REVIEW ARTICLE

Ascorbic acid in the prevention and treatment of cancer

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SUMMARY

This review is aimed at the systematic mapping of ascorbic acid in the prevention and/or treatment of cancer in clinical and non-clinical studies from 2011 to 2015, in order to understand dose-response variations as well as its mechanisms of action as an antioxidant and antitumor agent. Seventy-eight articles were retrieved from the PubMed/Bireme database, of which only 30 included ascorbic acid in the prevention and/or treatment of cancer. However, there are controversies regarding doses and a lack of clinical studies featuring its mechanism of action more clearly. Other studies are needed to understand dose-response variations, as well as its targeting mechanisms of action, both as an antioxidant and antitumor agent, to assist treatment and prevention of cancer, aiming at better quality of life for both patients and the general population.

Keywords: neoplasms, cancer, prevention, ascorbic acid, antioxidants.

INTRODUCTION

The word "cancer" derives from the Greek *Karkinos*, which means crab, a reference to the blood vessels infiltrated in the tumor as if they were the claws of this animal. Currently, cancer is characterized as a complex disease that involves the alteration of gene expression, sustains cell survival and proliferation, and can be modified by genomic and epigenomic factors.[1] Genomic factors are characterized by changes in the sites of the genes, promoting mutations, while epigenomic factors correspond to changes that do not alter the sequence of DNA bases but their conformation through changes in histone, methylations in DNA bases and nucleosome remodeling.[2,3]

Cancer cells operate under a high level of oxidative stress, due to high baseline levels of reactive oxygen species, oncogenic transformation, and metabolic reprogramming.[4] Oxidative stress occurs due to imbalance between the production of free radicals [superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), nitric oxide (NO), and more] and their elimination by antioxidant defense mechanisms [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), among others], which induces cell damage caused by lipid peroxidation generating derangement and loss of function and integrity of the cell membrane, as well as DNA dam-age, promoting genomic instability and cell proliferation, thereby increasing the somatic mutations and neoplastic transformation.[5,6]

According to the Brazilian National Cancer Institute (INCA, in the Portuguese acronym), in 2012 there were 14.1 million cases of cancer in the world, with a total of 8.2 million deaths from the disease. It is estimated that in 2030 the global burden of cancer will be 21.4 million new cases and 13.2 million deaths, mainly due to the growth and aging of the population.[7] Among the types of cancer, breast cancer is the second most common, with a worldwide estimate in 2012 of about 1.67 million new cases. In Brazil, the estimate is about 25% of all cancer types diagnosed in women. Mortality amounts to 70% and the 5-year survival ranges from 80 to 40% depending on the country's economic development. In Brazil, the number of new cases of breast cancer was estimated at 49,000 in 2010.[8]

The etiology of breast cancer is not fully understood; it is multifactorial and includes genetic, reproductive, and environmental factors. The World Health Organization (WHO) states that consumption of fruits and vegetables can help prevent cancer, due to its composition with nutrients such as vitamins, minerals and fiber.[9] According to the INCA (2011), antioxidant foods, rich in ascorbic acid (vitamin C), carotenoids (vitamin A) and tocopherol

(vitamin E), selenium, and flavonoids, are recommended due to their antagonistic action, following the requirements presented in the Dietary Reference Intake (DRI), as they can help in the prevention of cancer, inhibiting oxidation and free radical production, and also favoring oxidative stress and even promoting carcinogenesis.[10]

Ascorbic acid (vitamin C) has been widely used in the treatment and prevention of cancer; nevertheless, the clinical results are still inconclusive. At low concentrations, it has an antioxidant role, preventing oxidation, which induces apoptosis. However, its high content can increase the production of ATP (generated by mitochondria) inducing apoptosis in tumor cell lines, via a pro-oxidant mechanism.[11] Studies show dose-dependent antineoplastic activity with influence on apoptosis, cell cycle, and cell signaling, increasing the cytotoxicity of the antineoplastic agent in cell lines of breast cancer treated with mitoxantrone and ascorbic acid.[12]

However, there are still many controversies regarding the role of vitamin C in the prevention and treatment of cancer. This review is aimed at the systematic mapping of ascorbic acid in the prevention and/or treatment of cancer in clinical and non-clinical studies from 2011 to 2015, in order to understand dose-response variations as well as its mechanisms of action as an antioxidant and antitumor agent.

