

Review

Dietary mistletoe lectin supplementation and reduced growth of a murine non-Hodgkin lymphoma

I.F. Pryme¹, S. Bardocz², A. Pusztai² and S.W.B. Ewen³

¹Department of Biochemistry and Molecular Biology, University of Bergen, Bergen, Norway,

²Rowett Research Institute, Greenburn Road, Bucksburn, Scotland and ³Department of Pathology, University of Aberdeen, Scotland

Summary. The growth of a murine non-Hodgkin lymphoma (NHL) tumour has been shown to be reduced by incorporating mistletoe lectin (ML-1) into the diet. The morphological characteristics of NHL tumours in mice fed ML-1-supplemented diets were different from those in LA (control)-fed mice. The degree of mitotic activity was lower and nuclear area reduced. The degree of lymphocyte infiltration was increased in tumours from ML-1 fed mice and this was accompanied by a high incidence of apoptotic bodies. Visual observation of NHL tumours from individuals fed ML-1 diet showed a poorly developed blood supply in contrast to control-fed mice. A major reduction in number of blood capillaries in NHL tumours was confirmed by microscopic evaluation of tumour sections. The results suggested an anti-angiogenic response in ML-1-fed mice. The feeding of ML-1 compared to control diet thus provided several identifiable changes in the morphology of NHL tumours which were consistent with the observed reduction in tumour weight. There was no longer histological evidence of viable tumour in 25% mice fed the ML-1 diet for 11 days. Morphological studies of the small bowel indicated (a) that the lectin induces hyperplasia, and (b) that the lectin binds avidly to lymphoid tissue of Peyer's patches. There was evidence of limited endocytosis of the lectin. An experiment where ML-3 was added to the diet of mice three days after inoculation of tumour cells showed that the lectin was able to slow down further growth of an established tumour. The results show that ML lectins induce powerful anti-cancer effects when provided by the oral route.

Key words: Mistletoe, Lectin, Non-Hodgkin lymphoma, Peyer's patch, Apoptosis, Anti-angiogenesis

Introduction

The use of mistletoe extracts in cancer therapy has a history of more than 80 years. Due to the hitherto poor documentation of the actual nature and characterisation of potential active anti-cancer components the use of such extracts in cancer therapy has received heavy criticism. A major problem is the fact that the different extracts on the market are prepared by different procedures and there is a general lack of standardisation. Furthermore, the effects of mistletoe extracts seem to be related to the nature of the host tree and importantly, the composition of the different components present in the extracts has been shown to vary quite considerably, often as a consequence of seasonal climatic variation. Such variation is clearly shown by the results of Elsasser-Beile et al. (1998), where the biological effects of 12 different clinically applied mistletoe preparations were compared. They established that both apoptosis and cytokine production was induced differentially in leukocyte cultures by the various mistletoe preparations tested. These results indicate, therefore, that clinical studies performed using different mistletoe preparations are not readily comparable. Mistletoe extracts have very often been used under circumstances where all other methods of treatment have failed. It is not therefore surprising that the documented success rate with mistletoe extracts is not particularly high.

Due to the lack of conformity between individual mistletoe extracts and because of their innate complexity it has thus become important to perform studies using purified mistletoe components. This review covers the recent use of mistletoe lectins as dietary supplements in an animal tumour model system.

Eifler et al. (1993) have developed a procedure for the purification of lectins from the dried mistletoe plant. Three major mistletoe lectins have thus been identified: ML-1, ML-2 and ML-3. The best studied of these is ML-1 which has proved to be a type-2 RIP (ribosome-inactivating protein). It is composed of two chains – an A-chain which has N-glycosidase activity (accounting

for the property of inactivating ribosomes), and a B-chain possessing galactose-specific binding properties. The B-subunit is responsible for binding to appropriate cell surface receptors and thereafter enables the cellular entrance of the A-chain, which then exerts its cytotoxic properties. Recent studies by Vervecken et al. (2000) have shown that the individually separated A- and B-chains do not possess cytotoxic properties.

Mistletoe has a history of being generally considered to be extremely toxic. A recent study, however, concerning the outcome of 1,754 exposures to accidental intake of the plant, has shown no evidence of profound toxicity in humans (Krenzelok et al., 1997). It is therefore evident that mistletoe has an unfounded and totally undeserved reputation of being classified as toxic following oral intake. On the contrary, following the feeding of purified ML-1 to developing rats the lectin was shown to have growth-stimulatory rather than growth-inhibitory effects on crypt cells of the small intestine (Pusztai et al., 1998).

It is well known that the mistletoe lectins have the ability to initiate an immunomodulating response (Hajto et al., 1989). A lectin-enriched aqueous mistletoe extract was observed to cause a strong initial proliferation of peripheral blood mononuclear cells following administration to healthy adults (Stein and Berg, 1998; Stein et al., 1998). This was accompanied by increased production of both TNF and IL-6, and a less pronounced release of IFN- and IL-4. Heiny et al. (1998) and Baxevanis et al. (1998) independently reported enhanced activity of peripheral blood natural killer cells (NK) in response to mistletoe lectin. Increased β -endorphin plasma levels and stimulation of T-lymphocytes exhibiting expression of CD25/interleukin-2 receptors and HLA/DR-antigens, was also observed (Heiny et al., 1998). Baxevanis et al. (1998) demonstrated that ML-1 preferentially stimulated and expanded a population of CD8⁺ T cells which mediated the cytotoxic effect. Furthermore, an activation of PBL with both ML-1 and IL-2 resulted in simultaneous induction of T and CD56⁺ cell-mediated NK and LAK (lymphokine-activated killer) cytotoxicity. Schink (1997) states that isolated ML induce augmentation of both number and activity of NK in peripheral blood in a dose-dependent manner. Immunophenotyping of blood samples from lectin-treated patients has revealed increases in NK number, increased activity of peritoneal macrophages and NK cells as well as a response of splenic T-cells to mitogens (Gabijs et al., 1992).

Recently, Bussing et al. (1999) analysed mitochondrial alterations in human lymphocytes incubated with ML-1. They demonstrated the generation of reactive oxygen intermediates (ROI) and the induction of expression of newly described mitochondrial membrane proteins (Apo2.7). The cytotoxic response thus appears in part to be a result of a distinct 'death signal' causing a rapid induction of Apo2.7 molecules.

Frantz et al. (2000) have established that serum glycoproteins, particularly haptoglobin, but also alpha (1)-acid glycoprotein and transferrin, effectively inhibit the apoptosis-inducing properties of ML lectins. Interestingly, deglycosylated haptoglobin did not exhibit such a protective effect. These results probably account for the fact that when mistletoe lectins are administered to patients then no undesirable side-effects have been detected.

