

High Doses of Ascorbate (Vitamin C): A New Frontier in the Treatment of Intraocular Cancer

Introduction: Retinoblastoma and Uveal Melanoma

Retinoblastoma is a rare intraocular tumour affecting the retina of young children and infants [1]. Chemotherapeutic agents such as carboplatin and etoposide, have been shown to effectively reduce the volume of intraocular tumours in these children [2,3]. However, the toxicity of systemic chemotherapy, still represents an issue which deserves further investigation [4,5], particularly when genomic instability is involved, as in the case of retinoblastoma [6,7].

Uveal Melanoma is a malignant cancer of the uvea, affecting around 4.3-5 individuals per million, in the United States, the vast majority of which are Caucasian males [8]. Despite its paucity, uveal melanoma is the most common primary tumour of the eye in adults, with the choroid as the most commonly involved anatomical structure [9]. Up to 50% of patients develop metastatic disease in a time-lapse variable from five to 15 years after diagnosis, with preferential involvement of the liver, and about 90% of them will ultimately succumb to metastatic spread in less than three months [10-15].

Until the late eighties, the only treatment available for uveal melanoma was enucleation of the affected eye, though brachytherapy, thermotherapy and radiation therapy can be used to treat small/medium size tumours with preservation of the eye [16]. Several different chemotherapeutic agents have been used, such as Dacarbazine, alone or in combination with Interferon-alpha 2b, after primary treatment, to patients at high risk of developing metastatic disease, but they have not improved the outcome of these patients [17,18].

Globally, despite the improvements in the treatment of primary tumours and although very rarely patients show detectable metastasis at presentation, still half of the patients die of metastatic disease. Regarding the mechanisms of malignant transformation, in uveal melanoma, emphasis has been given to genetic changes, but, although both cytogenetics and genetics have enhanced prognosis, allowing the distinction of tumours with higher metastatic potential, metastatic disease remains the leading cause of death among patients with uveal melanoma [19].

In recent years, Reactive Oxygen Species (ROS), the key mediators in oxidative stress, have emerged as promising targets for anticancer drug discovery and extensive research has documented a causative involvement of alterations of the Red-Ox (reduction/oxidation) balance in tumour progression, particularly for oncological indications with little treatment options, including metastatic melanoma [20-24].

The Anticancer Properties of Vitamin C in High Concentrations

Among its innumerable physiologic properties, Vitamin C (in the form of both ascorbic acid or sodium ascorbate), when administered in high ("pharmacologic") concentrations, is toxic

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for cancer cells, both *in vitro* and *in vivo*, in a number of different ways. Although widely known as the founder of all antioxidants and free radical scavengers, Vitamin C is cytotoxic for cancer cells, *in vitro*, mainly because of its pro-oxidant properties [25-33], and this activity seems to be primarily mediated by hydrogen peroxide; therefore, Vitamin C in pharmacologic concentrations is considered a prodrug of hydrogen peroxide [34-42].

When oxidized, in biological systems, Vitamin C generates hydrogen peroxide, which can be considered a ROS; this action can be enhanced by divalent cations such as iron and copper [43-46]. The amount of hydrogen peroxide generated is usually proportional to the amount of Vitamin C administered [47-50]. However, other factors influence the formation of hydrogen peroxide, after administration of high doses of Vitamin C; in particular, cupric ion is a catalyst of the oxidation of Vitamin C, and, as such, it increases the production of hydrogen peroxide, starting from Vitamin C [51-54].

The increased levels hydrogen peroxide, upon administration of Vitamin C, leads to accumulation of this ROS in cancer cells, which have amount of catalase of 10-to 100 times lower than the normal ones, and this explains the selective toxicity of high concentration of Vitamin C for cancer cells, as compared to the normal ones [55-60]. In addition to Vitamin C per se, some products of Vitamin C metabolism have shown anticancer properties; among others: dehydroascorbic acid, 2,3-diketogulonic acid, and 5-methyl 1-3,4-dehydroxytetrone [27,28,60-63].

Secondary anticancer properties of Vitamin C include:

- i. Improved collagen synthesis with consequent limitation of cancer spread [64,65].
- ii. Improved immune competence, through enhanced synthesis of immunoglobulin's, phagocytosis and production of interferon [66-69].
- iii. Inhibition of prostaglandins and the release of arachidonic acid, which is supposed to represent a synergistic signal leading to cell proliferation [70,71].

iv. Stabilization of p53, a protein involved in the control of cell proliferation [72,73].

Current Chemotherapy for Retinoblastoma and High Doses of Vitamin C: Evidence from *in vitro* Studies

Chemotherapeutic agents such as carboplatin and etoposide, have been shown to effectively reduce the volume of intraocular tumours in children affected by retinoblastoma [74,75]. However, the toxicity of systemic chemotherapy, still represents an issue which deserves further investigation [76,77], particularly when genomic instability is involved, as in retinoblastoma [6,7]. In an effort to improve drug delivery to the tumour, and simultaneously reduce systemic toxicity, clinical researchers have more recently developed super selective Ophthalmic Artery Infusion (SOAI) of chemotherapeutic agents [78] which, although still controversial [79,80], promises a dramatic improvement in the rate of preservation of the affected eyes [81-83], particularly when different drugs are combined [84-86].

Although widely used, chemotherapy of retinoblastoma is based on studies testing only a few chemotherapeutic agents [87,88] using the clonogenic assay, which has been largely criticized, in the past [89, 90] and almost completely abandoned nowadays, given its low reliability [91]. More recently, the combination of carboplatin and cell immunotherapy has been demonstrated superior to carboplatin alone, in killing Y79 cells [92] and carboplatin-resistant retinoblastoma cells *in vitro* [93]. However, even at its best, cancer chemotherapy has limited value in the treatment of retinoblastoma while *in vitro* testing on Vitamin C, has already shown promising results and a clear superiority, when compared to conventional chemotherapy.

