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The *Viscum album* preparation *Isorel* inhibits the growth of melanoma B16F10 by influencing the tumour–host relationship

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The aim of this study was to analyse whether *Viscum album* (mistletoe; *Isorel*) modulates the tumour–host relationship and whether this might be a basic mechanism of the antitumorous activity of the drug. The effects of a single intraperitoneal injection of the drug (100 mg/kg single '*planta tota*' dose) were analysed for mice-bearing melanoma B16F10 growing in the hind limb. Injection of *Isorel* reduced the size of the tumour and caused abundant tumour necrosis with inflammatory response, oedema and destruction of the malignant tissue. Furthermore, the lymphocytes of saline-treated tumorous mice were not able to respond to the mitogenic lectin concanavalin A *in vitro*, while those of mistletoe extract-treated mice showed high reactivity to the mitogen, but only if cultured in the medium supplemented with the plasma of the mistletoe extract-treated mice. Moreover, melanoma cells exposed to the mistletoe extract were more sensitive to the cytotoxic activity of the lymphocytes than the control tumour cells, particularly in the presence of the plasma of mistletoe extract-treated mice. The plasma itself, however, did not show any cytotoxic activity. These results indicate that the antitumour activity of the mistletoe drug is due to a modulation of the tumour–host relationship, mediated by direct cytotoxicity of the drug to tumour cells and/or through a potentiation of immune response by certain, as yet unidentified, growth modifying humoral factors of the host.

Keywords: Mistletoe, *Viscum album*, melanoma, cytotoxicity, humoral factors, cytotoxic lymphocytes, plasma.

Introduction

Because of cytotoxic but not immunosuppressive effects, various *Viscum album* L. (mistletoe) preparations are used for the therapy of human diseases, particularly cancer. Several data point to a cytotoxic action of mistletoe extracts against different tumour cell lines [1–4]. Moreover, application of mistletoe extracts *in vivo* may have an immunomodulating effect, mainly stimulating the cytotoxic activity of the lymphocytes [1,5–7]. Different procedures used for isolation of the active components from mistletoe resulted in purification of numerous different substances, among which mistletoe lectins are considered as the most important since they often show similar biological activity to the plain plant extracts [8–11]. Thus, while the nature of the active principle of mistletoe is still unclear, the dual activity of the mistletoe preparations (cytotoxicity to tumour cells and enhancement of immune host defence against malignant cells) lends support for the practical use of these preparations in human medicine. This is also true for the mistletoe preparation *Isorel* [12–15] used in this study. The aim of this investigation was to analyse whether the modulation of the tumour–host relationship by the drug could be the basic principle of the antitumorous activity of the mistletoe extract *Isorel*.

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Materials and methods

Mistletoe extract

The original preparation of the apple tree fresh mistletoe extract *Isorel* M (Novipharm GmbH, Pörtschach, Austria) was used. The preparation is produced by the cold water extraction of the fresh entire plant, without homogenization of the plant or extract fermentation, and was donated by Novipharm for the purpose of this research.

Treatment of the tumour-bearing mice

Melanoma B16F10 cells were collected from established semiconfluent cultures and were injected subcutaneously into the hind limb of the four-month-old, C57BL/GoZgr male mice (14 animals, weight 25 g, 10^6 live tumour cells per animal). The animals were kept in plastic cages with food (Sljeme, Zagreb, Croatia) and water was given *ad libitum*. On the 14th day after tumour transplantation, the animals were divided into two groups and were injected intraperitoneally with 0.5 ml sterile saline or with 0.5 ml 10% v/v *Isorel*/saline solution, respectively. Thus, the mice treated with the mistletoe extract received the drug as a single dose of 100 mg/kg (*planta tota* dose). On the same day, animals were marked and the size of the growing tumour in each animal was measured by calliper. After 2 days, the size of

the respective tumour was measured again, the animals were anaesthetized by ether and sacrificed by cutting the neck with scissors under sterile conditions. The obtained blood was collected into test tubes with ethylenediamine tetra-acetic acid, allowing preparation of pooled plasma samples of the tumorous mice. From each of the animals, axillary and inguinal lymph nodes were taken and used for the preparation of the lymphocyte cultures (from the cell suspensions pooled according to the groups of mice). The samples of tumorous tissue were also collected as a pooled tissue sample per group under sterile conditions, while the remainder of the tumours were fixed in 10% buffered paraformaldehyde solution for histological analysis.