Purified mistletoe lectins and their effects on tumour cells *in vitro*

In a series of experiments Mockel et al. (1997) studied the effects of ML-1 on the human T-cell leukemia line MOLT-4, the monocytic line THP-1 and on human peripheral blood mononuclear cells (PBMC). They examined the parameters of cell viability and induction of apoptosis. In a sensitive serum-free cytotoxicity assay, time- and concentration-dependent direct toxicity towards MOLT-4 cells was determined with IC₅₀-values ranging from 20-40 pg/ml (300-600 fmol/l). The ratio of apoptotic to viable MOLT-4 cells was measured after treatment with ML-1 for 24hr. In the concentration range of low cytotoxicity of ML-1 it became evident that induced cell death is quantitatively due to mechanisms involving apoptosis. Mockel et al. (1997) also investigated the immunomodulatory activity of ML-1 *in vitro* by measuring cytokine release. At concentrations of low cytotoxicity ML-1 demonstrated immunostimulatory activity on PBMC and THP-1. Using RT-PCR with THP-1 cells these workers were able to confirm that cytokine induction by ML-1 is regulated at the transcriptional level. These findings suggest that in the blood cells investigated, both apoptosis and cellular signalling are induced by the same concentration range of ML-1. In their studies on various tumour cell lines and human lymphocytes Bussing et al. (1998) reported that ML-3 from *Viscum album* L. mediated a very potent apoptotic death signal resulting in the binding of Annexin-V and expression of mitochondrial proteins Apo2.7.

Ribereau-Gayon et al. (1997) have also demonstrated an inhibition of Molt 4 cell growth with lectin concentrations in the pg/ml range. They found that ML-3 was approximately 10 times more cytotoxic than ML-1. Early events were membrane perforation and protrusions, which are typical signs of apoptosis. Bantel et al. (1999) also showed that treatment of leukemic T- and B-cell lines with ML-1 triggered cell death through the induction of apoptosis. Interestingly, a peptide cascade inhibitor was able to almost completely block the observed effects of the lectin. ML-1 apparently had the ability to potentiate the effect of chemotherapeutic drugs.

Lenartz et al. (1998) studied the cytotoxic activity of ML-1 towards the anaplastic glioma cell line (F98) using a three dimensional spheroid model system. They

showed that F98 glioma cell spheroid growth was significantly inhibited following incubation with lectin concentrations of 10 and 100 ng/ml, demonstrating ML-1 dose-dependent cytotoxicity.

Yoon et al. (1999) have examined the chemical and biological properties of cytotoxic lectins (KML-C) isolated from an extract of Korean mistletoe [*Viscum album* C. (coloratum)]. The properties of KML-C were compared with a lectin (EML-1) prepared from European mistletoe [*Viscum album* L. (loranthaceae)]. Both lectins showed strong cytotoxicity against various human and murine tumour cells. The cytotoxic activity of KML-C, however, was higher than that of EML-1. In a similar manner to that observed by Mockel et al. (1997) and Ribereau-Gayon et al. (1997) for ML-1, tumour cells treated with KML-C also exhibited typical patterns of apoptotic cell death. Yoon et al. (1999) showed that the apoptosis-inducing activity of KML-C was blocked by addition of Zn^{2+} , an inhibitor of Ca^{2+}/Mg^{2+} -dependent endonucleases. Both Pae et al. (2000a) and Lyu et al. (2001) have recently demonstrated that incubation of human leukemia HL-60 cells with various doses of Korean mistletoe lectin causes an induction of apoptotic events. According to Pae et al. (2000a) apoptosis appeared to be prevented by activation of PKA or PKC. Lyu et al. (2001) have demonstrated the involvement of caspase-3 activation in apoptotic events in HL-60 cells. Park et al. (2000) have shown that mistletoe lectin II induced apoptosis in a variety of cell types including Jurkat T cells, RAW 264.7 cells, HL-60 cells, DLD-1 cells, and primary acute myelocytic leukemic cells. Interestingly, Pae et al. (2000b) have demonstrated that simultaneous treatment of human cervix carcinoma HeLa or breast carcinoma MCF-7 cells with MLs isolated from European or Korean mistletoe rendered the cells more sensitive to induction of apoptosis by TNF. Recently, it has been shown that intracellular expression of IL-4 and inhibition of IFN- γ production appear to be processes related to the induction of apoptosis in U-266 plasmacytoma cells (Stein et al., 2000a,b). Using human U-937 promonocytes Wenzel-Seifert et al. (1997) showed that ML-1 induced apoptosis through a signal mechanism involving intracellular Ca^{2+} . An important role of H-1 and C5a receptors in the regulation of the apoptotic process was indicated. It is thus evident that both Korean and European mistletoe lectins are able to initiate apoptosis in a series of cell lines.

In a different approach Kóteles et al. (1998) have shown using *in vitro* studies that incubation of weakly X-irradiated rabbit lymphocytes or human lung carcinoma cells (Calu-1) with ML-1, facilitated an apoptotic process in damaged cells rather than in non-injured cells. *In vivo* studies on X-irradiated (1 Gy) rabbits resulted in the same effect on damaged cells. The authors concluded that the combined effects of relatively low doses of ionizing radiation and MLs seem to be favourable because of the scavenging effect on damaged somatic cells.

Subcutaneously-, intravenously-, intraperitoneally- and intravesically-applied purified mistletoe lectins and effects on tumours *in vivo*

Lenartz et al. (1998) have investigated the *in vivo* cytotoxic efficacy of ML-1 using Fischer 344 rats intracerebrally implanted with F98 glioma cells. Animals were subjected to both local and systemic treatment with the lectin. From both histological and immunohistochemical evaluation a reduction in tumour volume was observed using both methods of treatment. The most pronounced and statistically significant effect was monitored following subcutaneous (immunomodulating) administration of $1 \text{ ng ML}^{-1}/\text{kg}$ body weight, and after low dose (10 ng ML^{-1} per application) local treatment. It appeared that the subcutaneous route, producing an immunomodulating response, was the most beneficial for glioma-bearing rats.

Using experimental models in mice Yoon et al. (1998) have studied the prophylactic effect of a Korean mistletoe (*Viscum album* coloratum) lectin (KM-110) on tumour metastasis produced by highly metastatic tumour cells (colon 26-M3.1 carcinoma, B16-BL6 melanoma and L5178Y-ML25 lymphoma cells). These workers showed that intravenous (i.v.) administration of KM-110 (100 mg/mouse) 2 days before tumour inoculation significantly inhibited lung metastasis of B16-BL6 and colon 26-M3.1 cells, and liver and spleen metastasis of L5178Y-ML25 cells. In addition, mice given KM-110 (100 mg) 2 days before the inoculation of tumour cells showed significantly prolonged survival rates compared with those mice which did not receive the lectin. In a time course analysis of NK activity, i.v. administration of the Korean lectin (100 mg) significantly promoted NK cytotoxicity to Yac-a tumour cells from 1 to 3 days after KM-110 treatment. Importantly, depletion of NK by injection of rabbit anti-asialo GM1 serum completely abolished the inhibitory effect of the lectin on lung metastasis of colon 26-M3.1 cells. Yoon et al. (1998) concluded that the immunopotentiating activity of KM-110 lectin enhances the host defence system against tumours, and furthermore, that its prophylactic effect on tumour metastasis is mediated through NK-activating mechanisms.

