Redox-Active Quinones and Ascorbate: An Innovative Cancer Therapy That Exploits the Vulnerability of Cancer Cells to Oxidative Stress

Julien Verrax[a], Raphael Beck[a], Nicolas Dejeans[a], Christophe Glorieux[a], Brice Sid[a], Rozangela C. Pedrosa[b], Julio Benites[c,e], David Vásquez[d,e], Jaime A. Valderrama[d,e] and Pedro Buc Calderon[a,c,e,*]

[a]*Toxicology and Cancer Biology Research Group, Louvain Drug Research Institute (LDRI), Université Catholique de Louvain, Brussels, Belgium;* [b]*Laboratorio de Bioquimica Experimental, Departamento de Bioquimica, Universidade Federal de Santa Catarina, Florianopolis, Brasil;* [c]*Departamento de Ciencias Quımicas y Farmacéuticas, Universidad Arturo Prat, Iquique, Chile;* [d]*Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile;* [e]*Instituto de EtnoFarmacología (IDE), Universidad Arturo Prat, Iquique, Chile*

Abstract: Cancer cells are particularly vulnerable to treatments impairing redox homeostasis. Reactive oxygen species (ROS) can indeed play an important role in the initiation and progression of cancer, and advanced stage tumors frequently exhibit high basal levels of ROS that stimulate cell proliferation and promote genetic instability. In addition, an inverse correlation between histological grade and antioxidant enzyme activities is frequently observed in human tumors, further supporting the existence of a redox dysregulation in cancer cells. This biochemical property can be exploited by using redox-modulating compounds, which represent an interesting approach to induce cancer cell death. Thus, we have developed a new strategy based on the use of pharmacologic concentrations of ascorbate and redox-active quinones. Ascorbate-driven quinone redox cycling leads to ROS formation and provokes an oxidative stress that preferentially kills cancer cells and spares healthy tissues. Cancer cell death occurs through necrosis and the underlying mechanism implies an energetic impairment (ATP depletion) that is likely due to glycolysis inhibition. Additional mechanisms that participate to cell death include calcium equilibrium impairment and oxidative cleavage of protein chaperone Hsp90. Given the low systemic toxicity of ascorbate and the impairment of crucial survival pathways when associated with redox-active quinones, these combinations could represent an original approach that could be combined to standard cancer therapy.

Keywords: Ascorbate, cancer, quinone, redox cycling, oxidative stress

I. CANCER, ROS SIGNALING AND ANTIOXIDANTS

ROS: Cause or Consequence of Cell Proliferation?

Cell signaling is part of a complex process of communication that governs the cell response to its microenvironment. If cell signaling was originally considered to be driven mainly by proteins such as kinases and phosphatases, it becomes clear that other molecules, as simple as ROS, can be involved in the signal transduction processes. Although ROS were originally considered as unavoidable by-products of normal cell metabolism, this point of view has recently changed and it is now widely accepted that physiological ROS generation is a tightly regulated process that plays a central role in cell signaling and participates in cell proliferation.

ROS and cell proliferation are intertwined in a complex web and it is difficult to know whether they are a cause or a consequence of proliferation. Since the main source of ROS is electron leakage from the mitochondrial transport chain, it is logical that increased proliferation (which requires energy and thus an increased metabolic activity) is accompanied by an increase in ROS generation. However, ROS themselves can also activate proliferation by different mechanisms.

First, ROS stimulate intracellular tyrosine phosphorylation by inhibiting various protein tyrosine phosphatases (PTPs) [1]. Indeed, PTPs contain an essential cysteine residue in their active site, which exists as a thiolate anion at neutral pH, and that is required to form a thiol-phosphate intermediate during the catalytic process. This cysteine residue is highly susceptible to oxidation by ROS, and especially by hydrogen peroxide, to form sulfenic acid or an intramolecular disulfide bond, leading to a reversible inhibition of the enzymatic activity. The former can be further oxidized to sulfinic and sulfonic acids, resulting in an irreversible inactivation of PTPs [2, 3]. This deactivation of phosphatases by ROS can lead to an increased activation of proliferation pathways such as the ERK MAPK pathway. Second, ROS can modulate the intracellular calcium homeostasis. Indeed, it has been shown in plants that mutations in NADPH oxidase impairs plant cell expansion by interfering with the activity of plasma membrane Ca^{++} channels and similar effects have been described in mammalian cells [4, 5]. Alternatively, ROS can also modulate Ca^{++} release from intracellular stores. For example, in human vascular endothelial cells, ROS derived from NADPH oxidase exhibit a critical role in the generation of high intracellular Ca^{++} concentrations in response to histamine, most probably by increasing the sensitivity of the endoplasmic reticulum to inositol 1,4,5 trisphosphate [6]. Third, ROS can activate several transcription factors such as NF-κB [7], of which the activation leads to a stimulation of cell proliferation.

Cancer cells, which exhibit a high proliferation rate, frequently present increased levels of ROS [8] and several mechanisms have been proposed to explain this phenomenon. These include the aberrant metabolism of cancer cells, but also mitochondrial dysfunctions or loss of functional p53 [9]. In addition, the activation of some oncoproteins has been shown to promote the generation of ROS. This is well-known for Bcr-Abl, although the precise mechanism, which seems to involve Nox4, still remains unclear [10, 11]. As a consequence of this ROS generation, Bcr-Abl bearing cells exhibit a high genetic instability [12]. The other oncogenic proteins for which the activity has been linked to increased intracellular levels of ROS are Ras [13], c-Met and c-myc [14]. Overall, ROS production promoted by the activity of oncogenic proteins induces cell proliferation and genetic instability, both of which are favorable for cancer progression. However, these are not the only processes that are facilitated by the high intracellular levels of ROS that cancer cells harbor. Indeed, ROS also promote angiogenesis, metastasis, and escape from immune attack. Thus, ROS plays an important role in mediating neovascularization during tumor growth. The H_2O_2 generated by Nox1 increases the expression of proteins such as VEGF (Vascular endothelial growth factor) and its receptor

*Address correspondence to this author at the avenue E. Mounier 73, 1200 Bruxelles, Belgium; Tel: 32-2-764.73.66; Fax: 32-2-764.73.59;
E-mail: pedro.buccalderon@uclouvain.be

1871-5206/11 $58.00+.00

(VEGFR), thereby promoting the vascularization and the rapid expansion of tumors. Explaining this pro-angiogenic effect, it has been shown that Nox-produced ROS (but also mitochondria-derived ROS) stabilize and activate the transcription factor HIF-1α [15-17]. ROS also regulate positively the metastatic potential of cancer cells [18], whereas antioxidant therapies could attenuate metastatic progress of cancer cells [19, 20]. In addition, it has been shown that pretreatment of highly metastatic tumor cells with ROS scavengers suppresses their metastatic potential in mice [21].

Antioxidants

Normal cells possess a variety of antioxidant systems to cope with ROS and prevent their damaging effects. However, the level of many cellular antioxidants is modified in cancer cells, as well as the activity of some antioxidant enzymes. For instance, decreased levels of manganese superoxide dismutase (MnSOD) have been reported in numerous cancer cell lines compared to the corresponding normal tissues and the same conclusion can be drawn for copper-zinc superoxide dismutase (CuZnSOD), although more variability is observed for this enzyme [22]. We also observed that global SOD activity was significantly decreased in hepatoma cells when compared to normal hepatocytes [23]. However, it is important to notice that a great discrepancy exists between the levels of antioxidant enzymes found in biopsies from cancer patients and those observed in tumor cell lines. Indeed, studies on SOD expression in human malignant diseases have shown variable results and no general conclusion can be drawn about the SOD activity in human cancerous tissue [24-28]. As for SOD, catalase is generally decreased in cancer cells grown in culture [29]. Accordingly, we observed that both the expression and the activity of catalase were decreased in hepatoma cells compared to normal hepatocytes, as well as and in leukemia cells compared to normal leukocytes [23, 30, 31]. Finally, low levels of glutathione peroxidase (GPx) are generally observed in cancer cells grown in culture [23, 30, 32]. However, once again, no general conclusion can be drawn about the expression of these two enzymes in human tumors because of the high variability observed in existing studies [33-35]. As nicely discussed by Kinnula and Crapo, several factors explain why the evaluation of antioxidant enzymes in tumor tissues can be tricky [25]. The localization and expression of antioxidants in tumor cells are often assayed using immunocytochemistry, which is a semiquantitative method directly influenced by the specificity of the antibody. Moreover, immunoreactivity does not necessarily correlate with the enzymatic activity. Tumor samples can also be heterogeneous both in terms of expression of a specific antioxidant enzyme and in terms of cell types and tissues (presence of vessels or immune cells). Finally, it is not easy to obtain normal control biopsies to compare the levels of antioxidant enzymes in malignant tumors with corresponding healthy tissues.