Melanoma B16F10 cell cultures

Grossly intact pieces of tumour (without a sign of necrosis), removed from the animal under sterile conditions, were used as a source of tumour cell suspension prepared by mechanical dispersion of the tissue. After washing the cells with RPMI 1640 medium three times at 150 **g** for 10 min each time, viability of the cells was tested by the trypan blue exclusion test. The number of viable tumour cells was adjusted to 2×10^5 cells/ml medium and the cells were seeded *in vitro*. The seeding density of the cells cultured in 96-well microcytoplates (Greiner, Frickenhausen, Germany) was 1×10^4 cells per culture. The cells were incubated in RPMI 1640 medium only or in medium supplemented with 5% of the murine plasma (prepared as described), and were cultured for 48 h at 37°C in a humidified air atmosphere with 5% CO_2. The uptake of ^{3}H-thymidine (specific activity 30 Ci/mmol, Amersham International plc, Buckinghamshire, UK) was determined for the last 24 h of cell growth *in vitro*, after which the cells were harvested with the flow harvester (PHD cell harvester, Cambridge Technology Inc., Watertown, USA). The intensity of the cell labelling was measured by a beta counter (Wallac 1209 Rackbeta liquid scintillation counter, Pharmacia, Sweden).

Murine lymphocyte cultures

Lymphocytes were obtained from the axillary and inguinal lymph nodes of the sacrificed tumorous mice. The tissues of seven mice were minced together (according to the groups of mice) and the crude cell suspension was prepared as for the melanoma B16F10 by mechanical dispersion of the tissue. After the viability of the cells was checked by the trypan blue exclusion test, the number of living cells was adjusted to 5×10^5 per ml of RPMI 1640 medium, and the cells were seeded *in vitro*. The seeding density of the lymphocytes cultured in 96-well microcytoplates was 2.5×10^5 cells per culture. The lymphocytes were incubated in RPMI 1640 plain medium or in medium supplemented with 5% of the murine plasma (prepared as described) and 5 μg/ml of the plant T cell mitogenic lectin concanavalin A (Sigma Chemical Co., St Louis, USA), and were cultured for 72 h at 37°C in a humidified air atmosphere with 5% CO_2. The incorporation of ^{3}H-thymidine was determined for the last 24 h of cell growth *in vitro*, as for the melanoma cells.

Mixed cell cultures

Melanoma B16F10 cells and murine lymphocytes were mixed in a 1 : 25 ratio and cultured in plain medium or in medium supplemented with the plasma of the sacrificed mice. Before mixing the cells, melanoma cells were kept in plastic Petri dishes for 2 h to allow adherence of the viable cells. The non-adherent cells were removed and the adhered tumour cells were harvested by intensive flow of fresh medium. The incidence of the trypan negative cells was determined. Afterwards, the cells were mixed with lymphocytes and incubated *in vitro* for 48 h under standard conditions. The intensity of ^{3}H-thymidine incorporation was determined for the last 24 h of cell growth *in vitro*.

