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Stimulation of antitumour immunity by intrapleural instillation of a *Viscum album* L. extract

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Intrapleural administration of mistletoe extracts is reported to result in pleurodesis in cancer patients with malignant pleural effusions. In a recent study, 20 consecutive cancer patients with malignant pleural effusions were treated intrapleurally with the mistletoe extract *Helixor*. The overall response rate for pleurodesis was 72%, with only 1.2% displaying side effects of the World Health Organization classification I. The decline of tumour cells in the effusion liquid correlated negatively with the number of instillations. However, the elimination of tumour cells was associated with a transient increase in macrophages and eosinophils, and a constant increase in CD8+T cells. Compared to the responder group, the non-responders exhibited high proportions of macrophages, CD8+T cells and T cells with human leukocyte antigens with DR specificity (HLA-DR) in the effusion liquid, compatible with a disturbance of macrophage/T cell co-operation and thus failure to eliminate the malignant cells. The preliminary results suggest that mistletoe-mediated pleurodesis is due to a stimulation of antitumour immunity rather than mechanical sclerosis.

Keywords: Viscum album L., malignant pleural effusion, pleurodesis, macrophages, human leukocyte antigen with DR specificity, expression, cytotoxic T cells.

Introduction

Since the appearance of a malignant pleural effusion in most cases indicates that the underlying cancer has advanced beyond the stage of curability, and these effusions obviously reduce the general well-being of the cancer patient, the first aim of any therapeutic approach must be an improvement of the clinical situation. Thus, effective control of malignant pleural effusions can greatly improve the quality of life of the cancer patient, but may not lengthen the patient's survival in most instances. Different treatment modalities with varying results have been used for controlling this common complication of malignant disease. Instillation of various agents such as talc, bleomycin, doxycycline, mitoxantrone, interferons and *Corynebacterium parvum*, into the pleural cavity to obliterate the occupied space is a common approach to management [1,2]. A review of the literature shows that chemical pleurodesis produced a complete response in 64% of patients [2]; however, the success rate of the agents varied from 0% with etoposide to 93% with talc. The most commonly reported adverse effects were pain (23%) and fever (19%) [2]. The multiplicity of treatment confirms the lack of an optimal therapy. The ideal treatment should be 100% effective, safe, and convenient. Whatever agent is used, the effectiveness of therapy should be correlated to the degree of pleurodesis achieved and to the side effects and complications of these techniques.

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Pleurodesis by mistletoe extracts

A number of investigations have shown that intrapleural instillation of the *Viscum album* L. (mistletoe) extract Iscador is effective in reducing the number of malignant cells in malignant pleural effusions [1–6]. In 197 cancer patients, a response rate of 92% was reported. The efficacy was suggested to be due to the cytostatic and immunomodulatory properties of the intrapleurally applied mistletoe extract, since Salzer and Popp observed an increase of eosinophils, T-helper cells, and natural killer cells in the effusions [7,8].

In a recent report from our group [9], 20 cancer patients with malignant pleural effusions were prospectively investigated and intrapleurally treated with the aqueous mistletoe extract *Helixor*. Here, 11 out of 18 patients who could be evaluated showed pleurodesis, while two patients had a partial remission after the treatment. In four out of the 18 patients, the therapy failed. The number of tumour cells in the malignant pleural effusions significantly decreased with application of the drug. The overall response rate of 72% is comparable to the treatment of malignant pleural effusions with tetracyclines, with only 1.2% displaying side effects of the World Health Organization classification I in mistletoe extract-treated patients. Thus, pleurodesis by mistletoe extracts is methodically simple, effective and has low side effects.

Activation of immunity by *Viscum album* L.?

Tumour cells, immune competent cells, and anti-mistletoe lectin antibodies were monitored by investigation of the effusion liquid [9,10]. Anti-mistletoe lectin antibodies were found in large amounts in the effusion liquid of the cancer patients because of subcutaneous pretreatment of the patients with the mistletoe extract. These specific antibodies were reported to neutralize the cytotoxic activity of mistletoe lectins [11]. Thus, a direct cytotoxic effect of the apoptosis-inducing mistletoe lectins [12] to the malignant cells seems unlikely. What are the mechanisms of tumour cell elimination?

Mistletoe extracts and purified components are reported to non-specifically stimulate the immune system, as they increase the number and activity of natural killer cells and neutrophils [13–15], induce cytokines such as tumour necrosis factor-α, interferon-γ, interleukin-1 and interleukin-6 [14–16]. Since intrapleural administration of interferon-γ in patients with malignant pleural mesotheliomas was associated with an activation of macrophages and cytotoxic T lymphocytes [17], we cannot exclude the possibility that the intrapleural application of the mistletoe extract may have stimulated antitumoral immunity and/or directly decreased the proliferation of malignant cells.