In comparison to three major antioxidant enzymes described above, the activity of other antioxidant enzymes is rather increased in cancer cells. For instance, thioredoxin reductases (TrxR) are frequently overexpressed in cancer cells and are thus considered as therapeutic targets by several authors [36-37]. On the other hand, recent studies have demonstrated that Trx overexpression (immunohistochemically detected) is not necessarily associated to an increase of activity [38]. Regarding peroxiredoxins (PRDX), their expression is also increased in many human cancers, even if differences exist between the different isoforms and the type of cancer tissue [39-42]. We have conversely observed that leukemia cells had highly decreased levels of PRDX2 and PRDX6 when compared to normal leukocytes (unpublished results). Glutaredoxins (Grx) seem to be induced in several cancer types, but differences also exist between the different isoforms [43]. Glutathione reductase activity is variable from one to another study [44] but the expression of glutathione S-transferases is generally increased [45, 46]. Finally, the expression of flavin-dependent quinone reductases (NQO) seems to be increased in many types of tumors but some studies have shown no modification of its expression [47, 48], and NQO expression is even lost in certain cancer cells [49].

Briefly, it is impossible to draw a general conclusion about the expression of antioxidant enzymes in cancer cells. If many cancer cell lines seem to have decreased levels of SOD, catalase and Gpx, things are more variable for samples obtained from cancer patients. Moreover, certain antioxidant enzymes seem to be overexpressed in cancer cells. Actually, it is likely that each cancer cell type has a different pattern of antioxidant enzymes.

As for enzymatic defenses, variable results are encountered in studies performed on small antioxidant molecules. For instance, glutathione (GSH) levels are often higher in cancer cells than in normal cells, contributing to chemotherapy resistance [50-52]. However, the levels of other antioxidants are rather decreased. Serum tocopherol is for example decreased in patients with head and neck squamous cell carcinoma [53] and low carotenoid levels are routinely observed in cancer patients, compared to healthy individuals [54, 55]. However, caution should be taken with these results because other studies failed to demonstrate a correlation between serum concentrations and cancer progression [56, 57]. Regarding ascorbate (vitamin C), decreased plasma levels are frequently observed in cancer patients and many studies have suggested an inverse relationship between ascorbate intake and the development of cancer [58]. However, it is worth noting that cancer patients often experience side effects from chemotherapy such as nausea, vomiting, diarrhea, and loss of appetite, leading to a lower intake of dietary constituents which likely explains this deficiency [59, 60]. Some studies have suggested the intracellular accumulation of ascorbate in cancer cells [30, 61]. This could be explained by the glycolytic metabolism presented by most cancer cells and which requires the overexpression of glucose transporters (GLUTs) [62]. Indeed, dehydroascorbic acid can be transported by GLUTs, then reduced intracellularly, leading to the accumulation of ascorbate in tumors [63]. However, there is no general consensus about the ascorbate levels in tumors because other studies have reported decreased levels in cancer cells compared with normal tissue [64]. Interestingly, it has been recently demonstrated that the intracellular levels of ascorbate dramatically affect tumor metabolism by controlling the activity of the transcription factor hypoxia-inducible factor (HIF)-1. Low ascorbate levels were associated with elevated VEGF, GLUT-1, and BNIP3 protein levels, increased activation of the HIF-1 pathway and with increased tumor size; whereas tumors with high ascorbate levels had lesser levels of HIF-1 activation [65].

II. OXIDATIVE STRESS IN CANCER TREATMENT

Anticancer Compounds Based on Redox Mechanisms

Cancer cells exhibit an abnormal redox status associated with increased basal levels of ROS and frequent alterations of the antioxidant systems. Thus the use of redox-modulating compounds appears as an interesting therapeutic approach taking advantage of this biochemical particularity to selectively target tumors. Indeed, normal cells maintain redox homeostasis with a low level of basal ROS because they tightly control the balance between ROS generation and elimination. As a consequence, normal cells can tolerate a certain level of exogenous ROS whereas cancer cells cannot [9]. As illustrated in Fig. (**1**), two strategies can be employed to disturb the cellular redox equilibrium: (1) the use of exogenous ROS-generating agents; (2) the use of compounds that inhibit the antioxidant systems.

Many agents inducing cancer cell death through redox mechanisms are actually under preclinical and/or clinical evaluation (Table **1**). For instance, agents such as arsenic trioxide (As_2O_3) which impair the function of the respiratory chain are known to increase the production of superoxide [66]. In addition, As_2O_3

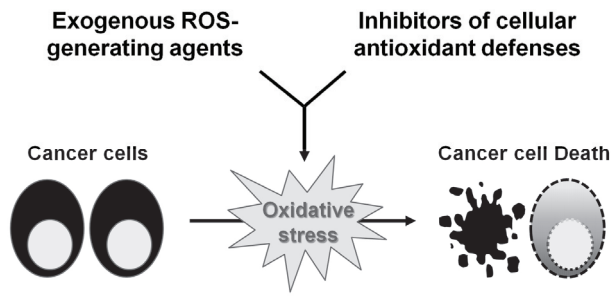

Fig. (1). Strategies to target cancer cells through ROS-mediated mechanisms. Exogenous agents that increase ROS generation or decrease ROS elimination can lead to significant ROS accumulation in cancer cells, leading to oxidative damage and subsequent cell death, which occurs either by necrosis (cell death represented on the right) or apoptosis (cell death represented on the left).

strongly inhibits the thioredoxin antioxidant system thereby providing a second mechanism by which this compound exerts pro-oxidative effects [67]. Redox cyclers such as daunorubicin, doxorubicin, or other quinones such as menadione generate superoxide anion by reducing molecular oxygen.

Isothiocyanate compounds such as benzylisothiocyanate (BITC), phenylethylisothiocyanate (PEITC), and sulphoraphane rapidly conjugate with GSH, the major ROS-scavenging system in cells, causing a depletion of the GSH pool [9]. Alternatively, it is possible to deplete GSH by inhibiting its synthesis. This is achieved by molecules like buthionine sulphoximine that inhibit γ-glutamylcysteine synthetase, the rate-limiting enzyme in GSH synthesis [68]. Sulphasalazine, an inhibitor of the cystine/glutamate antiporter, can also provoke GSH depletion by inhibiting the uptake of cystine, the precursor of cysteine, which is a rate-limiting substrate for GSH synthesis [69]. Adaphostin is another ROS-generating agent that induces cancer cell death by inhibiting the complex III of the mitochondrial respiratory chain, leading to mitochondrial ROS generation [70].

The Ascorbate/Menadione Combination

Ascorbate is a powerful reducing agent. It can donate either one or two electrons in redox reactions, yielding ascorbate free radical (ascorbyl radical) and dehydroascorbic acid, respectively [71]. Along with high mM intracellular levels, these properties allow ascorbate to directly scavenge physiologically relevant ROS and RNS such as hydroxyl radicals, superoxide anions, aqueous peroxyl radicals, hypochlorous acid, singlet oxygen and peroxynitrite. In addition, ascorbate can regenerate other small molecule antioxidants, such as α-tocopherol, glutathione (GSH), and β-carotene, from their respective radical species. Ascorbate can be regenerated from ascorbyl radical and dehydroascorbate by enzyme-dependent and independent pathways: the ascorbyl radical can be reduced by an NADH-dependent semidehydroascorbate reductase (in plants) and the NADPH-dependent selenoenzyme thioredoxin reductase, whereas dehydroascorbate can be reduced back to ascorbate nonenzymatically by GSH and lipoic acid as well as by thioredoxin reductase and the GSH-dependent enzyme glutaredoxin [72].