METHOD

The survey was conducted based on a literature review on Pubmed/Bireme databases, of scientific articles derived from clinical and non-clinical studies carried out between 2011 and 2015, using the keywords "cancer" and "ascorbic acid". Seventy-six articles were retrieved, of which 30 were used in the study as they met the inclusion criteria: clinical and non-clinical studies using ascorbic acid in the prevention and/or treatment of cancer between 2011 and 2015; and not the exclusion criteria: studies that do not use ascorbic acid in the treatment and/or prevention of cancer and who are outside the established time range (Figure 1). Of the articles selected for analysis, 12 used ascorbic acid as antioxidant: two clinical and ten non-clinical studies; 18 used it as pro-oxidant, of which only one was clinical, while the remaining (17) were non-clinical studies.

RESULTS AND DISCUSSION

Ascorbic acid as antioxidant

Ascorbic acid is an essential micronutrient for human health, having antioxidant activity and participating in the production of proteins such as collagen, norepinephrine and serotonin. It is acquired through the ingestion of various plants, especially citrus fruits such as lemon and orange, and vegetables including tomatoes and broccoli, with recommended daily doses of 90 mg for men and 75 mg for women.[13]

Table 1 shows the clinical and non-clinical studies on the use of ascorbic acid as an antioxidant in the treatment and/or prevention of cancer, describing its use in several types of cancer or cell lines at different doses/concentrations and their mechanisms of action. The clinical studies (2) involved different types of cancer (pancreas, breast, kidney, lung, liver, bladder, lymphoma, prostate, colon, brain, leukemia, stomach, ovary, skin, and uterus) and doses of ascorbic acid (0.04-0.28 mM; 1-10 mM); the non-clinical studies (10) used different tumor cell lineages (MCF-7 cells in breast cancer; renal carcinoma; B16FO
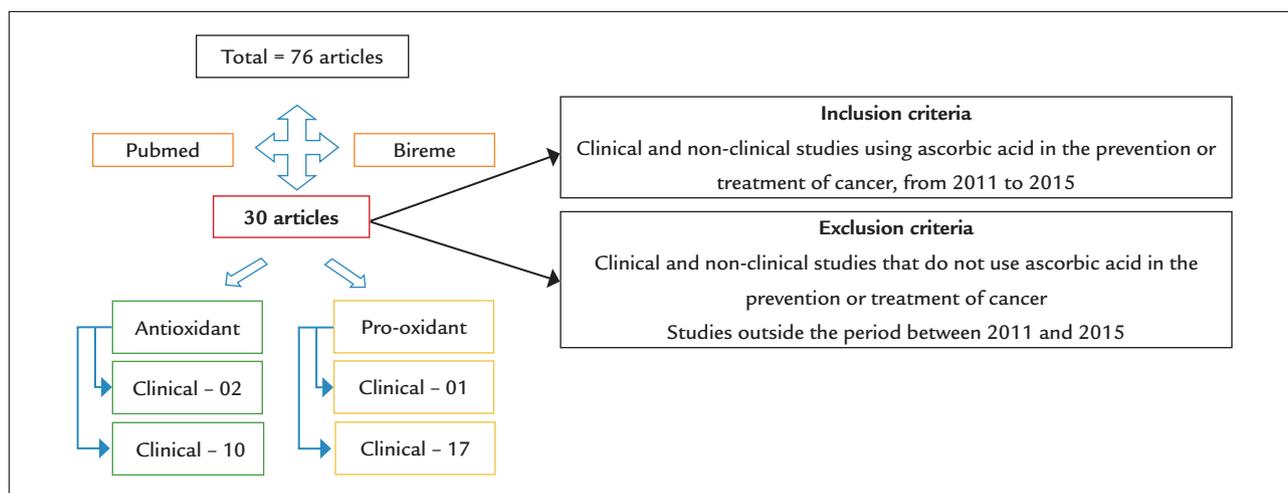

FIGURE 1 Literature search design with inclusion and exclusion criteria.

