Evaluation of the In Vitro and In Vivo Antitumor Activities of Vitamin C and K-3 Combinations against Human Prostate Cancer

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Neoplasms of the urinary tract and male genital tract account for ~28% of new cancers in males. Prostatic carcinoma is one of the most prevalent malignant tumors of the male reproductive organs, with an estimated 179,300 new cases and 37,000 deaths during the year 2000 in the United States (Greenlee et al. 2000). Treatment options for prostate cancer range from watchful waiting to radical prostectomy and include brachytherapy, external beam irradiation and hormonal ablation therapy (Foster et al. 1997). Although prostate cancer in its early stages is responsive to these standard treatments, patients with hormone-refractory prostate cancer have an overall median survival of 9–18 mo, and no currently available treatment produces a survival advantage (Chen et al. 1998). The lack of efficacy of the eliciting protocols points to the need for the development of effective regimens for treating or preventing these tumors.

Vitamins C and K-3 as antitumor agents

Because of their low systemic toxicity, several vitamins, including vitamin C (VC) and vitamin K-3 (VK-3), have been evaluated for their abilities to prevent and treat cancer. The results of in vitro studies demonstrate that vitamin C exhibits selective toxicity toward malignant melanoma cells, human leukemia cells, neuroblastoma cells, tumor ascites cells as well as acute lymphoblastic leukemia, epidermoid carcinoma and fibrosarcoma, with VC acting as a prooxidant (De Laurenzi et al. 1995). In the form of ascorbate, VC can be oxidized either by single- or two-electron transfer and can be converted back to ascorbate by NADH-dependent semidehydroascorbate reductase or glutathione-dependent dehydroascorbate reductase (De Laurenzi et al. 1995). This cycling process generates intracellular H₂O₂ and other reactive oxygen species (ROS) that deplete cellular thiol levels, initiate membrane lipid peroxidation and result in tumor cell death.

Vitamin K-3 (menadione, 2-methyl-1,4-naphthoquinone) is a synthetic derivative of vitamin K-1, which exhibits antitumor activity against liver, cervix, nasopharynx, colon, lung, stomach, breast, leukemia and lymphoma cell lines (Nutter et al. 1991, Wu et al. 1993a and 1993b). Vitamin K-3 is reduced intracellularly via one- or two-electron transfer. The two-electron reduction of quinone to hydroquinone can form non-toxic conjugates or slowly autoxidize to reform quinone. After the single-electron reduction of the quinone to semiquinone, the semiquinone reduces oxygen to the superoxide radical and regenerates the quinone. As a result, redox cycling can ensure and produce large amounts of superoxide, which can dismutate via superoxide dismutase to form H₂O₂ and O₂ or take part in metal-catalyzed reactions to form more toxic species of active oxygen. Therefore, if the single-electron reduction pathway predominates and the rate of redox cycling of VK-3 exceeds the capacity of the detoxifying enzymes, oxidative stress occurs (Stubberfield and Cohen 1989). This oxidative stress produces a variety of effects on cells, including reduction of NADP and ATP pools, depletion of glutathione, induction of single-stranded DNA breaks and oxidation of sulfhydryl groups of cytoskeletal proteins (Gant et al. 1988, Mirabelli et al. 1989).

The combination of vitamins C and K-3 as an antitumor agent

Redox cycling oxidants such as VK-3 may trigger apoptosis or cause cell necrosis, depending on the dose and duration of exposure and the subsequent amount of oxidative stress (Juan and Wu 1993, McConkey et al. 1988, Wu et al. 1993a). When VC and VK-3 are combined, their interaction fosters the reduction of VK-3 via one-electron reduction and increases the rate of redox cycling of the quinone (Jarabak and Jarabak 1995, Pething et al. 1983). These results suggest that coadministration of VC can increase the potential toxicity of VK-3. In fact, when Taper and co-workers (Noto et al. 1989) combined VC and VK-3 in a ratio (VC/VK-3) of 100:1, the combination exhibited tumor-specific antitumor activity against human breast, oral epidermoid and endometrial tumor cell lines at doses that were 10–50 times lower than when...
either vitamin was administered alone. Additional studies using a murine ascites transplantable liver tumor model showed that the VC-VK-3 combination is an effective chemosensitizer and radiosensitizer that induces little systemic or major organ pathology (Taper et al. 1987 and 1996, Taper and Roberfroid 1992). A single intraperitoneal (i.p.) injection of the VC-VK-3 combination into immunocompetent mice increased life span by 46%. Furthermore, administration of the VC-VK-3 combination 3 h before administration of a variety of chemotherapeutic agents increased life expectancy up to 143%. Although the mechanism of action of these vitamins has not been elucidated, their antitumor activity has been attributed to redox cycling of the vitamins and the possible generation of peroxides and other ROS followed by membrane lipid alteration, DNA activation and DNA destruction by the VC-VK-3 combination in the catalase-deficient cancer cells (Noto et al. 1989, Taper et al. 1987 and 1992).