The values for ^{3}H-thymidine incorporation obtained for the mixed cultures were compared with those of separate cultures of respective tumour cells and lymphocytes. Since the ^{3}H-thymidine uptake of lymphocytes ranged from 90–350 cpm/well, compared to 2.190–35.432 cpm/well for the melanoma cells (depending on the cells incubated and the culture conditions), it was reasonable to assume that the majority (at least 95% on average) of the ^{3}H-thymidine uptake registered in the mixed cell cultures was due to incorporation into the tumour cells. Hence, the results were analysed by comparison of the obtained values of ^{3}H-thymidine uptake and the expected values of ^{3}H-thymidine incorporation (cpm/well), calculated as a sum of the obtained values for the separately incubated lymphocytes (without any mitogen) and for the respective tumour cells. The relative change of ^{3}H-thymidine uptake was calculated as:

$$\text{Relative } ^3\text{H-thymidine uptake} = \frac{\text{Obtained value}}{\text{Expected value}} \times 100\ (\%)$$

Based on the degree of competition for ^{3}H-thymidine, it was possible to determine cytotoxic activity of the lymphocytes against the tumour cells as well as the enhancement of the growth of the respective tumour *in vitro*.

Statistics

The results obtained for the quadruplicates of cultures and for the tumour volumes of the seven mice per group were analysed using the Mann-Whitney U-test.

Results

The effect of a single intraperitoneal injection of mistletoe extract on the size of melanoma B16F10 that developed in the hind limb of mice is shown in Fig. 1. While the volume of the tumour increased in the saline-treated control mice, the size of the tumours decreased with the injection of mistletoe extract (in comparison to the initial value, $P < 0.05$). Thus, although the tumour size did not differ in the groups before the treatment (on the 14th day, $P > 0.1$), the tumours of the mice injected with *Isorel* were smaller than the control tumours after the treatment (on the 16th day; $P < 0.002$). The toxicity of mistletoe towards the melanoma cells was verified by a decrease of trypan blue negative (viable) cells in the tumour cell suspension (data not presented). The melanoma cell suspension prepared from the tumours of the control group contained more than 73% trypan blue negative (viable) cells, while the tumour cell suspension of the mistletoe extract-treated mice contained 29% viable tumour cells.

Histology of the tumours (Fig. 2) showed marked differences in the structure between the control and *Isorel*-treated tumours. In the controls, most of the tumours appeared as compact malignant tissue with abundant mitosis of malignant cells, infiltrating surrounding tissue with only moderate inflammation. In contrast, tumours of the mistletoe extract-treated mice showed intensive tumour necrosis with tissue oedema and abundant inflammatory response, particularly at the zone of tumour invasion, resulted in softening of the tumorous tissue, which indicates obvious decay of the tumours.

Cultured tumour cells, both of the control and mistletoe groups, showed a high affinity for ^{3}H-thymidine (Fig. 3). The growth of the tumour cells *in vitro* increased, however, by the addition of murine plasma obtained from the tumorous mice treated with the mistletoe extract. Plasma from control mice did not increase the growth of the tumour cells when compared with the

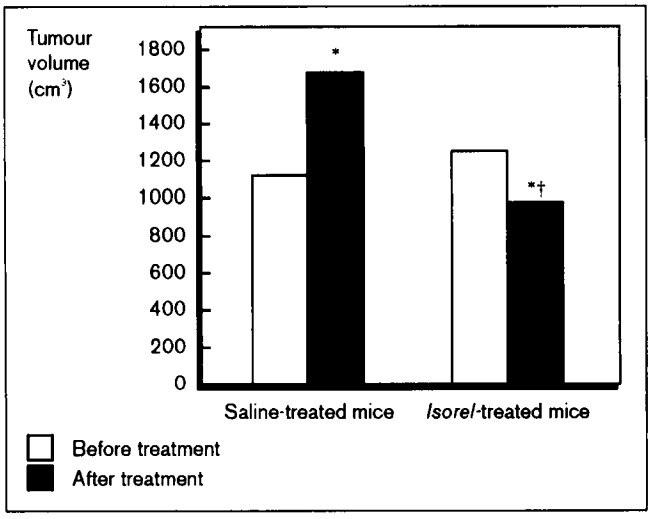

Figure 1. The effects of a single intraperitoneal injection of 100 mg/kg ('*planta tota*' dose) mistletoe extract on the volume of melanoma B16F10 growing in the hind limb of syngeneic C57BL/GoZgr mice. The animals received mistletoe extract (*Isorel*) on the 14th day after tumour transplantation. The size of the tumours was measured by calliper before treatment with the mistletoe extract and 2 days after. Results are means from seven mice per group (SD <28% of the respective mean value). *$P < 0.05$, versus pretreatment; †$P < 0.002$, versus saline group after treatment (by Mann-Whitney U-test).

