PHYTOTHERAPY RESEARCH
Phytother. Res. **23**, 407–411 (2009)
Published online 11 November 2008 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.2643

Immunological Response to Mistletoe (*Viscum album* L.) in Cancer Patients: A Four-Case Series

Nilo Esvalter Gardin*
Rua Carlos Weber, 601/201-C 05303-000 São Paulo/SP, Brazil

European mistletoe (*Viscum album*) has been used in complementary cancer treatment, but little is known concerning its effects on immunological parameters, although there is evidence that *Viscum* may stimulate the immune system. In this study, a trial was conducted with cancer patients to determine whether *Viscum album* extracts could improve the results of immune tests. These were: white blood cell count (leukocytes, neutrophils, lymphocytes), CD4+ and CD8+ T-lymphocytes, intradermal tests of delayed hypersensitivity (candidin, trichophytin, purified protein derivative-PPD), complement C3 and C4, and immunoglobulin A, G and M. Four patients received seven doses of subcutaneous *Viscum album* 20 mg, twice weekly. Immunological tests were carried out before and after treatment, and an increase in several parameters of humoral and cellular immunity were shown. Apart from reactions around the injection sites, treatment was well tolerated and all patients benefited from it. These results suggest that *Viscum album* can enhance humoral and cellular immune responses in cancer patients, but further studies attesting to the possible clinical impact of these immunological effects are necessary. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: Viscum album; *Loranthaceae*; mistletoe; cancer; lectins; viscotoxins.

INTRODUCTION

Viscum album Linnaeus (VA) is a hemiparasitic plant of the *Loranthaceae* family that grows wild on trees, bushes and other plants, from Northern Europe to Northwest Africa, Southwest and Central Asia and Japan (Becker, 1986). Although it has been used in these regions for decades (Franz, 1986), VA was first used as a treatment for cancer in 1917 by Steiner and Wegman, founders of anthroposophic medicine, a complementary medicine system practiced worldwide (Leroi and Leroi, 1987), and since then, more than 100 000 patients have been treated with VA. Within the past 30 years it has become one of the most widely used complementary cancer therapies in Europe (Moschèn *et al.*, 2001). Extracts are made from fresh leafy shoots and berries from VA obtained from different species of host tree such as oak (*Quercus, Qu*), apple tree (*Malus, M*), pine (*Pinus, P*) and others. Dosage and route of application vary individually, depending on the reaction of the patient and the stage of disease. Several studies have assessed its cytotoxic (Siegle *et al.*, 2001; Ribéreau-Gayon *et al.*, 1986; Holtskog *et al.*, 1988; Kuttan *et al.*, 1990; Jung *et al.*, 1990; Jurin *et al.*, 1993) and immunomodulatory (Jurin *et al.*, 1993; Pelletier *et al.*, 2001; Chernyshov *et al.*, 2000; Stein *et al.*, 1999a, 1999b; Büssing *et al.*, 2005; Kovacs, 2000; Rentea *et al.*, 1981; Bloksma *et al.*, 1982; Hajto, 1986) properties. In 2001, a large study with 10 226 cancer patients showed that VA prolonged overall survival of patients with colon, rectum, breast and lung (small-cell) cancer (Grossarth-Maticek *et al.*, 2001). However, to date, no clinical trials evaluating immunological indices before and after the use of VA that could attest to its stimulating effects on cellular and humoral immune system have been reported. Understanding immunosurveillance is important for developing efficient antitumor immunological treatments. Antitumor responses of the immune system include T lymphocytes, B lymphocytes, natural killer cells, macrophages, dendritic cells and granulocytic cells (Boon *et al.*, 2000). These immune mechanisms, if stimulated, can enhance tumor destruction or reduction.

Impairment in immune antitumor function, which has been seen in cancer patients, can help to explain tumor appearance and spread. In addition, cancer treatment with chemotherapy and radiotherapy generally leads to further immunosuppression, so prevention of this would be beneficial for these patients. For this reason a small trial with cancer patients was conducted to determine whether VA can improve immune tests that had previously been altered. There is also a special significance for patients with malignant neoplasia affecting the immune system, for example lymphoma and multiple myeloma: in these disorders, immune parameters generally have great impact on clinical outcome.

