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Intratumoral injection of a *Viscum album* L. preparation in a case of unresectable, occlusive carcinoma of the esophagus

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Viscum album L. (VAL) exerts both, DNA-stabilizing and apoptosis-inducing properties *in vitro*. However, the cytotoxic effects are not observed in cancer patients treated subcutaneously or even intravenously with VAL extracts. Since patients with metastatic or bulky unresectable carcinoma of the esophagus have a poor prognosis, with a median survival of 6 month, and often require palliation of dysphagia, a male patient (GW, *1926) with disseminated adenocarcinoma of esophagus was treated intratumorally with an aqueous extract produced from mistletoes grown on pine trees (*Helixor* P). The carcinoma of the stomach was treated in summer 1992 with surgery, chemotherapy and irradiation. The first relaps was observed several month after surgery in 1992, the second 4 years later. In 5/96, the esophagus was completely occluded by unresectable bulky tumor masses. Placement of a feeding tube or stint was rigorously rejected by the patient. Due to the apoptosis-inducing properties of mistletoe extracts, namely their toxic lectins (ML), and putative immunomodulating properties, the ML III-rich drug extract *Helixor* P was choosen. 9 endoscopically injections with increasing concentrations were applied within a period of 5 weeks. Only 5 mg of the drug (ML II/III: 650 ng/ml) resulted in necrosis of the upper tumor mass without pain or a bleeding. By increasing the concentration up to 100 mg, the occluding tumor masses were restrained, so oral ingestion was possible and life quality improved.. This effect was associated with a transient increase of peripheral $CD28^- CD8^+$ putative suppressor cells, C-reactive protein and cortisol in the serum, while the number of granulocytes, monocytes and other lymphocyte subsets remained almost stable. However, biopsy of the tumor revealed an infiltration mainly with granulocytes and T lymphocytes ($CD4^+$ *vs.* $CD8^+$ T cells 2:1). In conclusion, intratumoral injection of a cytotoxic drug extract from VAL is tolerable and sufficient when the primary goal is palliation of dysphagia near the end of life. Obviously, we were unable to cure the malignat disease. Further investigation is currently under way.

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THE IMPACT OF INTRAPERITONEAL ZYMOSAN ON EARLY POSTOPERATIVE PERIOD IN COLORECTAL CANCER PATIENTS

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The aim of our study was to detect the impact of intraoperative intraperitoneal (2.0 ml 1% suspension) and postoperative intradermal Zymosan application on immunological changes in patients with colorectal cancer during early postoperative period.
Altogether 30 patients were operated for histologically confirmed colorectal cancer. Thereafter 14 patients received Zymosan and 16 - remained control for the follow-up period of 3 months. Peripheral blood lymphocyte subpopulations CD3+, CD4+, CD8+, CD19+, CD38+ were detected by flow cytometry, tumor markers CEA, CA 19-9 and CA-125 were assessed by EIA (Hoffmann - La Roche). Routine blood tests were performed to both controls and patients on Zymosan therapy.
First three postoperative days were characterized by more expressed monocytosis and leucocytosis in Zymosan treated group. CD4+ and CD38+ cell number increases significantly in Zymosan group ($p< 0.05$) on the 10th postoperative day and then during the 3 month observation period. CA-125 was equally elevated in both groups.
These results allow us to suggest that Zymosan intraperitoneal application during the operation followed by intradermal administration activates leucopoesis and induces inflammatory reaction in abdominal cavity which thereafter stimulates activation of the immune system during early postoperative period.

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Aqueous mistletoe extracts and vesicles induce T-cell proliferation of sensitized lymphocytes in patients treated with mistletoe extracts

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Mistletoe extracts are widely used in adjuvant cancer therapy. Multiple compounds with diverse biological effects may contribute to the overall response to mistletoe treatment. We have investigated the in vitro responsiveness of T-cells from mistletoe-treated cancer patients. Proliferation of peripheral blood mononuclear cells from treated patients but not from untreated patients was observed in response to therapeutically used mistletoe extracts prepared from apple (*mali*) and pine (*pini*) host trees. The proliferation maximum was measured in a wide individual range between 0.5 and 500 µg/ml extract. The strongest proliferation was induced by vesicles of *mali* extract in a range between 2,5 and 10 µg/ml. Using a newly developed flow cytometry assay, we determined that cell growth was restricted to CD4 T-helper-cells. Analysis with a panel of monoclonal antibodies against the variable region of the T-cell-receptor ß chain (Vß) revealed an oligoclonal pattern of CD4 T-cell activation.

Using a whole extract and not defined components of the mistletoe is often reviewed. For the first time, we show strong amplifying synergistic effects of lectins and vesicles in relation to proliferation of lymphocytes and an antagonistic effect in relation to cytototxicity. In addition these synergistic effects are measurable with biochemical methods for example in ELISA's. These results demonstrate a significant influence on all commonly used methods for the determination of mistletoe lectins.

Our results demonstrate that mistletoe components other than lectins are potent activators of cellular immune responses in vitro. These results indicate that therapeutic administration of mistletoe extracts sensitized a restricted set of CD4 T-lymphocytes in mistletoe-treated patients. Lectins and vesicles showed synergistic effects in relation to CD4 T-cell proliferation and cytotoxicity.

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Effect of Bleomycin and Asparaginase on Leukotriene Production in vitro and in vivo.

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Mast cells are implicated as effector cells in allergic and inflammatory responses due to their distribution in sensitive tissues and their capacity to synthesize and secrete a number of potent mediators including leukotrienes. Bleomycin and asparaginase are well known for their allergic and inflammatory side effects ranging from mild febrile responses to severe pneumonitis or anaphylactic shock.
We studied the effect of bleomycin and asparaginase on leukotriene production in murine bone-marrow derived mast cells (BMMC) and in patients. LTC_4 production by BMMC was determined by radioimmunoassay (RIA). Leukotriene production in vivo was assessed by determining leukotriene E_4 and N-acetyl-leukotriene E_4 in urine by means of combined high-performance liquid chromatography (HPLC) and RIA.
Bleomycin induced a bell-shaped increase in LTC_4 production both in unstimulated and in calcium-ionophore stimulated mast cells. Bleomycin enhanced the endogenous leukotriene production in 3 of 7 patients suffering from high-grade non-Hodgkin's lymphoma who were treated with bleomycin on day 14 of a polychemotherapy regimen. Enhanced leukotriene production was associated with febrile reactions and was most pronounced in a patient who developed an ARDS. Asparaginase increased leukotriene production in stimulated but not in unstimulated BMMC. In a patient who developed an anaphylactic reaction after treatment with asparaginase, a pronounced increase in the urinary leukotriene concentration was observed. In contrast to asparaginase or bleomycin a number of other cytostatic agents were without effect on leukotriene production by BMMC.
Our data indicate that some of the inflammatory and allergic side effects of bleomycin and asparaginase could be mediated by leukotrienes, a possible source of which may be mast cells.
Key words: leukotrienes; bleomycin; asparaginase; mast cells; side effects.