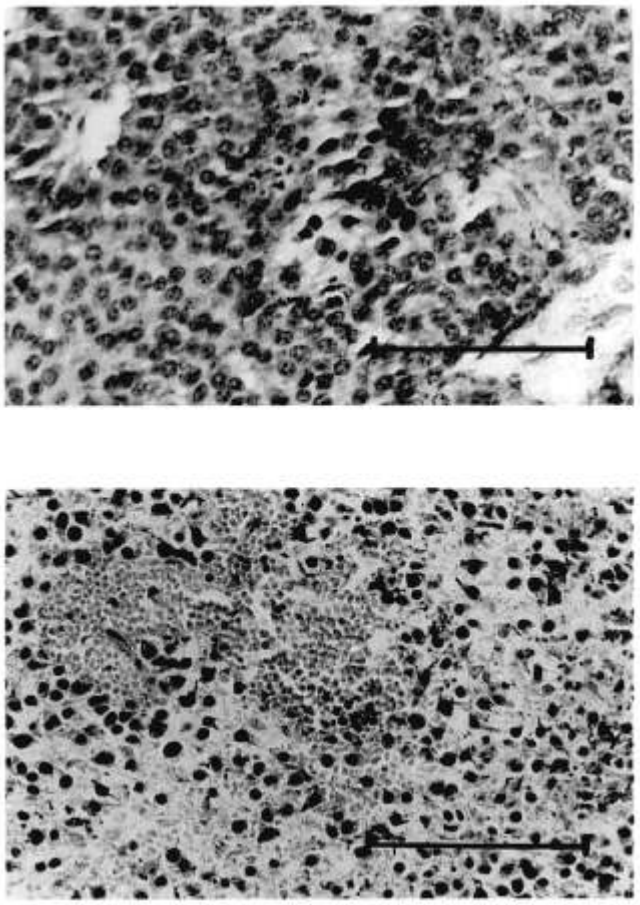

Figure 2. The effects of a single intraperitoneal injection of 100 mg/kg ('*planta tota*' dose) mistletoe extract on the histological structure of melanoma B16F10 growing in the hind limb of syngeneic C57BL/GoZgr mice. The tumours from control mice (top) consisted of compact anaplastic tissue with mitosis of the tumour cells (in the centre of the photomicrograph) and without apparent presence of inflammatory cells. The tumours of the mice injected with the mistletoe extract (bottom) were softening, with obvious oedema, haemorrhagic necrosis and abundant presence of inflammatory cells (mainly mononuclear cells with some granulocytes). Haematoxylin eosin stain; 100×, bar = 100 μm.

