Preclinical report

Absence of tumor growth stimulation in a panel of 16 human tumor cell lines by mistletoe extracts in vitro

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Extracts of Viscum album (mistletoe) are widely used as complementary cancer therapies in Europe. The mistletoe lectins have been identified as the main active principle of mistletoe extracts. They have been shown to exhibit cytotoxic effects as well as immunomodulatory activities. The latter is exemplified by induction of cytokine secretion and increased activity of natural killer cells. Recent reports however, indicated possible tumor growth stimulation by mistletoe extracts. Therefore, the three aqueous mistletoe extracts (Iscador M special, Iscador Qu special and Iscador P) were evaluated for antiproliferative and/or stimulatory effects in a panel of 16 human tumor cell lines in vitro using a cellular proliferation assay. The results show no evidence of stimulation of tumor growth by any of the three Iscador preparations, comprising central nervous system, gastric, non-small cell lung, mammary, prostate, renal and uterine cancer cell lines, as well as cell lines from hematological malignancies and melanomas. On the contrary, Iscador preparations containing a high lectin concentration (Iscador M special and Iscador Qu special) showed antitumor activity in the mammary cancer cell line MAXF 401NL at the 15 μg/ml dose level with a more than 70% growth inhibition compared to untreated control cells. In addition, a slight antitumor activity (growth inhibition 30–70%) was found in three tumor cell lines for Iscador M special and in seven tumor cell lines for Iscador Qu special, respectively. Iscador P, which contains no mistletoe lectin I, showed no antiproliferative activity. [© 2002 Lippincott Williams & Wilkins]

Key words: Antiproliferative activity, human tumor cell lines, mistletoe extracts, stimulation of tumor growth.

Introduction

Aqueous extracts of the European mistletoe (Viscum album L.) have been widely used for decades as alternative treatment and adjuvant cancer therapy, particularly in Germany, Austria and Switzerland.1-5 The main components of mistletoe extract are lectins, viscostoxins and alkaloids. The mechanism of action is probably 2-fold. On the one hand, mistletoe lectins can stimulate immunological relevant effector cells like macrophages, natural killer cells, and B and T lymphocytes with subsequent release of cytokines [interleukin (IL)-1, IL-6, IL-10, tumor necrosis factor-α and granulocyte macrophage colony stimulating factor];1-8 on the other hand, mistletoe lectins have shown direct growth inhibitory effects on tumor cells. Depending on the concentration, treatment with mistletoe lectins results in death via apoptosis or necrosis.9-13 Moreover, preclinical activity of aqueous mistletoe extracts has been shown in transplantable murine tumor models in vivo.14

In particular, the stimulation of immunological effector cells could be also associated with a potential growth stimulatory effect on hematological malignancies which are derived from the immune system like non-Hodgkin’s or Hodgkin’s lymphomas as well as acute leukemias. There is a case report that mistletoe extracts can enhance the growth of non-Hodgkin’s lymphomas.15 The majority of patients, however, appear to have benefited from an additional therapy with mistletoe.1-5 Furthermore, Gäbius et al. described stimulation of melanoma and sarcoma cell lines by a purified mistletoe lectin in vitro.16

One of the oldest mistletoe preparations is Iscador. Iscador is extracted from mistletoe plants growing on different host trees like apple (Iscador M special), oak (Iscador Qu special) and pine (Iscador P). The aqueous extracts are biologically and biochemically standardized. Iscador M special contains 250 ng total lectins/ml, Iscador Qu special contains 375 ng total lectins/ml, whereas Iscador P contains only trace amounts of lectins.
In order to study direct effects of Iscador preparations on the growth of tumor cells *in vitro*, a panel of 16 human tumor cell lines was investigated in a cellular proliferation assay.

**Materials and methods**

**Cell lines**

Characteristics of the 16 tumor cell lines are shown in Table 1. Ten cell lines were established from human tumor xenografts as described by Roth *et al.* The origin of the donor xenografts was described by Fiebig *et al.* They comprise the following tumor types: GXF 251L (gastric), LXFA 629L, LXFL 529L and LXFE 66NL (lung, non-small-cell), MAXF 401NL (mammary), MEXF 462NL and MEXF 514L (melanoma), RXF 944L and RXF 393NL (renal), and UXF 1138L (uterine). The cell lines SF268 (glioblastoma), H460 (lung, non-small-cell), MCF7 (mammary) and PC3M (prostate), as well as the hematological cell lines HL60 (promyelocytic leukemia) and RPMI 8226 (myeloma) were kindly provided by the US National Cancer Institute. Cells were routinely passaged once or twice weekly. They are maintained no longer than 20 passages in culture. All cells were grown at 37 °C in a humidified atmosphere (95% air, 5% CO₂) in RPMI 1640 medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum (Sigma, Deisenhofen, Germany) and 0.1% gentamicin (Invitrogen).

