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Prolonged cytotoxic effect of aqueous extracts from dried *Viscum album* on bladder cancer cells

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Aqueous extracts from whole dried mistletoe (*Viscum album* L., Iscucin®) are often used in anti-cancer treatment. We studied the effect of extracts obtained from mistletoe bushes that grew on different host trees on bladder cancer cells by means of MTT-colorimetric cell proliferation/survival assays. The extracts possessed concentration-dependent cytotoxic properties whose extent varied with the host tree, but did not always correlate with the corresponding mistletoe lectin content. A 2-hours treatment of bladder cancer cells triggered a later, strong cytotoxic effect. This prolonged effect suggests that instillation with Iscucin® has therapeutic potential for bladder cancer patients.

Viscum album (mistletoe) extracts are often used as an anti-cancer therapy in complementary medicine (Molassiotis et al. 2006). Besides being cytotoxic to a variety of cancer cells, through the induction of apoptosis and/or necrosis (Knopfl-Sidler et al. 2005; Zuzak et al. 2006), these extracts have also been shown to possess immunomodulatory properties (Stein et al. 1998; Huber et al. 2005; Pryme et al. 2006; Zuzak et al. 2006). Whereas the *in vitro* toxicity of the extracts is likely to be caused at least in part by mistletoe lectin, the extent of the effect of *Viscum album* extracts on cancer cells does not always correlate with their lectin content (Eggenschwiler et al. 2006; Zuzak et al. 2006), suggesting that contrarily to what was initially assumed other components might also play a role.

Bladder cancer is the sixth most common malignant disease and the ninth leading cause of cancer-related deaths (Roach et al. 2001). Although most (ca. 75%) of the patients suffer from superficial bladder cancer, this has a strong tendency to recur and patients with unresectable or metastatic disease have low long-term survival prospects (Roach et al. 2001; American Cancer Society 2005). To avoid recurrence of the superficial forms, there are several therapeutic possibilities, namely instillation with drugs that can have either a chemotherapeutic or an immunomodulating effect. Since mistletoe extracts can lead to these two types of responses, they are likely to have potential in the therapy of bladder cancer.

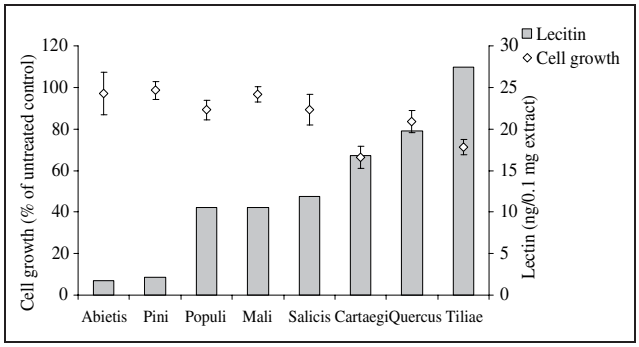

Fig. 1: Effect of Iscucin® Abietis, Pini, Populi, Mali, Salicis, Crataegi, Quercus and Tiliae on VM-CUB-1 bladder carcinoma cell growth. The cells, platted in the evening before at 10^4 cells/well of a 96-well plate, were incubated with 0.1 mg/ml of the various extracts (WALA Heilmittel GmbH, Germany) during 48 h. Thereafter cell growth was determined using a colorimetric assay based on the reduction of MTT (3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide) by the mitochondrial reductases (Eggenschwiler et al. 2006; Zuzak et al. 2006); VM-CUB-1 cell growth data are shown as mean of 6 experiments ± S.D. and are expressed as percentage of the values obtained with untreated cells. Each cell growth experiment included 8 determinations. The lectin concentrations are shown as bars; the extracts were analyzed at a *Viscum album* concentration of 50 mg/ml. All materials were purchased from common commercial sources; the cells were cultured as advised by the suppliers.

We now focused on the cytotoxic effect of aqueous extracts of dried, entire mistletoe – Iscucin® – on bladder cancer cell lines. Firstly, we compared the effects of mistletoe extracts prepared from bushes growing on seven different hosts (Fig. 1, 0.1 mg/ml) on the *in vitro* growth of a well-known bladder cancer cell line (VM-CUB-1), by means of MTT-cell growth assays (see legend to Fig. 1) essentially as previously described (Eggenschwiler et al. 2006; Zuzak et al. 2006). The two extracts that had the strongest cytotoxic effects were those corresponding to the host trees *Crataegus* (whitethorn) and *Tilia* (basswood). At the lower concentrations tested (0.01–0.0001 mg/ml) no effect of the Iscucin® extracts on VM-CUB-1 cell growth could be detected (data not shown), revealing that these cells are relatively resistant to the treatment.

The lectin levels in the various mistletoe extracts were determined and are depicted as well in Fig. 1. The highest values achieved were 8400, 9900 and 13700 ng lectin/ml in the cases of Iscucin® Crataegi, Quercus and Tiliae, respectively. While the extracts with the highest mistletoe lectin levels were relatively potent, the most effective one – Iscucin® Crataegi – had merely 61% of the maximal level detected. This suggests that the observed cytotoxicity of the Iscucin® extracts cannot be merely attributed to the mistletoe lectin; other components are likely to be present in Iscucin® Crataegi which are relevant in the case of these bladder cancer cells.