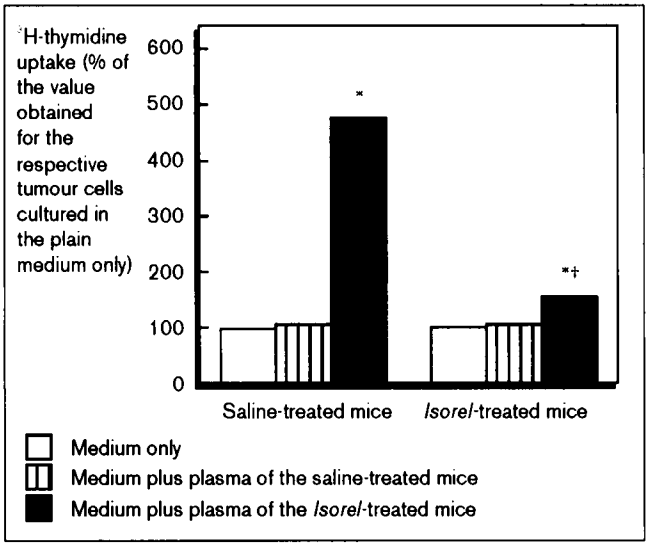

Figure 3. The *in vitro* growth of the melanoma B16F10 cells. The cells were obtained from animals killed 2 days after treatment of melanoma B16F10 bearing mice with a single intraperitoneal injection of 100 mg/kg ('*planta tota*' dose) mistletoe extract or with saline. The cells were incubated in plain medium or in medium supplemented with 5% of the respective mouse plasma. The intensity of ^{3}H-thymidine incorporation was measured for the last 24 h of 2 days incubation. Mean values obtained for quadruplicates of cultures are presented (SD <17% of the respective mean). $^*P < 0.05$, versus medium only and medium plus plasma; $^\dagger P < 0.05$, versus *Isorel* group (by Mann-Whitney U-test).

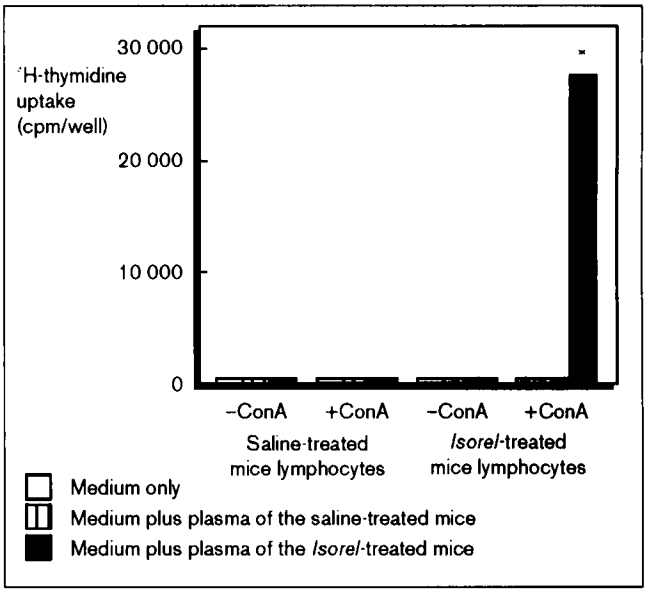

Figure 4. Proliferation of lymph node lymphocytes from tumour-bearing mice. The cells were obtained from animals killed 2 days after treatment of melanoma B16F10 bearing mice with a single intraperitoneal injection of 100 mg/kg ('*planta tota*' dose) mistletoe extract or with saline. Lymphocytes were incubated in plain medium or in medium supplemented with 5% of the respective mouse plasma. The intensity of ^{3}H-thymidine incorporation was measured for the last 24 h period at the end of 3 days incubation with or without concanavalin A. Mean values obtained for quadruplicates of cultures are presented (SD <14% of the respective mean). $^*P < 0.05$, versus all other values by Mann-Whitney U-test.

plain medium only ($P > 0.05$). By contrast, in the presence of plasma from mistletoe extract-treated mice, the tumours of both the control and *Isorel* group grew better than in the presence of the medium only or in the presence of the medium with the control tumorous mouse plasma (for both tumours in both cases, $P < 0.02$). However, the growth of the control tumour cells was more pronounced ($P < 0.002$).

Similarly, the growth of the lymphocytes *in vitro* was strongly stimulated by the addition of plasma from mistletoe extract-treated tumorous mice (Fig. 4). However, the growth promoting effect was observed only for the lymphocytes of the *Isorel*-treated mice when cultured in the presence of the mitogenic lectin concanavalin A. The opposite was seen in the plasma from *Isorel*-treated mice when looking at the growth of tumour cells cultured in the presence of lymphocytes (mixed cell cultures, Fig. 5). Plasma of the saline-treated mice did not influence the growth of tumour cells or leukocytes (mixed cell cultures) from control mice, whereas in the presence of plasma from mistletoe extract-treated mice, an obvious inhibition of the ^{3}H-thymidine incorporation was observed in mixed cell cultures from control and *Isorel*-treated mice ($P < 0.05$), indicating cytotoxic activity of lymphocytes to the tumour cells.

