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Congenital fibrosarcoma in complete remission with Somatostatin, Retinoids, Vitamin D3, Vitamin E, Vitamin C, Melatonin, Calcium, Chondroitin sulfate associated with low doses of Cyclophosphamide in a 14-year Follow Up

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Abstract

At birth, a male child presented a 6 cm tumour in the right leg. The tumour was partially removed after just 12 days. Histology showed a congenital fibrosarcoma associated with reactive lymphadenitis. A first cycle of adjuvant chemotherapy did not prevent the rapid progression of the disease. Subsequent evaluation for surgical removal raised serious concerns due to the need for a major operation involving total amputation of the right leg and hemipelvectomy. Since surgery could not exclude the possibility of disease recurrence and since the traditional cycles of chemotherapy did not offer any possibility of a cure, the parents opted for the Di Bella Method. The combined use of Somatostatin, Melatonin, Retinoids solubilized in Vit. E, Vit. C, Vit. D3, Calcium, and Chondroitin sulfate associated with low doses of Cyclophosphamide resulted in a complete objective response, still present 14 years later, with no toxicity and without the need for hospitalization, allowing a normal quality of life and perfectly normal adolescent psychophysical development.

CASE REPORT

INTRODUCTION

Fibrosarcoma is a malignant tumour which develops from fibroblasts and affects above all the skin, tendons, fasciae musculares and periosteum. It is part of the group of soft tissue sarcomas, representing 10% of pediatric cases and is the most common soft tissue tumour in infants below the age of one year. The treatment of choice is surgical removal, since as in all solid tumours chemotherapy is unable to eradicate the neoformation. The tumoral mass is removed, except for cases involving the metaphysis, in which amputation is necessary. Surgery is thus of fundamental importance and may be the only treatment that can offer a cure. In some cases, depending on the characteristics of the tumour, cycles of chemotherapy are administered before surgery in order to reduce the size of the primary tumour.

The most active and investigated drugs are ifosfamide and doxorubicin or adriamycin. In infantile forms, in view of the better prognosis and

age of the patient, other potentially less toxic drugs are also used, such as vincristine and actinomycin. None of these protocols is, however, able to provide significant results, and the effects are limited to a temporary reduction in size of the tumour which is only achieved in a small percentage of cases.

The use of radiotherapy is reserved for selected cases due to the possible side effects of the treatment (for example in very small children). The possibilities of cure depend on surgery alone and are linked to the complete removal of the tumour (influenced by the site of the tumour, its size and its relationship to the surrounding organs).

We report this case since it documents, for the first time, the possibility of a complete objective and stable response of a congenital fibrosarcoma, obtained and maintained for over 14 years by means of the biological Di Bella Method (DBM). Other significant aspects include the absence of toxicity of this treatment and the possibility of following it at home.

CLINICAL FINDINGS –DIAGNOSTIC FOCUS AND ASSESSMENT

The child was born on 28.12.1998 by normal delivery and presented an inguinal neoformation measuring around 6 cm, extending to the proximal anteromedial part of the right leg.

11.01.1999 – Histology showed: "Neoplasia consisting of a monomorphous population of spindle cells with modest cellular pleomorphism attributable to fibroblasts and small lymphocyte-like rounded cells. Low mitototic index …" Immunohistochemistry:
Vimentin (+) – Desmin (–) – CK (–) – Smooth muscle actin (–) – Striated muscle actin (–) Lymph gland (H 15 B-99): Reactive lymphadenitis

15.01.1999 – Discharged from hospital following partial removal of congenital Fibrosarcoma on the right leg.

04.02.1999 – Adjuvant chemotherapy (4 cycles of VA), which did not prevent subsequent rapid progression.

05.03.1999 – US scan: "…the previously described tumour located at the proximal third of the right thigh appears to have slightly increased in size, maintaining the same echo-structural features as before. Dimensions: 47×23 mm…"

The consideration of further surgery gives rise to serious concerns due to the extent of such an operation, involving total amputation of the right leg and hemipelvectomy.

THERAPEUTIC FOCUS AND ASSESSMENT

In view of the fact that, despite its invalidating nature, the operation could not exclude the possibility of recurrence of the disease and that the traditional cycles of chemotherapy had no possibility of achieving a cure, the parents opted for the Di Bella Method (DBM).

