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DNA-STABILISING EFFECTS OF VISCUM ALBUM L.: ARE MISTLETOE EXTRACTS INDICATED AS ADJUVANT TREATMENT DURING CONVENTIONAL CHEMOTHERAPY?

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Summary

Viscum album L. (VAL) extracts have been used for years in the adjuvant therapy of cancer. As well as having a non-specific immunostimulatory effect, these preparations display pronounced cytostatic and cytotoxic effects that are mediated by mistletoe lectins. A further important aspect of VAL extracts is their anti-mutagenic action, which appears to unfold primarily in peripheral blood mononuclear cells (PBMC). In contrast, cytostatic effects tend to predominate in malignant cells and cultured amniotic fluid cells. It has been demonstrated that both spontaneous as well as cyclophosphamide-induced DNA lesions are significantly reduced in PBMC by 10 μg/ml of the whole-plant extract *Helixor* A. At the expression level, too, the VAL extract *Helixor* A brings about a significant improvement in cyclophosphamide-induced depression of the activation-associated molecules CD25 (interleukin-2 receptor α chain) and CD71 (transferrin receptor) on the surface of cultured T-cells. In contrast, the purified components, such as mistletoe lectins I and II/III or viscotoxins, did not prevent the depression of activation markers mediated by cyclophosphamide. In cultured leukaemic Jurkat T-cells and in a mouse model, VAL did not exhibit any protective effects; however, marked cytotoxic effects were observed against malignant cells. The concrete results presented and discussed here thus provide a scientific basis to justify the adjuvant administration of mistletoe extracts as a useful therapeutic modality during and/or after conventional chemotherapy.

Key words: *Viscum album* L. – cyclophosphamide – DNA stabilisation – cytotoxicity

Actions of *Viscum album* L.

Viscum album L. (VAL) extracts have been used for years in the adjuvant therapy of cancer. Despite the misgivings of some mainstream medical scientists [12, 13], there is increasing acceptance of VAL extracts, which have been shown to contain a wide range of constituents such as viscotoxins, lectins, sugars/alcohols, polysaccharides, and amines etc. This greater acceptance has been due on the one hand to rising confidence on the part of patients, but also to the increasing body of exact scientific results that has elucidated the mechanisms of action of VAL and its constituents. The following actions have been attributed to VAL: induction of acute-phase proteins [1], enhancement of the number and activity of granulocytes and of natural and lymphokine-dependent killer cells [14, 15, 16, 30], induction of cytokines such as interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α), interleukin-1 (IL-1) and IL-6 [15, 31, 37], as well as cytostatic/ cytotoxic activity. The cytostatic/cytotoxic effects of VAL against cultured tumour cells and lymphocytes have been documented in numerous publications [6, 11, 18 – 20, 22, 39]. Cell death occurs both as a result of the induction of programmed cell death (apoptosis; Figure 1) [7, 19] as well as following direct/ indirect cell membrane damage [7]. Naturally, the observed non-specific stimulation of the immune system should not be equated with therapeutic efficacy of VAL extracts in the treatment of malignant disease. Various studies have sought to confirm a benefit of mistletoe therapy for cancer patients [review in 21]. Although the quality of these studies is frequently viewed as unsatisfactory and some critics have refused to concede that they have any relevance due to methodological shortcomings [17], some controlled studies are nevertheless characterised both by robust design and by statistically significant results [review in 21]. Is there a rational basis therefore that would justify the adjuvant administration of VAL extracts?

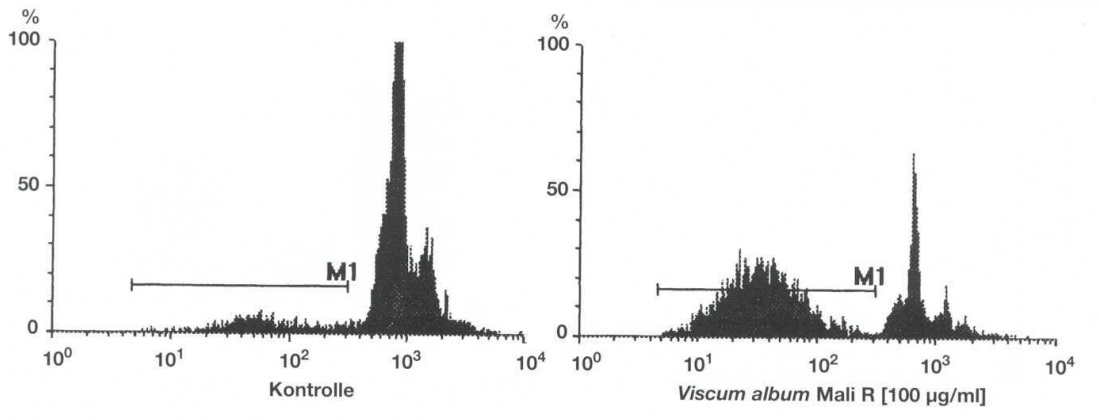

Figure 1: DNA fluorescence (propidium iodide) of lymphocytes that have been cultured for 72 h. Apoptotic cells have a hypodiploid DNA content and are shown as a sub-G_1 peak (M1). Following addition of *Viscum album* Mali R, the proportion of apoptotic cells is increased from 9 % to 67 % by comparison with the control, while the proportion of lymphocytes in the G_1 phase and in the S-G_2 phase of the cell cycle is reduced accordingly.

