R. TRIVISONNE

Supportive treatments and whey proteins in cancer patients

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Interventi di supporto e proteine del siero di latte nel paziente oncologico

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Medical Oncology - "Mazzoni Hospital" z.t.13 - ASUR Marche
Ascoli Piceno, Italy

Address for correspondence:
Dr. Raffaele Trivisonne
Medical Oncology - "Mazzoni Hospital"
z.t.13 - ASUR Marche
Ascoli Piceno, Italy
E-mail: r.trivisonne@asl13.marche.it

Summary
In the treatment of neoplastic patients many toxicities caused by chemo or radiotherapy are related to the formation of free radicals and to the oxidative stress and have been treated with antioxidants products. Besides some vitamins (A, E, C, carotenoids, etc) few specific antioxidants are used in the supportive treatment in medical oncology: amifostine, dexrazoxane, sodium 2-sulfanylethanesulfonate (MESNA), glutathione (GSH). The aminoacids included in the milk whey proteins (cysteine and glutamic acid) as precursors of GSH can increase the tissue concentration of GSH, stimulate the immunologic system, detoxify potential carcinogens and neutralize the Reactive Oxygen Species (ROS). The milk proteins are used in the supportive treatment of the neoplastic patient especially for the antioxidants properties of GSH. A whey protein concentrate supplemented with a high content of cysteine and glutamic acid, as precursors of GSH, given to breast cancer patients under chemo or radiotherapy, has led to an increase of BAP test (plasma anti-oxidant capacity) and of d-ROMs test (plasma oxidant capacity). Cysteine as precursor of milk proteins as well as of GSH may also be considered an important agent in the supportive treatment of neoplastic cachexia. We still need controlled clinical trials and reliable guide lines in order to use natural or pharmaceutical supplements in medical oncology.

Riassunto
Molte delle tossicità da chemioterapia e da radioterapia correlate alla formazione di radicali liberi e allo stress ossidativo sono state trattate con l'impiego di anti-ossidanti. Oltre alle vitamine (A, E, C, carotenoidi, etc) ci sono alcuni particolari anti-ossidanti, che vengono impiegati nel trattamento di supporto in chemioterapia oncologica: amifostina, dexrazoxane, sodio 2-sulfaniletanosulfonato (MESNA), glutatione (GSH). Gli aminoacidi contenuti nelle proteine del siero di latte (cisteina e acido glutammico) in quanto precursori del GSH sono in grado di aumentare in maniera rilevante la concentrazione tissutale di GSH, di stimolare il sistema immunitario, di detossificare i potenziali cancerogeni e di distruggere le specie reattive centrate sull'ossigeno (Reactive Oxygen Species, ROS). Le proteine del siero di latte sono impiegate nel trattamento di supporto del paziente oncolo-

gico specie in riferimento alle capacità antiossidanti del GSH. Un concentrato di proteine di siero di latte addizionato di cisteina e acido glutammico (come principali precursori del GSH) in aggiunta al trattamento oncologico standard (radioterapia e chemioterapia) in pazienti con neoplasia della mammella ha fatto riscontrare un incremento della capacità anti-ossidante del plasma (misurata con il BAP test) e della capacità ossidante del plasma (misurata con il d-ROMs test). La cisteina come precursore sia delle proteine sieriche che del GSH (antiossidante cellulare) può essere considerata un importante supporto nella cachessia neoplastica. Mancano tuttora studi clinici controllati e linee guida accreditate per l'impiego degli integratori naturali o farmacologici in ambito oncologico.

Introduction

Treatment of the cancer patient consists in the use of therapeutical strategies that aim at containing, on the one hand, the evolution of the neoplastic disease and, on the other, the side effects of the treatments used.

In oncology, therapeutic treatments are often integrated and consist of surgical interventions, chemotherapy treatment with antiblastic drugs (associated or not with molecular target biological drugs) and radiotherapy treatment. The approach to the cancer patient is today defined as being a "global approach" in that not only is the medical treatment (chemotherapy, hormone therapy) or radiotherapy evaluated but also the following interrelated aspects are considered:

- control of early and long-term side effects,
- control of iatrogenic effects due to treatment,
- prevention of relapses (tertiary prevention),
- psychosocial aspects,
- psychophysical rehabilitation,
- treatment of clinical symptoms and support care in advanced disease.