Remarkably, ascorbate can also reduce some quinones, generating a semiquinone form that can be re-oxidized by molecular oxygen. As shown in Fig. (**2**), the final result of this reaction is the production of superoxide anion, which is then converted into hydrogen peroxide. Based on this observation, our laboratory has developed a combination of ascorbate with such a redox active quinone, namely menadione, as a ROS-generating system that induces the death of various cancer cell types. This combination exhibits a synergistic effect which clearly indicates that redox cycling

Table 1. Clinical Trials of ROS-Modulating Agents for Cancer

ROS-Modulating Agents	Mechanism of Action	Current Status
Inhibitors of the antioxidant defenses		
Phenylethylisothiocyanate	• Inhibition of GPx • Conjugation to GSH	• Phase I trials in lymphoproliferative disorders
Buthionine sulphoximine	• Inhibition of GSH synthesis	• Phase I studies in combination with melphalan in neuroblasoma and melanoma
2-methoxyestradiol	• Increases superoxide levels in tumor cells	• Phase II trials in multiple myeloma, glioblastoma, prostate, ovarian and renal cancers
ROS-generating agents		
β-lapachone	• Production of $O_2^{\cdot-}$ and H_2O_2 through redox-cycling	• Phase II trials in squamous cell carcinoma of the head and neck, leiomyosarcoma and pancreatic cancer.
Ascorbic acid	• Generation of H_2O_2 through its oxidation by a metalloprotein catalyst	• Phase I/II studies in pancreas and prostate cancers, non-Hodgkin's lymphoma and myeloproliferative disorders, either alone or in combination with standard chemotherapies
Motexafin gadolinium	• Inhibition of TrxR • Production of $O_2^{\cdot-}$ and H_2O_2 through redox-cycling	• Phase I/II/III studies in brain and central nervous system tumors, in combination with radiation therapy • Phase I/II studies in non-Hodgkin's lymphoma, pancreatic and kidney cancers
Based on other mechanisms		
Arsenic trioxide (As_2O_3)	• Inhibition of TrxR • Inhibits the mitochondrial transport chain	• Standard of care in relapsed or refractory acute promyelocytic leukemia • Phase I/II trials in melanoma, multiple myeloma, brain and endometrium tumors
Bleomycin	• Formation of a Fe^{3+} hydroperoxide complex (BLM-Fe(III)-OOH) that cleaves DNA by hydrogen abstraction.	• Approved to treat malignant pleural effusion • Used in Hodgkin lymphoma, head, neck and testicular cancers

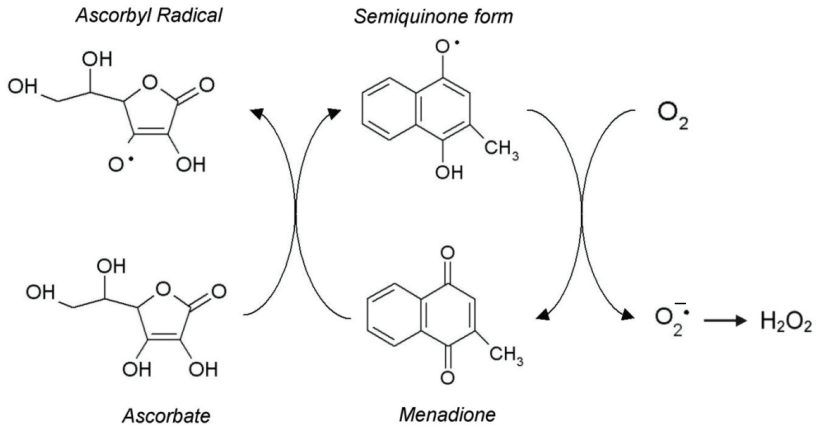

Fig. (2). The ascorbate-driven menadione redox cycling. From Beck *et al* [31].

is the major event in the mechanism of cytotoxicity. However, it should be noted that menadione, which is a naphtoquinone with a double bond α to a keto group, can undergo a Michael addition to form adducts with sulfhydryls and primary amines that also lead to cell injury and cell death. To discriminate which of both pathways (redox cycling or covalent binding) are involved in the cytotoxicity induced by the ascorbate/menadione combination (asc/men), we used DMNQ (2,3-dimethoxy-naphtoquinone), a menadione analog without arylation sites. The combination between ascorbate and DMNQ produced the same profile of cytotoxicity as observed with asc/men, underlining the key role of the redox cycling pathway [73].

The first *in vivo* studies with asc/men were performed in the eighties, using a single dose of 1g/kg and 10 mg/kg for ascorbate and menadione, respectively. These studies demonstrated a potent antitumor effect of the combination, as well as a potentiation of chemotherapy in a model of hepatoma-bearing mice (TLT, transplantable liver tumor) [74]. Interestingly, the potentiating effect was not specific for a particular class of chemotherapeutic agents. Indeed, the five classes tested (alkylating agents, antimetabolites, mitotic inhibitors, intercalating agents, and asparaginase) were potentialized by asc/men. It rapidly became clear that hydrogen peroxide (H_2O_2) was involved in the asc/men antitumor effects because the addition of catalase totally suppressed the toxicity of asc/men in cultured cancer cells [75]. These first studies also demonstrated that asc/men was also able to potentiate radiotherapy [76]. From these experiments, it can be concluded that neither ascorbate nor menadione alone induced an inhibitory effect on tumor growth. Actually, results obtained in further studies have shown that repeated i.v. injections of very high dosage of ascorbate (up to 4g/kg, every day) can decrease but do not suppress tumor growth rate, as shown by preclinical and clinical data [77-78]. The advantages of combining ascorbate and menadione compared of using each compound by separate therefore rely on a stronger antitumoral effect, due to the synergism, and the possibility to reduce the doses of each compound, thus decreasing the possibility of undesirable side effects.

Further studies were performed to elucidate the molecular mechanisms involved in the cancer cell death induced by asc/men. These data demonstrated that asc/men-induced cell death was indeed dependent on the formation of H_2O_2 and was necrotic rather than apoptotic [23, 79, 80]. It was also shown that cell death occurred because of the inhibition of glycolysis and the subsequent depletion in intracellular ATP levels. This inhibition of glycolysis is due to a depletion in NAD levels, itself provoked by a poly(ADPribose) polymerase (PARP) activation that occurs in response to DNA damage [81]. However, further studies have shown that other events were involved in the molecular mechanisms leading to cell death in asc/men-treated cancer cells. Indeed, asc/men also induces a dysregulation of calcium homeostasis and an endoplasmic reticulum (ER) stress in cancer cells [82]. Calcium is rapidly released from the endoplasmic reticulum to the cytosol following treatment of cells with asc/men, inducing an ER stress that participates in the death of cancer cells. Accordingly, we observed that BAPTA-AM, a calcium chelator, partially inhibited cell death in asc/men-treated cancer cells [83]. These results indicate that asc/men disrupts ER calcium homeostasis, a mechanism that also participates in asc/men toxicity, even if it is not the main process explaining cell death.

In addition, we also showed that asc/men affects the MAPK pathway and the activity of the chaperone protein HSP90. Asc/men induces a strong deactivation of the ERK proliferation pathway (namely ERK1/2 and its upstream kinase c-Raf) and the activation of p38 [83]. Since the ERK pathway, which promotes cell proliferation, is activated in many cancers [84], we hypothesized that its inhibition could contribute to the cancer cell death induced by asc/men. We further showed that asc/men induced the cleavage of HSP90 and the degradation of its client proteins, such as RIP, Akt, or Bcr-Abl [85]. Due to the major role played by HSP90 in the stabilization of many oncogenic proteins, its cleavage by asc/men could have a potential interest in anticancer therapy. We postulated that the mechanism by which asc/men induces the cleavage of HSP90 relies on the in situ formation of ROS by a Fenton-type reaction located at the N-terminal nucleotide binding pocket of HSP90. The local generation of hydroxyl radical forms a Hsp90 protein radical, which, by rearrangement, causes the rupture of the peptide backbone, prevents the chaperone activity and leads to the degradation of client proteins that are critical for cancer cells [Beck *et al*., submitted]. Given that HSP90 stabilizes oncoproteins such as Bcr-Abl, and due to the appearance of mutations in Bcr-Abl, rendering cells resistant to standard small molecule inhibitors (i.e. imatinib), we hypothesized if asc/men could be cytotoxic in this particular context. For that purpose, we used cells expressing mutated (and thus resistant) forms of Bcr-Abl. Our results showed that asc/men, by inducing an oxidative cleavage of HSP90, is cytotoxic against cells expressing either the wild type or mutated forms of Bcr-Abl, therefore representing a novel therapeutic strategy to overcome resistance to classical Bcr-Abl inhibitors [31].