At the age of 4 months, with the tumour progressing, the child started the DBM, consisting of:
Solution of:

- All-Trans-Retinoic Acid 0.5 g
- Palmitate axerophthol 0.5 g
- Beta carotene 2 g
- Alpha-tocopherol-acetate 1000 g

(one 2 ml teaspoonful of Solution every 3 hours) together with:
Dihydrotachysterol (one drop in the teaspoon for each administration)
Bromocriptine 2.5 mg tablets (1/4 of a tablet twice a day, morning and evening)
Melatonin 12%, Adenosine 51%, Glycine 37% (one 5 mg vial diluted in water)
Chondroitin sulfate (one 500 mg vial orally with the evening feed/meal)
Cyclophosphamide 50 mg tablets (1/4 of a tablet with the main feed/meal)
Somatostatin – 14 amino acids (one 0.25 mg vial slowly injected subcutaneously in the evening).

FOLLOW-UP AND OUTCOMES

07.05.1999 – US scan: "…on the inner surface at the top of the thigh an egg-shaped neoformation can be seen with a solid non-homogeneous echo-structure and a max. diameter of 41 mm. The transverse diameter of 24mm. located between the subcutaneous plane from which it seems separated by its wall and the muscular plane from which it does not appear to be clearly detachable…"

01.12.1999 – US scan: "…the pathological formation at the top of the right thigh appears to be slightly smaller in size, with a longitudinal diameter of around 35 mm. Its echo-structural features are unchanged …"

28.06.2000 – US scan: "…formation with a max diameter of 24 mm, non-homogeneous, in the compartment of the adductors…"

12.03.2001 – US scan: "…the formation at the top of the thigh, in the compartment of the adductors, presents unchanged echo-structural features with reduction of the longitudinal diameter (approx. 18 mm.)…"

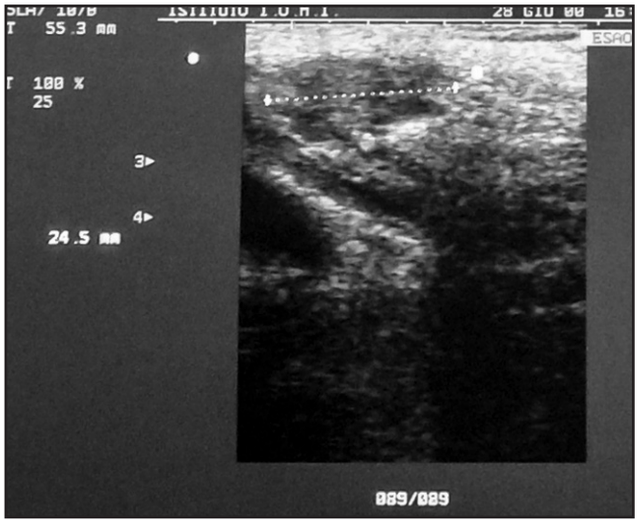
09.11.2001 – US scan: "…the formation appears to be slightly smaller in size, with a max diameter of around 12 mm…"

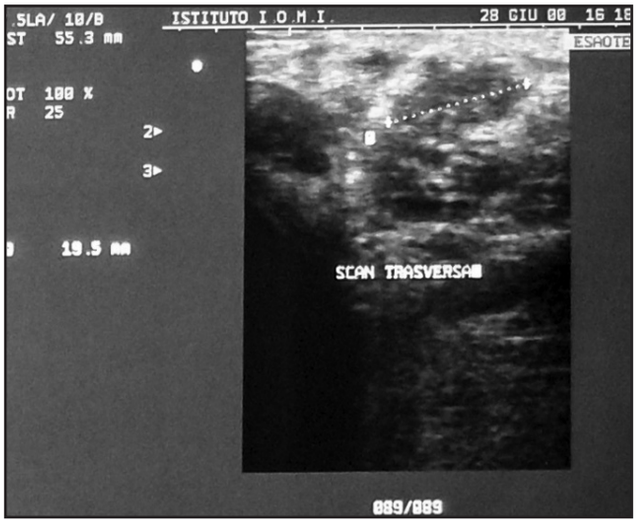
09.02.2002 – MRI "…compared with the previous investigations performed in 1999 and 2000, the nodular formation of pathological tissue can no longer be seen….there is slight atrophy of the venter musculi in the compartment compared to the contralateral ones …"

