Case Reports of Sarcoma Patients with Optimized Lectin-Oriented Mistletoe Extract Therapy

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Abstract

Background: Mistletoe (Viscum album L) extracts (ME) are widespread as immunomodulatory therapeutic agents in alternative tumor treatment. Assessing the often-controversial clinical results is rather difficult since the effects of ME on the immune system cannot be equally reproduced. Mistletoe lectins (ML) are the only mistletoe ingredients also found in vivo that are capable of having a positive effect on the immune balance of patients with tumors. Other components have only been tested in vitro, and the removal of mistletoe lectins ML from the extract can put an end to the immunological efficacy of ME. Preclinical investigations in the tumor models (using nude mice xenotransplanted with human leiomyosarcoma and interleukin-12-deficient C57BL6 mice) show that without immunological reactions, ME induce less antitumor efficacy. ML, functioning as ligands for pattern recognition receptors of the natural immune system, are docked to ganglioside molecules (CD75) of monocytes and granulocytes, thereby stimulating the natural antitumor mechanisms.

Objectives: The aim of this article is to present and discuss several favorable clinical responses of patients who had sarcoma and who were treated with immunologically effective ME preparations.

Course of therapy and results: In accordance with the bell-shaped dose–response relationship of ML, the patients with sarcoma were treated with ME preparations, standardized for the active sugar-binding lectin contents. Thus, an optimal dose of 0.75–1.0 ng/kg ML was given twice a week subcutaneously. In this report, the clinical progress of 6 patients with sarcoma showed remissions of tumor symptoms.

Conclusions: It seems that this disease is beneficially influenced by optimized lectin-oriented ME therapy since patients with sarcoma may react especially well to the improved balance of natural immunological mechanisms. These case reports require further clinical studies with patients with sarcoma.

Introduction

Mistletoe (Viscum album L) is a parasitic plant that grows on various trees. Aqueous extracts of mistletoe plant (ME) have been used for a long time as a complementary medicine with immunomodulatory effects in tumor therapy. However, the immunological efficacy of various ME preparations can differ considerably from each other. The immunomodulatory effect of ME therapy is based on lectin–sugar interactions on the cell membrane of the innate immune system.1–4 As is known, the natural immune cells, such as granulocytes, macrophages, dendritic cells, and natural killer (NK) cells, are able to enhance their basic activity against infections and tumor cells, if their appropriate pattern recognition receptors (PRR) are bound by pathogen-associated molecular pattern (PAMP) ligands. Growing evidence suggest that mistletoe lectins (ML) also belong to these PAMP ligands. ML are able to bind PRR molecules on phagocytes, and this binding shows some similarities with interactions between Toll-like receptors and appropriate PAMP ligands of microorganisms.1–4 ML belong to the family of ribosome-inhibiting proteins (RIP), and they have highly conserved parts of their molecules since between the members of RIP many sequence homologies were established.1 The most important ML isomer, ML-I, consists of an apoptosis-stimulating A-chain with a molecular weight (MW) of 29 kDa and a carbohydrate-binding B-chain with a MW of 34 kDa. Therefore, the B-chain is the immunologically effective lectin part of the whole molecule.1

Not only lectins but other mistletoe components have also been suggested by several authors to participate in the immunomodulatory efficacy of ME.1–8 However, up to now, these other mistletoe components (such as viscotoxins,5 peptides,6 polysaccharides,7 and vesicles8) have only been tested in vitro and only ML have also been verified in vivo as

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substances responsible for the immunological effects of ME.\textsuperscript{1,4} In a previous study, all types of ML were completely removed from a commercially available ME preparation by chromatographic procedures without any other further alteration in the composition of the extract. The removal of ML from immunologically effective mistletoe extract resulted in immunosuppressive responses in healthy volunteers injected with such lectin-free preparations.\textsuperscript{3} This residual immunotoxicity of lectin-free extracts may be related to visco-toxins, which can cytolytically damage cell membranes,\textsuperscript{9} but other components, such as viscin,\textsuperscript{10} may also be involved.