Given the encouraging results obtained in preclinical studies, the use of asc/men for the treatment of metastatic or locally advanced, inoperable transitional cell carcinoma of the urothelium (stage III and IV bladder cancer) has been granted by the FDA. Recently, an oral combination of ascorbate and menadione (Apatone©) has been evaluated as an anticancer agent in a clinical study in prostate cancer patients who had failed standard therapy. Promis-

ing delay in the biochemical progression of the disease was observed in Apatone-treated patients. Indeed, a significant increase in the PSA (Prostate Serum Antigen) doubling time was induced by Apatone treatment. More importantly, no dose-limiting adverse effects were observed [86]. Up to now, this study is the first that explored the toxicity profile of the asc/men combination, however, many data are available for each compound used separately. Thus, pharmacokinetic studies have demonstrated that ascorbate concentrations in plasma and tissue are tightly controlled as a function of the oral dose. Therefore, oral administration of ascorbate cannot achieve plasma concentrations higher than 50–100 μM [87, 88]. However, parenteral administration of high doses of ascorbate, similar to that used in preclinical trials with the asc/men combination (~1g/kg), bypasses tight control and produces plasma concentrations up to 20 mM [77, 78, 89]. At these pharmacologic concentrations, ascorbic acid can potentially induce some adverse effects: hyperoxaluria, urine acidification or hemolysis in patients suffering from glucose-6-phosphate dehydrogenase deficiency [90-92]. Nevertheless, the first rigorous phase I trial of i.v. ascorbate, performed in cancer patients, showed a good tolerability and minimal adverse effects [78]. Concerning menadione, phase I and II trials that were conducted in the 90's established the maximum tolerated dose at 2.5 g/m^2, when given as a continuous intravenous infusion [93-95]. The adverse effects consisted of hypersensitive reactions, facial flushing, burning feelings, chest pain and dyspnea. At higher doses (4 and 8 g/m^2), menadione induced hemolysis, despite the presence of red blood cell glucose-6-phosphate dehydrogenase. Even if the doses described above are 5 to 10 times higher than those used with the asc/men combination, it is tempting to replace menadione by other, less toxic, redox active compounds bearing a quinone moiety. The rationale is that the combination of both the reducing agent and the redox cycler is more potent than each compound by separate. We have thus developed a research project exploring new molecules acting as redox-cyclers.

Since the higher redox potential of quinones has been correlated with enhanced DNA strand breaks [96], we explored the ability of several diversely substituted 1,4-benzoquinones, 1,4-naphthoquinones and pyrimido[4,5-c]isoquinoline-7,10-quinones to induce cell death, when used in combination with ascorbate. Briefly, the results we obtained may be summarized as follows:

- A first set of results clearly showed that cancer cell death induced by the cycling of ascorbate with redox active quinone relies on the generation of ROS. This oxidative stress-mediated cytotoxicity may be predicted on the basis of the reduction potentials of quinone compounds [79].
- The antitumoral effects of Euryfuryl-1,4-benzoquinones (Fig. (**3**)), are potentiated by ascorbate, leading to necrotic-like cancer cell death in TLT, a murine hepatoma cell line [97].
- Since we had the possibility to use a variety of furan-2-yl 1,4-quinones having a wide range of redox capability [98, 99], we investigated the mechanism of cytotoxicity of these quinones in combination with ascorbate. To this end, we focused on 2-furyl-1,4-naphthoquinones and its 5- and 5,8-hydroxyderivatives. Our hypothesis was that the electron donor effect of the *peri*-hydroxyl substituent on furylnaphthoquinones and the hydrogen bond between the peri-hydroxy and the quinone carbonyl groups (Fig. (**4**)) will influence the electron-acceptor capability of the quinone nucleus and thus modifies the electron transfer from ascorbate to the electroactive quinone nucleus [100].

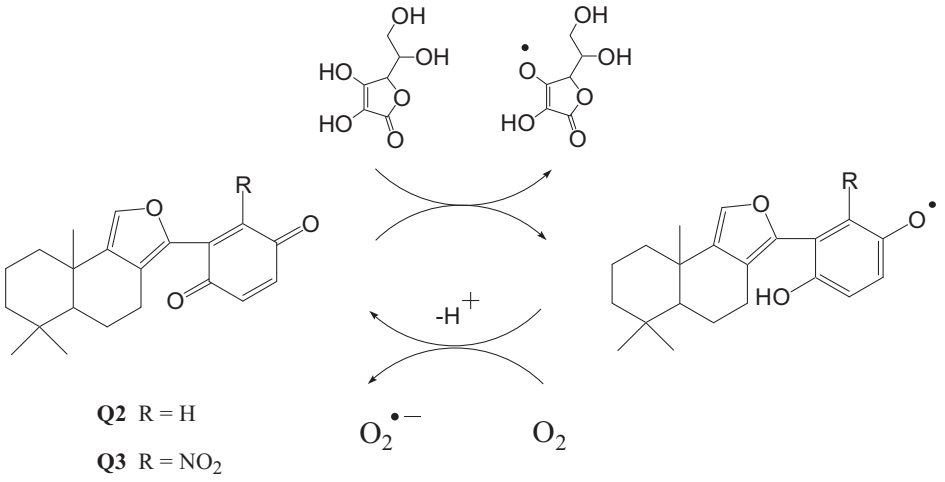

Fig. (3). Redox-cycling of 2-euryfuryl- and 2-euryfuryl-3-nitro-1,4-benzoquinone (compounds Q2 and Q3) in the presence of ascorbate

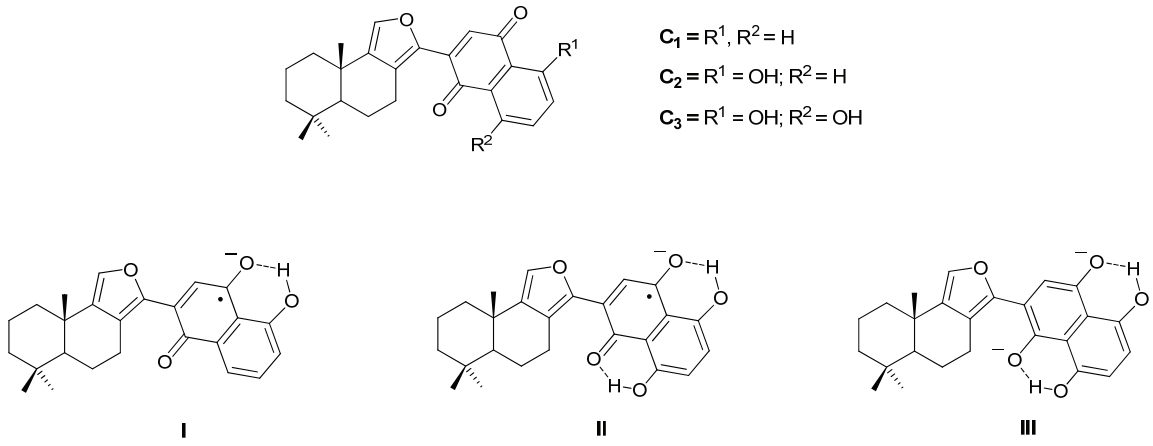

Fig. (4). Probable structure of the radical anion semiquinones and hydroquinone dianion generated from C_2 and C_3.

Based on previous data acquired on the aminoquinone scaffold from a variety of synthetic and natural antitumor agents [101-104], we also explored whether synthetic molecules derived of 8-phenylaminopyrimido[4,5-c]isoquinolinequinone, may also act as redox-cyclers inducing a cytotoxic activity in cancer cells. We observed that the addition of ascorbate strongly enhances the cytotoxicity of quinones leading to cancer cell death [101]. The quinones for which the cytotoxic effect was potentiated by ascorbate had their first half wave reduction potential ($E^{I}_{1/2}$) values within the range -480 to -680 mV vs NHE [as measured in non-aqueous solutions by Vasquez *et al*., submitted]. For the aqueous systems more relevant to *in vivo* medium, the values for menadione were reported to be -335 or -203 mV vs SHE for quinone/semiquinone redox couple (aqueous medium, 1 M concentration, pH 7,…) [105, 106]. While thermodynamics would not favor coupling of quinone with ascorbate, the fast oxidation of semiquinone is driving cycling with ascorbate [107].