**Cell proliferation assay**

A modified propidium iodide assay was used to assess the effects of Iscador extracts on the growth of the human tumor cell lines. Briefly, cells were harvested from exponential phase cultures by trypsinization (not for hematological cells), counted and plated in 96-well flat-bottomed microtiter plates at a cell density dependent on the cell line (5–12 000 viable cells/well). After 24 h recovery to allow the cells to resume exponential growth, 10 μl of culture medium (six control wells per plate) or culture medium containing Iscador extracts was added to the wells. Each concentration was plated in triplicate. Iscador preparations were applied in five concentrations ranging from 0.0015 to 15 μg plant extract/ml (Iscador M special and Qu special) and 0.003 to 30 μg plant extract/ml (Iscador P), respectively. Following 4 days of continuous drug exposure, cell culture medium was replaced by 200 μl aqueous propidium iodide (PI) solution (7 μg/ml). Since PI only passes leaky or lysed cell membranes, DNA of dead cells can be stained and measured, while living cells will not be stained. To measure the proportion of living cells, cells were permeabilized by freezing the plates, resulting in death of all cells. After thawing of the plates, fluorescence was measured using the Cytofluor 4000 microplate reader (excitation 530 nm/emission 620 nm), giving a direct relationship to the total cell number. The assay included untreated and positive controls (doxorubicin and vindesine).

**Table 1. Human tumor cell lines used for testing Iscador extracts**

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Cell line</th>
<th>Histology in nude mice</th>
<th>Doubling time (h)</th>
<th>Tumor formation in vivo</th>
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<tr>
<td>CNS</td>
<td>SF268</td>
<td>undifferentiated glioblastoma</td>
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<td>Hematologic</td>
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<td>Lung, non-small cell</td>
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<td></td>
<td>H460</td>
<td>large cell</td>
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<td></td>
<td>LXFA 629L</td>
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<td>LXFL 529L</td>
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<td>LXFE 66NL</td>
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<td>MAXF 401NL</td>
<td>papillary adenocarcinoma, well differentiated</td>
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<td>Melanoma</td>
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<td>RXF 393NL</td>
<td>clear cell carcinoma</td>
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<td>Uterine</td>
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Cell lines developed in Freiburg were described by Roth *et al.*

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Growth inhibition/stimulation was expressed as treated/control × 100 (%T/C). Antitumor activity was defined as inhibition of tumor growth to less than 30% compared to the medium-treated control cells. Coefficient of variation [SD/mean × 100 (%)] was experiments below 20% in nearly all. Experiments were performed 3 times and T/C values are shown as mean of three experiments (Figures 1–3).

**Figure 1.** *In vitro* growth effects of Iscador M in a panel of 16 tumor cell lines. Growth inhibition/stimulation was expressed as treated/control × 100 (% T/C). Results were presented as mean of three experiments. (A) Hematologic, melanoma and renal. (B) Gastric and lung. (C) Central nervous system, mammary, prostate and uterine.
Mistletoe preparations

Iscador M special, Iscador Qu special and Iscador P were kindly provided by Weleda (LOCATION???, Germany). The ampoules contain 1 ml of an aqueous extract from 5 mg total plant of *V. album* (Mali) with 250 ng total lectins/ml, 5 mg total plant of *V. album* (Quercus) with 375 ng total lectins/ml or 10 mg total plant of *V. album* (Pini), respectively. Referring to these original amounts of plant material, maximum

Figure 2. *In vitro* growth effects of Iscador Qu in a panel of 16 tumor cell lines. Growth inhibition/stimulation was expressed as treated/control × 100 (% T/C). Results were presented as mean of three experiments. (A) Hematologic, melanoma and renal. (B) Gastric and lung. (C) Central nervous system, mammary, prostate and uterine.
Results

The effects of the three mistletoe extracts on tumor growth were investigated in a panel of 16 human
tumor cell lines comprising nine different tumor types. Iscador M special and Iscador Qu special were applied in dose levels ranging from 1.5 ng/ml to 15 μg/ml total plant extract under continuous drug exposure for 4 days. Iscador P was added to the cells in dose levels from 3 ng/ml to 30 μg/ml total plant extract. The results are summarized in Figures 1–3 for Iscador M special, Iscador Qu special and Iscador P, respectively. There was no evidence of stimulation of tumor growth by any of the three Iscador preparations. Independent on the dose level of Iscador preparations, none of the cells lines demonstrated a T/C > 120% compared to the medium-treated control cells.

