates of cancer patients including chemotherapy, radiotherapy, photodynamic therapy and recently catalytic therapy (CT). CT is a cancer treatment modality based on the generation of reactive oxygen species (ROS) such as the hydroxyl radical using a redox active mixture of ascorbate (vitamin C)/medicinal herbal extracts and copper [1, 2]. Although ascorbic acid has an antioxidant function in living sys-

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tems, it also acts as a prooxidant generating ROS in the presence of transition metal ions such as copper. This is a property similar to phytochemicals such as polyphenols which are constituent of many herbal extracts [3, 4]. Along with ascorbic acid, phytochemicals have been identified as potent anticancer and tumor-suppressing agents [5, 6]. The idea behind CT involves a prooxidant action of these compounds in the presence of transition metal ions such as copper [7, 8]. Since tumor cells have an altered antioxidant system they may be more vulnerable to oxidative stress as they function with a heightened level of ROS due to an increased rate of growth and metabolism [9]. Studies with ROS-producing systems such as hypoxanthine/xanthine oxidase have shown that their administration leads to cytotoxic effects which are limited to cancer cells with low or no toxicity to normal cells [10]. The first attempt using an approach similar to CT was reported in 1983 by Kimoto et al. [11] where they showed enhancement of antitumor activity of ascorbate by a copper:glycylglycyl complex. Ascorbate is considered the most suitable substrate for CT as many tumors accumulate ascorbate to a greater extent than do normal cells, possibly due to the high rates of metabolism [12].

In this review based on our work and those of others, we propose a mechanism for the cytotoxic properties of ascorbate which forms the basis of CT for cancer. We show that ascorbate in the presence of copper leads to a prooxidant action and consequent cell killing. When these agents are used alone they are able to mobilize endogenous (possibly chromatin bound) copper ions, which are known to be significantly elevated in cancer cells [13]. Such enhanced copper levels in cancer cells define their selective killing at relatively low concentrations of ascorbate [14].

#### **Evidence for the Copper-Dependent Prooxidant Action of Ascorbic Acid as an Anticancer Mechanism**

We give below several lines of indirect evidence in the literature, which strongly suggest that the prooxidant action of ascorbic acid rather than its antioxidant activity may be an important mechanism for its anticancer and apoptosis-inducing properties.

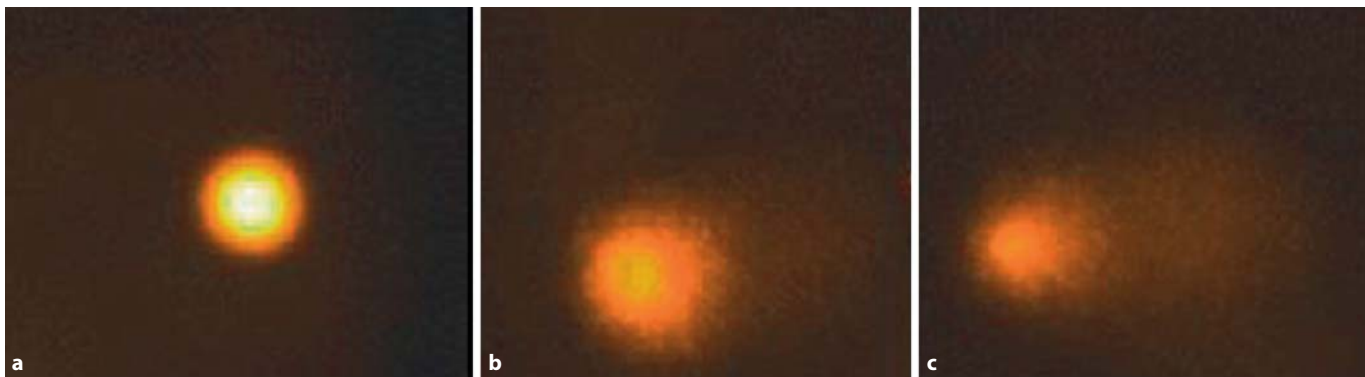
The apoptosis-inducing activity of ascorbic acid has been ascribed to its prooxidant action and is inhibited by catalase, antioxidants like N-acetylcysteine and GSH,  $\text{Ca}^{2+}$  and  $\text{Fe}^{3+}$  depletion but stimulated by  $\text{H}_2\text{O}_2$  and  $\text{Cu}^{2+}$  [15]. Certain properties of naturally occurring antioxidants including ascorbic acid, such as cleavage of DNA and the generation of ROS in the presence of transition

