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The Adjuvant Nutritional Intervention in Cancer (ANICA) Trial

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Adjuvant Nutritional Intervention in Cancer (ANICA) was a clinical study carried out in Denmark in the 1990s with 32 typical patients with breast cancer, aged 32–81 yr and classified high risk because of tumor spread to the lymph nodes. The patients received standard therapy for their breast cancer, but got from the start additionally an adjuvant therapy in form of a cocktail consisting of vitamin C (2,850 mg/day), vitamin E (2,500 IU/day), beta-carotene (32.5 IU/day), selenium (Se; 387 micrograms/day), various other vitamins and essential trace elements, essential fatty acids (1.2 g gamma-linolenic acid/day and 3.5 g omega-3 PUFAs/day), and coenzyme Q_{10} (CoQ_{10}, 90 mg/day). The protocol was later changed, with reduction of the Se intake and more coenzyme Q_{10} than when the study was started. The average survival of high-risk breast patients in the study was 50% after 5 yr, whereas for low-risk breast cancer patients (without metastases in the axilla when treatment was started), the average survival was 90% after ten years. The main investigator died, and the final report from the ANICA study was therefore never written. However, the published preliminary results from the trial were very promising; it seems, therefore, important to follow-up this study.

INTRODUCTION

Adjuvant Nutritional Intervention in Cancer (ANICA) was a clinical study that was carried out with a group of so-called high-risk breast-cancer patients in Copenhagen, Denmark in the 1990s (1–3). The patients received standard therapy for their breast cancer but got an adjuvant therapy additionally in the form of a cocktail of dietary supplements. This cocktail was a combination of antioxidant nutrients, other B-group vitamins and essential fatty acids.

The physician who was responsible for the adjuvant therapy in the ANICA trial, Knud Lockwood (1906–2002), had no responsibility for the ordinary treatment of the same patients. He was a retired surgeon who, before he became a pensioner, had been working at Rikshospitalet (the State Hospital) in Copenhagen. The ANICA study was carried out in collaboration with Professor Karl Folkers (1906–1997) in the United States.

If coenzyme Q_{10} supplementation is actually clinically useful, it is possible that in most cases it should be given as part of a cocktail together with other nutrients also deficient rather than as monotherapy. But it was precisely as one of the ingredients in a cocktail and not as monotherapy that coenzyme Q_{10} was used in the ANICA trial. If similar, new clinical studies were planned today, it might, however, seem natural to enhance the number of ingredients in the cocktail even more.

Karl Folkers and his collaborators had studied blood concentrations of coenzyme Q_{10} in cancer patients (4,5), and had found lower than normal average blood concentrations of coenzyme Q_{10} in the group. Folkers and collaborators could report about 10 case histories of cancer patients supporting the statement that therapy of cancer patients with CoQ_{10}, which they said has no significant side effects, had allowed survival on an exploratory basis for periods of 5 to 15 yr (6). This was a treatment similar to what had been used for breast cancer in the ANICA trial, but with the addition of 50 g fish protein concentrate type B (FPC per day) to compensate for enhanced rates of tissue protein degradation, from late winter 1994 onwards.

In a study by Folkers and collaborators of blood levels of coenzyme Q_{10} in 116 cancer patients, an incidence of 23.1% of patients ($n = 17$) was found in breast cancer patients whose blood levels of coenzyme Q_{10} were below 0.5 μg/ml. The incidence of breast cancer cases with levels of coenzyme Q_{10} below 0.6 μg/ml was 38.5%. This incidence of low blood plasma coenzyme Q_{10} concentration was found to be higher ($P < 0.05$) than for a group of ordinary people serving as controls (5). Patients ($n = 15$) with myeloma showed a mean blood level of 0.67 +/− 0.17 μg/ml. The incidence of a coenzyme Q_{10} blood level below 0.7 μg/ml for these 15 cases of myeloma was 53.3%, which was also higher ($P < 0.05$) than the 24.5% incidence found for a group of ordinary people (5).

THE ANICA TRIAL

The ANICA trial was carried out with 32 typical patients with breast cancer, aged 32–81 yr and classified as high risk

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because of tumor spread to the lymph nodes in the axilla (2). The average survival of high-risk breast patients was 50% after 5 years, whereas for low-risk breast cancer patients (without metastases in the axilla when treatment was started), the average survival was 90% after 10 years. The experiment was initially planned to go over a period of 5 yr, using historical controls.

There are some published reports about the preliminary results before the trial was finished, but a final report about the results after 5 yr was never written because Knud Lockwood, who had the direct contact with the patients, died. He was still alive after more than 5 yr after the experiment had started but wanted to follow the patients over more than 5 yr because the results were so good and became better and better the longer he followed his patients with remarkably few deaths. Two of the deaths, however, were not because of breast cancer; one was because of suicide and the other a fatal reaction to therapy with doxorubicin because the doctors responsible for the ordinary therapy suspected liver metastases. The patient died on the same day after the doxorubicin treatment had started, but on autopsy, no metastases at all were found (Olav Albert Christophersen, personal communication, July 28, 2013). This was the reason he wanted to wait to write the final report, but before he had written it, he died himself.