In a recent study Mengs et al. (2000) have investigated the effects of a locally applied lectin-standardised aqueous mistletoe extract (AME) on the growth of urinary bladder carcinoma (MB49) in an orthotopic murine model. On day 1, a total of 4×10^4 tumour cells was implanted into the bladder of female C57BL/6J mice. The animals were then randomly allocated into three groups (13 mice per group). From day 2 onwards, AME was given intravesically 3 days a week for 4 consecutive weeks at concentrations related to 30 or 300 ng bioactive mistletoe lectin (ML)/ml. In the control group, 39% of the mice survived to the end of the scheduled study period in comparison to 69% and 85% in the groups treated with 30 or 300 ng ML/ml, respectively. At necropsy, 80% of the surviving control

animals showed a visible solid bladder tumour, whereas only 56% and 18% respectively, had tumours in the lectin-treated groups. In both cases the differences were statistically significant at the high concentration in comparison to controls ($p < 0.05$). A non-significant effect was observed regarding the formation of multiple metastases (40% in controls versus 33% and 18% in the treated groups). Based on the results, the authors concluded that under the conditions described, ML shows anti-tumoural activity. They considered this to be mainly due to the established cytotoxic properties of mistletoe lectins. In a related study Elsasser-Beile et al. (2001) investigated the effect of intravesically-applied recombinant, galactoside-specific mistletoe lectin (rML) on chemically-induced (N-methyl-N-nitrosourea – NSU) tumour development in the urinary bladder of rats. Atypical hyperplasia and neoplastic transformation was found in 82% of animals in the control group and about 50% in four rML treated groups. Their data provide evidence for an inhibitory effect of rML on experimental urothelial carcinogenesis that does not seem to be due to interferon- and/or interleukin-10-dependent mechanisms.

Schumacher et al. (2000) have also studied the anti-cancer potential of rML on the growth of SoTu3 human ovarian cancer cells injected intraperitoneally into SCID mice. Eighty-four days after inoculation only 10% animals in the control group survived and were tumour-free while in the experimental group which had received 500 ng/kg rML daily, 65% survived and there was no evidence of tumour cells in the peritoneum at the end of the experiment. The authors concluded that clinical studies with post-operative installation of rML in ovarian cancer patients should be encouraged in order to provide clinical evidence for the effectiveness of rML treatment.

In a recent report Lenartz et al. (2000) presented results from a study where malignant glioma patients were prospectively enrolled into a clinical trial. All the patients received internationally recommended oncologic standard treatment (neurosurgery, radiation, basic clinical care according to protocol and indication). The patients were randomly divided into a treatment group (receiving complementary immunotherapy with a galactoside-specific lectin from mistletoe, ML-1) and a control group (without additional complementary treatment). An evaluation of relapse free/overall survival was performed after a 50-month period of follow-up. Non-stratified analysis of all the patients revealed non-relevant prolongation of relapse-free intervals/overall survival time for the treatment group. However, analysis of stratified stage III/IV glioma patients demonstrated (i) a tendency for a prolongation of relapse-free survival for patients in the treatment group (17.43 ± 8.2 months) versus the control group (10.45 ± 3.9 months), and (ii) a statistically significant (BRESLOW $p = 0.035$) prolongation of the overall survival for the treatment group (20.05 ± 3.5 months) as compared to the control group (9.90 ± 2.1 months). The authors state that the

promising data warrant confirmation in a GCP-based prospectively randomized (multicentre) study and that this is currently being designed.

Orally-supplied lectins in cancer therapy in animal model systems

Phytohaemagglutinin

Because of the intrinsic nature of the molecule, phytohaemagglutinin (PHA), the lectin present in the raw kidney bean (*Phaseolus vulgaris*), is resistant to proteolytic degradation by the digestive enzymes of the gut and thus retains its biological activity during passage through the intestine (Pusztai et al., 1990). This property is shared by many lectins, including for example those of mistletoe. It has been shown in both rats and mice that the feeding of a diet containing raw kidney bean protein results in a time-dependent hyperplastic growth of the gut, and diets containing an equivalent amount of purified PHA produce essentially the same results (de Oliveira et al., 1988; Bardocz et al., 1994a,b, 1995; Pryme et al., 1996a). Despite a 20% loss in body weight during one week of feeding PHA, the long-term health of the mice was not affected since a rapid gain of weight was observed when the mice were returned to a normal diet (Pryme et al., 1996a). The rapid loss of body weight was essentially due to the lipolytic effect of PHA (Bardocz et al., 1994a). Prior to the occurrence of gut hyperplasia which immediately follows the feeding of PHA, a marked sequestration of polyamines in the intestinal tissue has been observed (Bardocz et al., 1990a,b; Pusztai, et al., 1989; Pusztai, 1991). The accumulation of polyamines would appear to be a result of an increased uptake from exogenous sources since no major elevation in ODC activity has been detected in the gut tissue (Koninkx et al., 1996). In addition, ODC activity was shown to be low in 19-day-old differentiated Caco-2 cells and no increase was observed upon PHA stimulation (Koninkx et al., 1996). It has been shown, furthermore, that PHA stimulates a rapid passage of polyamines from the blood circulation through the basolateral membrane of epithelial cells (Pusztai et al., 1989; Bardocz et al., 1990a). Not only do rapidly growing tissues require a range of important nutrients in order to sustain their growth but in addition need a large supply of polyamines (Bardocz, 1989; Seiler et al., 1996). The gut appears to contain the most active tissue in the body, illustrated by the fact that some 40% of the total protein synthesizing activity in the body per day occurs in this organ (Bardocz et al., 1990a).