Key:
[Left-hand X axis:] Control
[Right-hand X axis:] *Viscum album* Mali R [100 µg/ml]

DNA stabilisation by *Viscum album* L. extracts

A marked improvement in survival time of gamma-irradiated mice has been observed in experimental animal studies following injection of polysaccharides from VAL [32]. Kuttan & Kuttan [24] also demonstrated in an experimental animal study that radiation- and cyclophosphamide-induced leukopenia in mice could be improved by injection of VAL. UV-irradiated lymphocytes from women with breast cancer treated with a fermented VAL extract (*Iscador* M) following previous chemotherapy and/or radiotherapy showed an increase in [^{3}H]-thymidine incorporation into DNA within an observation period of 7 – 9 days after the first dose [23]. These studies underline the experiences of oncologists involved in patient care that radiotherapy or chemotherapy is clearly better tolerated when treatment with VAL extracts is given.

As recently described by our research team, an important clue in accounting for the suspected anti-mutagenic activity of VAL extracts could be the DNA-stabilising effect which appears to be limited to peripheral blood mononuclear cells (PBMC) [2, 3]. Co-culturing of mononuclear cells from the peripheral blood of healthy subjects with VAL extracts (7 – 14 µg/ml *Helixor* A and *Helixor* P) led to a significant reduction in sister chromatid exchange-inducing DNA lesions [2]. Although the precise mechanism underlying its development is unclear, sister chromatid exchange (SCE) is regarded as a sensitive indicator of DNA damage and mutagenicity [review in 9]. Perry & Evans [35] have shown that various mutagens induce SCE even at concentrations that do not yet produce chromosome aberration. SCE develops following breakage of parental DNA and subsequent rejoining with the daughter strand of the sister chromatids [25]. There were no demonstrable changes in lymphocyte subpopulations, cytostatic effects or alterations to the proliferation pattern of the cultured cells that might be suspected as causes of the observed SCE reduction. When rapidly proliferating amniotic fluid cells were used, a reduction in SCE frequency was only observed following addition of very high VAL concentrations (0.2 – 2 mg/ml *Iscador* P) [3].

Protective effects of *Viscum album* L. extracts for cyclophosphamide-induced DNA lesions and activation marker expression

On the assumption that this DNA-stabilising effect of VAL extracts might possibly improve chemotherapy-induced immunosuppression, we cultured PBMC from healthy subjects in an in-vitro model in the presence of the alkylating cytostatic agent cyclophosphamide (CP). The subsequently observed induction of SCE was significantly improved by simultaneous administration of an aqueous whole-plant extract (10 µg/ml *Helixor* A) [4, 5]. Simple pharmacological inactivation of the CP-activating lymphocytic cytochrome P-450 could be excluded because in 2 subjects SCE induction was actually increased by combined administration of CP and VAL. However, these experiments were not designed to abolish the effect of a potent agent such as CP, but to estimate the anti-

mutagenic potency of VAL. In further investigations we were able to show that there was dramatic SCE induction in children with acute lymphoblastic leukaemia 1 day after CP administration [27]. Over the course of one week SCE frequency fell again but remained at a clearly elevated level. This correlated with marked T-cell depletion. Thus, SCE-inducing DNA lesions that are incompatible with life appear to lead to cell death whereas less serious lesions appear to allow immunocompetent cells to survive. However, are the resultant surviving cells also functionally competent?

In order to address this question we investigated the expression of activation/ proliferation markers, such as the low-affinity IL-2 receptor (CD25) and the transferrin receptor (CD71), on the surface of cultured T-cells. Following a 72-h incubation period in the presence of CP, there was marked depression of these molecules – a phenomenon that was significantly improved again by VAL (10 µg/ml *Helixor* A) [4]. Since the sequential expression of these receptors and the binding of IL-2 and transferrin to their respective receptors are essential for the transition from the G_1 phase to the S phase of the cell cycle of activated T-cells [33], these results are indicative of the stabilisation of DNA and, consequently, also of protein synthesis.