In vitro studies with human prostate cancer cell lines

In previous experiments conducted in our laboratory, the VC-VK-3 combination exhibited synergistic antitumor activity against two androgen-independent human prostate cancer cell lines (DU145 and PC3) and a battery of seven other urologic tumor cell lines. Although the individual vitamins exhibited antitumor activity at high concentrations, coadministration of the vitamins in a VC/VK-3 ratio of 100:1 potentiates antitumor activity 4- to 61-fold even when exposure times were as short as 1 h. Exogenous catalase destroyed this antitumor activity and implicated H2O2 and other ROS in the antitumor mechanism of these vitamins (Venugopal et al. 1996a and 1996b). Electron microscopy revealed vitamin-induced perturbation of nucleolar, mitochondrial and lysosomal structure, alteration in stress filaments and other cytoskeletal structures (Gilloteaux et al. 1995, Jamison et al. 1996). Despite the mitochondrial damage, tumor cells did not die from ATP depletion. However, vitamin treatment decreased DNA synthesis, slightly increased protein synthesis, induced a G1 phase block in the cell cycle, triggered the degradation of DNA and decreased cellular thiol levels (Jamison et al. 1996, Venugopal et al. 1996a and 1996b). These results suggest that redox cycling of the vitamin combination increased oxidative stress until it surpassed the reducing ability of the cellular thiols and cellular or genetic damage ensued.

In vivo antitumor activity of the vitamin C–vitamin K-3 combination

In recent in vivo studies designed to determine the effect of vitamin administration on the life span of nude mice, DU145 cells were given by i.p. injection; the vitamin combination was administered orally for 1 wk before tumor implantation in a single i.p. injection 48 h after tumor implantation or both orally and by i.p. injection. Sham-treated mice lived an average of 60 ± 4.7 d. Mice receiving i.p. vitamin and mice receiving oral vitamin survived 66 ± 12 and 71 ± 15 d, respectively. Mice receiving both oral and i.p. vitamin lived an average of 69 ± 4.6 d. The difference in mean survival time of the control mice and the mice receiving oral and i.p. vitamin is significant (P < 0.01). In addition, 25% of the mice receiving oral vitamins were long-term survivors. One month after the death of the last control mouse, surviving mice were killed and autopsied. These mice showed little if any tumor burden.

The results of additional in vivo studies, designed to determine the effect of vitamin administration on the growth of solid tumors in nude mice, demonstrated that administration of clinically attainable doses of oral vitamins given with free access in drinking water could significantly reduce the growth rate of solid tumors in nude mice (P < 0.05). These results suggested that the continuous presence or periodic reintroduction of vitamins into the host to maintain elevated circulating levels of vitamins may be required to obtain the optimum antitumor activity and probably mirrors the liability of the vitamins.

Analysis of sections of tumors taken from mice used in solid-tumor growth experiments indicate that the vitamin combination induced a novel type of cell death called autoschizis (Gilloteaux et al. 1998), with degradation of tumor cell DNA (RNA) induced by alkaline and acid DNase (and possibly RNase) as one of the principal effectors of tumor cell death (Taper et al. 2000). Furthermore, nude mice receiving the vitamin combination by oral gavage for 4 wk did not exhibit any significant bone marrow toxicity, changes in organ weight or pathologic changes of these organs. Because the vitamin combination is a chemosensitizer (Taper et al. 1987) and a radiosensitizer (Taper et al. 1996), combined VC and VK-3 administration may be considered a new nontoxic adjuvant cancer therapy that can be easily introduced into the classical protocols of clinical cancer therapy without any substantial risk for patients (Taper et al. 2000).

LITERATURE CITED