It has recently been shown that the development of transplantable murine non-Hodgkin lymphoma (NHL) tumours, growing either intraperitoneally or subcutaneously in NMRI mice (Bardocz et al., 1994a; Pryme et al., 1994a,b, 1995, 1996a-c, 1998a,b, 1999a,b), and the growth of MPC-11 plasmacytoma tumours growing subcutaneously in Balb/c mice (Pryme et al., 1996d), can be greatly retarded by the inclusion of PHA

in the diet. It was suggested that competition occurs for nutrients, growth factors, polyamines, etc from the body pool between the gut epithelium undergoing hyperplasia and the developing tumour and may be an important factor with respect to the initial low level of tumour growth (Bardocz et al., 1997, 1999; Pryme et al., 1998b, 1999c). NMRI mice injected subcutaneously with Krebs II NHL cells and fed on a semi-artificial diet containing PHA within the range 0.45-7.0 mg/g diet, developed tumours during a 10-day-period which on average were only 35% of the dry weight of those which grew in control-fed (lactalbumin [LA]-based diet) animals (Pryme et al., 1996c). The reduction in growth occurred in a dose-dependent manner in the range 0.45-3.5 mg/g diet. The degree of hyperplasia of the small intestine in response to feeding the PHA diets was higher in non-injected compared to injected mice, indicating competition between gut and tumour for nutrients/polyamines, etc (Pryme et al., 2000a). We have recently shown that when mice which had been fed for 7 days on a non-protein containing diet, were then switched to a normal LA diet and injected with NHL cells on the same day, then tumour growth was highly stimulated. The inclusion of PHA in the diet, however, greatly reduced the tumour mass (Pryme et al., 2000b).

Mistletoe lectins

The effects of ML-1 on growth, gut and body metabolism and composition following the feeding of rats diets containing 67 or 200 mg ML-1/kg body weight

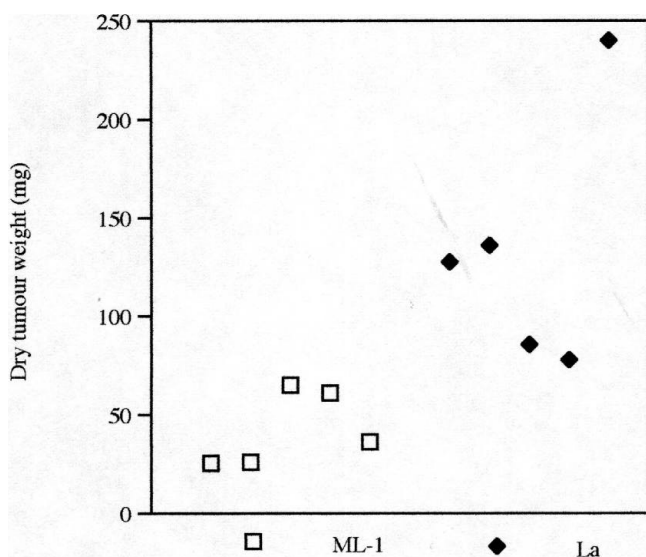


Fig. 1. The mass of NHL tumours in two groups of NMRI mice (5 individuals/group) after 11 days of growth following the s.c. injection of 2×10^6 Krebs II non-Hodgkin lymphoma (NHL) cells. One group was fed on an LA (control) diet (for composition see Pryme et al. 1996c) and one on a diet supplemented with ML-1 lectin (1.67mg/g diet) from the day of injection. Results are expressed as dry tissue weight (mg) for the individual tumours.

for 10 days were studied by Pusztai et al. (1998). Although the voluntary feed intake of rats was depressed following addition of ML-1 to the diet and a reduced growth rate was observed, none of the rats lost weight during the experimental period. The digestibility of proteins or other dietary components was not apparently affected. At the highest level tested ML-1 was seen to increase urinary nitrogen loss, thus reducing the overall N balance and total body N content. Faecal weight and its N content, however, were not affected. Serum insulin levels were reduced as earlier observed where PHA was used (Pusztai, 1991; Bardocz et al., 1996). This presumably was the reason for the observed loss of body fat. As for PHA, ML-1 also induced dose-dependent hyperplasia of the small intestine. Thirty hours after feeding an ML-1-containing diet a significant increase in plasma TNF was detected, and plasma interleukin-1 β was also elevated. Thus the release of cytokines that is considered to be an essential step in immunomodulation leading to decreased tumour growth (Mannel et al., 1991), was promoted following the oral presentation of mistletoe lectin. An avid binding of ML-1 to M cells of Peyer's patch in the rat small intestine has been reported (Pusztai et al., 1998). It would seem likely that this is implicated with the observed increase in plasma cytokines. Since a diet containing ML-1 was without apparent detrimental effects Pusztai et al. (1998) were able to conclude that the lectin should be tested for anti-tumour properties in animal experiments.

A major advantage of providing mistletoe lectins orally is that large amounts of the lectins, through their binding to the gut mucosa, are ultimately presented to lymphocytes of Peyer's patches (M cells) and are thus able to induce a major cytokine response. Within 24 hr of feeding ML-1 to mice we have observed a 14% reduction in the weight of the spleen. It is possible that this may be a result of a mass release of lymphocytes into the blood circulation in response to increased plasma cytokine levels. That such a response would be promoted by the small quantities of lectins which could reach the lymphatic tissue when mistletoe extracts or lectins are injected subcutaneously, would seem highly unlikely. It is also important to note that NK activity is far higher in the spleen than for example in the lymph nodes. Although most (>95%) ML-1 passes through the gut unchanged, small amounts are taken up by endocytosis and ultimately pass into the blood circulation. Oral presentation of the lectin has been shown to stimulate the production of specific serum IgG and IgA antibody after three oral doses (Lavelle et al., 2000). In addition to the production of antibodies against MLs, a further important complication of presenting MLs via the blood is the fact that certain serum glycoproteins (e.g. haptoglobin, alpha (1)-acid glycoprotein and transferrin) inhibit the apoptosis-inducing properties of ML lectins *in vitro* (Frantz et al., 2000). It is therefore uncertain how effectively the lectins will be able to exert their biological properties when presented in this manner.

Based on the impressive effects described above of orally-presented PHA in reducing growth of a transplantable murine non-Hodgkin lymphoma tumour, similar experiments have been performed using pure ML-1. The lectin was incorporated into semi-synthetic diets as for PHA (Pusztai et al., 1998). The effect of feeding mice the LA control diet, or one containing ML-1 lectin is shown in Figure 1 where tumour mass in individual mice is expressed. In experiments where a concentration range of the lectin (0.42-1.67 mg/g diet) was tested, even at the lowest lectin concentration tested an appreciable effect on tumour growth was evident in that the mass of the tumour was reduced by about 25%. At the highest concentration of ML-1 the tumour mass was reduced by approximately 40% (Pryme et al., 1998a). The results indicated that the reduction in tumour growth occurs in an apparent dose-dependent manner. The observations were similar to those seen earlier with the NHL tumour growing s.c. in NMRI mice fed PHA in the range 0.45-7.0 mg/g diet (Pryme et al., 1996c).