CONCLUSION

Taken together, the data we have acquired during these past ten years strongly suggest that targeting the altered redox status of cancer cells by the combinations of redox-active quinones and pharmacologic doses of ascorbate is an interesting approach to potentiate chemotherapy. However, to favor the effectiveness of this strategy and define the proper chemotherapeutic association, it is important to understand the biological consequences of the chronic exposure of cancer cells to ROS. Studies are therefore in progress to explore this critical issue.

ABBREVIATIONS

Asc/men	=	Ascorbate and menadione combination
ATP	=	Adenosine triphosphate
BITC	=	Benzylisothiocyanate
BLM	=	Bleomycin
ER	=	Endoplasmic reticulum
GLUT	=	Glucose transporter
GPx	=	Glutathione peroxidase
Grx	=	Glutaredoxin
GSH	=	Glutathione
HIF-1	=	Hypoxia-inducible factor-1
NQO	=	NADPH: quinone oxidoreductase
PARP	=	Poly(ADPribose) polymerase
PEITC	=	Phenylethylisothiocyanate
PRDX	=	Peroxiredoxin
PSA	=	Prostate specific antigen
PTP	=	Protein tyrosine phosphatase
ROS	=	Reactive oxygen species
RNS	=	Reactive nitrogen species
SOD	=	Superoxide dismutase
TLT	=	Transplantable liver tumor
TrxR	=	Thioredoxin reductase
Trx	=	Thioredoxin
VEGF	=	Vascular endothelial growth factor

REFERENCES

[1] Heffetz, D.; Bushkin, I.; Dror, R.; Zick, Y. The insulinomimetic agents H_2O_2 and vanadate stimulate protein tyrosine phosphorylation in intact-cells. *J. Biol. Chem.,* **1990**, *265*(5), 2896-2902.

[2] Cho, S. H.; Lee, C. H.; Ahn, Y.; Kim, H.; Kim, H.; Ahn, C. Y.; Yang, K. S.; Lee, S. R. Redox regulation of PTEN and protein tyrosine phosphatases in H_2O_2-mediated cell signaling. *FEBS Lett.,* **2004**, *560*(1-3), 7-13.

[3] Chan, E. C.; Jiang, F.; Peshavariya, H. M.; Dusting, G. J. Regulation of cell proliferation by NADPH oxidase-mediated signaling: Potential roles in tissue repair, regenerative medicine and tissue engineering. *Pharmacol. Therapeut.,* **2009**, *122*(2), 97-108.

[4] Foreman, J.; Demidchik, V.; Bothwell, J. H. F.; Mylona, P.; Miedema, H.; Torres, M. A.; Linstead, P.; Costa, S.; Brownlee, C.; Jones, J. D. G.; Davies, J. M.; Dolan, L. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature,* **2003**, *422*(6930), 442-446.

[5] Hudasek, K.; Brown, S. T.; Fearon, I. M. H_2O_2 regulates recombinant Ca^{2+} channel alpha(1c) subunits but does not mediate their sensitivity to acute hypoxia. *Biochem. Bioph. Res. Co.,* **2004**, *318*(1), 135-141.

[6] Hu, Q. H.; Yu, Z. X.; Ferrans, V. J.; Takeda, K.; Irani, K.; Ziegelstein, R. C. Critical role of NADPH oxidase-derived reactive oxygen species in generating Ca2+ oscillations in human aortic endothelial cells stimulated by histamine. *J. Biol. Chem.,* **2002**, *277*(36), 32546-32551.

[7] Schreck, R.; Rieber, P.; Baeuerle, P. A. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO J.,* **1991**, *10*(8), 2247-2258.

[8] Trachootham, D.; Zhou, Y.; Zhang, H.; Demizu, Y.; Chen, Z.; Pelicano, H.; Chiao, P.; Achanta, G.; Arlinghaus, R.; Liu, J.; Huang, P. Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. *Cancer Cell,* **2006**, *10*(3), 241-252.

[9] Trachootham, D.; Alexandre, J.; Huang, P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat. Rev. Drug Discov.,* **2009**, *8*(7), 579-591.

[10] Sattler, M.; Verma, S.; Shrikhande, G.; Byrne, C.; Pride, Y.; Winkler, T.; Greenfield, E.; Salgia, R.; Griffin, J. The BCR/ABL tyrosine kinase induces production of reactive oxygen species in hematopoietic cells. *J. Biol. Chem.,* **2000**, *275*(32), 24273-24278.

[11] Naughton, R.; Quiney, C.; Turner, S. D.; Cotter, T. G. Bcr-Abl-mediated redox regulation of the PI3K/AKT pathway. *Leukemia,* **2009**, *23*(8), 1432-1440.

[12] Koptyra, M.; Falinski, R.; Nowicki, M. O.; Stoklosa, T.; Majsterek, I.; Nieborowska-Skorska, M.; Blasiak, J.; Skorski, T. BCR/ABL kinase induces self-mutagenesis *via* reactive oxygen species to encode imatinib resistance. *Blood,* **2006**, *108*(1), 319-327.

[13] Lee, A. C.; Fenster, B. E.; Ito, H.; Takeda, K.; Bae, N. S.; Hirai, T.; Yu, Z. X.; Ferrans, V. J.; Howard, B. H.; Finkel, T. Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. *J. Biol. Chem.,* **1999**, *274*(12), 7936-7940.

[14] Vafa, O.; Wade, M.; Kern, S.; Beeche, M.; Pandita, T.; Hampton, G.; Wahl, G. c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. *Mol. Cell,* **2002**, *9*(5), 1031-1044.

[15] Komatsu, D.; Kato, M.; Nakayama, J.; Miyagawa, S.; Kamata, T. NADPH oxidase 1 plays a critical mediating role in oncogenic Ras-induced vascular endothelial growth factor expression. *Oncogene,* **2008**, *27*(34), 4724-4732.

[16] Kizaki, M.; Xian, M.; Sagawa, M.; Ikeda, Y. Induction of apoptosis *via* the modulation of reactive oxygen species (ROS) production in the treatment of myeloid leukemia. *Curr. Pharm. Biotechnol.,* **2006**, *7*(5), 323-329.

[17] Sanjuan-Pla, A.; Cervera, A. M.; Apostolova, N.; Garcia-Bou, R.; Victor, V. M.; Murphy, M. P.; McCreath, K. J. A targeted antioxidant reveals the importance of mitochondrial reactive oxygen species in the hypoxic signaling of HIF-1alpha. *FEBS Lett.,* **2005**, *579*(12), 2669-2674.

[18] Lim, S. D.; Sun, C.; Lambeth, J. D.; Marshall, F.; Amin, M.; Chung, L.; Petros, J. A.; Arnold, R. S. Increased Nox1 and hydrogen peroxide in prostate cancer. *Prostate,* **2005**, *62*(2), 200-207.

[19] Wang, J.; Yi, J. Cancer cell killing *via* ROS: to increase or decrease, that is the question. *Cancer Biol. Ther.,* **2008**, *7*(12), 1875-1884.

[20] Ferraro, D.; Corso, S.; Fasano, E.; Panieri, E.; Santangelo, R.; Borrello, S.; Giordano, S.; Pani, G.; Galeotti, T. Pro-metastatic signaling by c-Met through RAC-1 and reactive oxygen species (ROS). *Oncogene,* **2006**, *25*(26), 3689-3698.

[21] Ishikawa, K.; Takenaga, K.; Akimoto, M.; Koshikawa, N.; Yamaguchi, A.; Imanishi, H.; Nakada, K.; Honma, Y.; Hayashi, J. ROS-

generating mitochondrial DNA mutations can regulate tumor cell metastasis. *Science,* **2008**, *320*(5876), 661-664.

[22] Weydert, C. J.; Waugh, T. A.; Ritchie, J. M.; Iyer, K. S.; Smith, J. L.; Li, L.; Spitz, D. R.; Oberley, L. W. Overexpression of manganese or copper-zinc superoxide dismutase inhibits breast cancer growth. *Free Radic. Biol. Med.,* **2006**, *41*(2), 226-237.

[23] Verrax, J.; Cadrobbi, J.; Marques, C.; Taper, H.; Habraken, Y.; Piette, J.; Calderon, P. Ascorbate potentiates the cytotoxicity of menadione leading to an oxidative stress that kills cancer cells by a non-apoptotic caspase-3 independent form of cell death. *Apoptosis,* **2004**, *9*(2), 223-233.