TABLE 1 Clinical and non-clinical studies using ascorbic acid as antioxidant in the treatment and/or prevention of cancer.

Study type	Type of cancer/cell	Mechanism of action	Dose/concentration	References
Clinical	Pancreas, breast, kidney, lung, liver, bladder, lymphoma, prostate, colon, and stomach	Decrease in levels of C-reactive protein and proinflammatory cytokines	0.04-0.28 mM	(25)
Non-clinical	Breast cancer in mice	Hinders metastases, tumor growth and secretion of inflammatory cytokines, and enhances tumoral encapsulation	0.85 mM	(26)
Non-clinical	MCF-7 cells in breast cancer	Attenuation of cytotoxicity, decrease in apoptosis, protection of neoplastic cells against lipid peroxidation	0.05 and 0.5 mM	(27)
Non-clinical	MCF-7 cells in breast cancer	Decrease in cytotoxicity	0.1 mM	(28)
Clinical	Pancreas, breast, kidney, lung, liver, bladder, lymphoma, prostate, colon, brain, leukemia, stomach, ovary, skin, and uterus	Decreased inflammation	1-10 mM	(29)
Non-clinical	Cells from renal carcinoma	Anti-apoptotic activity by recruiting Bcl-2	0.11 and 2.28 mM	(30)
Non-clinical	Cells from melanoma (B16FO)	Hinders metastases and tumor growth	4 mM	(31)
Non-clinical	Cells from cervical cancer (HeLa)	Modulation of markers for cancer proliferation (Ki67), invasion and metastasis (MMP-2 and -9), angiogenesis (VEGF), apoptosis (TUNEL and Bcl-2), and inflammation (COX-2, iNOS and GSTπ)	4 mM	(32)
Non-clinical	Lung cancer in ferrets	Protection against lung injury induced by exposure to cigarette smoke, by inhibiting the expression of cyclin D1	1.2 mM	(33)
Non-clinical	Cells from neuroblastoma	Anti-apoptotic activity	0.1-0.4 mM	(34)
Non-clinical	Cells from acute lymphoblastic leukemia	Decreased production of reactive oxygen species caused by 4-(hydroxyphenyl) retinamide (4-HPR)	100 µM	(35)
Non-clinical	Cells from osteosarcoma (143-B)	Limitation of the invasive potential	0.28 mM	(36)

Source: Literature search.

melanoma cells; HeLa cervical cancer cells; lung; neuroblastoma; cells from acute lymphoblastic leukemia; 143-B osteosarcoma cells), at varied doses/concentrations (0.85 mM; 0.05 and 0.5 mM; 0.1 mM; 0.11 and 0.28 mM; 4 mM; 1.2 mM; 0.1-0.4 mM; 0.28 mM).

Plants and most animals synthesize ascorbic acid using glucose. Humans, however, do not synthesize this compound as the L-gulonolactone oxidase gene does not function, and thus this vitamin is obtained through the diet as ascorbate and dehydroascorbic acid (DHA). The normal concentration of ascorbic acid in human plasma is about 40 to 80 µM, and it is at this concentration range that endogenous vitamin C acts as an antioxidant. Physiological concentrations of ascorbate demonstrated inhibition of LDL oxidation and a synergistic action with vitamin E preventing lipid oxidation of cell membranes.[14] Studies describe that intravenous ascorbic acid is more effective for raising serum levels of ascorbate than the form administered orally.[15]

Clinical studies showed that reductions in the levels of C-reactive protein and proinflammatory cytokines, resulting in decreased inflammation, are the main mechanisms antioxidant. Non-clinical studies, in turn, revealed attenuation of cytotoxicity, reduced apoptosis, protection of neoplastic cells against lipid peroxidation, modulation of markers of cancer proliferation (Ki67), invasion and metastasis (MMP-2 and -9), angiogenesis (VEGF), apoptosis (TUNEL and Bcl-2) and inflammation (COX-2, iNOS and GSTπ), decreased production of reactive oxygen species generated by 4-(hydroxyphenyl)retinamide (4-HPR), limitation of the invasive potential, also hindering metastases, tumoral growth and secretion of inflammatory cytokines, and enhancing tumor encapsulation, anti-apoptotic activity through Bcl-2 recruitment and protection against lung

injury induced by exposure to tobacco smoke, by inhibiting the expression of cyclin D1 (Table 1).