From the start of the trial, the patients received a cocktail consisting of vitamin C (2850 mg/day), vitamin E (2500 IU/day), beta-carotene (32.5 IU/day), Se (387 mg/day), various other vitamins and essential trace elements, essential fatty acids (1.2 g gamma-linolenic acid/day and 3.5 g omega-3 PUFAs/day), and coenzyme Q_{10} (CoQ_{10}, 90 mg/day) (2). But later the protocol was changed, with reduction of the Se intake and more coenzyme Q_{10} than when the study was started. The ANICA protocol was based on a working hypothesis that cancer may be synergistically related to a combination of diverse biochemical dysfunctions and vitamin deficiencies (2). The objective was therefore to test the possibility of a synergistic effect of those various categories of nutritional supplements, including coenzyme Q_{10}, which in earlier studies had been shown to be deficient and/or to be of therapeutic value as single elements in diverse forms of cancer (2). Biochemical markers, clinical condition, tumor spread, quality of life parameters, and survival were followed during the trial (2).

A preliminary report was given at a meeting about coenzyme Q_{10} in Stockholm during the fall of 1993, at which time the patients had been followed over a period of 18 months since the study was started (2). The main observations were 1) none of the patients had died during the study period (the expected number was 4) (2); 2) none of the patients showed signs of further distant metastases (2); 3) the quality of life was improved (no weight loss, reduced use of pain killers) (2); And 4) 6 patients showed apparent partial remission (2). It was also told at the Stockholm meeting that in 2 patients, the metastases had shrunk until they had completely disappeared (3). Lockwood had through his entire career as a cancer surgeon never seen anything similar before (Olav Albert Christophersen, personal communication, July 28, 2013).

Later, there were some deaths, but not more than 5 or 6 (rather than 16 as expected from historical controls) at the time when the last written report describing the results was made in form of a poster presented at a meeting in Italy after 4.5 yr. And 2 of the deaths did not happen as a direct result of cancer. In a majority of the patients, tumors were still present, but their progression had stopped, or they had started to shrink, although they had not disappeared entirely. And it is, of course, possible for a patient to live happily with cancer, when the disease is under control in the sense that there is no progressive development of the tumors any more—when they have stopped to grow, and there are no new metastases.

In one of those six cases that had shown partial tumor regression after 18 months, the dosage of CoQ_{10} was increased to 390 mg/day (2). In 1 mo, the tumor was no longer palpable and in another month, mammography confirmed the absence of tumor (2). Encouraged by this observation, another case having a verified breast tumor, after non-radical surgery and with verified residual tumor in the tumor bed was then treated with 300 mg CoQ_{10} (2). After 3 mo, the patient was in excellent clinical condition, and there was no residual tumor tissue (2). In an attempt to try to explain these observations, the authors believed that the bioenergetic activity of CoQ_{10}, expressed as hematological or immunological activity, might have been dominant, but not the sole molecular mechanism causing the regression of breast cancer (2).

In a later report, more information is given in these cases plus yet another with complete tumor regression (3). The numerous metastases in the liver of a 44-year-old patient "disappeared," and no signs of metastases were found elsewhere (3). A 49-year-old patient, on a dosage of 390 mg of CoQ_{10}/day, revealed no signs of tumor in the pleural cavity after 6 mo, and her condition was excellent (3). A 75-year-old patient with carcinoma in one breast showed after lumpectomy and 390 mg of CoQ_{10}/day no cancer in the tumor bed or metastases (3). Blood levels of CoQ_{10} of 0.83–0.97 increased from ranging from 0.62 μg/ml to 3.34–3.64 before the intervention to 3.77 μg/ml, respectively, on therapy with CoQ_{10} (3).

DISCUSSION

It is reasonable to believe that part of the explanation for the good results obtained in the ANICA trial must have been suppression of prostaglandin production in the tumor cells in a fairly high proportion of the patients, and perhaps all, because of a synergistic interaction between the supplementation with long-chain omega-3 PUFAs at a high dosage level, Se and some small-molecular antioxidants, both the endogenous antioxidant coenzyme Q_{10} and the exogenous ones vitamin C and vitamin E, all at high levels. The rate of prostaglandin synthesis depends strongly on the dietary omega-6/omega-3 PUFA ratio (7), but also on the intake of antioxidant nutrients such as

Se and GSH precursor amino acids because of strong redox regulation both of the activity of phospholipase A_2, of COX-1 and COX-2, and of the expression of COX-2 (7).