Ultimately, today the oncologist deals with guaranteeing the cancer patient an adequate "quality of life" within each stage of the neoplastic disease.

Toxic effects of cancer treatments

One of the major problems encountered in oncology assistance programs is represented by management of treatment toxicity.

The toxic effects of chemotherapy and radiotherapy are due to the action exerted collaterally on healthy organs and body systems. The most common forms of toxicity are:

- Hematologic toxicity (leukopenia, piastrinopenia, anaemia)
- Gastroenteric toxicity (mucositis, nausea, vomiting, diarrhoea, constipation)
- Cardiac toxicity (changes in heart rhythm and heart function, pericarditis)
- Neurological toxicity (altered consciousness, peripheral neuropathy)
- Nephrotoxicity (changes in creatinine levels, azotaemia, cystitis)
- Hepatotoxicity (changes in bilirubin levels, ALT, AST)
- Cutaneous toxicity (erythema, desquamation, folliculitis, alopecia)
- Fever and infections.

Whilst the prevention measures and treatment of specific forms of toxicity of certain chemotherapic drugs and subsequent complica-

tions are well known, it has long been hypothesized that the administration of antioxidants during chemotherapy and/or radiotherapy can protect normal tissues against the toxic side effects of these treatments.

The main therapeutic effect of radiotherapy is given by the biological effect of the ionization of water molecules present in the cellular cytoplasm from which ROS (reactive oxygen species), such as superoxides and hydroxylic radicals (free radicals) originate. Similarly, many antiblastic agents [anthracyclines (doxorubicin, epidoxorubicin), platinum compounds (cisplatin, carboplatin, oxaliplatin), alkylating agents (cyclophosphamide, ifosfamide) and antibiotics (bleomycin, mitomycin-C)] manifest their cytotoxic effect through the production of ROS.

Many forms of chemotherapy and radiotherapy toxicities have been treated with antioxidants as correlated with the formation of free radicals and oxidative stress and have been the subject of many studies (1).

Under normal conditions the human body is able to neutralise the damage caused by free radicals thanks to natural antioxidant defence mechanisms. The body produces endogenous antioxidants (superoxide dismutase, catalase, glutathione peroxidase, coenzyme Q, melatonin, transferrin, alpha-lipoic acid, ubiquinone, etc.) and exogenous antioxidants present in food or in nutritional supplements (ascorbic acid, alpha-tocopherol, carotenoids etc.)

Antioxidants have been used in oncology not only as supportive care agents in the prevention and contrast of antineoplastic-treatment-related effects, but also as cancer preventive agents.

Although antioxidants by definition clean up free radicals, one should bear in mind that they can also act through other mechanisms of action that involve cellular proliferation, apoptosis, angiogenesis, neoplastic growth and metastatization processes (2).

In vitro and *in vivo* studies carried out both in experimental animals as well as in Humans, have shown that antioxidants such as retinoids (vitamin A), alpha-tocopherol (vitamin E), sodium ascorbate (vitamin C) and carotenoids, alone or in combination, induce cellular differentiation, increase the inhibitory effects of neoplastic growth due to radiation and antiblastic drugs through complex mechanisms and are capable of reducing the toxicity of many antiblastic drugs (3).

Reviews of these studies evaluating the clinical use of antioxidants in oncology have underlined some important aspects: studies carried out on the use of antioxidants in neoplastic patients greatly differ according to study design, statistical power, type of intervention, duration of observation, type of tumour etc. and therefore, caution must be taken when considering antioxidant therapy and use of nutritional supplements in general (4-6).

In a thorough review of 22 studies that observed patients with breast cancer undergoing radiotherapy, chemotherapy or hormone therapy in which several nutritional supplements were used (vitamin C, vitamin E, multivitamins, glutamine, glutathione, melatonin, soy isoflavones, antioxidant combinations) it was impossible to reach any conclusion on the antitumoural efficacy of these specific treatments on prevention of disease relapses and on survival. Some of the studies analysed reported that supplementation with antioxidants may reduce some of the side effects due to these treatments and in particular vitamin E has proved to have a positive effect on hot flushes following hormone therapy and glutamine on chemotherapy-induced oral mucositis. Therefore, the studies carried out to date (clinical and observational) have proven to be inadequate in demonstrating the actual usefulness of antioxidant supplements in breast cancer treatment and further clinical trials and adequately powered observational studies are needed in order to reach a conclusion (7).