Having identified the two most potent Iscucin® extracts, we included four additional bladder cancer cell lines in the next step of our study and adapted the experimental conditions to mimic the instillation procedure often used in the treatment of bladder cancer, similarly to what has been previously described (Urech et al. 2006). Fig. 2 shows that a 2 h treatment with Iscucin® Crataegi or Tiliae at 8 mg/ml, i.e. at a concentration easy to achieve within an instillation procedure and during the standard period of time that an instillation lasts, was able to trigger a strong cytotoxic effect. Most remarkably, the magnitude of this effect increased with time up to 72 h and persisted for at least 144 h (6 days). Again Iscucin® Crataegi turned out

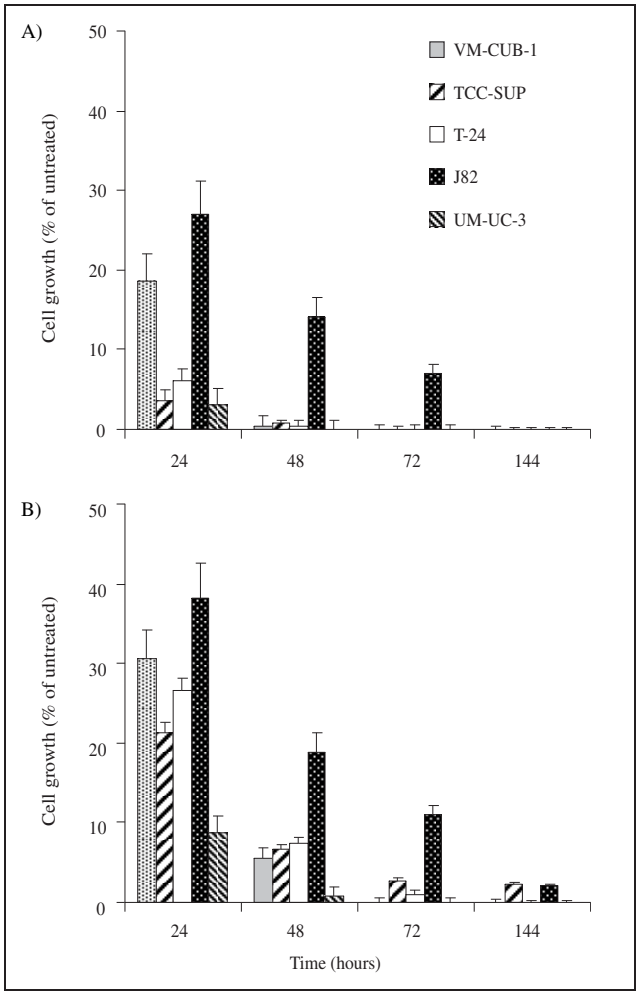

Fig. 2: Prolonged effect of Iscucin® Crataegi (A) and Tiliae (B) on bladder cancer cell lines. VM-CUB1, TCC-SUP, T-24, J82 and UM-UC-3 cells were incubated for 2 h with 8 mg/ml of either Iscucin® Crataegi (A) or Tiliae (B). After cautiously removing the supernatant, the cells were washed with 100 µl phosphate-buffered saline and 150 µl of fresh medium were added to each well. The MTT assay was performed after 24, 48, 72 and 144 h. Data are shown as mean of 3 experiments ± S.D. and are expressed as percentage of the values obtained with untreated cells. Each experiment included 16 determinations. Materials and MTT-assay as in the legend to Fig. 1.

to be more potent than Iscucin® Tiliae. In both cases, lower concentrations of the extracts (4 and 2 mg/ml) could reduce the growth of each of the cell lines, but to a lower extent than 8 mg/ml. A higher concentration (16 mg/ml) caused a more rapid inhibitory effect. Comparable experiments performed with Iscucin® Abietis, Populi and Quercus revealed a comparable tendency, but the effects were quantitatively smaller than those obtained with Iscucin® Crataegi and Tiliae (data not shown), as to be expected from the data depicted in Fig. 1.

Our observations corroborate previous data obtained with fermented preparations of fresh mistletoe – Iscador® – which revealed an antiproliferative effect of these preparations on bladder cancer cells (Urech et al. 2006). A first phase I/II clinical trial indicated that the recurrence rate for superficial bladder cancer after instillation with a standardized mistletoe preparation was comparable to that of an historical control (Elsässer-Beile et al. 2005).

In conclusion, our observations show that Iscucin® extracts, especially Iscucin® Crataegi und Iscucin Tiliae®, at a concentration of 8 mg/ml and more, strongly inhibit the growth of bladder cancer cells in a long-lasting way, under experimental conditions which correspond to the instillation procedure. Therefore the data point towards a clear-cut therapeutic potential of Iscucin® extracts in the prevention of recurrence and treatment of superficial bladder cancer and emphasize the urgent need for prospective, controlled clinical trials.

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