[24] Sanchez, M.; Torres, J. V.; Tormos, C.; Iradi, A.; Muniz, P.; Espinosa, O.; Salvador, A.; Rodriguez-Delgado, J.; Fandos, M.; Saez, G. T. Impairment of antioxidant enzymes, lipid peroxidation and 8-oxo-2'-deoxyguanosine in advanced epithelial ovarian carcinoma of a Spanish community. *Cancer Lett.,* **2006**, *233*(1), 28-35.

[25] Kinnula, V.; Crapo, J. Superoxide dismutases in malignant cells and human tumors. *Free Radic. Biol. Med.,* **2004**, *36*(6), 718-744.

[26] Sander, C. S.; Hamm, F.; Elsner, P.; Thiele, J. J. Oxidative stress in malignant melanoma and non-melanoma skin cancer. *Br. J. Dermatol.,* **2003**, *148*(5), 913-922.

[27] Monari, M.; Foschi, J.; Calabrese, C.; Liguori, G.; Di Febo, G.; Rizzello, F.; Gionchetti, P.; Trinchero, A.; Serrazanetti, G. P. Implications of antioxidant enzymes in human gastric neoplasms. *Int. J. Mol. Med.,* **2009**, *24*(5), 693-700.

[28] Hwang, T. S.; Choi, H. K.; Han, H. S. Differential expression of manganese superoxide dismutase, copper/zinc superoxide dismutase, and catalase in gastric adenocarcinoma and normal gastric mucosa. *Eur. J. Surg. Oncol.,* **2007**, *33*(4), 474-479.

[29] Sato, K.; Ito, K.; Kohara, H.; Yamaguchi, Y.; Adachi, K.; Endo, H. Negative regulation of catalase gene expression in hepatoma cells. *Mol. Cell. Biol.,* **1992**, *12*(6), 2525-2533.

[30] Verrax, J.; Pedrosa, R.; Beck, R.; Dejeans, N.; Taper, H.; Calderon, P. In situ modulation of oxidative stress: a novel and efficient strategy to kill cancer cells. *Curr. Med. Chem.,* **2009**, *16*(15), 1821-1830.

[31] Beck, R.; Pedrosa, R. C.; Dejeans, N.; Glorieux, C.; Leveque, P.; Gallez, B.; Taper, H.; Eeckhoudt, S.; Knoops, L.; Calderon, P. B.; Verrax, J. Ascorbate/menadione-induced oxidative stress kills cancer cells that express normal or mutated forms of the oncogenic protein Bcr-Abl. An *in vitro* and *in vivo* mechanistic study. *Invest. New Drugs,* **2010**.

[32] Sun, Y.; Oberley, L.; Elwell, J.; Sierra-Rivera, E. Antioxidant enzyme activities in normal and transformed mouse liver cells. *Int. J. Cancer,* **1989**, *44*(6), 1028-1033.

[33] Pljesa-Ercegovac, M.; Mimic-Oka, J.; Dragicevic, D.; Savic-Radojevic, A.; Opacic, M.; Pljesa, S.; Radosavljevic, R.; Simic, T. Altered antioxidant capacity in human renal cell carcinoma: role of glutathione associated enzymes. *Urol. Oncol.,* **2008**, *26*(2), 175-181.

[34] Rainis, T.; Maor, I.; Lanir, A.; Shnizer, S.; Lavy, A. Enhanced oxidative stress and leucocyte activation in neoplastic tissues of the colon. *Dig. Dis. Sci.,* **2007**, *52*(2), 526-530.

[35] Zelen, I.; Djurdjevic, P.; Popovic, S.; Stojanovic, M.; Jakovljevic, V.; Radivojevic, S.; Baskic, D.; Arsenijevic, N. Antioxidant enzymes activities and plasma levels of oxidative stress markers in B-chronic lymphocytic leukemia patients. *J. BUON,* **2010**, *15*(2), 330-336.

[36] Lincoln, D. T.; Ali Emadi, E. M.; Tonissen, K. F.; Clarke, F. M. The thioredoxin-thioredoxin reductase system: over-expression in human cancer. *Anticancer Res.,* **2003**, *23*(3B), 2425-2433.

[37] Biaglow, J. E.; Miller, R. A. The thioredoxin reductase/thioredoxin system: novel redox targets for cancer therapy. *Cancer Biol. Ther.,* **2005**, *4*(1), 6-13.

[38] Shan, W.; Zhong, W.; Zhao, R.; Oberley, T. D. Thioredoxin 1 as a subcellular biomarker of redox imbalance in human prostate cancer progression. *Free Radic. Biol. Med.,* **2010**, *49*(12), 2078-2087.

[39] Yanagawa, T.; Iwasa, S.; Ishii, T.; Tabuchi, K.; Yusa, H.; Onizawa, K.; Omura, K.; Harada, H.; Suzuki, H.; Yoshida, H. Peroxiredoxin I expression in oral cancer: a potential new tumor marker. *Cancer Lett.,* **2000**, *156*(1), 27-35.

[40] Noh, D. Y.; Ahn, S. J.; Lee, R. A.; Kim, S. W.; Park, I. A.; Chae, H. Z. Overexpression of peroxiredoxin in human breast cancer. *Anticancer Res.,* **2001**, *21*(3B), 2085-2090.

[41] Chang, J. W.; Jeon, H. B.; Lee, J. H.; Yoo, J. S.; Chun, J. S.; Kim, J. H.; Yoo, Y. J. Augmented expression of peroxiredoxin I in lung cancer. *Biochem. Biophys. Res. Commun.,* **2001**, *289*(2), 507-512.

[42] Kinnula, V. L.; Lehtonen, S.; Sormunen, R.; Kaarteenaho-Wiik, R.; Kang, S. W.; Rhee, S. G.; Soini, Y. Overexpression of peroxiredoxins I, II, III, V, and VI in malignant mesothelioma. *J. Pathol.,* **2002**, *196*(3), 316-323.

[43] Cha, M. K.; Kim, I. H. Preferential overexpression of glutaredoxin3 in human colon and lung carcinoma. *Cancer Epidemiol.,* **2009**, *33*(3-4), 281-287.

[44] Singh, S. V.; Brunnert, S. R.; Roberts, B.; Krishan, A. Differential expression of glutathione S-transferase, glutathione peroxidase and glutathione reductase in normal and malignant human breast tissues. *Cancer Lett.,* **1990**, *51*(1), 43-48.

[45] Peters, W. H.; Nagengast, F. M.; Wobbes, T. Glutathione S-transferases in normal and cancerous human colon tissue. *Carcinogenesis,* **1989**, *10*(12), 2371-2374.

[46] Tsuchida, S.; Sekine, Y.; Shineha, R.; Nishihira, T.; Sato, K. Elevation of the placental glutathione S-transferase form (GST-pi) in tumor tissues and the levels in sera of patients with cancer. *Cancer Res.,* **1989**, *49*(18), 5225-5229.

[47] Cheng, Y.; Li, J.; Martinka, M.; Li, G. The expression of NAD(P)H:quinone oxidoreductase 1 is increased along with NF-kappaB p105/p50 in human cutaneous melanomas. *Oncol. Rep.,* **2010**, *23*(4), 973-979.

[48] Marjani, H. A.; Biramijamal, F.; Rakhshani, N.; Hossein-Nezhad, A.; Malekzadeh, R. Investigation of NQO1 genetic polymorphism, NQO1 gene expression and PAH-DNA adducts in ESCC. A case-control study from Iran. *Genet. Mol. Res.,* **2010**, *9*(1), 239-249.

[49] Bruge, F.; Virgili, S.; Cacciamani, T.; Principi, F.; Tiano, L.; Littarru, G. P. NAD(P)H:quinone oxidoreductase (NQO1) loss of function in Burkitt's lymphoma cell lines. *Biofactors,* **2008**, *32*(1-4), 71-81.

[50] Russo, A.; DeGraff, W.; Friedman, N.; Mitchell, J. B. Selective modulation of glutathione levels in human normal versus tumor cells and subsequent differential response to chemotherapy drugs. *Cancer Res.,* **1986**, *46*(6), 2845-2848.