Discussion

Treatment of C57BL/GoZgr male mice with the mistletoe extract *Isorel* increased the cytotoxic activity of the lymphocytes of tumour-bearing mice and/or increased sensitivity of the tumour cells against the cytotoxic activity of the lymphocytes. Furthermore, treatment with *Isorel* also changed the growth modifying activity of plasma of the tumorous mice, which itself was not cytotoxic but promoted the toxicity of the lymphocytes against the tumour cells. These results confirm previous studies that the fresh mistletoe plant extract *Isorel* is effective in tumour growth inhibition and in stimulating immune activity [12–16]. *In vitro*, *Isorel* inhibits the growth of malignant cells more than the growth of normal cells [12–14]. However, the biologically active components of mistletoe remain to be clarified. Previous investigations indicate a synergistic, or at least additive, activity of different mistletoe components [3,10,11,16–

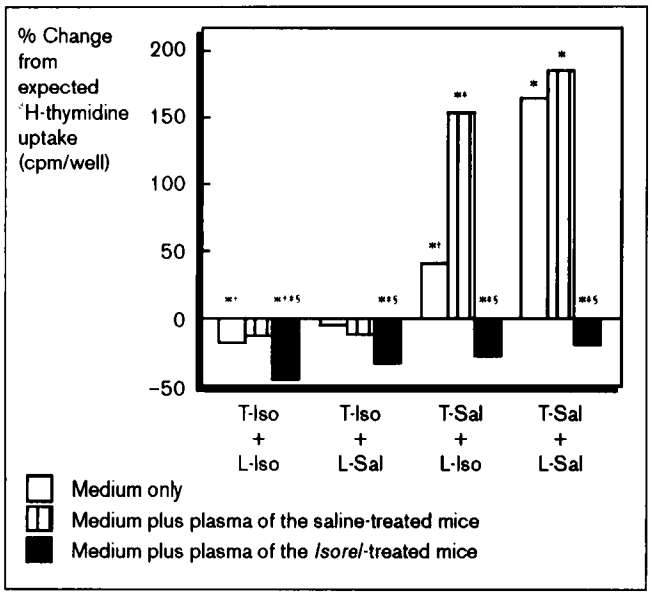

Figure 5. Proliferation of the mixed cultures of melanoma B16F10 cells and lymph node leukocytes. The cells were obtained from animals sacrificed 2 days after treatment of melanoma B16F10 bearing mice with a single intraperitoneal injection of 100 mg/kg ('*planta tota*' dose) mistletoe extract or with saline. The cells were incubated in plain medium or in medium supplemented with 5% of the respective mouse plasma. The intensity of ^{3}H-thymidine incorporation was measured for the last 24 h of 2 days of cultures and compared to the values for the separately cultured tumour and lymph node cells (as described in Materials and methods) to analyse the inhibition of the tumour growth due to the *in vitro* cytotoxic activity of the lymph node lymphocytes. Mean values obtained for quadruplicates of cultures are presented (SD <22% of the respective mean). $^*P < 0.05$, versus the expected values; $^\dagger P < 0.05$, versus values obtained from the same tumour cells cultured with control mouse lymphocytes; $^\ddagger P < 0.05$, versus values obtained from the same 'mixed cell' cultures incubated without any plasma supplementation; $^\S P < 0.05$, versus values obtained from the same 'mixed cell' cultures incubated with control mouse plasma (by Mann-Whitney U-test).