The NHL tumour in LA- or PHA-fed mice has characteristically an extremely well-developed blood capillary system and are thus extremely blood-rich in appearance upon dissection. Interestingly, the NHL tumours removed from ML-1 fed mice, in addition to being smaller, were far less bloody in nature with macroscopic evidence of a less profusely developed vascularisation. Morphological studies of tumour

sections confirmed a greatly reduced incidence of tumour vascularisation (Ewen et al., 1999). These results corroborated well with the initial "naked eye" observations of the NHL tumours, and would suggest that one of the responses to oral ML-1 is an induction of the production of anti-angiogenic factors. These observations were fully in line with the results of Yoon et al. (1995); using an extract of Korean mistletoe on a series of murine tumour cell lines they proposed that the anti-metastatic effect of the lectin was in part due to an inhibition of tumour-induced angiogenesis.

In earlier experiments PHA was shown to cause hyperplasia of the small intestine and the results have indicated a relationship between the stimulation of a fully reversible "normal" growth and the depressed growth of the tumour. These observations suggested a competition between the two types of cell proliferation for nutrients and growth factors from a common body pool (Bardocz et al., 1999; Pryme et al., 1999c). Based on tissue mass measurements ML-1 was seen to promote gut hyperplasia as previously observed with PHA (Pryme et al. 1998a). These results were confirmed by histological analysis of the small intestine where the crypts show approximately 50% elongation, whereas the villi, apart from slight broadening, were not altered in appearance (Fig. 2). There was a simultaneous increase in crypt length of the jejunum with increasing intake of ML-1 (Ewen et al., 1998). The data suggested that ML-1 exhibited similar effects on both tumour growth and on

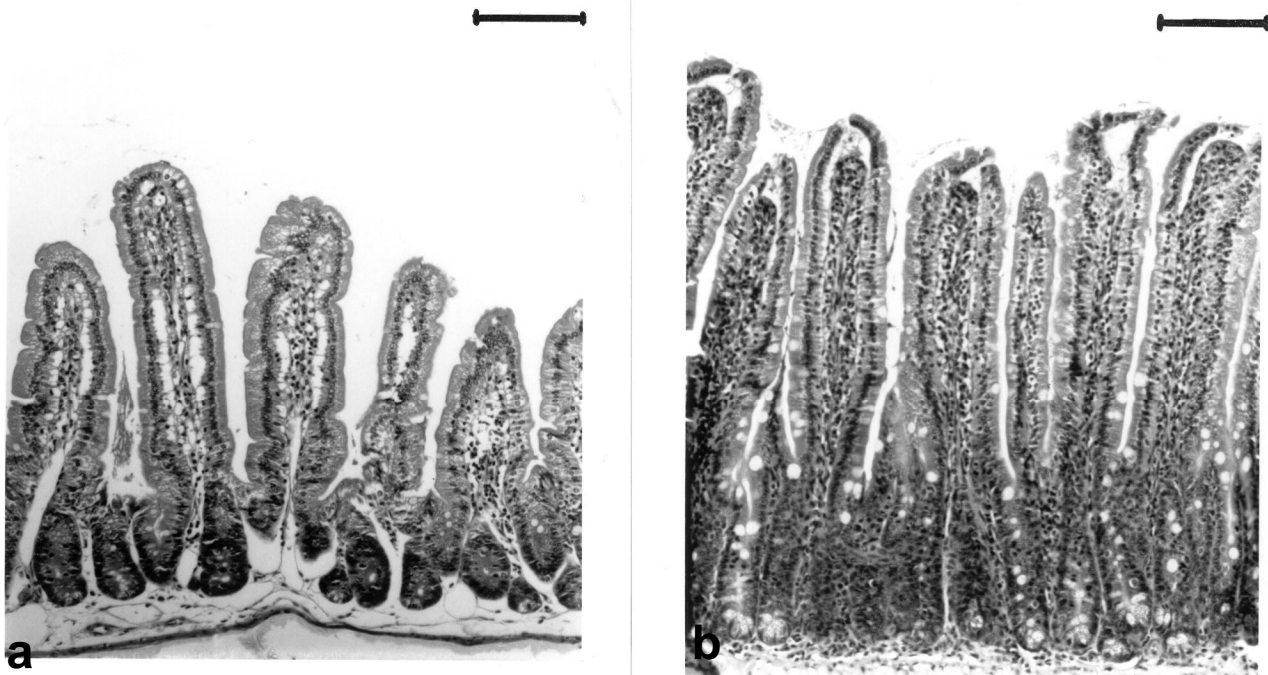


Fig. 2. Histology of the jejunum from LA, control-fed mice (**a**), and ML-1-fed mice (**b**). Sections stained with haematoxylin and eosin. Crypt elongation is evident in (b). Bar : 100 μ m.

the gut as earlier described for PHA.

Detailed studies have been performed to examine the characteristics of the NHL tumour at the microscopic level following the feeding of ML-1-supplemented diets to mice. Typically, the tumour in LA-fed mice consists of fairly large blast-type cells with many mitotic figures, whereas after ML-1 feeding the tumour cells are smaller, consistent with increased differentiation (Fig. 3). Morphological studies of NHL tumours obtained from ML-1-fed mice indicated an intense lymphoid host response within the tumour, compared to a patchy and sparse lymphoid reaction in the control-fed animals (see Fig. 3b, Ewen et al., 1999). Increased numbers of apoptotic cells were observed with an increased area of serpiginous irregular dead cells, and the non-viable cells occupied a two-fold increased area in the mice fed the lectin (Ewen et al., 1999). Apoptoses were particularly identified around areas of non-viable tumour cells, at the advancing edge of the tumour and within intense lymphoid aggregates. A decrease in the nuclear area of the tumour cells was observed following feeding ML-1 (mean decrease 21%). The number of tumour cell mitoses was reduced from 7.4 (control) to 1.7 in tumours from mice with the highest daily intake of ML-1. Importantly, the results showed that dietary

supplementation with ML-1 resulted in complete histological ablation of the NHL tumour in 6 out of 25 mice. Such an effect was not observed when mice were fed PHA. The impressive effect of ML-1 may well be related to the apparent anti-angiogenic effect promoted by oral intake of this lectin.

Experiments with ML-1 were performed such that mice were injected with tumour cells on the same day as the control diet was replaced by one containing the lectin. In a recent experiment performed with purified ML-3 the tumour cells were first injected three days before replacing the control diet with a lectin-supplemented diet. The results showed that feeding of the lectin to mice bearing an established NHL tumour was extremely effective in slowing down further tumour growth (Fig. 4). It is thus apparent that growth of an already established tumour can be curtailed by switching from a control to an ML-3-supplemented diet. For comparative purposes it can be seen that there were no effects on the mass of three selected organs.