15.05.2002 – US scan: "…does not show signs of nodular type lesions…"

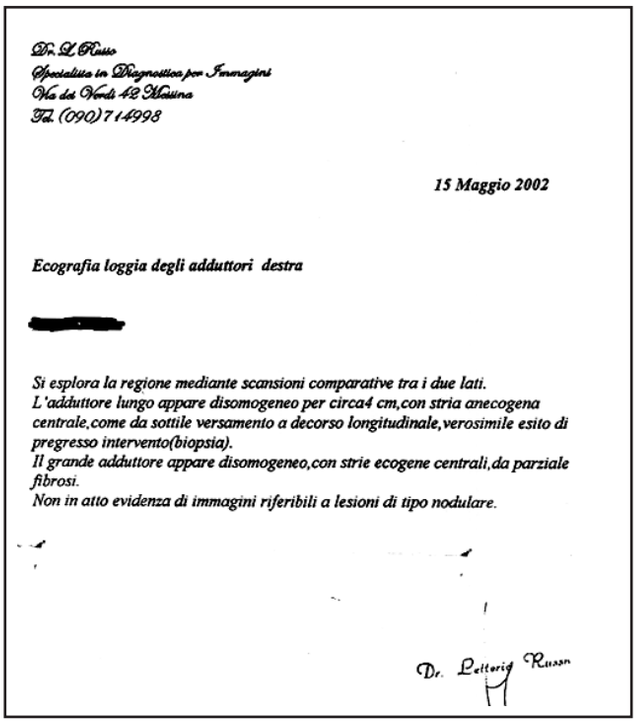
Subsequent investigations, up to the present day, have not revealed structural modifications.

The combined use of Somatostatin, Melatonin, Retinoids solubilized in Vitamin E, Vitamin C, Vitamin D3, with Calcium, Chondroitin sulfate, and low doses





of Cyclophosphamide produced a complete objective response, maintained for over 14 years. Without the need for hospitalization and in the absence of toxicity, the DBM first had a cytostatic effect and after 8 months it progressively reduced and then eliminated the large tumoral mass, allowing a normal quality of life and a perfectly regular psycho-physical development. The rationale and the molecular mechanisms of action of the therapy, which has a differentiating, immunomodulating, cytostatic, pro-apoptotic effect, are discussed. The treatment preserves and enhances the trophism and functionality of organs and tissues, and immune and antiblastic homeostasis. This result is in agreement with the positive results already published on the use of the DBM in lymphoproliferative diseases, in stage 3 and 4 lung cancer, in breast cancer and in tumours of the upper aerodigestive epithelia.



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15 Maggio 2002

Ecografia loggia degli adduttori destra

[redacted]

Si esplora la regione mediante scansioni comparative tra i due lati.
L'adduttore lungo appare disomogeneo per circa4 cm,con stria anecogena centrale,come da sottile versamento a decorso longitudinale,verosimile esito di pregresso intervento(biopsia).
Il grande adduttore appare disomogeneo,con strie ecogene centrali,da parziale fibrosi.
Non in atto evidenza di immagini riferibili a lesioni di tipo nodulare.

Dr. Letterio Russo

DISCUSSION

Cellular proliferation is strictly dependent on prolactin (Ben-Jonathan *et al.* 2002) on GH (Lincoln *et al.* 1998), the greatest inducer of growth and on GH-dependent mitogenic molecules, positively regulated by GH, such as EGF, FGF, HGF, IGF1-2, NGF, PDGF, TGF, VEGF (Kath & Höffken 2000). Numerous studies indicate that the pituitary hormones GH and PRL play a crucial role in the development and progression of human tumours. Their receptorial expression is ubiquitary (De Souza *et al.* 1974; Hooghe *et al.* 1998) with a dose/dependent relationship between the receptorial expression of GH and the processes of tumour induction and progression, detected histochemically and through Western Blot techniques, in situ hybridization and qPCR. The reports of markedly higher concentrations of GHR in tumoral tissues with respect to physiological and peritumoral tissues confirm its potent mitogenic role (Lincoln *et al.* 1998; Zeitler & Siriwardana 2000; Gebre-Medhin & Kindblom 2001). The temporal mechanics of this etiopathogenetic process is currently under investigation: the most likely hypotheses include the hypophyseal secretion of GH and PRL, and probable mechanisms of autocrine and/or paracrine signalling, on the basis of detection of local production of GH, GHR, PRL, PRLR and IGFI in the tissues of many types or tumours, including tumours of the nervous system.