COX-2 is often, but far from always, expressed in the tumor cells of women with breast cancer. Aberrant upregulation of COX-2 resulting in accumulation of PGE_2 in a cancer cell environment is a marker for progression of many cancers, including breast cancer (8). COX-2 expression in breast cancer tumor cells has been found to play a role in the establishment of metastatic tumors (8), to be positively related to the invasiveness of the tumors (9), to recruit regulatory T cells (Tregs) to the tumor (10), and to promote radioresistance (11) via p38/MAPK-mediated cellular anti-apoptosis and invasiveness (11), although inhibition of COX-2 in breast cancer cells has been reported to decrease breast cancer cell motility and migration (12,13), metastasis (14–16), invasiveness (12,13), matrix metalloproteinase expression (13), tumor angiogenesis (12,15), tumor lymphangiogenesis (15), and growth (15), as well as enhancing tumor-induced osteoclastic lesions in breast cancer bone metastasis (17). There is now much evidence suggesting that COX-2 may be an Achilles' heel for CoX-2-dependent tumors (18). PGE_2 inhibits antitumor leukocytes (7), such as NK cells (19–22) and stimulates tumor angiogenesis (7).

It can be concluded that it is reasonable to believe that reduction of prostaglandin biosynthesis in the tumor may have been an important part of the explanation for the good results observed in the ANICA trial. However, the results were too impressive that it is reasonable that inhibition of prostaglandin biosynthesis can alone be a sufficient explanation for the good results.

Because an apparent effect could be noticed on some of the patients after the daily dose of coenzyme Q_{10} was changed from 90 mg/day to 390 mg/day, one gains at face value the impression that coenzyme Q_{10} may have been important for the recovery (Olav Albert Christophersen, personal communication, July 28, 2013). One possible explanation might be local depletion of coenzyme Q_{10} in the tumor because of high oxidant stress leading to fast coenzyme Q_{10} degradation by peroxidation processes inside the tumor, which might perhaps have been combined with impaired coenzyme Q_{10} synthesis inside the tumor because of too much oxidative stress or because of inadequate intra-tumor supply of some of those numerous vitamins that are needed for normal synthesis of coenzyme Q_{10}. Depletion of mitochondrial coenzyme Q_{10} below normal must be expected to lead to enhancement of the ratio between ratios of ROS and ATP production, which must be considered harmful to most normal cells, but especially so when it happens in male germ cells (23). However, for a tumor cell it is not necessarily only harmful if the rate of mitochondrial ROS production is enhanced.

Retrospectively, it is an attractive working hypothesis that the substances most important for the total effect of the dietary supplement cocktail that were given to the patients in the ANICA trial may have been coenzyme Q_{10}, Se, and the fish oil. The possible role of vitamin E is open to question because there are reports of experiments with alpha-tocopherol for treatments of other diseases giving disappointing results. It is possible that one of the reasons for this might be differences in the biological activity for different natural forms of vitamin E, and that alpha-tocopherol alone is not the same as natural vitamin E. However, it is possible that part of the explanation also may be found in the observations of Stocker and his group that vitamin E alone functions as a prooxidant catalyst of the oxidation of low density lipoprotein (LDL), although various so-called co-antioxidants, including coenzyme Q_{10}, change the function of vitamin E from being a prooxidant to become instead an antioxidant reducing the rate of LDL oxidation (24–27). So it is possible that a vitamin E/coenzyme Q_{10} combination may work better as biological antioxidants than each of them given alone.

Selenium has opposite effects on living cells in a physiological and toxic dose range, being important as part of antioxidative enzymes when present at physiological levels, but a prooxidant producing much ROS in the toxic range (28). Reports about Se causing death of tumor cells reflect these opposite functions because Se can act like an ordinary cytotoxic agent in tumor cells when the concentration is high enough (29,30), but it can sometimes also induce the death of tumor cells when given at high physiological levels, where it must be expected to exert mainly an antioxidant effect also inside the tumor cells (31).

In one such study, sodium selenite-induced apoptosis in murine B-lymphoma cells was found to be associated with inhibition of protein kinase C-delta, nuclear factor kappa B, and inhibitor of apoptosis protein (31). In another study, it was found that sodium selenite and a novel synthetic compound, methylseleninic acid, that served as a precursor of the putative active monomethyl metabolite methylselenol inhibited cell growth and induced apoptosis in prostate carcinoma cells. It was also found that these substances, when given in cytotoxic doses, inhibited NF-kappa B DNA binding induced by tumor necrosis factor-alpha and lipopolysaccharide in prostate cells (**32**). Selenium has, moreover, also been reported to inhibit the expression of various peptide signal substances that can be secreted by prostate cancer cells and either may help to stimulate the growth of the tumor or in other ways may be harmful to the rest of the body, such as VEGF, $TGFbeta_1$, and IL-6 (**33**).

In the ANICA trial, Se was not given at a toxic level, but at physiological levels high enough (especially during the first part of the study) that a synergistic interaction between Se and coenzyme Q_{10} is not implausible (Olav Albert Christophersen, personal communication, July 28, 2013).

The final report from the ANICA study was as already mentioned never written. However, the published preliminary results from the trial were very promising; it seems, therefore, important to follow up this study. There is a need for more

research to evaluate the therapeutic effect of the combined use of antioxidative vitamins, coenzyme Q10, Se, and essential fatty acids in cancer patients.

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