Determination of active, PRR-binding lectin content renders possible an immunological standardization of commercially available ME.\textsuperscript{1,3,11,12} Using the enzyme-linked lectin assay (ELLA), the sugar binding capacity of ME can be quantified. Previous studies with ME revealed that the immunomodulatory effects and the sugar-binding activity of an extract have a close relationship and standardized ME exhibited a bell-shaped dose–response relationship.\textsuperscript{1,3}

In this article, several case reports of patients with different types of sarcoma are presented who were treated with standardized ME. Given in ME, the ML doses corresponded to 0.75–1.0 ng/kg, which were repeatedly able to induce the most effective immune responses in animal models and in healthy volunteers. The favorable results suggest that a ML-induced improvement of immune balance in patients with sarcoma may have clinical benefit. Until now, only a few clinical studies with optimized immunological efficacy using standardized ME have been reported.\textsuperscript{13,14} Consequently, these reports may stimulate further clinical investigations.

Materials and Methods

Mistletoe extract

Iscador is a fermented aqueous mistletoe plant extract manufactured and supplied by Weleda AG (CH-4144 Arlesheim, Switzerland). The active (sugar-binding) lectin content of commercially available Iscador preparations was measured in the research laboratory of Pharmacochemical Department of Medical University Pécs.

Standardization of ME with ELLA

The determination of sugar-binding ML level in ME was carried out by an optimized ELLA technique as published previously.\textsuperscript{15} Briefly, the method is based on the binding of lectin to an immobilized oligosaccharide ligand (asialofetuin) and subsequent binding of specific (polyclonal) antibody to the bound lectin. The specific binding of rabbit antibodies was quantitatively assessed using goat anti-rabbit peroxidase and the subsequent generation of a colored product from the substrate o-phenylenediamine hydrochloride. The measurements were carried out in an enzyme-linked immunosorbent assay plate reader at 492 nm. Standard lectin was isolated from fresh plants using affinity chromatography, and then it was lyophilized as described previously.\textsuperscript{15}

Dose of standardized ME preparations

ME-induced cellular responses of innate immune system in Balb/c mice and in healthy volunteers were repeatedly investigated. Standardized ME exhibited a bell-shaped dose–response relationship and 0.75–1.0 ng/kg lectin doses were found to be most effective, as it was always determined previously using healthy volunteers. Since 3 therapy-free days were found to be necessary for an immunological optimum effect, the subcutaneous ME injections twice a week were regularly given. Consequently, in the treatment of patients with sarcoma, applied lectin-oriented doses of ME corresponded to this regimen.

Case Reports

In Table 1, 6 patients with sarcoma are listed for the period of observation.

Case 1

In a 52-year-old patient, a tumor extirpation of a liposarcoma with infiltration of the fascia tissue and the adjoining musculature (thigh left) was carried out in October 1987. In the computed tomography (CT) scan from July 1989, multiple coin lesions were found for the first time in segment II in the right upper lung, in segment V and VII on the left side, and in the paravertebral sinus on both sides. The average size of the metastases was 3 cm each. At this time the patient was without symptoms and refused the proposed chemotherapy. From November 1989, lectin-standardized ME therapy was given. The patient was regularly investigated by CT of the thorax and radiograph. Within 15 months a nearly total remission could be observed. After abandoning the

| Table 1. Brief Summary of 6 Case Reports of Various Patients with Sarcoma |
|---|---|---|---|---|---|
| Case 1 | Liposarcoma (thigh) | Lung | + | Hormone therapy | 21 years | CR |
| Case 2 | Stroma sarcoma (uterus) | Peritoneum metastasis | + | Radiotherapy (4 years prior to the discovery of sarcoma) | 10 years | PR |
| Case 3 | Angiosarcoma (right mammary) | – | + | Radiotherapy (5 years prior to the discovery of sarcoma) | 4 years | CR |
| Case 4 | Angiosarcoma (right mammary) | – | + | Six cycles doxorubicin (in the first 6 months) | 3 years | CR |
| Case 5 | Myxoid liposarcoma (retroperitoneum) | Inoperable peritoneum + para-aortal lymph node metastasis | + | Five cycles epirubicin (in the first 5 months) | 18 months | PR |
| Case 6 | Stroma sarcoma (uterus) | Lung metastasis | + | – | 9 months | CR |

Complete remissions (CR) and partial remissions (PR) are defined conventionally.
precise lectin dose in late 1992, new metastases were found in the posterior mediastinum and in the left lung (segment V) in July 1993. In December 1993 the same lectin-standardized optimized dose was restarted. From October 1994 no tumor progression has been found, in April 1995 a remission of the coin lesion in the left lung and in December 1995 a remission of the coin lesion in segment V (left side) and of the metastasis in the posterior mediastinum were observed. A further remission of the coin lesions was found in June 1996 and in September 1997. From April 2002 until 2010, there have not been any signs of intrapulmonary tumor lesions in the CT. The patient has continued the ME therapy without interruption until the present. Figure 1 represents several X-ray pictures indicating the remission of the disease between 1989 and 2005.