PATIENTS AND METHODS

The Ethical Committee of Edmundo Vasconcelos Hospital in São Paulo, Brazil approved the study, and all participants provided written informed consent before enrolling in the study according to institutional guidelines.

Patients. Patients were recruited from the Hematology and Oncology ambulatory of Edmundo Vasconcelos Hospital. Eligibility was limited to those above 18 years old, with a diagnosis of malignant neoplasia confirmed

* Correspondence to: Nilo Esvalter Gardin, Rua Luminarias 154-05439-000 São Paulo/SP, Brazil.
E-mail: nilogardin@superig.com.br

Received 20 June 2006
Accepted 16 June 2008

by histological or cytological tests, with a deficit of cellular or humoral immunity demonstrated by laboratory tests, and with adequate renal and hepatic function values (respectively: serum creatinine 1.5 mg/dL or lower, serum bilirubin 2 mg/dL or lower). Other inclusion criteria were the absence of chemotherapy, radiotherapy, immunotherapy with corticosteroids or other immunosuppressive drugs, or any other experimental treatment in the 30 days before study entry, and the absence of granulocyte or granulocyte-macrophage colony-stimulating factors given in the previous 10 days. Patients were ineligible if they had had a recent positive pregnancy test, were breast-feeding, there was a possibility of a future pregnancy, or if they had a psychiatric or neurological disorder including dementia that could affect the compliance with the protocol.

Laboratory evaluation. Patients underwent laboratory analysis after clinical evaluation. Tests included white blood cell count, T lymphocyte count, CD4+ and CD8+ T cell subsets, plasma concentration of complement component C3 and C4, measurement of serum immunoglobulin (Ig) A, IgG and IgM, and then intradermal tests of delayed hypersensitivity with antigens inoculation (candidin, trichophytin and purified protein derivative – PPD). Skin test indurations were measured 48 to 72 h after inoculation, and after this VA treatment began. All the tests were performed before the VA treatment and 2 or 3 days after. Then 4 weeks after the last dose of VA, blood tests were performed again. Skin tests were made only twice because of sensitization risks.

VA treatment. Ampoules of 20 mg of VA *Qu* were supplied by Weleda do Brasil Laboratório & Farmácia Ltda (São Paulo, Brazil). The mistletoe extract *Viscum album Qu* (*Quercus*) 20 mg is an aqueous sterile preparation derived from *Viscum album* L. grown on oak and fermented with *Lactobacillus plantarum.* VA was subcutaneously injected in the abdominal or gluteal region, twice a week, with an interval of 3 or 4 days; a total of seven injections. All patients were monitored weekly by the responsible investigator and adverse events were checked closely at each visit. Common terminology criteria for adverse events were used to classify reactions associated with the use of VA (National Cancer Institute, 2003).

RESULTS

Four patients were enrolled in this study and their characteristics are shown in Table 1. The first patient was also diagnosed with squamous cell carcinoma of the epiglottis, stage II, treated with surgery and radiotherapy 24 months before the VA tests; and basal cell carcinoma of the nose and upper limb, treated with surgery 2 months before the VA tests.

The second patient was the only smoker (8 cigarettes/day for 20 years), and the fourth patient was the only one taking other drugs during the VA tests (atenolol 100 mg and nifedipine 40 mg daily for hypertension). This patient had to be admitted to the hospital 2 weeks after the second test because of a new chemotherapy cycle and was not submitted to the third test.

Immunological response

Tables 2–5 show all laboratory parameters examined before the VA use ('before'), 2 or 3 days after the VA treatment ('second tests') and 4 weeks after the last dose of VA ('third tests'). Values out of the normal range are in bold type. The fourth patient was not submitted to PPD for a second test because he had a strong positive reaction to the first. The first patient showed the best response, resulting in the enhancement of all analysed parameters apart from the intradermal tests. Similarly, the fourth patient showed an increase in all indices except trichophytin inoculation. All parameters, which were initially below the normal range, had increased by the final tests, except for some intradermal reactions (three skin tests of the first and second patients and one of the third and fourth patients).

Adverse events

The VA treatment was well tolerated and were administered in the outpatient setting. Systemic symptoms did not occur. All patients reported mild induration on the injection site (Table 6). Only one patient had moderate pain and erythema. All toxicities reversed spontaneously without sequela, generally after one day. There was no need to use symptomatic medication, interrupting VA, or changing VA dose or frequency.