Vitamin C is an excellent reducing agent, which undergoes two successive oxidations to form the ascorbate radical (Asc·⁻). Ascorbate is relatively unreactive due to the stability of the unpaired electron and oxidizes ascorbic acid to DHA; this reducing agent function is what maintains the structure of enzymes, thus allowing the biochemical machinery of cells and tissues functioning normally.[14]

Low electron potential and resonance stability is what makes it an antioxidant. The authors also reported that vitamin C plays the role of collecting reactive oxygen species, acting as an antioxidant for maintaining the intracellular redox balance and minimizing the oxidative damage caused by these free radicals.[16] Corroborating these studies, other researchers cite that nutrients like vitamin A, C, and E can neutralize reactive oxygen species, derived from the imbalance between antioxidant defenses and oxidative stress caused by diseases such as cancer or its treatment. Therefore, antioxidants, such as ascorbic acid, may assist in the prevention of cancer or its treatment, reducing side effects related to chemotherapy.[17]

Ascorbic acid as an antitumor agent

Vitamin supplementation can improve the benefits and quality of life of cancer patients. However, the literature shows controversy over the treatment of cancer including ascorbic acid. Researchers report that *in vitro* studies of neuroblastoma, bladder cancer, pancreatic cancer, and other tumor types showed cytotoxic effect of ascorbic acid, while *in vivo* studies supported this anti-cancer potential of the vitamin C.[18]

Table 2 shows the clinical and non-clinical studies on the use of ascorbic acid as an antitumor agent in the treatment and/or prevention of cancer, describing its use in several types of cancer or cell lines at different doses/concentrations and mechanisms of action. Of these, only one clinical study showed patients with metastatic pancreatic cancer treated with ascorbic acid at 0.28; 0.34 and 0.56 mM doses.

Regarding the non-clinical studies (17), they used different cancer cell lineages [IOSE-385, OVCAR-3, SKOV-3 and OVCA-432 for ovary cancer; esophageal squamous cells and CP-A, CP-B, CP-C and CP-D for esophageal cancer; 23132/87 for gastric carcinoma, HT-29 colon cells, SKOV-3 ovary cells, BXPC-3 pancreatic cells, BT-20, MDA-MB-468, MDA-MB-231 and MCF-7 breast cells, U-13898, U-87 and U-251 glioblastoma cells, umbilical vein endothelial cells (HUVECs) and NHDF cells; HeLa for cervical cancer; colon carcinoma; and HEp-2 cells for laryngeal carcinoma; SK-N-MC neuroblastoma cells; PANC-1, AsPC-1, BxPC-3 and MIA PaCa-2 for pancreatic cancer; Epstein-Barr virus (EBV)-positive Burkitt's lymphoma and lymphoblastoid cells; PC3 prostate cancer cells; malignant pleural mesothelioma; NSCLC epithelial lung cancer; solid Ehrlich carcinoma; RKO and SW480 for colon cancer], in various concentrations of ascorbic acid (0.25 mM; 0.1-2 mM; 0.3 mM; 0.5 mM; 0.005-0.1 mM; 4 mM; 28.39 mM; 3-10 mM; 1-6 mM; 4.26 mM; 22.71 mM; 0.4 mM; 11.36 mM; 0.5-5 mM; 0.68 mM; 1-3 mM).