Case 2

Because of a low-grade stroma sarcoma of the uterus, a total abdominal hysterectomy and adnexectomy were carried out in November 1998 in a now 63-year-old patient. Peritoneal tumor manifestations remained as evidenced by CT scan. From December 1998 until April 2001, palliative medroxyprogesterone therapy (dependent on serum level <1.0 g Farlutal/day) was given, which is able to block the estrogen-2 receptors in tumor tissue. From November 2000, the patient was given lectin standardized ME therapy. In January 2001, magnetic resonance imaging (MRI) as well as sonography showed three new implantation metastases in the abdominal musculature. In April 2001 the patient had to be operated on again because of an ileus in the small intestine. By way of adhesiolysis, an abdominal metastasis was removed at the same time, and biopsies of peritoneal metastases were taken. Histologically, the metastases of the stroma sarcoma with estrogen positivity could be proved. In June 2001, the hormone therapy was changed to an anti-
estrogen drug (2.5 mg Femara/day). After that, regular sonography and MRI controls showed the remission of the peritoneal carcinosis, while the solid subcutaneous lesions in the ventral pelvis remained stationary. Under hormone and ME therapy, the subcutaneous metastases in the pelvis showed partial remission. In the latest MRI data from 2010, neither relapse nor progression has been visible.

Case 3

In the now 60-year-old patient, a tumorectomy and axillary revision was carried out in November 2001 because of invasive ductal mammary carcinoma (right side). Following the operation, four cycles of Farmorubicin (75 mg/m²) and Endoxan (750 mg/m²) were given by February 2002 as adjuvant treatment. From March 2002 until February 2007, hormone therapy with tamoxifen (20 mg/day) was applied, followed by Femara (2.5 mg/day) until March 2009. From April 2002 until May 2002, irradiation of the right anterior thorax with 59.4 Gy was applied. Following the patient falling in February 2006, a large hematoma in the right breast developed that did not disappear. Mammography and ultrasonography of both breasts revealed inconspicuous results. An MRI showed a thickening of the cutis with asymmetry and intake of the cutis in the upper lateral quadrant (right side), with no hint of a tumor relapse or second tumor. After a dramatic increase of the skin alteration similar to hematoma, a mastectomy was carried out in December 2006 because of a well-differentiated angiosarcoma with infiltration of the glands that was predominantly confined to the cutis. Standardized ME therapy was begun in November 2006. In November 2007, a malignant melanoma in the lower part of the left leg was excised in an initial stage. Since the ME therapy was applied, the general condition of the patient

FIG. 1. Radiographs of a patient with liposarcoma (case 1) showing lung metastasis. From November 1989, he was treated with lectin-standardized ME therapy without other oncotherapeutic modalities. The dates of x-ray investigations were the following: A. November 1989. B. September 1990. C. September 1997. D. May 2005. As shown with arrows (A and B), within 10 months a partial remission could be observed. In September 1997 (C) and in May 2005 (D) a complete remission was found.
has gradually improved, and by 2010 no relapse or metastases have occurred, as evidenced by ultrasonography and MRI.

Case 4

Because this 52-year-old patient had mammary carcinoma (right), a tumorectomy in December 1997, and lymphadenectomy on the right side in January 1998, subsequently 50-Gy irradiation was carried out from February to April 1998. ME therapy without lectin-oriented dose was given from April 2001 until December 2002 since non-standardized preparations were used. In May 2003, because of an angiosarcoma grade II in the radiation area, a modified radical mastectomy was performed. At the same time, standardized ME therapy was started and regularly injected until summer 2006. After an interruption of about 3 months, the patient again was injected with standardized ME irregularly until in January 2007, when a discoloring of the skin in the scar area developed, suggesting initial local relapse of angiosarcoma. The relapse was completely removed in April 2007; the large skin defect was covered by inverse abdominal plastic surgery. The histology showed a relapse of the postactinic angiosarcoma at the cranial, lateral, and pectoral resection line with a maximal diameter of 5 cm. Since January 2007, the standardized ML therapy has again been performed regularly. The patient has remained relapse free solely under ME therapy, as evidenced by the latest MRI and positron emission tomography/CT scans in February 2010.