One must also bear in mind that antioxidants can interfere with the effects of radiotherapy and antiblastic agents by neutralising or reducing their therapeutic effect. Therefore, great caution is recommended when considering the potential effect that some antioxidants could have in reducing therapy efficacy (1).

On the other hand, a recent review dealt with the topic of antioxidants in a "positive and enthusiastic" way and concluded by stating that: antioxidants and other supplements when administered with concomitant chemotherapy and/or radiotherapy: 1. do not interfere with the effect of the therapy; 2. increase the cytotoxic effect of chemo and radiotherapy; 3. have a protective effect on normal tissues; 4. increase patient survival and "the use of antioxidants and other dietary supplements" is in fact recommended " as they are safe and capable of improving cancer patient treatment" (8).

Then, there are particular antioxidants such as amifostin, dexrazoxane, MESNA and glutathione that are different to vitamins and that have become part of common clinical practice in the supportive treatment of oncologic chemotherapy as they have been shown to have clinical efficacy.

Amifostin (Ethyol™) is indicated for the prevention of both early and late onset of cisplatin-induced nephrotoxicity in patients who have undergone cervico-facial radiotherapy (9).

Dexrazoxane (Cardioxane™) is indicated in association with anthracycline chemotherapy (doxorubicin, epidoxorubicin) to reduce cardiac toxicity (9).

Furthermore, dexrazoxane (Savene™ - Spepharm) is indicated in the treatment of the local necrotizing effect due to accidental extravasation during venous infusion of anthracyclines leading to a likely ferrochelating effect similar to that caused by EDTA from which dexrazoxane originates (10).

MESNA (Uromitexan™) is a thiol compound which acts as an antioxidant providing protection against inflammatory-haemorrhagic phenomena on the bladder mucosa caused by acrolein and other degradation metabolites of the antiblastic agents cyclophosphamide and ifosfamide (11).

Glutathione (GSH) has been shown to reduce toxicity of platinum-derived chemotherapy agents (cisplatin, carboplatin, oxaliplatin) probably due to the affinity of cysteine's SH-thiol group for heavy metals. Glutathione is a tripeptide (glycine, cysteine and glutamine) that plays an important role in the antioxidant mechanism as it acts at the cytoplasmic level near the mitochondrial apparatus, where most ROS is produced. Glutathione, spontaneously or through the intervention of peroxidase, releases the necessary H+ ions to neutralize free radicals. The active element in the reaction is represented by cysteine's SH-thiol group that is oxidized to cysteine disulphide when performing its antioxidant activity.

Glutathione was used in a randomised clinical phase III trial studying 151 patients with ovarian cancer, treated with cisplatin chemotherapy. Of these, 74 patients were also treated with 3g/m^2 of GSH every 3 weeks: the addition of GSH reduced peripheral nephrotoxicity, cisplatin-induced nephrotoxicity and ototoxicity, improved quality of life and reduced weight loss (there was an average weight loss of 2 Kg in patients who did not receive GSH treatment) (12).

In another phase III clinical study GSH was used in patients with metastatic colorectal cancer and treated with oxaliplatin-fluorouracil chemotherapy. An intravenous dose of GSH of 1.5 g/m^2 every 2 weeks was able to reduce the peripheral oxaliplatin-induced nephrotoxicity without reducing the clinical efficacy of oxaliplatin (13).

Therefore, GSH is used in some protocols in association with chemotherapy mainly in the prevention of neurologic toxicity.

Whey proteins

Many of the effects of whey proteins have been studied in several

pathological conditions associated with cancerogenesis, chemo/radiotherapy toxicity, neoplastic cachexia, as well as with immune processes and pathological conditions of other organs and body systems: bone, muscle, kidney, pancreas, nervous system, vascular system etc (14).