At low millimolar concentrations, ascorbic acid is able to "kill" some cell lines *in vitro*, while *in vivo* it generates superoxide radicals, hydrogen peroxide, and extracellular ascorbyl responsible for its cytotoxic activity; however, concentrations as high as 20 mM did not pose any risk to the lineage of non-malignant cells.[19] Other studies confirm that high doses of ascorbic acid are effective in cell death as seen in *in vitro* studies as well as *in vivo* tumor growth inhibition.[20] Corroborating this study, researchers describe that vitamin C can be toxic in a selective manner in some types of tumor cells as a pro-oxidant, since concentrations above physiological (0.1 mM), between 1 mM and 10 mM, are toxic for neoplastic cells *in vitro*, for example, for melanoma and neuroblastoma cells, where concentrations from 10 nM to 1 mM can induce apoptosis.[21]

Regarding antitumor mechanism of action, the clinical study reported decreased tumor size. The non-clinical studies reported inhibited cell progression by increasing the levels of H_2O_2; anti-proliferative effect of tumor cells, through interference with cell cycle (G_0/G_1) and generation of H_2O_2; cytotoxicity; modification of proteins related to apoptosis; reduction and inhibition of cell growth; reduction of serotonin levels, increasing degree of hemorrhagic necrosis and endothelial permeability; production of reactive oxygen species through the release of Ca_2^+; induced apoptosis; induced aponecrosis in cells resistant to apoptosis; inhibited cell proliferation and secretion of MMP-2 and -9, and increased secretion of TIMP-2; induced autophagy; tumor suppression; blockage of tumor progression and metastasis; activation of apoptosis and reactive oxygen species-dependent mechanisms; loss of cell viability; increased expression of p53; down-regulation of proteins (Sp1, Sp3 and Sp4) and decreased expression of genes that involve cell proliferation and angiogenesis (Table 2).

Vitamin C has been occasionally used to complement the treatment of cancer since 1974, aiding in patients' survival and quality of life. Studies in humans, animals, and *in vitro* show that antioxidants such as ascorbic acid, tocopherols, and carotenoids can inhibit the growth of neoplastic cells, inducing apoptosis, boost cell differen-

TABLE 2 Clinical and non-clinical studies using ascorbic acid as antitumor agent in the treatment and/or prevention of cancer.

Study type	Type of cancer/cell	Mechanism of action	Dose/concentration	References
Non-clinical	Cells from ovarian cancer (IOSE-385, OVCAR-3, SKOV-3 and OVCA-432)	Inhibition of cell progression by increasing the levels of H_2O_2	0.25 mM	(37)
Non-clinical	Different cell lines	Antiproliferative effect on tumor cells, through interference with cell cycle (G_0/G_1) and production of H_2O_2	0.1-2 mM	(38)
Non-clinical	AGS cells	Inhibition of cell progression, cytotoxicity, modification of proteins related to apoptosis	0.3 mM	(39)
Non-clinical	Esophageal squamous cells and Barrett's esophagus cells (CP-A, CP-B, CP-C and CP-D)	Decreased cell growth	0.5 mM	(40)
Non-clinical	Cells from gastric (23132/87), colon (HT-29), ovary (SKOV-3), pancreas (BXPC-3) and breast (BT-20, MDA-MB-468, MDA-MB-231 and MCF-7) carcinoma, glioblastoma (U-13898, U-87 and U-251), endothelial cells (HUVEC) and fibroblasts (NHDF)	Production of H_2O_2	0.005-0.1 mM	(41)
Non-clinical	Cells from cervical cancer (HeLa)	Decreased cell growth	4 mM	(32)
Non-clinical	Colon carcinoma in mice	Decreased levels of serotonin, increased level of hemorrhagic necrosis and permeability of the endothelium	28.39 mM	(42)
Non-clinical	Laryngeal carcinoma (HEp-2)	Production of reactive oxygen species through the release of Ca_2^+	3-10 mM	(43)
Non-clinical	Cells from neuroblastoma	Induction of apoptosis	1-6 mM	(34)
Non-clinical	Cells from pancreatic cancer (PANC-1, AsPC-1, BxPC-3 and MIA PaCa-2)	Aponecrosis induced in cells resistant to apoptosis	4.26 mM	(44)
Non-clinical	Cells from Epstein-Barr virus-positive Burkitt's lymphoma and lymphoblastoid cells	Production of reactive oxygen species and induction of cell death	22.71 mM	(45)
Non-clinical	Cells from neuroblastoma (SK-N-MC) in vitro and in vivo	in vitro – induction of apoptosis, inhibition of cell proliferation, secretion of MMP-2 and -9, and increased secretion of TIMP-2; in vivo – inhibition of tumor growth	Not reported	(46)
Clinical	Metastatic pancreatic cancer	Decreased tumor size	0.28; 0.43 and 0.5 mM	(47)
Non-clinical	Cells from prostate cancer (PC3) in mice	Production of reactive oxygen species, induced autophagy, tumor suppression	0.4 mM	(48)
Non-clinical	Cells from malignant pleural mesothelioma in rats	in vitro – synergism in the mechanism of cytotoxicity; in vivo – blocking of tumor progression and metastasis, reduction in tumor size	11.36 mM	(49)
Non-clinical	Epithelial cells from lung cancer (NSCLC)	Induction of cell death by activation of apoptosis and via mechanism of production of reactive oxygen species, loss of cell viability	0.5-5 mM	(50)
Non-clinical	Solid Ehrlich carcinoma in mice	Inhibition of tumor growth and increased expression of p53	0.68 mM	(51)
Non-clinical	Cells from colon cancer (RKO and SW480)	Decreased cell proliferation, induced apoptosis and necrosis, down-regulation of proteins (Sp1, Sp3 e Sp4) and decreased expression of genes that involve cell proliferation and angiogenesis	1-3 mM	(52)