As a matter of fact, Medina and Schweigerer, in 1993-4, showed that Ascorbate has cytotoxic effect on Y79 cells in long-term incubations in the presence of limited amounts of serum in the medium [94,95], while other Authors have demonstrated that a mixture, containing, among others, ascorbic acid, induces apoptosis on Y79 cells, *in vitro* [96]. In our experience, Y79 cells are highly sensitive to sodium ascorbate, when exposed to this vitamin for only one hour, under standard culture conditions (RPMI 1640, supplemented with antibiotics, Glutamine, 10% heat inactivated Fetal Bovine Serum in an atmosphere of 5% CO₂ and 95% air). In particular, after exposure for one hour to millimolar (mM) concentrations of sodium ascorbate, about 90% of Y79 cells in culture died, at a concentration of Vitamin C of 5 mM [97]. Melphalan at the standard dose, used to treat retinoblastoma, had no effect on Y79 cells treated for comparison.

The rationale behind the use of ascorbate in the treatment of retinoblastoma, is not only to be found in the metabolic properties of this molecule, but also on evidence showing that a maternal diet and nutrition rich in vegetables and fruits, may prevent the development of this tumour [98]. On the other hand, the role of ROS in the genesis of retinoblastoma is very well known and documented by different Authors [99-101].

Current Chemotherapy for Uveal Melanoma and High Doses of Vitamin C: Evidence From *in vitro* Studies

When uveal melanoma is limited to the eye, surgical resection may lead to some improvement of the overall survival [102];

other forms of local treatment applied to uveal melanoma, are: intra-arterial chemotherapy, trans-arterial percutaneous chemoembolization, selective internal radiation therapy, and radiofrequency ablation [103]. However, as previously mentioned, up to 50% of patients develop metastatic disease, with spread of tumour cells to liver (89%), lung (29%), bone (17%), and other organs [104,105], and this event represents a clear indication to systemic chemotherapy. Unfortunately, although various chemotherapeutic agents have been tested so far, like dacarbazine, fotemustine, or gemcitabine/treosulfan, the impact of systemic chemotherapy on patients' survival is questionable [106,107].

Skin melanoma is supposed to depend on exposure to ultraviolet light [108,109], but the evidence of the role of ultraviolet light in the pathogenesis of uveal melanoma is still controversial [110,111]. However, it is quite clear that both uveal and skin melanoma, depend on a dysregulation of the cellular Red-Ox state [112-115] with an increase in the production of ROS, which represents an indication for prevention with antioxidant and treatment with pro-oxidant molecules, a role which can be fully interpreted by ascorbate, depending on the dose administered.

In our experience, uveal melanoma cell lines (C918 and OCM1) exposed for two hours to increasing concentrations of ascorbate in the culture medium, are highly sensitive to the cytotoxic effects of the vitamin. In particular, at 7mM of sodium ascorbate in the medium, the percentage of live cells is well below the 10%, and, in most cases, below the 5%. Arsenic Trioxide (ATO), which is known to induce apoptotic/necrotic cell death through production of hydrogen peroxide (H₂O₂), has been used in phase II clinical trials in uveal melanoma, even if with limited results [116]; interestingly, in our experience, adding arsenic trioxide to the culture, significantly increased the cytotoxic effects of ascorbate on uveal melanoma cell lines [117].

Concluding Remarks

In spite of the great number of reports showing a strong anticancer activity of high concentration of ascorbate *in vitro* [118,119] the *in vivo* antitumor effect of high doses of the vitamin is still controversial and further controlled clinical trials are needed to fully elucidate it. However, ascorbate administered in high doses by intravenous route, has great potentialities in the treatment of cancer, once a few technical issues are further clarified.

In a recent, excellent review on this matter, Gonzalez and Coll. enumerate the variables that may interfere with the clinical response to high doses of intravenous ascorbate [120]. In particular the Authors underscore the level of tissue oxygenation, the level of blood glucose, and the physiological Red-Ox balance, as the most relevant physiological/cellular variables to take into account, to fully exploit the anticancer potential of high doses of ascorbate *in vivo*. In 1956, Otto Warburg clarified that cancer cells are metabolically different from their normal counterpart, since they not only survive under adverse conditions such as hypoxia, but are also capable of proliferation and distant invasion (metastasis) [121,122].

The so called "Warburg effect" (or phenomenon), is represented by a metabolic shift which compels cancer cells to rely on the inefficient glycolytic mode of ATP synthesis (2 ATPs/

glucose), rather than respiration that produces substantially more ATP/glucose (about 36 ATPs/glucose). As a consequence, cancer cells must adopt a mode of increased glucose import to meet their energy demands, and this explains why they increase their levels of glucose transporters (GLUT) [123] and crave for glucose [124-127]. Taken together, these data indicate that, since high concentrations of ascorbate seem to kill cancer cells essentially by increasing the local production of hydrogen peroxide (H₂O₂), it is clear that a good local oxygenation and a reduced intake of glucose are fundamental pre-requisites to optimize the cytotoxic effect of the vitamin on cancer cells. Once optimized as suggested by the metabolic changes occurring in cancer cells, the intravenous injection of high doses of Vitamin C promises to radically improve the outcome and the quality of life of patient affected by both retinoblastoma and uveal melanoma, since:

- I. In both its oral and intravenous (IV) formulations, ascorbate is remarkably safe.
- II. It improves the quality of life of cancer patients.
- III. It reduces the many complications of the disease.
- IV. It enhances the cytotoxic effects and reduces the side effects of chemotherapy.
- V. As a pro-drug of hydrogen peroxide, it is toxic only for cancer cells.

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