The aminoacids contained in whey proteins (in particular cysteine and glutamic acid) being GSH precursors, are able to do the following:

1. markedly increase GSH tissue levels
2 boost the immune system
3. detoxify potential carcinogens
4. destroy free radicals (ROS)
5. maintain proteins in a reduced state (15).

Whey proteins have been widely studied in animal models (rats) for cancer prevention and dietary supplementation with whey protein leads to an increase in serum and tissue levels of GSH, an increased splenic lymphocyte proliferation, an increased phagocytosis of natural killer T helper cells and an increase in T cell cytotoxic activity (16).

The effects of whey on colon and breast tumours induced by carcinogens have been studied in animal models. Dietary supplementation with whey was able to reduce the incidence of tumours in rats and it is thought that lactoferrin, in particular, may be responsible for the anti-carcinogenic effect (17, 18).

In particular, lactoferrin has attracted a lot of attention as it seems to act through various anti-neoplastic mechanisms inducing apoptosis, inhibiting angiogenesis, modulating enzymes that metabolize carcinogenic agents and acting as an iron chelating agent. In particular, whey seems to be able to exert an antineoplastic activity also due lactoferrin's iron-chelating ability, as iron is considered a mutagen agent capable of causing oxidative tissue damage (19).

An *in vitro* study demonstrated that a whey protein concentrate compared to a preparation of casein proteins was able to increase GSH synthesis thus protecting human prostate cells from cell death induced by an oxidant effect (20).

To date, the ability of whey proteins to contrast cancerogenesis has been demonstrated in numerous animal models.

Supportive care in the cancer patient

Whey proteins seem to play a key role in the support care of the cancer patient especially in relation to GSH antioxidant properties.

Bounous demonstrated that whey proteins have elevated levels of cysteine residues (serum albumin, alpha-lactalbumin, lactoferrin) that are easily absorbed at intracellular level increasing the level of glutathione (GSH) that in turn expresses its antioxidant action by destroying free radicals, detoxifying potential carcinogens and stimulating the immune system (21).

Oxidative stress is the condition under which tissue levels of ROS are produced in excess compared to the body's natural ability to remove them. Therefore, the use of antioxidants in neoplastic cells, that have a hyper production of ROS, may be accompanied by a reduction in neoplastic growth.

On the basis of these considerations, Bounous reported the results related to the use of a whey protein concentrate enriched with casein in cancer patients. In 13 out of 15 patients with prostate cancer, during the administration of the whey protein concentrate a progressive reduction of PSA was observed; in 1 patient with renal cancer a significant reduction of hepatic and pulmonary metastasis was observed and this lasted 3 years; in 1 patient with bladder cancer after 1 year of treatment with whey proteins no recurrence was observed (15).

In a preliminary study, Bounous and colleagues used a whey protein concentrate in a small series of cancer patients following the hypothesis according to which

whey proteins can reduce the level of GSH in tumour cells (that contain elevated levels of GSH) making them susceptible to the antineoplastic effect of chemotherapy (22).

Regarding the antioxidant ability of GSH, a recent study considered a whey protein concentrate enriched with cysteine and glutamic acid (as principal precursors of GSH) (Prother™) used in addition to a standard cancer treatment (radiotherapy and chemotherapy) in patients with breast cancer (23) In 20 patients treated for 12 months with a daily oral dose of 10 g of whey proteins enriched with cysteine and whose oxidative balance was evaluated every two months, both an increase in the antioxidant capacity of plasma (measured with the BAP test) and in the oxidant capacity of plasma (measured with d-ROMs test) were recorded. In the patients with breast cancer and undergoing chemo/radiotherapy as treatment it was observed that an increase in the consumption of antioxidants reduced the plasmatic antioxidant barrier (4), therefore the oral supplement therapy with the whey proteins used in this study was able to progressively reestablish the potential antioxidant effect of plasma that returned to its optimal levels (23).

Neoplastic cachexia

Another very important clinical aspect in oncology is represented by neoplastic cachexia. A standard treatment of neoplastic cachexia does not exist and many efforts have been made to improve conditions in patients with cachexia. The following were used: medroxyprogesterone acetate, glucocorticoids, antiserotonins to contrast anorexia in the cachectic patient and stimulate appetite, prokinetic agents and antinausea drugs to contrast a sense of satiety and nausea due to opioids, branched chain aminoacids and eicosapentanoic acid as integrated support (24, 25).