The cytokines IL-1 and TNF-α are thought to possess a haematoprotective effect during radiation and against 4-hydroperoxy-CP [28, 29, 40]. A prolonged survival time has also been noted in lethally irradiated mice following administration of these substances [34]. Since induction of IFN-γ, TNF-α, IL-1 and IL-6 has been described following VAL administration [15, 31, 37], we investigated whether these cytokines might possibly be responsible for the effects observed by us. However, apart from slightly improved TNF-α activity following VAL administration, we were unable to detect any increased release of TNF-α or IL-1β in the supernatants of cultured mononuclear cells that had been treated with CP [5]. We were also unable [5] to observe induction of SCE-reducing cytokines, such as IFN-α and IFN-β [26, 38].

Although mistletoe lectin I (ML I) is regarded as the most important biologically active component of VAL [1, 12, 13, 15], the addition of purified toxic proteins, such as β-galactoside-specific ML I or N-acetyl-galactosamine-specific ML II/III, or of viscotoxins did not achieve any improvement at all in the CP-induced depression of activation markers [4]. The fact that the lectin content is not of primary importance in the phenomenon of SCE reduction is emphasised by the observation that *Viscum album* Abietis R, an aqueous whole-plant extract of fir-grown mistletoe with a relatively higher ML content than *Helixor* A and a clearly higher killing potency, also leads to SCE reduction (Figure 2A). At a dose of 14.3 μg/ml, *Helixor* M (the lectin content of which is approximately the same as that of *Helixor* A) even shows a slight increase again in the frequency of SCE (Figure 2B). Therefore either other VAL constituents or else complex interactions of these components in the whole-plant extract appear to be responsible for the effects described.

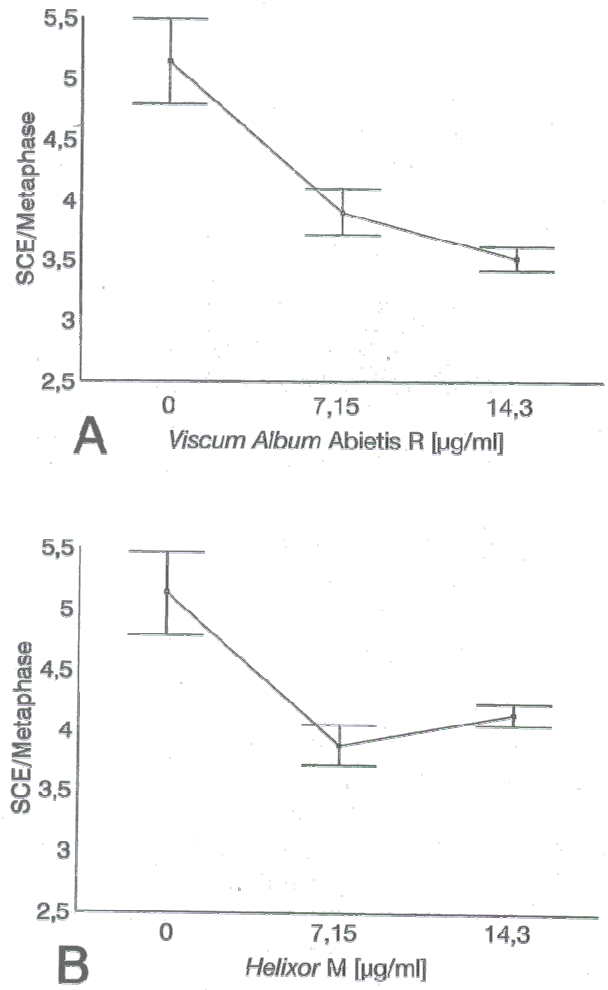

Figure 2: Sister chromatid exchanges (SCE) of mononuclear cells from healthy subjects (n = 3) cultured for 72 h following addition of the VAL extract *Viscum album* Abietis R (**A**) or Helixor M (**B**). As described in [2], at least 25 M2 metaphases were counted per experiment and design. With reference to body weight and body fluid, the VAL concentrations used here correspond approximately to those received by tumour patients as daily subcutaneous injections. Mean values ± standard deviations are shown.

Key:

[Figure 2A, Y axis:] SCE/metaphase

[Figure 2A, X axis:] *Viscum album* Abietis R [µg/ml]

[Figure 2A, Y axis:] SCE/metaphase

[Figure 2A, X axis:] *Helixor* M [µg/ml]

No protective effects for cultured leukaemic cells and transfected mammary carcinoma cells in a murine model

The observations described here are only of clinical relevance if the protective effect of VAL extracts is selective for normal PBMC and not for malignant cells. However, VAL-induced protective effects could not be achieved in cultured leukaemic B-cells and in the Jurkat T-cell line [8]. When leukaemic Jurkat cells were used, markedly increased cytotoxicity was detected after culturing for 96 h following administration of CP together with VAL [4].