SOMATOSTATIN

The use of somatostatin and its analogues, acting on the main oncogenes, is rationally indicated in all tumours (Schally *et al.* 2001; Massa 2004; Guillermet-Guibert *et al.* 2007; Lee *et al.* 2008; Di Bella *et al.* 1979; Verhoef

2008; Di Bella 2010; Di Bella & Di Bella 2015; Di Bella *et al.* 2015; Di Bella *et al.* 2013; Di Bella *et al.* 2013. The PRL/GH/GF axis has a certain influence on the biological development of a tumour, thus providing logical and rational support for the synergic and interactive anticancer use of anti-prolactinemic agonists of D2R with biological antagonists of GH, such as Somatostatin and its analogues, which extend their negative regulation to highly mitogenic, GH-dependent growth factors, EGF (Watt HL. 2008) and to the respective receptorial signalling pathways, with consequent antiproliferative, pro-apoptotic, differentiating and anti-angiogenic consequences, such as IGF1-2 (Taslipinar 2009). The IGFR respond mitogenically to IGF, and the suppressive effect of SST and its analogues on the serum levels of IGF1 is both direct (through inhibition of the IGF gene) and indirect, through suppression of the GH and thus of its hepatic induction of IGF1(Hagemeister & Sheridan 2008). The antiproliferative effect of the analogues of somatostatin on sarcomas, as on other tumours, thus takes place also through mechanisms that involve the suppression of the IGF system.

A receptorial expression for Somatostatin has been documented in many non-endocrine tumours (Friend 2000; Alberini 2000; Stetak 2001; Orlando 2001; Florio 2008; Ioannou & Khanna 2008; Pisarek 2009; Ruscica 2010). A markedly higher expression of GH and GHR has also been confirmed in tumoral tissues than in healthy tissues (Lincoln *et al.* 1998). The hypothalamic hormone GHRH stimulates the synthesis and release of GH by the pituitary gland, and its mRNA has been found at much higher concentrations in a wide variety of tumours compared to healthy tissues. Various studies have shown that the GHRH antagonist analogues of somatostatin also cross the blood-brain barrier without difficulty (Kovács 2010). Until the cells that make up the first tumoral mass of just a few millimetres are able to create their own system of blood vessels (neoplastic angiogenesis), they grow very slowly and will remain at the stage of "in situ cancer". The expansion of the tumour takes place only when angiogenesis occurs, i.e. when a network of blood vessels is formed to guarantee the supply of nutritional substances and the elimination of metabolic waste. The literature shows that all the steps of angiogenesis and the molecules synergically involved in this process (both promoters of angiogenesis and angiogenic growth factors) are negatively regulated by somatostatin and its analogues and, albeit to a lesser extent, by all the other components of the DBM. While angiogenesis is an obligatory step in tumoral expansion and angiogenesis is totally inhibited by somatostatin, the literature clearly shows that somatostatin is indicated in all tumours, whether or not its receptor is present.

Local situations of anoxia and acidosis favour angiogenesis, and are to a good extent corrected by the improvement in blood-tissue exchanges induced by the differentiating components of the DBM.