DISCUSSION

The present study investigated immune stimulation by VA in four cancer patients who had immune impairment. These patients, who received seven subcutaneous doses of VA 20 mg, showed improvement in several laboratory parameters, confirming that VA can improve the immune response and restore suppressed cellular and humoral immunity to some extent. There is evidence, supported by clinical studies, that VA has positive benefits for

Table 1. Patient characteristics

Patient	Gender	Age (years)	Cancer diagnosis	Stage	Months from diagnosis[a]	Treatment	Months from end of treatment[a]
1	Male	57	Hodgkin disease (nodular sclerosis)	III-B	43	CT + RT	31
2	Male	18	Hodgkin disease (mixed cellularity)	II-A	50	CT + RT	33
3	Female	44	Breast (infiltrative ductal)	II	30	SR + RT + CT	20
4	Male	46	Multiple myeloma (IgG)	III-A	16	CT	4

[a] To VA tests.

CT, chemotherapy; RT, radiotherapy; SR, surgery.

DOI: 10.1002/ptr

Table 2. Results of patient 1

Parameter	Normal range	Before VA	2–3 days after VA	4 weeks after VA	Conclusion
Leucocytes × 10^9/L	4000–11000	**1670**	**2280**	**3440**	Improvement
Neutrophils × 10^9/L	1800–7700	**868**	**1254**	2476	Improvement
Lymphocytes × 10^9/L	800–4950	**701**	889	**791**	Improvement
CD4 (cells/μL)	240–1800	**109**	Not done[a]	**126**	Improvement
CD8 (cells/μL)	120–1110	244	Not done[a]	249	Kept normal
CD4/CD8 ratio	0.9–2.2	**0.45**	Not done[a]	**0.51**	Improvement
C3 (mg/dL)	50–120	66	84	94	Normal
C4 (mg/dL)	10–40	21	26	26	Kept normal
IgA (mg/dL)	60–400	188	227	210	Kept normal
IgG (mg/dL)	900–1500	**882**	963	968	Improvement
IgM (mg/dL)	70–320	**63**	**63**	78	Improvement
Candidin (mm)	5–10	**0**	**0**	–	Unaltered
PPD (mm)	5–10	**0**	**0**	–	Unaltered
Trichophytin (mm)	5–10	**0**	**0**	–	Unaltered
Normal/Evaluated[b]		4/14	5/11	7/11	Improvement

[a] Due to a technical problem.
[b] Number of normal parameters/number of parameters evaluated.

Table 3. Results of patient 2

Parameter	Normal range	Before VA	2–3 days after VA	4 weeks after VA	Conclusion
Leucocytes × 10^9/L	4000–11000	**3840**	**3600**	4730	Improvement
Neutrophils × 10^9/L	1800–7700	1958	2160	2606	Kept normal
Lymphocytes × 10^9/L	800–4950	1612	1116	1466	Kept normal
CD4 (cells/μL)	240–1800	324	**234**	408	Kept normal
CD8 (cells/μL)	120–1110	555	333	522	Kept normal
CD4/CD8 ratio	0.9–2.2	**0.58**	**0.7**	**0.78**	Improvement
C3 (mg/dL)	50–120	65	98	76	Kept normal
C4 (mg/dL)	10–40	24	21	20	Kept normal
IgA (mg/dL)	60–400	213	216	211	Kept normal
IgG (mg/dL)	900–1500	1460	1430	1250	Kept normal
IgM (mg/dL)	70–320	109	119	128	Kept normal
Candidin (mm)	5–10	**0**	**0**	–	Unaltered
PPD (mm)	5–10	**0**	**0**	–	Unaltered
Trichophytin (mm)	5–10	**0**	**0**	–	Unaltered
Normal/Evaluated[a]		9/14	8/14	10/11	Improvement

[a] Number of normal parameters/number of parameters evaluated.