To address this question, we investigated the immune cells in the effusion liquid and observed obvious differences between the responders and non-responders [18]. Within the group of patients as a whole, the number of tumour cells significantly declined [9,10]; however, the proportions of CD4+ T-helper cells, CD8+ suppressor/cytotoxic T cells, CD16+CD56+ natural killer cells and macrophages did not differ between the first and last day of treatment in the groups (Table 1), while the relative amount of eosinophils and neutrophils increased within the observation period. As shown in Table 2, high amounts of macrophages, T cells with human leukocyte antigen with DR specificity (HLA-DR) and CD8+ suppressor/cytotoxic T cells were observed in the effusion liquid of non-responders prior to therapy, while the relative amounts of these cells were significantly lower in responders. Surprisingly, during the observation period, the high level of cell numbers remained almost unchanged in the non-responders and partial responders.

A simple comparison (first day versus last day of treatment) does not show a comparable individual immune time-course response (Fig. 1). Intensive immunological investigations of cells in the effusion liquid from eight patients indicate that the decline in tumour cells was associated with a transient rise in macrophages and eosinophils (Fig. 1); however, the peak for macrophages and eosinophils is not seen in the non-responders (Fig. 2). In the case of non-responders, high numbers of macrophages, CD8+ T cells and HLA-DR+ T cells were present in the malignant pleural effusions during the observation

Table 1. Cancer patients with malignant pleural effusions treated with *Helixor* intrapleurally

	First day of treatment		Last day of treatment	
	NR + PR	CR	NR + PR	CR
Tumour cells (Units 1–4)	2.3 ± 1.5	1.3 ± 1.4	1.0 ± 1.1	0.4 ± 1.1
Macrophages (%)	15.7 ± 25.4	1.8 ± 3.5	16.0 ± 24.9	3.4 ± 6.3
Eosinophils (%)	0.8 ± 1.3	3.5 ± 6.1	4.5 ± 5.5	13.5 ± 25.0
Neutrophils (%)	12.3 ± 22.7	4.7 ± 8.8	28.8 ± 30.6	13.5 ± 25.0
HLA-DR+ T cells (%)	20.5 ± 12.7	14.7 ± 9.0	29.7 ± 22.4	16.0 ± 9.3
CD3+CD8+ T cells (%)	44.6 ± 7.0	25.9 ± 12.4	39.0 ± 9.8	24.0 ± 4.4
CD3+CD4+ T cells (%)	44.6 ± 7.2	56.1 ± 15.4	41.8 ± 9.0	53.8 ± 15.3
CD16+CD56+ NK cells (%)	9.5 ± 4.0	9.0 ± 4.0	8.8 ± 2.8	6.1 ± 3.2

Results are means ± SD. NR, non-responder; PR, partial responder; CR, complete responder; HLA-DR, human leukocyte antigen with DR specificity; NK, natural killer.

Table 2. Malignant pleural effusions of cancer patients prior to therapy

	NR (n = 4)	PR (n = 2)	CR (n = 12)
Tumour cells (Units 1–4)	3.3 ± 0.5 (3–4)	0.5 ± 0.7 (0–1)	1.3 ± 1.4 (0–4)
Macrophages (%)	22.8 ± 29.5 (0–62)	1.5 ± 0.7 (1–2)	1.8 ± 3.5 (0–12)
Eosinophils (%)	1.3 ± 1.5 (0–3)	0.0 ± 0.0 (0)	3.5 ± 6.1 (0–17)
HLA-DR+ T cells (%)	24.0 ± 14.9 (3–38)	13.5 ± 0.7 (13–14)	14.7 ± 9.0 (5–34)
CD3+CD8+ T cells (%)	49.3 ± 2.9 (46–51)	37.5 ± 3.5 (35–40)	25.9 ± 12.4 (9–42)
CD3+CD4+ T cells (%)	43.3 ± 9.7 (35–54)	46.5 ± 2.1 (45–48)	56.1 ± 15.4 (40–80)
CD16+CD56+ NK cells (%)	8.0 ± 2.2 (7–11)	12.5 ± 6.4 (8–17)	9.0 ± 8.7 (2–27)

Results are means ± SD (range). NR, non-responder; PR, partial responder; CR, complete responder; HLA-DR, human leukocyte antigen with DR specificity; NK, natural killer.