18]. Some of these components may exert a direct cytotoxic effect on the tumour cells (for example the mistletoe lectins), as observed by the histological analysis of the tumours performed in this study. This probably induces further inflammatory responses, as noticed in this study. On the other hand, mistletoe contains both biologically active components which mediate cytostatic/cytotoxic activities, such as the mistletoe lectins, and non-lectin components preventing even genotoxicity in the lymphocytes [19,20]. While serum attenuates the lectin-mediated toxicity, plasma of the mistletoe extract-treated mice did not show higher toxicity *in vitro* than the control plasma. Thus, serum itself may not have the same attenuating effect on the other active factors of the fresh mistletoe plant extracts [21]. As reported recently, a non-lectin lymphocyte stimulating factor was observed in a bacterially fermented mistletoe extract [17]. Further investigations are therefore necessary to evaluate the underlying mechanisms. It may be possible that these mistletoe factors could induce programmed cell death (apoptosis) and thus inhibit metastatic ability of the tumour cells. Indeed, such activity was observed for mistletoe extracts and purified mistletoe lectins *in vitro* [22,23]. However, it remains to be clarified whether an induction of apoptosis by mistletoe extracts may occur also in tumour-bearing mice and which factors influence the sensitivity of tumour cells to the cytotoxic activity of the lymphocytes (perforin/granzymes, APO-1/Fas ligation, tumour necrosis factor-α, interferon-γ) observed in this study. An increased cytotoxic activity of the lymphocytes to the tumour cells has been shown in different studies performed both *in vitro* and *in vivo* as well as in cancer patients [1,2,4,6,9].

The influence of the plasma of mistletoe extract-treated mice on tumour growth and cytotoxic activity of lymphocytes against tumour cells suggests that the active principle of mistletoe extract is related to certain growth regulating humoral factors. The nature of these factors and their relevance for the tumour–host relationship and the therapeutic efficiency of the mistletoe preparation have yet to be studied.

Conclusions

(1) Treatment with a single intraperitoneal injection of 100 mg/kg ('*planta tota*' dose) of the mistletoe extract *Isorel* inhibited the growth of melanoma B16F10 cells injected into the hind limb of syngeneic C57BL/GoZgr mice; (2) inhibition of tumour growth was accompanied by abundant inflammatory reaction and necrosis; (3) injecting the mice with mistletoe extract-treated mice plasma increased the cytotoxicity of lymphocytes against the tumour cells and their reactivity to concanavalin A mitogen; (4) these *in vitro* effects were dependent on humoral factors present in the plasma of mistletoe extract-treated mice whereas the plasma itself lacked any cytotoxic activity at all; and (5) these findings indicate that the mistletoe extract *Isorel* modifies the tumour–host relationship by increasing the host defence against the tumour through certain, as yet unidentified, humoral growth modifying factors and inflammatory reactions causing tumour decay