Source: Literature search.

tiation and inhibiting the activity of protein kinase C and adenylyl cyclase, which proves its antitumor effect, also affirming that a high-dose therapy can benefit patients by improving their prognosis and therapeutic efficacy.[22]

Studies have reported that the pro-oxidant mechanisms of ascorbic acid include an ability to reduce metal ions such as Fe^{3+} and Cu^{3+}, a process that generates free radicals such as hydroxyl radical, which interact with DNA, causing breaks in the phosphodiester bonds in addition modifications in the bases, generating induced cytotoxicity.[23] Some research indicate another antitumor mechanism of action, the proliferation of natural killers (NK) cells without affecting its normal functions. According to them, these cells have the ability to "kill" tumor cells without the need for sensitization of direction, and that ascorbic acid promotes their proliferation.[24]

Conclusion

Studies have reported the use of ascorbic acid in the prevention and treatment of cancer. However, there is controversy about its antioxidant and antitumor role. This study revealed that there are reports in the literature of the effects of ascorbic acid at different doses/concentrations as antioxidant acting by several mechanisms, including the attenuation of cytotoxicity, reduced apoptosis, protection of neoplastic cells against lipid peroxidation, decrease in tumor growth and inflammatory cytokine secretion. And as an antitumor agent, ascorbic acid acts through the inhibition of cell progression, increased levels of H_2O_2, antiproliferative effect of tumor cells, cytotoxicity, induction of apoptosis, and more. There are also incompatibilities with regard to doses/concentration of ascorbic acid, as well as the need for characterization of clinical studies and mechanisms of action. Thus, other studies are needed to understand dose-response variations, as well as its targeting mechanisms of action, both as an antioxidant and antitumor agent, to assist treatment and prevention of cancer, aiming at better quality of life for both patients and the general population.

Acknowledgments

Resumo

Ácido ascórbico na prevenção e no tratamento do câncer

Este estudo de revisão teve como objetivo fazer o mapeamento sistemático do ácido ascórbico na prevenção e/ou no tratamento do câncer como antioxidante e/ou pró-oxidante em estudos clínicos e não clínicos, entre 2011 e 2015, para o entendimento das variações de dose-resposta, bem como dos seus mecanismos de ação como agente antioxidante e antitumoral. Nas bases de dados Pubmed e Bireme, foram identificados 78 artigos, dos quais apenas 30 apontavam o ácido ascórbico na prevenção e/ou no tratamento do câncer. Contudo, há controvérsias sobre as doses utilizadas e faltam estudos clínicos que caracterizem melhor o seu mecanismo de ação. Outros estudos devem ser realizados para o entendimento das variações de dose-resposta, bem como de seus mecanismos de ação, como agente antioxidante ou antitumoral, para auxiliar o tratamento e a prevenção do câncer, visando à melhor qualidade de vida dos pacientes e da população em geral.

Palavras-chave: neoplasias, câncer, prevenção, ácido ascórbico, antioxidantes.

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