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.

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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 (Leroy and Leroy, 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 (Moschén 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) proper-
ties. 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 stimulat-
ing effects on cellular and humoral immune system have been reported. Understanding immunosurveillance is important for developing efficient antitumor immuno-
logical 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 deter-
mine whether VA can improve immune tests that had previously been altered. There is also a special signifi-
cance for patients with malignant neoplasia affecting the immune system, for example lymphoma. The Ethical Committee of Edmundo Vasconcelos Hospital in São Paulo, Brazil approved the study, and all particip-
ants provided written informed consent before enrolling in the study according to institutional guidelines.

**PATIENTS AND METHODS**

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**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 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, immuno-
therapy with corticosteroids or other immunosuppres-
sive 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 preg-
ancy, or if they had a psychiatric or neurological disor-
der 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 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 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, visco toxins 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.

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408 N. E. GARDIN

IMMUNE RESPONSE TO MISTLETOE 411

REFERENCES


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