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Clinical relevance of immunoactive mistletoe lectin-I

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Recent investigations have shown that defined, non-toxic doses of the galactoside-specific mistletoe lectin (mistletoe lectin-I , a constituent of clinically approved plant extracts) have immunomodulatory potencies. The obvious ability of certain lectins (e.g. mistletoe lectin-I) to activate (non-)specific defence mechanisms supports the assumption that lectin–carbohydrate interactions may induce clinically beneficial immunomodulation. Initial clinical trials were promising and currently prospectively randomized multicentre trials are being performed to evaluate the ability of complementary mistletoe lectin-I treatment to reduce the rate of tumour recurrences and metastases, to improve the overall survival as well as the quality of life and to exert immunoprotection in cancer patients under tumour destructive therapy.

Keywords: Mistletoe lectin-I , mistletoe lectin-II, immunomodulation, BALB/c-mice, cancer patients, immunoprotection, quality of life.

Mistletoe lectin-I : experimental evaluation of immunomodulating efficacy

Specific immune responses against tumour-associated antigens mediated by T-lymphocytes and non-specific immune responses induced by cells of the mononuclear phagocyte system and natural killer cells appear to participate in naturally acquired resistance against neoplastic disease [1]. Increases in such mechanisms through immunomodulators, e.g. plant extracts, may thus be beneficial for therapy.

The obvious ability of certain lectins to activate (non)-specific defence mechanisms supports the assumption that lectin–carbohydrate interactions may induce clinically beneficial immunomodulation. Recent investigations have shown that lectins, especially small non-toxic doses of the galactoside-specific lectin from mistletoe (mistletoe lectin-I) have immunomodulatory potencies that comprise *in vitro*: (1) enhanced expression of lymphocyte (interleukin-2 receptor on T-cells; human leukocyte antigen-DQ antigen on B-cells) and monocyte/macrophage (MAC-3 antigen) activation markers [2]; (2) enhanced phagocytic activity of polymorphonuclear leukocytes towards *Staphylococcus aureus* [3]; (3) evident cytokine release (interleukin-1, interleukin-2, tumour necrosis factor-α, interferon-γ) by mononuclear immune cells [2]; and (4) no enhancement of tumour cell proliferation; however, dose-dependent cytotoxic effects are seen towards tumour cells, immune cells and parenchymal cells for high mistletoe lectin-I concentrations [4]. A regular subcutaneous administration of mistletoe lectin-I *in vivo* (murine model systems) yielded a dose-dependent optimum immunomodulation (1 ng mistletoe lectin-I /kg body weight) [5,6] and a moderate downregulation of defined immune parameters [7] after administration of non-optimal high concentrations (≥2.5 ng mistletoe lectin-I /kg body weight). To evaluate the immunomodulating capacity of mistletoe lectin-I , the optimal concentration was applied to BALB/c-mice and this induced: (1) increased counts and activities (expression of activation markers as determined by flow cytometry with fluorescence-activated cell sorter; FACScan, Becton Dickinson GmbH, Heidelberg, Germany) of peripheral blood leukocytes, lymphocytes, and monocytes [7]; (2) enhanced thymocyte proliferation, maturation, and emigration [6]; (3) enhanced organ weight (spleen), cell counts and activity of the mononuclear phagocyte system [5]; (4) statistically significant antimetastatic effects in different experimental tumour models (BALB/c-mouse, L-1 sarcoma, RAW 117 H-10 lymphosarcoma) [5]; (5) relevant immunoprotection after cortisone treatment [8]; and (6) evidently reduced lethality after experimental *Listeria monocytogenes* infection [9].

In order to investigate the immunoactive ability of mistletoe lectin-I in more detail, a commercially available mistletoe lectin-I standardized mistletoe extract was administered (optimal dose and timing scheme) to BALB/c-mice and compared with the analogue mistletoe lectin-I depleted extract with otherwise identical components [10]. This study indicated that mistletoe lectin-I is the immunoactive component of the extract investigated since after mistletoe lectin-I depletion all parameters tested (e.g. spleen/thymus weight and cell counts, peripheral blood/peritoneal macrophage cell counts and activities) were comparable to control values after analogue buffer treatment. Mistletoe lectin-I standardized extract, however, induced the well known activation of the lymphatic and mononuclear phagocyte system after administration of the optimal dose. Although these studies warrant further experimental consolidation (e.g. more extended kinetics concerning time schedule and extract concentration, and evaluation of other clinically

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relevant mistletoe extracts differing in production) they suggest that mistletoe lectin-I is the immunoactive component of the mistletoe extract investigated [10].