metal ions [16] are similar to those of several known anticancer drugs [17].  $\text{Fe}^{3+}$  and  $\text{Cu}^{2+}$  are the most redox-active of the metal ions in living cells. However, the major metal ions in the nucleus are copper and zinc [18]. Burkitt et al. [19] suggested that the internucleosomal DNA fragmentation might be caused not only by endonuclease but also by metal-chelating agents such as 1,10-phenanthroline (OP), which promotes the redox activity of endogenous copper ions and the resulting production of hydroxyl radicals. Thus, the internucleosomal DNA 'laddering' often used as an indicator of apoptosis may also reflect DNA fragmentation by non-enzymatic processes.

The complex formed between 1,10-phenanthroline and  $\text{Cu}^{2+}$  has a redox potential ( $E^\circ$  for  $\text{Cu}^{2+}/\text{Cu}^+$ ) of 0.17 V that favors redox cycling, whereas that for  $\text{Fe}^{3+}/\text{Fe}^{2+}$  is 1.1 V, presumably because of stabilization in the ferrous state. Various studies have shown that while zinc, iron and selenium concentrations were significantly lower in cancer patients, the copper concentrations were almost always found to be either elevated or significantly elevated (up to 2- to 3-fold) compared to age-matched samples from normal tissue [13]. It therefore appears that the copper-dependent redox status is an important element in the prooxidant activity of these compounds in selectively targeting cancer cells.

#### **Oxidative DNA Cleavage by Ascorbate in vitro and in Human Lymphocytes**

Studies in our laboratory have shown that a number of antioxidants including ascorbate cause oxidative strand breakage in DNA either alone or in the presence of transition metal ions such as copper [3, 6, 20]. In an earlier study site-specific DNA cleavage by ascorbic acid in the presence of Cu(II) was described [21]. Using a cellular system of lymphocytes, we have shown that ascorbate (200  $\mu\text{M}$ ) in the presence of exogenously added 20  $\mu\text{M}$  cupric chloride (Cu(II)) gives a degree of DNA breakage which was considerably greater than that caused by ascorbate alone (fig. 1) [6]. Copper ions are known to interact with both DNA phosphates and the bases through coordination binding [22]. These findings demonstrate that the ascorbate-Cu(II) system for DNA breakage is physiologically feasible and could be of biological significance. We have further shown that ascorbate is able to cause DNA breakage in isolated lymphocytes in the absence of exogenously added copper. Such cellular DNA breakage is the result of mobilizing endogenous chromatin-bound copper ions [6, 23].



**Fig. 1.** Single cell gel electrophoresis of human peripheral lymphocytes showing comets (100×) after treatment with: untreated (**a**), ascorbic acid (200 μM; **b**) and ascorbic acid (200 μM) + Cu(II) (20 μM; **c**).

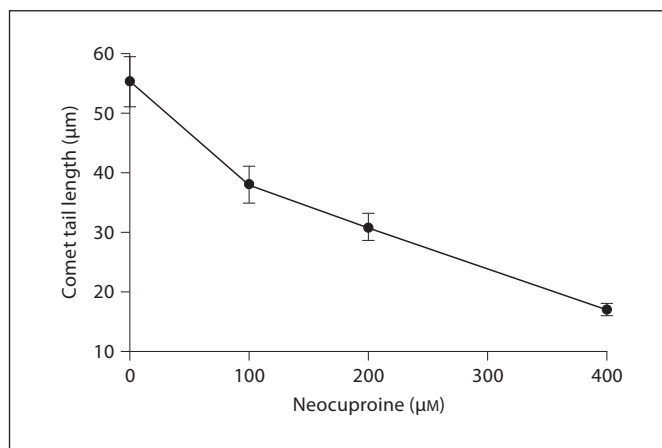
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### Ascorbate-Induced Cellular DNA Breakage Involves Redox Cycling of Endogenous Copper and Generation of ROS

In order to examine the role of endogenous copper ions in ascorbate-induced DNA breakage, we used a membrane permeable Cu(I)-specific chelator, neocuproine [24]. Figure 2 gives the results of an experiment where three progressively increasing concentrations of neocuproine (100–400 μM) were tested on ascorbic acid- (100 μM) induced DNA breakage in lymphocytes [6]. A progressive decrease in the tail length as a function of increasing neocuproine concentration is seen. From the results we may conclude that the DNA breakage by ascorbic acid involves endogenous copper ions and that Cu(I) is an intermediate in the pathway that leads to DNA breakage. In table 1 the effects are shown of three scavengers of ROS, namely superoxide dismutase, catalase and thiourea, on ascorbate-induced lymphocyte DNA breakage. All three cause significant inhibition of DNA breakage [6]. These results indicate that ascorbic acid-mediated DNA breakage in lymphocytes involves copper ions and the generation of ROS.