In a recent experiment mice were fed both PHA and ML-1 lectins (Pryme et al., 2001). Tumour mass was much lower in the lectin-fed compared to LA-fed mice; the mitotic rate in tumour cells in mice fed a lectin-supplemented diet was higher than that in control-fed

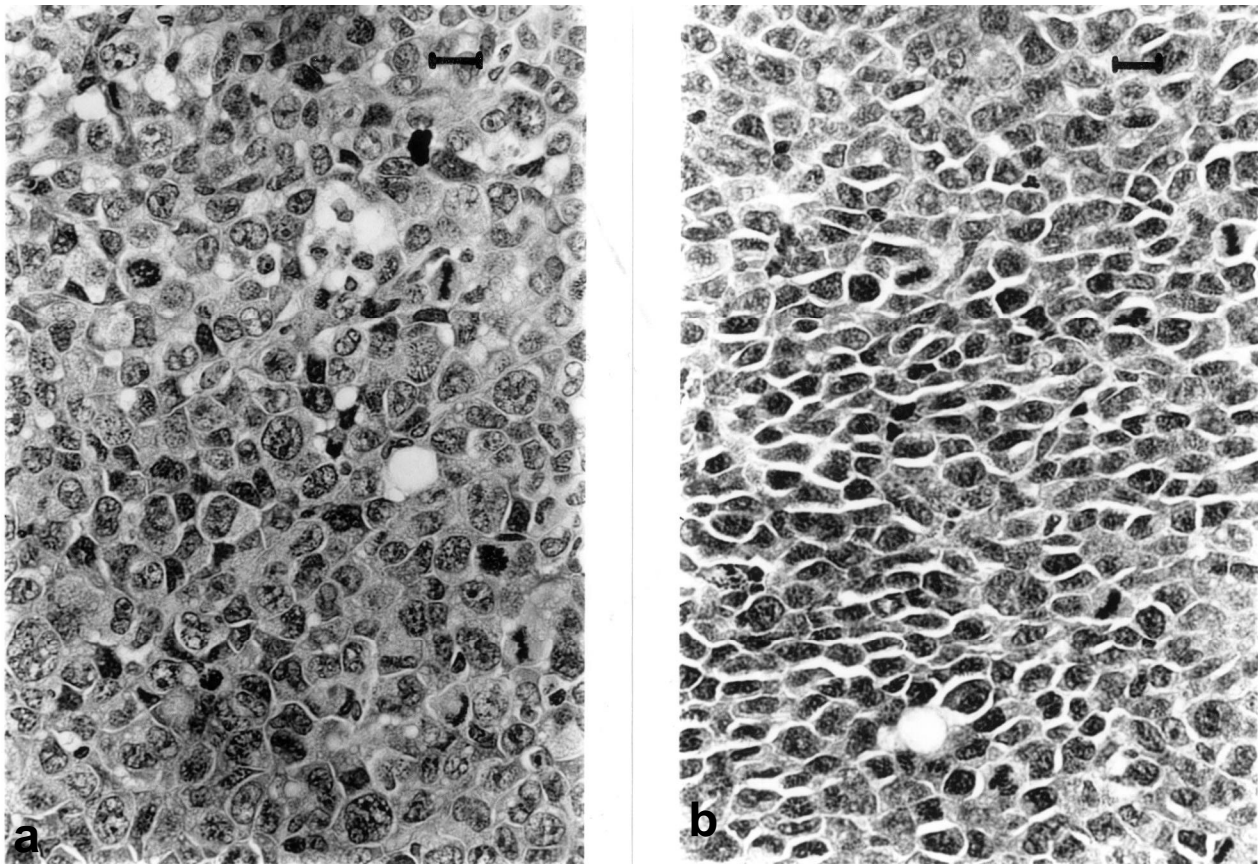


Fig. 3. Histology of the NHL tumour from LA, control-fed mice (**a**), and ML-1-fed mice (**b**). Large blast-type cells are evident in (**a**), while smaller cells consistent with increased differentiation are typical in (**b**). Bar: 10µm.

individuals; the level of lymphocyte infiltration in tumours was more extensive in lectin-fed than LA-fed mice, and the degree of necrosis was higher in lectin-fed compared to control mice. The results indicated that the successive feeding of two different lectins did not appear to cause any interference with respect to observed biological response.

Stages in the effects of dietary-supplemented mistletoe lectins on tumour growth

Based on observations from our animal model system, and published data, the following sequence of events is suggested following the oral intake of mistletoe lectins: 1. MLs bind to Peyer's patch M cells through galactose/N-acetyl glucosamine receptors. 2. Stimulation of cytokine production and their release: increased plasma levels. 3. Activation and release of splenic lymphocytes and macrophages, activation of NK. 4. Tumour infiltration by lymphocytes, NK and macrophages. 5. Production and release of anti-angiogenic factors: reduced tumour vascularisation. 6. Reduced availability of nutrients for tumour growth and decreased oxygen supplies. 7. Cytotoxic effects on tumour cells. 8. Induction of apoptosis. 9. Tumour cell death

Conclusions and future perspectives.

The extremely promising results obtained using

mistletoe lectins presented as dietary supplements with respect to causing reduced growth of a transplantable murine non-Hodgkin lymphoma would suggest a new avenue of opportunity for the establishment of a novel form of cancer treatment. Taken from the point of view of the cancer patient the oral ingestion of a lectin preparation, with negligible side-effects, would represent a highly preferable alternative to conventional present-day anti-cancer treatment (e.g. chemotherapy, radiotherapy) which is very often associated with extensive discomfort and major trauma. It would seem likely that the crypt-stimulating properties of ML could be utilised both in a protective and synergistic manner with respect to the known damaging effects of chemo- and radiotherapy on crypt structure. It would thus appear that ML would be a perfect dietary supplement before, during and after chemotherapy and/or radiotherapy. Further experimentation is warranted such that detailed knowledge of the actual mechanisms involved with respect to the oral effects of mistletoe lectins as anti-metastatic agents can be obtained. Now that a series of plant lectins have been purified future work should be devoted to studies incorporating these into diets for in-depth animal studies and examination of their possible anti-tumour potential.

Acknowledgements. The collaborative work was performed in the EC COST 98 and 917 programmes. Purified ML-1 and ML-3 were kindly supplied by Professor Uwe Prüller, University of Witten/Herdecke. The Norwegian Cancer Society is thanked for financial support.