Another experimental *in vivo* approach (BALB/c-mice) compared the immunomodulating ability of the galactoside-specific mistletoe lectin-I to the *N*-acetyl-galactosamine-specific mistletoe lectin-II [11]. Compared to mistletoe lectin-I, mistletoe lectin-II administration (same concentration at 1 ng/kg body weight and same time schedule) induced a statistically significant downregulation of thymocyte counts, resulting from the reduction of immature cells. Peripheral blood cell counts (leukocytes, lymphocytes and monocytes) and activities, however, were not influenced by mistletoe lectin-II treatment with values comparable to buffer-treated control mice [1]. These data also suggest that mistletoe lectin-I is the immunoactive component of mistletoe extracts. However, the conclusions require further consolidation (concerning optimal mistletoe lectin-II concentration and timing of administration).

Clinical efficacy of mistletoe lectin-I in oncology

In order to assess its immunomodulatory capacity in tumour patients, the effect of administration of mistletoe lectin-I standardized mistletoe extract on peripheral blood lymphocyte subsets and activity were monitored. Mistletoe lectin-I standardized mistletoe extract was subcutaneously injected at the optimal concentration (1 ng/kg body weight) twice a week for 3 months, followed by a 2 month break. This therapeutic procedure proved to be promising for further studies [12–16].

Blood was drawn by standard methods at defined times before and during therapy. No significant side effects could be seen in these treated patients. Rubor at the injection site (about 2% of patients) generally disappeared within 12 h. With this therapeutic schedule, counts of peripheral blood lymphocytes increased significantly in tumour patients, especially natural killer cells, helper T cells and cytotoxic T cells. To gain information on the status of activation of lymphatic cells, the expression of interleukin-2 receptors and HLA-DQ antigens were determined during therapy and their significant upregulation confirmed the immunoactive potency of the treatment [12,13].

There is some evidence that β-endorphins (endogenous neuropeptides with morphine-like activity) may serve as modulators of pain and mood, e.g. in cancer patients [17,18]. Accordingly, the question arose whether changes of mood and altered perception of pain after immunomodulatory mistletoe lectin-I treatment could be β-endorphin-mediated. Regular mistletoe lectin-I administration to patients with breast carcinoma on a standard chemotherapy regimen was found to modulate the immuno–neuroendocrine axis since significantly increased β-endorphin plasma levels in patients treated in this way correlated positively with stabilization of chemotherapy-induced lymphopenia and cytokine release [15]. Adequate modulation of the immuno–neuroendocrine axis may improve the quality of life in cancer patients. A questionnaire for patients treated with mistletoe lectin-I confirmed this empirical observation and showed an increased quality of life during immunomodulation [15]. Complementary mistletoe lectin-I administration to cancer patients could be shown to stimulate certain immune functions (necessarily involved in antitumour resistance) and the neuroendocrine system (which, among other things, determines the quality of life) [19].

In order to investigate immunomodulation with mistletoe-lectin-I , patients suffering from malignant stage III/IV glioma (n = 35) were enrolled into a prospectively randomized, clinical trial. All patients were provided with standard oncological treatment (neurosurgery, radiation, basic clinical care according to protocol and indication) and randomly divided into either a treatment group (receiving subcutaneous injections of mistletoe lectin-I standardized mistletoe extract at 1 ng/mistletoe lectin-I /kg body weight, twice a week for 3 months, starting on day 1 postsurgery) or a control group without additional complementary treatment. Immunophenotyping of peripheral blood leukocytes was regularly performed by flow cytometry to evaluate the immunomodulating capacity of mistletoe lectin-I standardized mistletoe extract. Standard tumour destructive treatment of glioma patients proved to be suppressive for peripheral blood lymphocytes since all subsets tested revealed statistically significant downregulation. Unlike the lymphocyte counts and activities of patients from the control group who presented with preoperative values after 3–6 months, mistletoe treatment induced a statistically significant upregulation of cell counts (pan T cells, CD-3; helper/inducer T cells, CD-4; suppressor/cytotoxic T cells and CD-8) and activities (interleukin-2 receptor, CD-25; HLA-DR antigen positive cells) after 3 months compared to preoperative values. A strong immunoprotective/immunostimulatory effect was induced by the treatment of glioma patients with mistletoe lectin-I standardized mistletoe extract [19].

A standard questionnaire (SPITZER) was used in an attempt to investigate a possible quality of life benefit of immunotherapy with mistletoe lectin-I standardized mistletoe extract. Although no obvious difference between the control and treatment groups could be shown initially (3 months postoperatively), patients in the mistletoe lectin-I treated group presented considerably higher questionnaire scores (correlating with an improved qual-

ity of life) after a 6 month follow-up period compared to patients in the control group [19]. Accordingly, co-stimulation of the neuro–immuno–endocrine system can be anticipated, as shown for breast cancer patients recently [15].

Further prospectively randomized clinical (multicentre) studies are currently under investigation to extend these data. So far, no further relevant conclusion can be drawn because of the limited (still ongoing) patient recruitment and follow-up. However, the preliminary data suggest that mistletoe lectin-I standardized mistletoe extract can be recommended for oncological baseline therapy.

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