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References

1. Hajto T, Hostanska K, Gabius HJ: Modulatory potency of the β-galactoside-specific lectin from mistletoe extract (*Iscador*) on the host defence system *in vivo* in rabbits and patients. *Cancer Res* 1989, **49**: 4803–4814.
2. Hajto T, Hostanska K, Fischer J, Lentzen H: Investigations of cellular parameters to establish the response of a biomodulator: galactoside-specific lectin from *Viscum album* plant extract. *Phytomedicine* 1996, **3**: 129–137.
3. Jung ML, Baudino S, Ribéreau-Gayon G, Beck JP: Characterisation of cytotoxic proteins from mistletoe (*Viscum album* L.). *Cancer Lett* 1990, **51**: 103–114.
4. Heiny BM, Beuth J: Mistletoe lectin as an immunomodulator: effects on β-endorphin and the release of cytokines in patients with breast cancer [in German]. *Dtsch Zschr Onkol* 1994, **26**: 103–108.
5. Beuth J, Ko HL, Tunggal L, *et al.*: Dose-dependency of the immune activity of mistletoe lectin-1 [in German]. *Arzneim-Forsch/Drug Res* 1994, **44**: 21–25.
6. Beuth J, Stoffel B, Ko HL, Buss B, Tunggal L, Pulverer G: Immunostimulating activity of different dosages of mistletoe lectin-1 in patients with mammary carcinoma. *Arzneim-Forsch/Drug Res* 1995, **45**: 505–507.
7. Franz H: Request for an impartial discussion of the so-called mistletoe therapy. *Oncology* 1986, **43** (suppl 1): 1–15.
8. Ganguly C, Das S: Plant lectins as inhibitors of tumor growth and modulators of host immune response. *Chemotherapy* 1994, **40**: 272–278.
9. Hülsen H, Mechelke F: *In vitro* effectiveness of a mistletoe preparation on cytostatic-drug-resistant human leukemia cells. *Naturwiss* 1987, **74**: 144–152.
10. Khwaja TA, Dias CB, Pentecost S: Recent studies on the anticancer activities of mistletoe and its alkaloids *Oncology* 1986, **43** (suppl 1): 42–49.
11. Kuttan G, Vasudevan D M, Kuttan R: Isolation and identification of a tumor reducing components from mistletoe extract (*Iscador*). *Cancer Lett* 1988, **41**: 307–314.
12. Jurin M, Zarkovic N, Hrzenjak M, Ilic Z: Antitumorous and immuno-modulatory effects of *Viscum album* preparation *Isorel*. *Oncology* 1993, **50**: 393–398.
13. Kissel D, Jurin M, Zarkovic N: The cytostatic and immunological effects of *Viscum album* [in German]. *Erfahrungsheilkunde* 1990, **39**: 59–63.
14. Kissel D, Jurin M, Zarkovic N: Combined tumour therapy: an experimental study on the activity of *Viscum album* [in German]. *Dtsch Zschr Onkol* 1990, **22**: 134–136.
15. Zarkovic N, Trbojevic-Cepe M, Ilic Z, Hrzenjak M, Grainca S, Jurin M: Comparison of the effects of high and low concentrations of the separated *Viscum album* L. lectins and of the plain mistletoe plant preparation (*Isorel*) on the growth of normal and tumor cells *in vitro*. *Period Biol* 1995, **97**: 61–67.
16. Jurin M, Zarkovic N, Borovic S, Kissel D: Immunomodulation by the *Viscum album L.* preparation *Isorel* and its antitumorous effects. In: *Grundlagen der Misteltherapie*. Edited by Scheer R, Becker H, Berg PA. Stuttgart: Hippocrates Verlag; 1996: 315–323.
17. Stein G, Berg PA: Non-lectin component in a fermented extract from *Viscum album* L. grown on pines induces proliferation of lymphocytes from healthy and allergic individuals *in vitro*. *Eur J Clin Pharm* 1994, **47**: 33–38.
18. Zarkovic N, Jurin M, Dittrich W, Hartleb M, Sladoljev S, Kissel D: Comparison of the antitumorous effects of *Viscum album* lectins and the plain or fractional fresh plant preparation *Isorel*. In: *Grundlagen der Misteltherapie*. Edited by Scheer R, Becker H, Berg PA. Stuttgart: Hippocrates Verlag; 1996: 325–336.
19. Büssing A, Jungmann H, Suzart K, Schweitzer K: Suppression of sister chromatid exchange-inducing DNA lesions in cultured peripheral blood mononuclear cells by *Viscum album* L. *J Exp Clin Cancer Res* 1996, **15**: 107–114.
20. Büssing A, Jurin M, Zarkovic N, Azhart T, Schweitzer K: DNA stabilizing activity of *Viscum album* L.: are mistletoe extracts indicated as supplements for conventional therapy? [in German]. *Forsch Komplementärmed* 1996, **3**: 244–248.
21. Ribéreau-Gayon G, Jung ML, Beck JP, Anton R: Effect of fetal calf serum on the cytotoxic activity of mistletoe (*Viscum album* L.) lectins in cell culture. *Phytother Res* 1995, **5**: 336–339.
22. Janssen O, Scheffler A, Kabelitz D: *In vitro* effects of mistletoe extracts and mistletoe lectins: cytotoxicity towards tumor cells due to the induction of programmed cell death (apoptosis). *Arzneim-Forsch/Drug Res* 1993, **43**: 1221–1227.
23. Büssing A: Induction of apoptosis by the mistletoe lectins: a review on the mechanisms of cytotoxicity by *Viscum album* L. *Apoptosis* 1996, **1**: 25–32.