[51] Perry, R. R.; Mazetta, J. A.; Levin, M.; Barranco, S. C. Glutathione levels and variability in breast tumors and normal tissue. *Cancer,* **1993**, *72*(3), 783-787.

[52] Lee, F. Y.; Allalunis-Turner, M. J.; Siemann, D. W. Depletion of tumour versus normal tissue glutathione by buthionine sulfoximine. *Br. J. Cancer,* **1987**, *56*(1), 33-38.

[53] Sakhi, A. K.; Russnes, K. M.; Thoresen, M.; Bastani, N. E.; Karlsen, A.; Smeland, S.; Blomhoff, R. Pre-radiotherapy plasma carotenoids and markers of oxidative stress are associated with survival in head and neck squamous cell carcinoma patients: a prospective study. *B.M.C. Cancer,* **2009**, *9*, 458.

[54] Ito, Y.; Gajalakshmi, K. C.; Sasaki, R.; Suzuki, K.; Shanta, V. A study on serum carotenoid levels in breast cancer patients of Indian women in Chennai (Madras), India. *J. Epidemiol.,* **1999**, *9*(5), 306-314.

[55] Palan, P. R.; Mikhail, M. S.; Goldberg, G. L.; Basu, J.; Runowicz, C. D.; Romney, S. L. Plasma levels of beta-carotene, lycopene, canthaxanthin, retinol, and alpha- and tau-tocopherol in cervical intraepithelial neoplasia and cancer. *Clin. Cancer Res.,* **1996**, *2*(1), 181-185.

[56] Venkitaraman, R.; Thomas, K.; Grace, P.; Dearnaley, D. P.; Horwich, A.; Huddart, R. A.; Parker, C. C. Serum micronutrient and antioxidant levels at baseline and the natural history of men with localised prostate cancer on active surveillance. *Tumour Biol.,* **2010**, *31*(2), 97-102.

[57] Yeum, K. J.; Ahn, S. H.; Rupp de Paiva, S. A.; Lee-Kim, Y. C.; Krinsky, N. I.; Russell, R. M. Correlation between carotenoid concentrations in serum and normal breast adipose tissue of women with benign breast tumor or breast cancer. *J. Nutr.,* **1998**, *128*(11), 1920-1926.

[58] Head, K. A. Ascorbic acid in the prevention and treatment of cancer. *Altern. Med. Rev.,* **1998**, *3*(3), 174-186.

[59] Mayland, C.; Bennett, M.; Allan, K. Vitamin C deficiency in cancer patients. *Palliat. Med.,* **2005**, *19*(1), 17-20.

[60] Georgiannos, S.; Weston, P.; Goode, A. Micronutrients in gastrointestinal cancer. *Br. J. Cancer,* **1993**, *68*(6), 1195-1198.

[61] Langemann, H.; Torhorst, J.; Kabiersch, A.; Krenger, W.; Honegger, C. Quantitative determination of water- and lipid-soluble antioxidants in neoplastic and non-neoplastic human breast tissue. *Int. J. Cancer,* **1989**, *43*(6), 1169-1173.

[62] Gatenby, R.; Gillies, R. Why do cancers have high aerobic glycolysis? *Nat. Rev. Cancer,* **2004**, *4*(11), 891-899.

[63] Agus, D.; Vera, J.; Golde, D. Stromal cell oxidation: a mechanism by which tumors obtain vitamin C. *Cancer Res.,* **1999**, *59*(18), 4555-4558.

[64] Landolt, H.; Langemann, H.; Probst, A.; Gratzl, O. Levels of water-soluble antioxidants in astrocytoma and in adjacent tumor-free tissue. *J. Neurooncol.,* **1994**, *21*(2), 127-133.

[65] Kuiper, C.; Molenaar, I. G.; Dachs, G. U.; Currie, M. J.; Sykes, P. H.; Vissers, M. C. Low ascorbate levels are associated with increased hypoxia-inducible factor-1 activity and an aggressive tumor phenotype in endometrial cancer. *Cancer Res.,* **2010**, *70*(14), 5749-5758.

[66] Pelicano, H.; Feng, L.; Zhou, Y.; Carew, J. S.; Hileman, E. O.; Plunkett, W.; Keating, M. J.; Huang, P. Inhibition of mitochondrial respiration: a novel strategy to enhance drug-induced apoptosis in human leukemia cells by a reactive oxygen species-mediated mechanism. *J. Biol. Chem.,* **2003**, *278*(39), 37832-37839.

[67] Lu, J.; Chew, E. H.; Holmgren, A. Targeting thioredoxin reductase is a basis for cancer therapy by arsenic trioxide. *Proc. Natl. Acad. Sci. U S A,* **2007**, *104*(30), 12288-12293.

[68] Griffith, O. W.; Meister, A. Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (S-n-butyl homocysteine sulfoximine). *J. Biol. Chem.,* **1979**, *254*(16), 7558-7560.

[69] Lo, M.; Wang, Y. Z.; Gout, P. W. The x(c)- cystine/glutamate antiporter: a potential target for therapy of cancer and other diseases. *J. Cell. Physiol.,* **2008**, *215*(3), 593-602.

[70] Le, S. B.; Hailer, M. K.; Buhrow, S.; Wang, Q.; Flatten, K.; Pediaditakis, P.; Bible, K. C.; Lewis, L. D.; Sausville, E. A.; Pang, Y. P.; Ames, M. M.; Lemasters, J. J.; Holmuhamedov, E. L.; Kaufmann, S. H. Inhibition of mitochondrial respiration as a source of adaphostin-induced reactive oxygen species and cytotoxicity. *J. Biol. Chem.,* **2007**, *282*(12), 8860-8872.

[71] May, J. M. Is ascorbic acid an antioxidant for the plasma membrane? *FASEB J.,* **1999**, *13*(9), 995-1006.

[72] Carr, A.; Frei, B. Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB J.,* **1999**, *13*(9), 1007-1024.

[73] Calderon, P.B.; Cadrobbi, J.; Marques, C.; Hong-Ngoc, N.; Jamison, J.M.; Gilloteaux, J.; Summers, J.L.; Taper, H.S. Potential therapeutic application of the association of vitamins C and K3 in cancer treatment. Curr. Med. Chem., **2002**, 9(24), 2271-2285.

[74] Taper, H.; de Gerlache, J.; Lans, M.; Roberfroid, M. Non-toxic potentiation of cancer chemotherapy by combined C and K3 vitamin pre-treatment. *Int. J. Cancer,* **1987**, *40*(4), 575-579.

[75] Noto, V.; Taper, H.; Jiang, Y.; Janssens, J.; Bonte, J.; De Loecker, W. Effects of sodium ascorbate (vitamin C) and 2-methyl-1,4-naphthoquinone (vitamin K3) treatment on human tumor cell growth *in vitro*. I. Synergism of combined vitamin C and K3 action. *Cancer,* **1989**, *63*(5), 901-906.

[76] Taper, H.; Keyeux, A.; Roberfroid, M. Potentiation of radiotherapy by nontoxic pretreatment with combined vitamins C and K3 in mice bearing solid transplantable tumor. *Anticancer Res.,* **1996**, *16*(1), 499-503.

[77] Verrax, J.; Calderon, P. Pharmacologic concentrations of ascorbate are achieved by parenteral administration and exhibit antitumoral effects. *Free Radic. Biol. Med.,* **2009**, *47*(1), 32-40.

[78] Hoffer, L.; Levine, M.; Assouline, S.; Melnychuk, D.; Paddayatty, S.; Rosadiuk, K.; Rousseau, C.; Robitaille, L.; Miller, W. J. Phase I clinical trial of i.v. ascorbic acid in advanced malignancy. *Ann. Oncol.,* **2008**, *19*(11), 1969-1974.

[79] Verrax, J.; Delvaux, M.; Beghein, N.; Taper, H.; Gallez, B.; Buc Calderon, P. Enhancement of quinone redox cycling by ascorbate induces a caspase-3 independent cell death in human leukaemia cells. An *in vitro* comparative study. *Free Radic. Res.,* **2005**, *39*(6), 649-657.

[80] Verrax, J.; Stockis, J.; Tison, A.; Taper, H.; Calderon, P. Oxidative stress by ascorbate/menadione association kills K562 human chronic myelogenous leukaemia cells and inhibits its tumour growth in nude mice. *Biochem. Pharmacol.,* **2006**, *72*(6), 671-680.