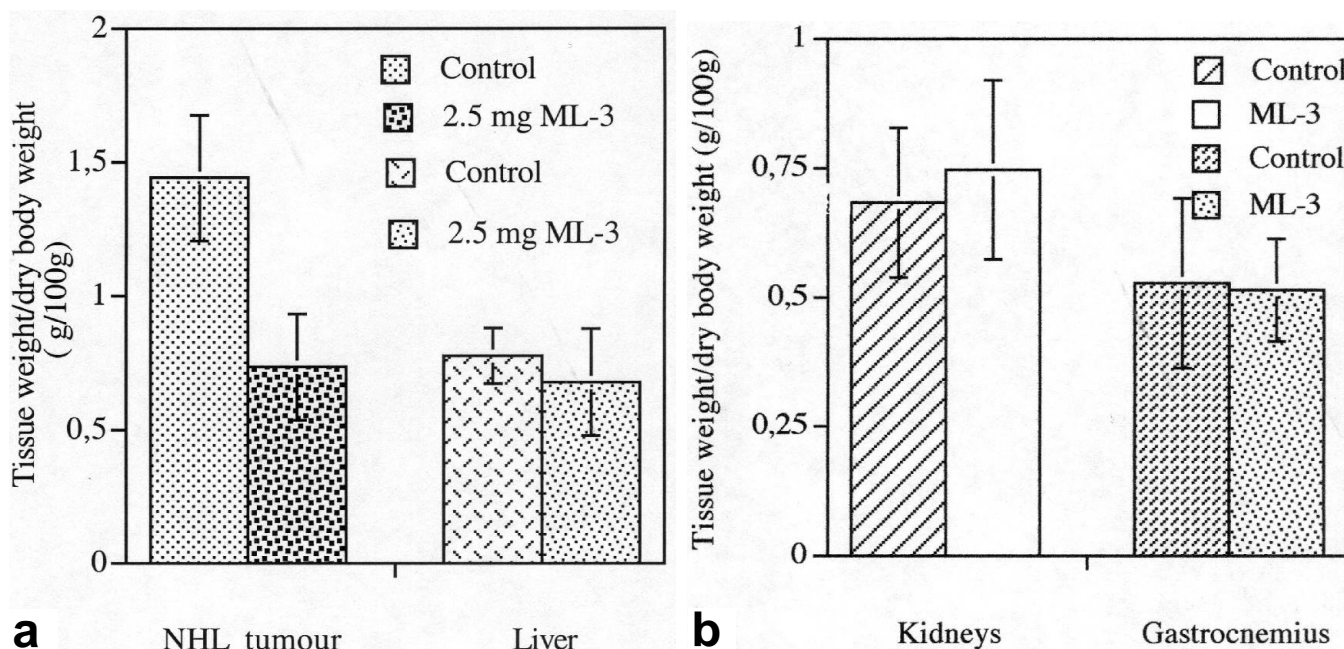


Fig. 4. Tissue mass of NHL tumour and liver (a), kidneys and gastrocnemius muscle (b) in two groups (5 individuals/group) of NMRI mice fed on an LA (control) diet or an ML-3-supplemented diet (2.5 mg/g diet). All mice in both groups were injected s.c. with 2×10^6 Krebs II NHL cells and initially fed on the LA diet for three days (period of tumour development). One group was then switched to the ML-3-supplemented diet while the other was continued on the control diet. All mice were sacrificed eight days later. Results are expressed as dry tissue weight/dry body weight (g/100g).