The neoplastic cachexia syndrome is strictly due to a state of oxidative stress confirmed by increased levels of ROS, reduced levels of glutathione peroxidase and an increase in serum proinflammatory cytokines IL-6 (interleukin 6) and TNF-alpha (tumour necrosis factor alpha). The use of antioxidant agents such as alpha-lipoic acid, carbocysteine, amifostin, glutathione (GSH) and vitamins A, C, E in advanced stage cancer patients leads to the reduction of oxidative stress and serum levels of ROS and proinflammatory cytokines and to an increase of glutathione peroxidase activity corresponding to a good correlation with performance status (26).

A similar study carried out on cachectic patients showed that by adding a cycloxigenase-2 inhibitor to the products already in use, there was an increase in lean body mass, weight, appetite, improvement of quality of life and a reduction of fatigue (27).

The impact of oral whey protein supplementation was evaluated also in neoplastic cachexia. A recent study evaluated 66 patients with pulmonary cancer and 35 patients with advanced colon cancer subjected to 6 months of oral supplementation with cysteine rich whey proteins versus an oral supplementation with casein. Patients treated with casein enriched whey proteins had a 2.5% increase in weight compared to a 2.6% loss in weight in patients supplemented with oral casein. Cysteine, being a precursor not only of serum proteins but also of GSH (a powerful intracellular antioxidant) may be considered as an important support therapy in fragile cancer patients with weight loss (neoplastic cachexia) and may correct the redox state of plasma swayed in an oxidative direction in the cancer patient in a neoplastic cachectic phase (28).

Conclusions

The use of natural or pharmacological nutritional supplements in oncology, though supported by in-

teresting data from *in vitro* and *in vivo* experimental animal models and some clinical and observational studies, has not yet been confirmed by controlled clinical trials that demonstrate the actual efficiency of their use in clinical practice.

We still lack accredited guide lines regulating the use of nutritional supplements as support care for cancer patients. Therefore, one should apply an ethical-professional principal "primum non nocere" by also using natural products (nutraceutics) like, for example, whey proteins in the support therapy to the cancer patient, especially during the more critical phases of the neoplastic illness.

Adequately designed clinical trials and observational studies are still needed to reach a conclusion on the actual benefit of integrating treatment of the cancer patient with natural or pharmacological products.