Table 4. Results of patient 3

Parameter	Normal range	Before VA	2–3 days after VA	4 weeks after VA	Conclusion
Leucocytes × 10^9/L	4000–11000	9670	9360	8230	Kept normal
Neutrophils × 10^9/L	1800–7700	6285	5616	4608	Kept normal
Lymphocytes × 10^9/L	800–4950	2417	2433	2798	Kept normal
CD4 (cells/μL)	240–1800	1184	1166	1556	Kept normal
CD8 (cells/μL)	120–1110	491	521	585	Kept normal
CD4/CD8 ratio	0.9–2.2	**2.41**	**2.24**	**2.66**	Kept high
C3 (mg/dL)	50–120	89	**128**	89	Kept normal
C4 (mg/dL)	10–40	30	32	26	Kept normal
IgA (mg/dL)	60–400	**455**	**478**	**449**	Kept high
IgG (mg/dL)	900–1500	1103	1490	1310	Kept normal
IgM (mg/dL)	70–320	131	135	125	Kept normal
Candidin (mm)	5–10	**0**	**0**	–	Unaltered
PPD (mm)	5–10	**2**	9	–	Improvement
Trichophytin (mm)	5–10	**2**	9	–	Improvement
Normal/Evaluated[a]		9/14	10/14	9/11	Improvement

[a] Number of normal parameters/number of parameters evaluated.

DOI: 10.1002/ptr

Table 5. Results of patient 4

Parameter	Normal range	Before VA	2–3 days after VA	Conclusion
Leucocytes × 10^9/L	4000–11000	4210	4570	Kept normal
Neutrophils × 10^9/L	1800–7700	2273	2467	Kept normal
Lymphocytes × 10^9/L	800–4950	1599	1965	Kept normal
CD4 (cells/μL)	240–1800	427	662	Kept normal
CD8 (cells/μL)	120–1110	677	792	Kept normal
CD4/CD8 ratio	0.9–2.2	**0.63**	**0.84**	Improvement
C3 (mg/dL)	50–120	117	**131**	Become high
C4 (mg/dL)	10–40	15	16	Kept normal
IgA (mg/dL)	60–400	63	72	Kept normal
IgG (mg/dL)	900–1500	**7380**	**7590**	Kept high
IgM (mg/dL)	70–320	**31**	**36**	Improvement
Candidin (mm)	5–10	**0**	5	Improvement
PPD (mm)	5–10	**30**	Not done	–
Trichophytin (mm)	5–10	**0**	**0**	Unaltered
Normal/Evaluated[a]		8/14	8/13	Improvement

[a] Number of normal parameters/number of parameters evaluated.

Table 6. Adverse events associated with the use of VA

Patient	Adverse event	Grade	Period	Duration
1	Induration and erythema	I	After dose 4	1 day
2	Pain and erythema	II	After dose 1	2 days
	Pain	I	After dose 2	1 day
	Induration	I	After dose 3	1 day
	Itching	I	After dose 5	1 day
3	Pain and erythema	I	After all 7 doses	1 day
	Induration	I	After all 7 doses	8–10 days
4	Pain	I	After all 7 doses	1 day
	Induration	I	After dose 1	1 day

some cancer patients although efficacy is still not considered to have been conclusively demonstrated (Ernst *et al.*, 2003). In 1989, Kiene published the first meta-analysis about VA clinical studies (Kiene, 1989), which included 46 studies and trials of VA therapy for carcinomatous diseases. There were 35 controlled studies, of which 12 were considered conclusive, and all of these showed an advantage of the mistletoe group in survival time and survival rate. Nine of those 12 studies were statistically significant. Kleijnen and Kipschild (1994) also analysed VA clinical studies, but were more critical about methodological aspects. They uncovered 11 controlled trials, four of which showed significance with a positive result for mistletoe as a treatment for cancer, six trials showed a positive trend and one with no benefit. Finally, in 2007 Kienle and Kiene evaluated only prospective clinical trials on the effectiveness of anthroposophic mistletoe therapy for cancer (Kienle and Kiene, 2007). Thirty seven studies were identified: 16 randomized, nine non-randomized and 12 single-arm cohort studies. Among 25 controlled trials evaluated for clinically relevant outcome measures, a statistically significant benefit for survival was reported in eight of 17 trials, for remission of tumor and malignant effusion in two of four controlled trials, for quality of life in three of five studies, and for quality of life and reduction of side effects of cytoreductive therapies (chemotherapy, radiation or surgery) in five of seven trials. Among 12 single-arm cohort studies, five of seven studies found substantial tumor remission, one study reported remission of carcinoma in intra-epithelial neoplasm, and four studies reported remission of malignant pleural effusion or ascites.