### Conclusion

Earlier studies carried out by Cameron and Pauling [25] reported clinical benefits and improved survival using both oral and intravenously administered ascorbic acid in the treatment of terminal cancer. In one of these studies, Kimoto et al. [11] also used a copper complex and demonstrated significant enhancement in the antitumor



**Fig. 2.** Effect of increasing concentrations of neocuproine on ascorbic acid- (100 μM) induced DNA breakage in human lymphocytes. Values reported are mean ± SEM of 3 independent experiments.

**Table 1.** Effect of scavengers of active oxygen species on ascorbic acid-induced lymphocyte DNA breakage

Dose	Tail length, μm	% Inhibition
Untreated	2.90 ± 0.08	–
Ascorbic acid (100 μM)	54.20 ± 3.1	–
+ SOD (100 μg/ml)	22.12 ± 2.3*	59
+ Catalase (100 μg/ml)	26.20 ± 2.1*	51
+ Thiourea (1 mM)	19.50 ± 2.7*	64

All values represent the mean ± SEM of 3 independent experiments. SOD = Superoxide dismutase.

\* p < 0.05 when compared to control.

activity of ascorbate. These findings were contradicted by Creagen et al. [26] and Moertal et al. [27] in 2 randomized placebo-controlled clinical trials at the Mayo Clinic where a high-dose oral administration of vitamin C was shown to have no benefits. These trials were considered definitive possibly because the difference in the in vivo levels of ascorbic acid achieved between the oral and intravenous administration was not adequately appreciated. It has been shown that ascorbic acid is toxic to a variety of cancer cell lines [28]. Recently, Chen et al. [29] demonstrated that pharmacologic ascorbic acid concentrations achievable through intravenous administration were cytotoxic to many types of cancer cells in vitro and significantly impeded tumor progression in vivo without toxicity to normal tissues. Plasma levels of ascorbic acid are tightly controlled and are around 50  $\mu\text{M}$  [30]. However, Padayatty et al. [31] have shown that intravenous administration of ascorbic acid bypasses such tight control and results in concentrations as much as 70-fold higher than those achieved by maximum oral consumption.

The concentration of copper in various tissues ranges from 10 to >100  $\mu\text{M}$  with 20% found in the nucleus [32]. Further, serum [33], tissue [34] and cellular [13] concentrations of copper are greatly increased in various malignancies. Copper ions from chromatin can be mobilized by metal-chelating agents giving rise to internucleosomal DNA fragmentation, a hallmark property of cells undergoing apoptosis [19]. Further, it has been proposed that most clinically used anticancer drugs can activate the late events of apoptosis (DNA degradation and morphological changes) and the essential signaling pathways differ

between pharmacological cell death and physiological induction of cell death [35]. Essentially this would be an alternative, non-enzymatic and copper-dependent pathway for the cytotoxic action of certain agents that are capable of mobilizing and reducing endogenous copper ions. As such, this would be independent of Fas and mitochondria-mediated programmed cell death. Such a mechanism may also lead to internucleosomal DNA breakage (as evidenced by DNA laddering on gels) as internucleosomal spacer DNA would be relatively more susceptible to cleavage by the ROS. The generation of hydroxyl radicals in the proximity of DNA is well established as a cause of strand scission. Among the oxygen radicals, the hydroxyl radical is the most electrophilic with high reactivity and therefore possesses a small diffusion radius. Thus in order to cleave DNA, it must be produced in the vicinity of DNA [36]. The location of redox-active metal is of utmost importance for the ultimate effect, because the hydroxyl radical, due to its extreme reactivity, interacts exclusively in the vicinity of the bound metal [38]. It may be presumed that the concentration of metals such as copper in cells is a decisive factor in driving an antioxidant such as ascorbate towards a prooxidant action. Further, the concentration of antioxidant required for such a cytotoxic action should be lower as the concentration of copper increases. Indeed, it has been shown that ascorbic acid is cytotoxic to leukemic cells (3- to 4-fold higher copper levels [22]) at a much lower concentration than normal lymphocytes [14]. This would also account for the observation that ascorbic acid selectively targets the cancer cells while normal cells are spared from such cytotoxic effects [29, 38].

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