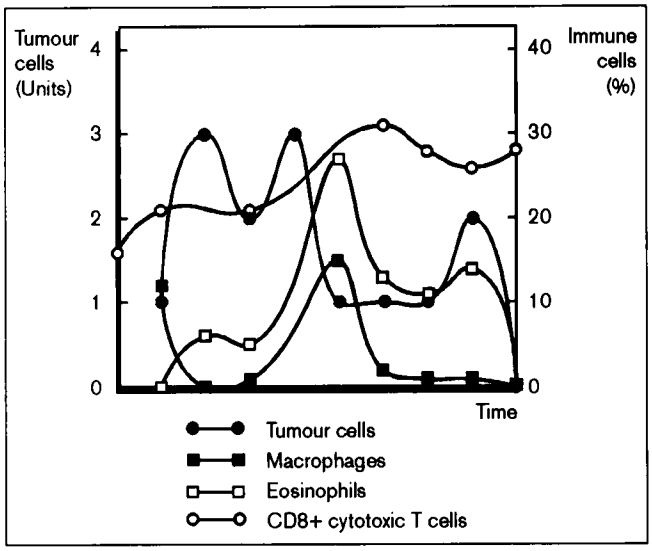

Figure 1. Tumour cells (Units) and macrophages, eosinophils, and CD8+ cytotoxic T cells (%) in the malignant pleural effusion of a female cancer patient (SZ230630) during therapy (3.5 months).

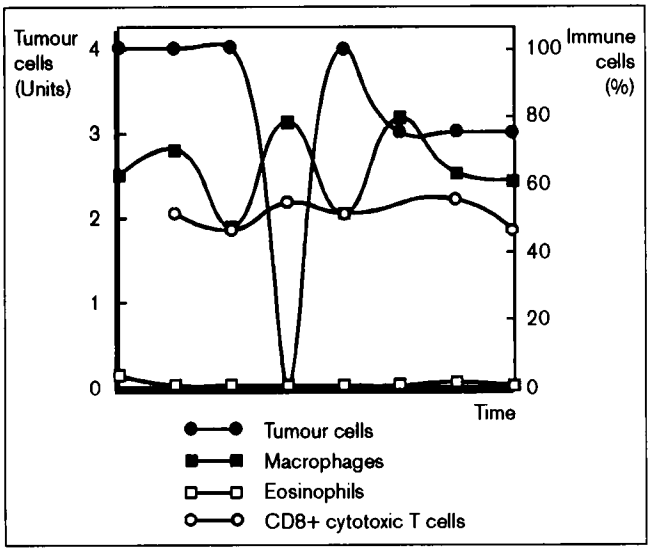

Figure 2. Tumour cells (Units) and macrophages, eosinophils, and CD8+ cytotoxic T cells (%) in the malignant pleural effusion of a female non-responder (BR 080756) during therapy (6 months).

period but the eosinophils did not respond to the treatment.

These observations suggest that the elimination of tumour cells in the malignant pleural effusion liquid is due to a non-specific activation of immune competent cells rather than a non-specific process of mechanical sclerosis. One may speculate that the macrophages and cytotoxic T cells of the non-responders are incompetent at recognizing malignant cells or in antigen presentation, and thus need to compensate this handicap by a higher cell number.

The findings of Gjomarkaj *et al.*[19] indicate that pleural mononuclear phagocytes are involved in tumour-associated inflammatory reactions in the pleural compartment by stimulating the proliferation of other inflammatory cells and by releasing inflammatory cytokines. It was proposed by Takahashi *et al.* [20] that the lymphokine-activated killer activity is augmented by pleural cavity macrophages. Thus, it was suggested that the immune function of cells in malignant effusions may be depressed due to a low population of cytotoxic T cells, low natural killer activity, and increased suppressor T cells. Indeed, an increase in CD8+ suppressor cells in pleural and peritoneal exudate cells and a decrease in natural killer activity was observed by Oka *et al.* [21]. Thus, whatever the exact mechanisms are, our preliminary results are compatible with a disturbance of macrophage/T cell co-operation in the non-responders. Because of this, we suggest that high amounts of macrophages, CD8+ T cells, and HLA-DR+ T cells (non-professional antigen-presenting cells?) in the effusion liquid prior to the instillation may predict an insufficient elimination of malignant cells and thus failure of pleurodesis despite a therapeutic approach. This is in agreement with previous observations that an increased amount of HLA-DR+ T cells and CD28–CD8+ suppressor cells is associated with a progressive decline in the general condition of cancer patients [22]. This effect was associated with a progression of the tumour or metastases.

Conclusion

We suggest that mistletoe-mediated pleurodesis is due to a stimulation of antitumour immunity rather than mechanical sclerosis because: (1) the tumour cells significantly declined by intrapleural administration of the mistletoe extract (negative correlation between number of tumour cells and number of instillations: $r = 0.3280$, $P < 0.01$); (2) this decline in tumour cells was associated with a transient peak of macrophages and eosinophils and a constant rise of CD8+ T cells in the effusion fluid of the responders while these changes were absent in the effusion liquid of the non-responders; and (3) the high proportions of macrophages, CD8+ T cells and HLA-DR+ T cells in the group of non-responders suggest a disturbance of functional competence and thus a failure to eliminate the malignant cells. However, these preliminary results need extended investigation, which is currently under way.

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