In the present study, almost all immune indices improved after VA therapy. This supports the work of Chernyshov *et al.* who showed previously that VA therapy reduced recurrent respiratory infections and improved immune parameters in more than 70% of 92 children living in areas exposed to the radioactive fallout from Chernobyl (Chernyshov *et al.*, 2000). The immunomodulating and anticancer activities of VA are attributed to its three main classes of biologically active components: lectins, viscotoxins and polysaccharides (Romagnoli *et al.*, 2003; Stein *et al.*, 1999b; Coulon *et al.*, 2003; Frantz *et al.*, 2000). The lectins especially have well recognized antitumor and immunomodulating activities.

The incidence of adverse effects was small, most of them transient and mild, and none systemic. Previous clinical studies showed the same results (Gorter *et al.*, 1999, Stein and Berg, 2000), consequently, complementary treatment with VA has been considered safe.

In conclusion, although this study has had only four cases, the VA therapy showed immune benefits in laboratory tests and suggests that VA can enhance humoral and cellular immune responses. However, new studies attesting to the clinical impact of these immunological effects in cancer patients are needed.

Acknowledgement

The author is grateful to Dr José Roberto Lazzarini Neves and Dr Bernardo Kaliks Litvak and Dr Ricardo Caponero for their scientific support.

REFERENCES

Becker H. 1986. Botany of European Mistletoe (*Viscum album* L.). *Oncology* **43** (Suppl. 1): 2–7.

Bloksma N, Schmiermann P, de Reuver M, van Dijk H, Willers J. 1982. Stimulation of humoral and cellular immunity by *Viscum* preparations. *Planta Med* **46**: 221–227.

Boon T, Coulie PG, Bruggen P, Baren N. 2000. Immunologic basis of cancer. In *Abeloff: Clinical Oncology*, 2nd edn. Churchill Livingstone: Orlando; 158–241.

Büssing A, Bischof M, Hatzmann W *et al.* 2005. Prevention of surgery-induced suppression of granulocyte function by intravenous application of a fermented extract from *Viscum album* L. in breast cancer patients. *Anticancer Res* **25**(6C): 4753–4757.

Chernyshov VP, Heusser P, Omelchenko LI *et al.* 2000. Immunomodulatory and clinical effects of *Viscum album* (Iscador M and Iscador P) in children with recurrent respiratory infections as a result of the Chernobyl nuclear accident. *Am J Ther* **7**: 195–203.

Coulon A, Mosbah A, Lopez A *et al.* 2003. Comparative membrane interaction study of viscotoxins A3, A2 and B from mistletoe (*Viscum album*) and connections with their structures. *Biochem J* **374**: 71–78.

Ernst E, Schmidt K, Steuer-Vogt MK. 2003. Mistletoe for cancer? A systematic review of randomised clinical trials. *Int J Cancer* **107**: 262–267.

Franz H. 1986. Request for an impartial discussion of the so-called mistletoe therapy. *Oncology* **43** (Suppl. 1): 1.

Frantz M, Jung ML, Ribereau-Gayon G, Anton R. 2000. Modulation of mistletoe (*Viscum album* L.) lectins cytotoxicity by carbohydrates and serum lycoproteins. *Arzneimittelforschung* **50**: 471–478.

Gorter RW, van Wely M, Reif M, Stoss M. 1999. Tolerability of an extract of European mistletoe among immunocompromised and healthy individuals. *Altern Ther Health Med* **5**: 37–44.

Grossarth-Maticek R, Kiene H, Baumgartner SM, Ziegler R. 2001. Use of Iscador, an extract of European mistletoe (*Viscum album*), in cancer treatment: prospective nonrandomized and randomized matched-pair studies nested within a cohort study. *Altern Ther Health Med* **7**: 57–66, 68–72, 74–76 passim.

Hajto T. 1986. Immunomodulatory effects of iscador: a *Viscum album* preparation. *Oncology* **43** (Suppl. 1): 51–65.