The phenomena of angiogenesis and neoangiogenesis, necessary conditions for growth of the tumour, like the cascade of monocytes, the paracrine release of interleukin 8 and the contribution of the growth factors (whose synergism is essential for angiogenesis), such as VEGF (Sall *et al.* 2004), TGF, IGF1, FGF, HGF, and PDGF (Cattaneo 1999), represent specific molecular targets negatively regulated by Somatostatin and its analogues (Albini *et al.* 1999; Cascinu *et al.* 2001; Florio *et al.* 2003; Jia *et al.* 2003). The inhibition of angiogenesis induced by SST is synergically reinforced by MLT (Lissoni *et al.* 2001), Retinoids (McMillan *et al.* 1999; Kini *et al.* (2001), vitamin D^3 (Kisker *et al.* (2003), Vitamin E (Neuzil *et al.* 2002), Vitamin C (Ashino *et al.* 2003), prolactin inhibitors (Turner *et al.* 2000) and by components of the extracellular matrix (Liu *et al.* 2005). Simultaneously, the same cytostatic, antiproliferative, and antimetastatic effects of Somatostatin are synergically increased by the other components of the DBM.

The inhibitory activity of SST on another potent mitogenic growth factor, EGF, through multiple mechanisms, is also widely documented:

- Block of the dose-dependent signalling (Inhibition of tyrosine phosphorylation) of EGFR (Mishima *et al.* 1999);
- Reduction of the expression of EGFR and its ligand (EGF) in tumour cells (Szepesházi *et al.* 1999);
- Total reduction of the plasma concentration of EGF (Castro 2000).

This inhibition is further reinforced by the concomitant administration of MLT and Vitamin D3, whose modulatory activity with respect to the epidermal growth factor is well known.

THE RECEPTORIAL EXPRESSION OF SOMATOSTATIN IN SARCOMAS

In a study on advanced-stage bone tissue sarcomas, 71% of patients had a positive scintigraphy demonstrating the tumoral expression of sst2 somatostatin receptors in vivo (Friedberg & Van den Abbeele 1999). Another study (Giannakenas *et al.* 2000) showed a positive scintigraphic finding for SSTR in 12/13 cases, while occult lesions were detected in 2 patients. Histological typing and the results of the bone scan were: fibrosarcoma (1/+1), embryogenic rhabdomyosarcoma (1/+1), leiomyosarcoma (3+/3), liposarcoma (2+/2), uterine sarcoma (2+/2), HIV-Kaposi's sarcoma (1+/1), osteosarcoma (1+/1), chondrosarcoma (1–/1) and neurogenic sarcoma. Cases of aggressive and rapidly growing fibromatosis have achieved protracted clinical benefit with isotopes radio-marked with octreotide (De Pas *et al.* 2003).

MELATONIN

The anti-cancer role of melatonin, one of the basic components of the DBM, is becoming increasingly evident in sarcomas, as in general in all other tumours.

Melatonin induces apoptosis in sarcomas through the increased expression of Fas and its ligand FasL. Inside the target cell, the Fas/FasL bond activates the enzymatic cascade that leads to apoptosis. Melatonin exerts a cytotoxic effect on tumour cells by means of the transitory increase of intracellular oxidants and acts on the nuclear transcription factor, NF-kB, apoptotically. The cytotoxic effects and the Fas/FasL regulation have been observed in all the studied lines of Ewing's Sarcoma, and not in the other cell lines, in which melatonin does not induce cell death (García-Santos & Martin 2012). As claimed by Prof Luigi Di Bella, MLT is an anti-cancer agent with differential toxicity, thus damaging the tumour cells while simultaneously having favourable effects on healthy cells.

(Casado-Zapico 2010) A study by *et al.* confirmed that MLT has a cytoprotective effect on healthy cells, with a two-stage differentiating toxic activity on tumour cells. MLT induces apoptosis extrinsically and cytotoxicity in SK-N-MC cells in Ewing's sarcoma cell lines. It also induces apoptosis when combined with chemotherapy agents, by activation of caspases 3 8 9.

The expression of MT1, in sarcomas, has been clearly shown, also justified by the significant role of Melatonin in bone metabolism (Toma *et al.* 2007).