Case 5

In a 48-year-old patient, a retroperitoneal myxoid liposarcoma (right) was removed by surgery in January 2007. In March 2008, a nephrectomy (left) and a hemicolecotomy (left) with a permanent ileostomy had to be carried out because of relapse. New progressive metastases could not be operated on in December 2008. Since February 2009, the patient has been given standardized ME in addition to chemotherapy (from January 2009 until June 2009 with six cycles of doxorubicin 60 mg/m²). In April 2009, a significant reduction of the peritoneal and para-aortal lymph nodes could already be found on a CT scan. The tumor in the upper right abdominal quadrant decreased from 2.1 cm to 1.5 cm. Between April and July 2009, no more change was observed under standardized ME therapy until the end of chemotherapy. From July 2009 until October 2009, solely under ME therapy, a further regression of the para-aortal lymph glands could be observed, whereas the retroperitoneal tumor remained unchanged. By February 2010, the tumor in the upper right abdominal quadrant had slightly increased from 1.5 cm to 2.1 cm, but further metastases or relapses in peritoneal and para-aortal lymph nodes have not developed so far. In June 2010, no progression or relapse was observed and the permanent ileostomy (existing since March 2008) was removed. The now amazingly good quality of life has been persisting, with the patient being able to work normally. Therefore, from July 2009 until August 2010, no further oncotherapy is being applied at present. Only standardized ME therapy (given since February 2009 without pause) was continued.

Case 6

In the now 19-year-old patient, first symptoms of sarcoma developed in September 2008. The first diagnosis and surgery for a low-grade stromal endometrium sarcoma took place in February 2009. In August 2009, a metastasis of the lung in segment III (left) proved by histology was excised. In October 2009, two additional metastases of the lung (right) were found in a CT scan, so that from November 2009 until March 2010, the patient was given chemotherapy (5 cycles of 90 mg/m² epirubicin) supported by simultaneous standardized ME therapy. Since after two cycles of chemotherapy a complete remission could be found in CT in January 2010 and in April 2010 as well as in MRI in March 2010, the planned sixth chemotherapy cycle was not given. She was regularly treated with standardized ME. Figure 2 illustrates this remission before and after therapy in CT.

Discussion

One hundred and twenty (120) years ago, a relationship between the clinical course and inflammatory events of patients with sarcoma had already been observed, since acute inflammations were occasionally able to improve their clinical state. In spite of these old experiences, the importance of the immune system in the pathogenesis of tumor disease was often understood insufficiently, and its correct judgment was controversial. However, there is growing experimental research evidence that basic activity of the innate immune system appears to be more important in tumor defense than the adoptive immune responses. For example, when the interaction between specific adaptive immune responses and nonspecific inflammatory reactions and their relation with prognosis of cancer patients were analyzed, results revealed that although there was a significant specific antitumor response as reflected by T cells, their effects on patient survival and local recurrence were less important when compared to effects of innate inflammatory responses. In addition, numerous experiments have attempted to find an answer to decreased activity of the innate immune system in patients with tumors, and there is agreement that soluble factors produced or induced by malignant cells and myeloid-derived suppressor cells play an important role in this depression. In spite of the fact that basic functions of the innate immune system are depressed in patients with tumors, its investigation is not in current use in clinical praxis, causing a continuous lack of clinical experience and emergence of concepts. This lack of understanding in medical praxis has hindered the development of various kinds of nonspecific immunotherapy modalities against cancer. Moreover, a periodic assessment of the suppressive nature of the tumor microenvironment would also be helpful but not practical for better interpretations of these unspecific immunotherapeutic interventions.

It must not be forgotten that inflammation can exhibit controversial effects. It may eradicate tumor cells but also, when it becomes chronic, may promote tumor growth. The effector cells of the innate immune system are committed in two directions: M1 macrophages and CD1a+ dendritic cells (DC1) generate interleukin-12 (IL-12), pro-inflammatory cytokines, and activate cytotoxic effector cells (such as natural killer [NK] and natural killer T [NKT] cells), which are potent inhibitors of tumor growth. However, they are defective in
patients with tumors. Available information suggests that tumor-associated macrophages belong to a prototypic M2 population. M2 generates IL-4 and IL-10, which facilitate the generation of Th2 cells and inhibits Th1 cells. M2 macrophages affect inflammation and promote cell proliferation by producing growth factors and products of the arginase pathway, as well as promoting angiogenesis and tissue repair.