Holtskog R, Sandvig K, Olsnes S. 1988. Characterization of a toxic lectin in Iscador, a mistletoe preparation with alleged cancerostatic properties. *Oncology* **45**: 172–179.

Jung ML, Baudino S, Ribéreau-Gayon G, Beck JP. 1990. Characterization of cytotoxic proteins from mistletoe (*Viscum album* L.). *Cancer Lett* **51**: 103–108.

Jurin M, Zarkovic N, Hrzenjak M, Ilic Z. 1993. Antitumorous and immunomodulatory effects of the *Viscum album* L. preparation Isorel. *Oncology* **50**: 393–398.

Kiene H. 1989. Klinische Studien zur Misteltherapie karzinomatöser Erkrankungen. *Therapeutikon* **6**: 347–353.

Kienle GS, Kiene H. 2007. Complementary cancer therapy: a systematic review of prospective clinical trials on anthroposophic mistletoe extracts. *Eur J Med Res* **12**: 103–119.

Kleijnen J, Knipschild P. 1994. Mistletoe treatment for cancer: Review of controlled trials in humans. *Phytomedicine* **1**: 255–260.

Kovacs E. 2000. Serum levels of IL-12 and the production of IFN-gamma, IL-2 and IL-4 by peripheral blood mononuclear cells (PBMC) in cancer patients treated with *Viscum album* extract. *Biomed Pharmacother* **54**: 305–310.

Kuttan G, Vasudevan DM, Kuttan R. 1990. Effect of a preparation from *Viscum album* on tumor development *in vitro* and in mice. *J Ethnopharmacol* **29**: 35–41.

Leroi A, Leroi R. 1987. Carcinoma: Etiologia e Terapêutica. In *A Imagem do Homem como Base da Arte Médica*, Husemann F, Wolff O. Associação Beneficente Tobias e Associação Brasileira de Medicina Antroposófica: São Paulo; 794–825.

Moschèn R, Kemmler G, Schweigkofler H *et al.* 2001. Use of alternative/complementary therapy in breast cancer patients – a psychological perspective. *Support Care Cancer* **9**: 267–274.

National Cancer Institute, Cancer Therapy Evaluation Program. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Publish Date December 12, 2003. Accessed March 18, 2004 at http://ctep.cancer.gov/reporting/ctc.html.

Pelletier M, Lavastre V, Savoie A *et al.* 2001. Modulation of interleukin-15-induced human neutrophil responses by the plant lectin *Viscum album* agglutinin-I. *Clin Immunol* **101**: 229–236.

Rentea R, Lyon E, Hunter R. 1981. Biologic properties of Iscador: a *Viscum album* preparation. I. Hyperplasia of the thymic cortex and accelerated regeneration of hematopoietic cells following x-irradiation. *Lab Invest* **44**: 43–48.

Ribéreau-Gayon G, Jung ML, Baudino S, Sallé G, Beck JP. 1986. Effects of mistletoe (*Viscum album* L.) extracts on cultured tumor cell. *Experientia* **42**: 594–599.

Romagnoli S, Fogolari F, Catalano M *et al.* 2003. NMR solution structure of viscotoxin C1 from *Viscum album* species *Coloratum ohwi*: toward a structure–function analysis of viscotoxins. *Biochemistry* **42**: 12503–12510.

Siegle I, Fritz P, McClellan M, Gutzeit S, Mürdter TE. 2001. Combined cytotoxic action of *Viscum album* agglutinin-1 and anticancer agents against human A549 lung cancer cells. *Anticancer Res* **21**(4A): 2687–2691.

Stein GM, Berg PA. 2000. Adverse effects during therapy with mistletoe extracts. In *Mistletoe, The Genus Viscum*, Büssing A (ed.). Harwood Academic Publishers: Amsterdam; 195–208.

Stein GM, Edlund U, Pfüller U, Büssing A, Schietzel M. 1999a. Influence of polysaccharides from *Viscum album* L. on human lymphocytes, monocytes and granulocytes *in vitro*. *Anticancer Res* **19**(5B): 3907–3914.

Stein GM, Schaller G, Pfüller U, Schietzel M, Büssing A. 1999b. Thionins from *Viscum album* L: influence of the viscotoxins on the activation of granulocytes. *Anticancer Res* **19**(2A): 1037–1042.