Many other studies (Roth *et al.* 1999) confirm the ability of MLT to induce differentiation of osteoblasts, showing how this pineal indole plays an essential role in regulating bone growth. Melatonin has shown oncostatic properties in a wide variety of tumours. Physiologically, the levels of melatonin start to decrease before puberty, and continue to diminish during the puberal stage. This could confirm a relationship between the rate of bone growth and the incidence of osteosarcoma, which has been found to be statistically higher, as far as the long bones of the legs are concerned, in the observational group of 10–14 year-olds. It is highly probable, therefore, that concomitant factors such as the reduction of Melatonin (with the consequent reduction of oncostatic protection) and the significant increase of GH (and of bone growth during puberty) can cause the greater incidence of osteosarcoma in the stages of maximum bone growth. Melatonin should thus be put to good use, also in combination with chemotherapy, since being non-toxic it reinforces the anti-cancer action of the chemotherapy drugs while reducing the side effects, and can therefore help to improve the prognosis of this too often fatal disease (Panzer 2012).

MLT negatively regulates tumour growth dependent on linoleic acid by blocking its absorption. A dose-dependent suppression of the tumoral production of cAMP, absorption of LA, release of 13-HODE, ERK1/2, MEK and Akt and of the incorporation of thymidine have been observed in leiomyosarcomas (Dauchy *et al.* 2009). Dosage of pituitary MLT showed that in the initial stages of tumour development the amount of melatonin increases with modification of the production profile, playing an active role in anti-cancer homeostasis, while a decrease has been observed in advanced stages, probably contributing to cachexia (Ferreira *et al.* 2004).

The onset of osteosarcoma coincides with the reduction in the secretion of melatonin. The decline in the levels of melatonin can therefore favour the incidence of osteosarcoma. Melatonin significantly reduced the proliferation of osteosarcoma MG-63 cells in a dose- and time-dependent way, and significantly increased the fraction of cells at G0/G1 phase of the cell cycle, while it simultaneously reduced the proportion of S and G2/M phases through reduction of cyclin D1, CDK4, cyclin B1 and CDK1 (Liu *et al.* 2013).

A study on 1400 patients (Lissoni 2002) showed that MLT prevents the toxicity induced by chemotherapy, reinforcing the anti-cancer effects through antiproliferative biological mechanisms, stimulation of antitumoral immunity, negative regulation of oncogene expression, and the anti-oxidating, anti-inflammatory and anti-angiogenic effects, in advanced stage tumours, cachexia, asthenia and thrombopoietic or lymphocytopenic deficits.

Treatment with melatonin reversed the alterations to the glucose and glutamine metabolism detected in tumours and stimulated the proliferation of lymphocytes. The results show that the effect of melatonin on the growth of the tumour involved stimulation of the immune system and causes metabolic homeostasis. It has been shown (Martins *et al.* 1998) that the use of melatonin, alone or combined with cyclophosphamide, led to a significant reduction in the expression of Bcl-2 in sarcoma cancer cells, with a marked decrease (93.61%) in tumour size (Ovsjanko *et al.* 2009).

RETINOIDS

Retinoids positively regulate the oncosuppressor genes RAR-beta2 and have proved to be effective in the treatment of skin lesions caused by Kaposi's Sarcoma and by cutaneous T cell lymphoma (Camacho 2003). As chemopreventive agents, retinoids have been shown to be effective in the regression of laryngeal papillomatosis and oral lesions of leukoplakia. The ability to prevent secondary malignancies in patients with tumours of the head and neck has been extensively documented, and the favourable differentiating effects in sarcomas (Cruz & Matushansky 2012) have been confirmed by numerous authors (Yang *et al.* 2012; Al-Tahan *et al.* 1999), who also showed the cytostatic and antiproliferative effect, and the cytotoxic and apoptotic activity by caspase 9, not only in sarcomas , but also in mesenchymal stem cells in a time- and dose-dependent manner. Retinoids increase the expression of the pro-apoptotic protein BAx, at the same time reducing the expression of the anti-apoptotic protein Bcl2 (Dozza *et al.* 2012).

By means of their conjugated nuclear receptors, retinoids exert a powerful effect on cell growth, on differentiation and on apoptosis and they show significant

promising activity for anti-cancer treatment and chemopreventive therapy (Siddikuzzaman 2011). Retinoids activate genes that provide resistance to the inhibitors of histone deacetylase (Epping *et al.* 2009; Ramp *et al.* 1995).

Not only Retinoic acid, but also its metabolites inhibit the proliferation and induce induction of cellular differentiation.

The over-expression of PPAR gamma, RARalpha, and RXRalpha, also in combination, inhibits tumour growth and in vivo proliferation and has been shown to promote the osteogenic differentiation of osteosarcoma and of mesenchymal stem cells.