References

1. Ratnam DV, Ankola DD, Bhardwaj V, et al. Role of antioxidants in prophylaxis and therapy: a pharmaceutical perspective. J Control Release 2006; 20(113): 189-207.
2. Blumberg J, Milbury P. Dietary flavonoids. In Bowman BA, Russell R eds. Present knowledge in nutrition. 9th ed. Washington DC:ILSI Press 2006; 361-70.
3. Prasad KN, Kumar A, Kochupialli V, et al. High doses of multiple antioxidant vitamins: essential ingredients in improving the efficacy of standard cancer therapy. J Am Coll Nutr 1999; 18, 13-25.
4. Ladas EJ, Jacobson JS, Kennedy DD et al. Antioxidants and cancer therapy: a systematic review. J Clin Oncol 2004; 22: 517-28.
5. Block KI, Koch AC, Mead MN, et al. Impact of antioxidant supplementation on chemotherapy efficacy: a systematic review of the evidence from randomized controlled trials. Cancer Treat Rev 2007; 33: 407-18.
6. Prasad KN. Rationale for using high-dose multiple dietary antioxidants as an adjunct to radiation therapy and chemotherapy. J Nutr 2004; 134: 3182S-3S.
7. Greenlee H, Hershman DL, Jacobson JS: Use of antioxidant supplements during breast cancer treatment: a comprehensive review. Breast Cancer Res Treat 2009; 115: 437-52.
8. Simone CB 2nd, Simone NL, Simone V, Simone CB: Antioxidants and other nutrients do not interfere with chemotherapy or radiation therapy and can increase kill and increase survival. Altern Ther Health Med 2007; 13: 22-28 part 1 and 40-46 part 2.
9. Mouridsen HT,Langer SW, et al. Treatment of anthracycline extravasation with Savene (dexrazoxane): results from two prospective clinical multicentre studies. Ann Oncol 2007; 18: 546-50.
10. Hensely ML,Hagerty KL, et al. American Society of Clinical Oncology 2008 Clinical Practice Guideline Update: Use of chemotherapy and radiation protectants. J Clin Oncol 2008; 27:127- 45.
11. Dorr R. T. Chemoprotectants for cancer chemotherapy. Semin Oncol 1991; 19(Suppl.2): 48-58.
12. Smyth JF, Bowman A, Perren T, et al. Glutathione reduces the toxicity and improves quality of life of women diagnosed with ovarian cancer treated with cisplatin: results of a double-blind, randomised trial. Ann of Oncol 1997; 8: 569-73.
13. Cascinu S, Catalano V, Cordella L et al. Neuroprotective effect of reduced glutathione on oxaliplatin-based chemotherapy in advanced colorectal cancer: a randomized, double-blind, placebo-controlled trial. J Clin Oncol 2002; 20, 3478-83.
14. Krissansen GW. Emerging health properties of whey proteins and thei clinical implications. A review. J Am Clin Nutrition 2007; 26: 713S-23S.
15. Bounous G. Whey protein concentrate (WPC) and gluthatione modulation in cancer treatment. Anticancer Res 2000; 20: 4785-92.
16. Parodi PW. A role for milk proteins and their peptides in cancer prevention. Curr Pharm Des 2007; 13: 813-28.
17. Tsuda H, Sekine K, Nakamura J, et al. Inhibition of azoxymethane initiated colon tumor and aberrant crypt foci development by bovine lactoferrin administration in F344 rats. Adv Exp Med Biol 1998; 443: 273-84.
18. Hakkar R, Korourian S, Shelnutt SR et al.Diets containing whey proteins or soy protein isolate protect against 7,12-dimethylbenz(a)anthracene-induced mammary tumors in female rats. Cancer Epidemiol Biomarkers Prev 2000; 9: 113-7.
19. Weinberg ED. The role of iron in cancer. Eur J Cancer Prev 1996; 5: 19-36.
20. Kent KD, Harper WJ, Bomser JA: Effect of whey protein isolate on intracellular glutathione and oxidant-induced cell death in human prostate epithelial cells. Toxicol *In Vitro* 2003; 17: 27-33.
21. Bounous G, Batist G, Gold P. Immunoenhancing property of dietary whey protein in mice: role of glutathione. Clin Invest Med 1989; 12: 154-61.
22. Kennedy RS, Konoki GP, Bounous G, et al. The use of a whey protein concentrate in the treatment of patients with metastatic carcinoma: a phase I-

II clinical study. Anticanc Res 1995; 15: 2643-50.
23. Landoni G, Mariani E, Oriani G, et al. Improvement of antioxidant status in women conventionally treated for breast cancer after 12 months of a cow milk whey-based supplementation A preliminary study. Mediterr. J. Nutr. Metab 2009; 2: 127-31.
24. Nelson KA: The cancer anorexia-cachexia syndrome. Semin Oncol 299; 27: 64-8.
25. Nitenberg G, Raynard B. Nutritional support of the cancer patient: issues and dilemmas. Crit Rev Oncol Hematol 200; 34: 137-68.
26. Mantovani G, Maccio A, Madeddu C et al. The impact of different antioxidant agents alone or in combination on reactive oxygen species, antioxidant enzymes and cytokines in a series of advanced cancer patients at different sites: correlation with disease progress. Free Radic Res 2003; 37: 213-23.
27. Mantovani G, Madeddu C, Macciò A. Cancer-related anorexia/cachexia syndrome and oxidative stress: an innovative approach beyond current treatment. Cancer Epidem Biomark Prev 2004; 13: 1651-9.
28. Tozer R, Tai P, Falconer W, et al. Cysteine-rich protein reverses weight loss in lung cancer patients receiving chemotherapy or radiotherapy. Antiox & Redox Signalling 2008; 10: 395-402.