Of course, it is then of interest to learn whether results from in-vitro models can also be transposed to an in-vivo model. For this purpose the effect of CP and of the VAL preparation *Isorel* A, an extract from fir-grown mistletoe, was investigated on pulmonary metastatic spread in CBA/HZgr mice. Following injection of vital mammary carcinoma cells (10^5 cells) into 4-month-old female CBA/HZgr mice (n = 7 per group), the animals each received 50 mg/kg body weight CP (Endoxan) one day later. On Day 2 the VAL extract *Isorel* A was administered in doses of 100 µg/kg body weight 3 times weekly by intraperitoneal injection. The mice in the control group received injections of physiological saline solution. Compared with the control group, 18 days after tumour transplantation, there was a significant reduction in pulmonary metastases following CP administration ($p < 0.001$), and this reduction was clearly exceeded in the group treated with CP and VAL ($p < 0.02$). VAL itself also led to a significant reduction in pulmonary metastases ($p < 0.001$), but this was clearly less pronounced than that induced by CP (Figure 3). In the mouse model too, therefore, there were no protective effects of VAL, but additive cytotoxic effects were detected for malignant cells. On the one hand, the cytotoxic potential of VAL appears to be evident here, mediated primarily by the lectins [7, 10, 19, 24], but also there is non-specific stimulation of the immune system [20, 22, 39].

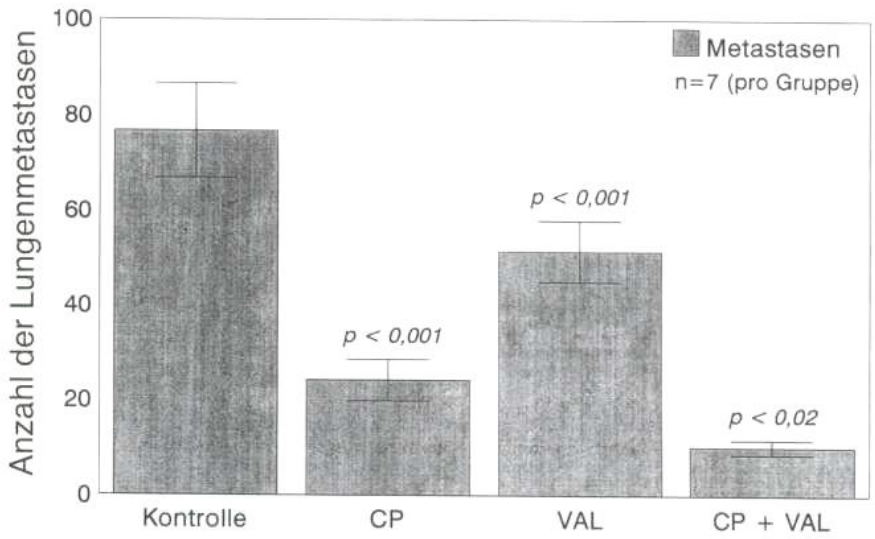

Figure 3: Effects of cyclophosphamide (CP) and the VAL preparation *Isorel* A on the development of induced pulmonary metastases of murine mammary carcinomas. Mean values ± standard deviations are presented for each group (n = 7). Compared with the group treated with CP alone, significantly fewer lung metastases were detected in the group treated with CP and VAL ($p < 0.02$; $p < 0.001$ compared with the control).

Key:
[Y axis:] Number of pulmonary metastases
[X axis:] Control CP VAL CP + VAL
[Text at top right:] Metastases n = 7 per group

Prospects

The balance between activation and killing appears to be of key importance for understanding the protective effects of VAL on the one hand and its cytostatic/ cytotoxic effects on the other. A prospective study in women with breast cancer treated with non-cytotoxic VAL extracts in addition to conventional chemotherapy is to be conducted to investigate whether the results presented are of clinical relevance. As Hauser has reminded us [17], there is indeed a 'need for the proper clinical investigation' of mistletoe preparations. However, since there are both VAL extracts with high killing potency and others with low cytotoxic activity [7], such clinical trials must consider which effect is to be tested with

which product. Standardisation to a single active component, e.g., ML I, appears questionable because this approach fails to appreciate the wide range of options for targeted and specific intervention in the complex interactions of the immune system as a basis for defence against infection and for tumour surveillance. The mistletoe lectins comprise only a few of the more than 1000 proteins that have been identified in mistletoe [36]. Although mistletoe lectins are certainly of key importance for the cytotoxicity of VAL, they do not appear to be of prime importance for the protective effects [4].

Taking these observations into account, the adjuvant administration of VAL extracts during and after conventional chemotherapy might therefore be a useful therapeutic approach in the treatment of cancer patients.

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