Patients with tumors can have up to 40% more M2 peripheral monocytes than healthy individuals, who have only 10% M2 monocytes. NKT cells can also have a similar opposing effect. In cancer, NKT-1 cells are protective by producing interferon-γ to activate M1 and DC1 dendritic cells, which produce IL-12, NKT-2 cells primarily inhibit tumor immunity, and these findings indicate an impaired balance of the innate immune system in patients with cancer. Consequently, learning to manipulate this balance along the regulatory axis may be critical to devising successful immune therapies against cancer.

Recently, a highly specific receptor, the CD75 ganglioside, was described, which was found in the PRR on several effector cells of the innate immune system. The existence of this PRR receptor may explain the selective binding capacity of neutrophils and monocytes to ML molecules, which may have a similar structure to the PAMP existing on the membrane of various microorganisms.

As known the effector cells of innate immune system do not posses a memory and the down regulation of their basic antitumor activity can often correlate with the progression of tumor disease. Preclinical experiments corroborate that ML stimulates the basic activity of M1 and DC1 cells and by this means can improve the disturbed balance of innate immune system in patients with tumors. Of note, ML in nanograms per kilogram dose range, which is four orders of magnitude lower than their toxic dosage (LD₅₀ = 160 μg/kg), can induce both apoptosis enhancing/cytostatic and immunomodulatory effects.

These case reports can also support several preclinical data that the immunomodulation is necessary for the anti-tumor efficacy of ML. Global judgment is difficult regarding the prognosis of patients with sarcoma, and it is strongly dependent on the extent of the disease. From the 6 patients with sarcoma described above, in 4 cases (cases 1 and 2 and 5 and 6) at the beginning of ME therapy, various metastases were found and their prognosis was judged to be poor. Two (2) patients with angiosarcoma (cases 3 and 4) had previously received radiotherapy (4 and 5 years prior to discovery of sarcoma, respectively), suggesting a causal relationship between them. All 6 patients with sarcoma showed remarkable remission under optimized lectin-oriented doses of ME, partly in addition to other oncotherapies. The first patient with metastases of liposarcoma (case 1) was regularly given mistletoe therapy without other treatments. His clinical progress reveals that the optimized mistletoe dose alone was able to bring about remission of metastases. Without a lectin-oriented dose, the metastases of the lung recurred, whereas under a strict lectin-oriented dose, regression took place. Currently, the patient has survived for 21 years with an excellent quality of life. In the other patient with liposarcoma with eventually inoperable peritoneal metastases and metastases of the lymph glands (case 5), simultaneous chemotherapy and lectin-oriented ME therapy brought about partial remission. Subsequently, with only ME therapy, the remission has persisted so far. Preclinical data in the tumor model with xenotransplanted sarcoma suggest that lectin-oriented ME therapy is capable of enhancing the efficacy of certain chemotherapies; however, further clinical investigations are necessary. The 2 patients with stroma sarcoma metastases were given lectin-oriented ME therapy in addition to hormone therapy (case 2) or chemotherapy (case 6).
The efficacy of hormone therapy on recurrent low-grade stroma sarcoma is controversial.\(^{20-31}\) Possibly, both therapies could support each other. In the second patient with stroma sarcoma (case 6), complete remission already occurred after two chemotherapy cycles so that the planned sixth cycle could be abandoned. Also in this case, the question of whether ME therapy given in a lectin-oriented dose improved the effect of cytostatics should be discussed.

The two patients suffering from angiosarcoma (cases 3 and 4) were on the lectin-oriented ME therapy following surgery. The 60-year-old patient described first (case 3) has remained without relapse for 3 years now, and the melanoma that was discovered and operated on 1 year later has remained without metastases. The second patient with angiosarcoma (case 4) interrupted the ME injections for 3 months; subsequently a still-operable relapse was found. Since that time, she has received regular ME treatment again and has remained without relapse for 3 years now.

Conclusions

Previous studies revealed that ML are able to bind pattern recognition receptors on cellular components of innate immune system, and they can improve the tumor-induced derangement of natural immune balance.

The 6 patients with sarcoma described above suggest that an optimum dose of ML between 0.75 and 1.0 ng/kg given by means of standardized subcutaneous ME injections twice a week can inhibit the progression of sarcoma. However, other ML doses or application of nonstandardized ME preparations are less effective, indicating the importance of the exact immunologically effective lectin dosage.

Since the remission rates of patients with sarcoma after chemotherapy are often found to be less than 40% and the duration of chemotherapy-induced remissions is relatively short, its combination with ML is promising.

The aim of these case reports is to attract attention, and it is also clear that further clinical investigations are necessary.

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