[81] Verrax, J.; Vanbever, S.; Stockis, J.; Taper, H.; Calderon, P. Role of glycolysis inhibition and poly(ADP-ribose) polymerase activation in necrotic-like cell death caused by ascorbate/menadione-induced oxidative stress in K562 human chronic myelogenous leukemic cells. *Int. J. Cancer,* **2007**, *120*(6), 1192-1197.

[82] Dejeans, N.; Tajeddine, N.; Beck, R.; Verrax, J.; Taper, H.; Gailly, P.; Calderon, P. B. Endoplasmic reticulum calcium release potentiates the ER stress and cell death caused by an oxidative stress in MCF-7 cells. *Biochem. Pharmacol.,* **2010**, *79*(9), 1221-1230.

[83] Beck, R.; Verrax, J.; Dejeans, N.; Taper, H.; Buc Calderon, P. Menadione reduction by pharmacological doses of ascorbate induces an oxidative stress that kills breast cancer cells. *Int. J. Toxicol.,* **2009**, *28*(1), 33-42.

[84] Dhillon, A. S.; Hagan, S.; Rath, O.; Kolch, W. MAP kinase signalling pathways in cancer. *Oncogene,* **2007**, *26*(22), 3279-3290.

[85] Beck, R.; Verrax, J.; Gonze, T.; Zappone, M.; Pedrosa, R.; Taper, H.; Feron, O.; Calderon, P. Hsp90 cleavage by an oxidative stress leads to its client proteins degradation and cancer cell death. *Biochem. Pharmacol.,* **2009**, *77*(3), 375-383.

[86] Tareen, B.; Summers, J.; Jamison, J.; Neal, D.; McGuire, K.; Gerson, L.; Diokno, A. A 12 week, open label, phase I/IIa study using apatone for the treatment of prostate cancer patients who have failed standard therapy. *Int. J. Med. Sci.,* **2008**, *5*(2), 62-67.

[87] Levine, M.; Conry-Cantilena, C.; Wang, Y.; Welch, R.; Washko, P.; Dhariwal, K.; Park, J.; Lazarev, A.; Graumlich, J.; King, J.; Cantilena, L. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. U S A,* **1996**, *93*(8), 3704-3709.

[88] Levine, M.; Wang, Y.; Padayatty, S.; Morrow, J. A new recommended dietary allowance of vitamin C for healthy young women. *Proc. Natl. Acad. Sci. U S A,* **2001**, *98*(17), 9842-9846.

[89] Chen, Q.; Espey, M.; Sun, A.; Lee, J.; Krishna, M.; Shacter, E.; Choyke, P.; Pooput, C.; Kirk, K.; Buettner, G.; Levine, M. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid *in vivo*. *Proc. Natl. Acad. Sci. U S A,* **2007**, *104*(21), 8749-8754.

[90] Rees, D.; Kelsey, H.; Richards, J. Acute haemolysis induced by high dose ascorbic acid in glucose-6-phosphate dehydrogenase deficiency. *B.M.J.,* **1993**, *306*(6881), 841-842.

[91] Peña de la Vega, L.; Lieske, J.; Milliner, D.; Gonyea, J.; Kelly, D. Urinary oxalate excretion increases in home parenteral nutrition patients on a higher intravenous ascorbic acid dose. *JPEN J. Parenter. Enteral. Nutr.,* **2004**, *28*(6), 435-438.

[92] Massey, L.; Liebman, M.; Kynast-Gales, S. Ascorbate increases human oxaluria and kidney stone risk. *J. Nutr.,* **2005**, *135*(7), 1673-1677.

[93] Margolin, K. A.; Akman, S. A.; Leong, L. A.; Morgan, R. J.; Somlo, G.; Raschko, J. W.; Ahn, C.; Doroshow, J. H. Phase I study of mitomycin C and menadione in advanced solid tumors. *Cancer Chemother. Pharmacol.,* **1995**, *36*(4), 293-298.

[94] Tetef, M.; Margolin, K.; Ahn, C.; Akman, S.; Chow, W.; Leong, L.; Morgan, R. J., Jr.; Raschko, J.; Somlo, G.; Doroshow, J. H. Mitomycin C and menadione for the treatment of lung cancer: a phase II trial. *Invest. New Drugs,* **1995**, *13*(2), 157-162.

[95] Lim, D.; Morgan, R. J., Jr.; Akman, S.; Margolin, K.; Carr, B. I.; Leong, L.; Odujinrin, O.; Doroshow, J. H. Phase I trial of menadiol diphosphate (vitamin K3) in advanced malignancy. *Invest. New Drugs,* **2005**, *23*(3), 235-239.

[96] Matsumoto, S. S.; Biggs, J.; Copp, B. R.; Holden, J. A.; Barrows, L. R. Mechanism of ascididemin-induced cytotoxicity. *Chem. Res. Toxicol.,* **2003**, *16*(2), 113-122.

[97] Benites, J.; Rojo, L.; Valderrama, J. A.; Taper, H.; Calderon, P. B. Part 1: Effect of vitamin C on the biological activity of two euryfurylbenzoquinones on TLT, a murine hepatoma cell line. *Eur. J. Med. Chem.,* **2008**, *43*(9), 1813-1817.

[98] Valderrama, J. A.; Benites, J.; Cortes, M.; Pessoa-Mahana, D.; Prina, E.; Fournet, A. Studies on quinones. Part 35: Access to antiprotozoal active euryfurylquinones and hydroquinones. *Tetrahedron,* **2002**, *58*(5), 881-886.

[99] Valderrama, J. A.; Benites, J.; Cortes, M.; Pessoa-Mahana, H.; Prina, E.; Fournet, A. Studies on quinones. Part 38: Synthesis and leishmanicidal activity of sesquiterpene 1,4-quinones. *Bioorg. Med. Chem.,* **2003**, *11*(22), 4713-4718.

[100] Benites, J.; Valderrama, J. A.; Taper, H.; Buc Calderon, P. Part 2: influence of 2-euryfuryl-1,4-naphthoquinone and its peri-hydroxy derivatives on both cell death and metabolism of TLT cells, a murine hepatoma cell line. modulation of cytotoxicity by vitamin C. *Chem. Pharm. Bull. (Tokyo),* **2009**, *57*(6), 615-619.

[101] Vasquez, D.; Rodriguez, J. A.; Theoduloz, C.; Verrax, J.; Calderon, P. B.; Valderrama, J. A. Synthesis and antitumor evaluation of 8-phenylaminopyrimido[4,5-c]isoquinolinequinones. *Bioorg. Med. Chem. Lett.,* **2009**, *19*(17), 5060-5062.

[102] Valderrama, J. A.; Ibacache, J. A.; Arancibia, V.; Rodriguez, J.; Theoduloz, C. Studies on quinones. Part 45: Novel 7-amino-isoquinoline-5,8-quinone derivatives with antitumor properties on cancer cell lines. *Bioorg. Med. Chem.,* **2009**, *17*(7), 2894-2901.

[103] Valderrama, J. A.; Ibacache, J. A. Regiochemical control in the amination reaction of phenanthridine-7,10-quinones. *Tetrahedron Lett.,* **2009**, *50*(30), 4361-4363.

Received: January 24, 2011 Revised: March 07, 2011 Accepted: March 08, 2011

[104] Vasquez, D.; Rodriguez, J. A.; Theoduloz, C.; Calderon, P. B.; Valderrama, J. A. Studies on quinones. Part 46. Synthesis and *in vitro* antitumor evaluation of aminopyrimidoisoquinolinequinones. *E. J. Med. Chem.,* **2010**, *45*(11), 5234-5242.

[105] Roginsky, VA.; Barsukova, T.K.; Stegmann, H.B. Kinetics of redox interaction between substituted quinones and ascorbate under aerobic conditions. *Chem. Biol. Interact.,* **1999**, *121*(2), 177-197.

[106] Song, Y.; Buettner, G.R. Thermodynamic and kinetic considerations for the reaction of semiquinone radicals to form superoxide and hydrogen peroxide. *Free Radic. Biol. Med.*, **2010**, *49*(6), 919-962.

[107] Jarabak, R.; Jarabak, J. Effect of ascorbate on DT-diaphorase-mediated redox cycling of 2-methyl-1,4-naphthoquinone. *Arch. Biochem. Biophys.*, **1995**, *318*(2), 418-423.