On the contrary, at the highest dose level, the mistletoe extracts containing standardized lectins (Iscador M special and Iscador Qu special) showed antitumor activity (T/C < 30%) in the mammary cancer cell line MAXF 401NL (Figures 1 and 2). In addition, a slight inhibition of growth (T/C = 30–70%) was found in three tumor cell lines following treatment with Iscador M special, i.e. leukemia RPMI 8226, non-small cell lung cancer LXFE 66NL and uterine cancer UXF 1138L (Figure 1). Iscador Qu special showed a slight inhibition of growth in seven tumor cell lines, comprising central nervous system cancer SF268, gastric cancer GXF 251L, non-small cell lung cancers LXFE 66NL and LXFL 529L, breast cancer MAXF 401NL, prostate cancer PC3M, renal cancer RXF 944L, and uterine cancer UXF 1138L (Figure 2). No growth inhibition was observed with Iscador P (Figure 3).

**Discussion**

Aqueous extracts from leaves and branches of the European mistletoe (V. album L.) exert antitumor activity via cytotoxic and immunological mechanisms.4–13 To exclude possible direct growth stimulatory effects on tumor cells, the activity of three standardized mistletoe extracts, Iscador M special, Iscador Qu special and Iscador P, was investigated in a panel of 16 human tumor cell lines in vitro by using a cellular proliferation assay. Doubling times of the cell lines ranged widely from 18–45 h and their chemosensitivity profiles were also diverse. For example, the slowly growing, chemosensitive cell line MAXF 401NL and the faster growing, resistant cell line RXF 944L were included in the studies.17,18,20,21

Our results showed no evidence for direct stimulation of tumor growth in vitro by all three Iscador extracts, comprising central nervous system, gastric, non-small cell lung, mammary, prostate, renal and uterine cancer cell lines as well as cell lines from hematological malignancies and melanomas.

Recently, Gabius et al.16 reported a slight enhancement of tumor growth by a purified mistletoe lectin in human tumor cell lines comprising the sarcoma Hs729, SK-UT-1B and SK-LMS-1 cell lines as well as the melanoma SK-MEL-28 and HT-144 cell lines. At a dose level of 50 pg/ml galactoside-binding mistletoe lectin they have found a very weak growth enhancement of 10 to maximal 40% compared to growth of untreated cells, in most cases at one incubation time point only, without a clear dose or time dependency. In our studies, Iscador M special was applied at dose levels ranging from 1.5 ng/ml up to 15 μg/ml plant extract, corresponding to a concentration of total lectin from 0.075 to 750 μg/ml. The lectin dose levels of the other lectin-containing product, Iscador Qu special, ranged from 0.11 to 1100 μg/ml in our experiments. Neither preparation showed any stimulatory effect at a lectin concentration of 50 μg/ml. We plan to examine the above-mentioned sarcoma and melanoma cell lines in our cell proliferation assay.

At the highest dose level studied, 15 μg/ml total plant extract, corresponding to a lectin concentration of 0.75 ng/ml (Iscador M special) and 1.1 ng/ml (Iscador Qu special), respectively, both extracts showed antiproliferative activity or slight inhibition of tumor growth in distinct cell lines, which is in agreement with results reported by other groups for a variety of aqueous mistletoe preparations standardized for bioactive mistletoe lectins.9–13 Moreover, Siegle et al.22 reported recently on the enhancement of cytotoxicity of purified mistletoe lectin in combination with standard therapeutic drugs, including doxorubicin, cisplatin and taxol in the human lung carcinoma cell line A549 in vitro, suggesting new clinical perspectives for mistletoe therapy.

**Conclusion**

In conclusion, the standardized mistletoe extracts Iscador M special, Iscador Qu special and Iscador P showed no tumor stimulatory properties in a panel of 16 human tumor cell lines. In contrast, the mistletoe extracts containing high amounts of lectins (Iscador M special and Qu special) showed antitumor activity in the mammary cancer cell line MAXF 401NL, which is an encouraging result that should be further exploited and confirmed in in vivo animal models.
No tumor growth stimulation by mistletoe extracts in vitro

References

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