VITAMIN D

The combined use of Vit D and retinoids has shown (Nozaki *et al.* 1999) a differentiating and antiproliferative effect in osteosarcoma cell lines through the negative regulation of interleukin 6 and 8 (Masood *et al.* 2000).

Other studies have achieved a dose-dependent antiproliferative, proapoptotic and antimetastatic effect on lines of osteosarcoma treated with Vit. D (Hara *et al.* 2001; Thompson *et al.* 2012). Treatments with the inhibitors of proteasome degradation (lactacystin) selectively stimulated the expression of GFP-VDR and YFP-RXR and also increased the endogenous levels of VDR and RXR. The expression of GFP-VDR did not have any effect on the sensitivity of RIOS cells to calcitriol. The increases in RXR levels by YFP-RXR expression, pharmacological treatments, or a combination of the two, however, restored the inhibitory growth effects of calcitriol 9-cis retinoic acid and the production of p21 by calcitriol (Prüfer *et al.* 2002).

It has been documented that calcitriol stops the proliferation and induces the differentiation of the osteoblastic cells that have a significant autocrine secretion of HGF, which calcitriol itself inhibits (Chattopadhyay *et al.* 2003).

Finally, it has been observed that the antiproliferative receptorial effects of Vit. D take place through reduction of the cyclin D1 and the accumulation of p27 (González-Pardo *et al.* 2013).

VITAMIN E

The excessive formation of reactive oxygen species (ROS), due to the alteration of the oxidation processes present in the tissues affected by the fibrosarcoma, and the cause of damage to the surrounding membrane structures, is effectively countered by the chemical-physical properties of Alpha tocopherol, which therefore contributes to the formation of a first defence barrier against proliferative extension.

A process of thymic involution has been observed in sarcoma cell lines, which induces apoptosis of the thymocytes and a consequent reduction in the production of T cells, to the detriment of the efficacy of the immune system. Vitamin E, a strong anti-oxidant, was administered orally to eliminate the ROS and evaluate the effect against thymic apoptosis. The recovery of thymic functionality and the reduction of thymocyte apoptosis were achieved after 3 weeks of treatment with vitamin E, normalizing ROS levels and thus achieving an anti-cancer effect through recovery of immunitary homeostasis (Liu & Liu 2012).

Cyclophosphamide, an alkylating agent currently used in the treatment of various types of tumours, alone or combined with other cytostatic drugs, has a detracting effect on lipid metabolism and causes hyperlipidemia in many patients . Alpha-tocopherol is known for its ability to reduce hyperlipidemia, and the effects of its addition to treatment with cyclophosphamide were therefore investigated. The study, performed on mice with fibrosarcoma, showed that Alpha tocopherol markedly reduced hyperlipidemia induced by cyclophosphamide and also reduced the lipid metabolism to the values observed in untreated control groups (Ilanchezhian *et al.* 1995).

Vit. E was shown to reduce oxidative stress damage and stimulated osteosclerosis and tumour cell apoptosis, both in vitro and in vivo (Badraoui *et al.* 2009).

LOW DOSES OF CYCLOPHOSPHAMIDE

An additional contribution is provided by the daily administration of low doses (50 mg/die per os) of cyclophosphamide (Endoxan®). As well as drastically reducing the known toxic/myelosuppressor effects of the drug, this dosage induces a marked shift in its mechanisms of action: triggering of the mitochondrial-dependent apoptotic cascade, anti-angiogenic action by reduction of the VEGF.

VITAMIN C

Vitamin C significantly prevented the induction of sarcomas in mice, thus demonstrating a good prophylactic (Abdel-Galil 1986) effect and playing an important role in the differentiation of osteoblasts (Sugimoto *et al.* 1986).

Cases of remission have been reported in sarcomas subjected to high doses of vit. C (Campbell *et al.* 1991), and the cytostatic effect of high doses of ascorbic acid by inhibition of angiogenic processes has been demonstrated (Yeom *et al.* 2009).

Finally, vit. C has an antimetastatic action by inhibiting the migration of tumour cells (Wybieralska *et al.* 2008).

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