References

- Bantel H., Engels I.H., Voelter W., Schulze-Osthoff K. and Wesselborg S. (1999). Mistletoe lectin activates caspase-8/FGLICE independently of death receptor signaling and enhances anticancer drug-induced apoptosis. *Cancer Res.* 59, 2083-2090.
- Bardocz S. (1989). Polyamines in tissue regeneration. In: *Physiology of polyamines*. Vol. 1. Bachrach U. and Heimer Y.M. (eds). CRC Press. Boca Raton, Florida pp 96-106.
- Bardocz S., Grant G., Brown D.S. and Pusztai A. (1990a). Luminal and basolateral polyamine uptake by rat small intestine stimulated to grow by *Phaseolus vulgaris* lectin phytohaemagglutinin *in vivo*. *Biochim. Biophys. Acta* 1034, 46-52.
- Bardocz S., Grant G., Brown D.S., Ewen S.W.B., Nevison I. and Pusztai A. (1990b). Polyamine metabolism and uptake during *Phaseolus vulgaris* lectin, PHA-induced growth of the rat small intestine. *Digestion* 46 (Suppl.), 360-366.
- Bardocz S., Grant G., Duguid T.J., Brown D.S., Pusztai A. and Pryme I.F. (1994a). Effect of phytohaemagglutinin on the growth of Krebs II tumour cells, body metabolism and internal organs of mice. *Int. J. Oncol.* 5, 1369-1374.
- Bardocz S., Grant G., Duguid T.J., Brown D.S., Sakhri M., Pusztai A., Pryme I.F., Mayer D. and Wayß K. (1994b). Phytohaemagglutinin in the diet induces growth of the gut and modifies some organ weights in mice. *Med. Sci. Res.* 22, 101-103.
- Bardocz S., Grant G., Ewen S.W.B., Duguid T.J., Brown D.S., Englyst K. and Pusztai A. (1995). Reversible effect of phytohaemagglutinin on the growth and metabolism of rat gastrointestinal tract. *Gut* 37, 353-360.
- Bardocz S., Grant G., Pusztai A., Franklin M.F. and Carvalho A. de FFU (1996). The effect of phytohaemagglutinin at different dietary concentrations on the growth, body composition and plasma insulin of the rat. *Brit. J. Nutr.* 76, 613-626.
- Bardocz S., Grant G., Duguid T.J., Brown D.S., Pusztai A. and Pryme I.F. (1997). Intracellular levels of polyamines in Krebs II lymphosarcoma cells in mice fed phytohaemagglutinin-containing diets are coupled with altered tumour growth. *Cancer Lett.* 121, 25-29.
- Bardocz S., Grant G., Hughes E.L., Brown D.S. and Pusztai A. (1999). Uptake, inter-organ distribution and metabolism of dietary polyamines in the rat. In: *Polyamines in health and nutrition*. Bardocz S. and White A. (eds). Kluwer Academic Publishers Boston/Dordrecht/London. pp 241-258.
- Baxevanis C.N., Voutsas I.F., Soler M.H.J., Gritzapis A.D., Tsitsilonis O.E., Stoeva S., Voelter W., Arsenis P. and Papamichail M. (1998). Mistletoe lectin I-induced effects on human cytotoxic lymphocytes. I. Synergism with IL-2 in the induction of enhanced LAK cytotoxicity. *Immunopharmacol. Immunotoxicol.* 20, 355-372.
- Bussing A., Multani A.S., Pathak S., Pfüller U. and Schietzel M. (1998). Induction of apoptosis by the N-acetyl-galactosamine-specific toxic lectin from *Viscum album* L. is associated with a decrease of nuclear p53 and Bcl-2 proteins and induction of telomeric associations. *Cancer Lett.* 130, 57-68.
- Bussing A., Wagner M., Wagner B., Stein G.M., Schietzel M., Schaller G. and Pfüller U. (1999). Induction of mitochondrial Apo2.7 molecules and generation of reactive oxygen-intermediates in cultured lymphocytes by the toxic proteins from *Viscum album* L. *Cancer Lett.* 139, 79-88.
- Eifler R., Pfüller K., Göckeritz W. and Pfüller U. (1993). Improved procedures for isolation of mistletoe lectins and their subunits: lectin pattern of the European mistletoe. In: *Lectins: Biology, biochemistry, clinical biochemistry*. Vol. 9. Basu J. Kundu M. and Chakrabarty P. (eds). Wiley Eastern Limited. New Delhi. pp 144-151.
- Elsasser-Beile U., Lusebrink S., Grussenmeyer T., Wetterauer U. and Schultze-Seemann W. (1998). Comparison of the effects of various clinically applied mistletoe preparations on peripheral blood leukocytes. *Drug Res.* 48, 1185-1189.
- Elsasser-Beile U., Ruhnau T., Freudenberg N., Wetterauer U. and Mengs U. (2001). Antitumoral effect of recombinant mistletoe lectin on chemically induced urinary bladder carcinogenesis in a rat model. *Cancer* 91, 998-1004.
- Ewen S.W.B., Bardocz S., Grant G., Pryme I.F. and Pusztai A. (1998). The effects of PHA and mistletoe lectin binding to epithelium of rat and mouse gut. In: *Effects of antinutrients on the nutritional value of legume diets*. Vol. 5. Bardocz S., Pfüller U. and Pusztai A. (eds). COST 98. EC Publication. Luxembourg. pp 221-225.
- Ewen S.W.B., Pryme I.F., Bardocz S. and Pusztai A. (1999). Does oral ingestion of ML-1 lectin ablate murine transplanted non-Hodgkin lymphoma by apoptosis or necrosis? In: *Effects of antinutrients on the nutritional value of legume diets*. Vol. 6. Bardocz S., Hajos G. and Pusztai A. (eds). COST 98. EC Publication. Luxembourg. pp 126-133.
- Frantz M., Jung M.L., Ribereau-Gayon G. and Anton R. (2000). Modulation of mistletoe (*Viscum album* L.) lectins cytotoxicity by carbohydrates and serum glycoproteins. *Drug Res.* 50, 471-478.
- Gabius S., Joshi S.S., Kayser K. and Gabius H.J. (1992). The galactoside-specific lectin from mistletoe as biological response modifier. *Int. J. Oncol.* 1, 705-708.
- Hajto T., Hostanska K. and Gabius H.J. (1989). Modulatory potency of the beta-galactoside-specific lectin from mistletoe extract (Iscador) on the host defense system *in vivo* in rabbits and patients. *Cancer Res.* 49, 4803-4808.
- Heiny B.M., Albrecht V. and Beuth J. (1998). Correlation of immune cell activities and beta-endorphin release in breast carcinoma patients treated with galactose-specific lectin standardized mistletoe extract. *Anticancer Res.* 18, 583-586.
- Koninx J.F.J.G., Brown D.S., Kok W., Hendriks H.G.C.J.M., Pusztai A. and Bardocz S. (1996). Polyamine metabolism of enterocyte-like Caco-2 cells after exposure to *Phaseolus vulgaris* lectin. *Gut* 38, 47-52.
- Köteles G.J., Kubasova T., Hurná E., Horváth Gy. and Pfüller U. (1998). Cellular and cytogenic approaches in testing toxic and safe concentrations of mistletoe lectins. In: *Effects of antinutrients on the nutritional value of legume diets*. Vol. 5. Bardocz S., Pfüller U. and Pusztai A. (eds). COST 98. EC Publication. Luxembourg. pp 81-86.
- Krenzelok T.D., Jacobsen E.P. and Aronis J. (1997). American mistletoe exposures. *Am. J. Emerg. Med.* 15, 516-520.
- Lavelle E.C., Grant G., Pusztai A., Pfüller U. and O'Hagan D.T. (2000). Mucosal immunogenicity of plant lectins in mice. *Immunology* 99, 30-37.
- Lenartz D., Andermahr J., Plum G., Menzel J. and Beuth J. (1998). Efficiency of treatment with galactoside-specific lectin from mistletoe against rat glioma. *Anticancer Res.* 18, 1011-1014.
- Lenartz D., Dott U., Menzel J., Schierholz J.M. and Beuth J. (2000). Survival of glioma patients after complementary treatment with galactoside-specific lectin from mistletoe. *Anticancer Res.* 20, 2073-2076.
- Lyu S.Y., Park W.B., Choi K.H. and Kim W.H. (2001). Involvement of

- caspase-3 in apoptosis induced by *Viscum album* var. *coloratum* agglutinin in HL-60 cells. *Biosci. Biotechnol. Biochem.* 65, 534-541.
- Mannel D.N., Becker H., Gundt A., Kist A. and Franz H. (1991). Induction of tumour necrosis factor expression by a lectin from *Viscum album*. *Cancer Immunol. Immunother.* 33, 177-182.
- Mengs U., Schwarz T., Bulitta M. and Weber K. (2000). Antitumoral effects of an intravesically applied aqueous mistletoe extract on urinary bladder carcinoma in MB49 mice. *Anticancer Res.* 20, 3565-3568.
- Mockel B., Schwarz T., Zinke H., Eck J., Langer M. and Lentzen H. (1997). Effects of mistletoe lectin I on human blood cell lines and peripheral blood cells. Cytotoxicity, apoptosis and induction of cytokines. *Drug Res.* 47, 1145-1151.
- de Oliveira J.T.A., Pusztai A. and Grant G. (1988). Changes in organs and tissues induced by feeding of purified kidney bean (*Phaseolus vulgaris*) lectins. *Nutr. Res.* 8, 943-947.
- Pae H.O., Seo W.G., Shin M.K., Lee H.S., Kim S.B. and Chung H.T. (2000a). Protein kinase A or C modulates the apoptosis induced by lectin II isolated from Korean mistletoe, *Viscum album* var. *Coloratum*, in the human leukemic HL-60 cells. *Immunopharmacol. Immunotoxicol.* 22, 279-295.
- Pae H.O., Seo W.G., Oh G.S., Shin M.K., Lee H.S., Lee H.S., Kim S.B. and Chung H.T. (2000b). Potentiation of tumor necrosis factor- α -induced apoptosis by mistletoe lectin. *Immunopharmacol. Immunotoxicol.* 22, 697-709.
- Park R., Kim M.S., So H.S., Jung B.H., Moon S.R., Chung S.Y., Ko C.B., Kim B.R. and Chung H.T. (2000). Activation of c-Jun N-terminal kinase 1 (JNK1) in mistletoe lectin II-induced apoptosis of human myeloleukemic U937 cells. *Biochem. Pharmacol.* 60, 1685-1691.
- Pryme I.F., Bardocz S. and Pusztai A. (1994a). A diet containing the lectin phytohaemagglutinin (PHA) slows down the proliferation of Krebs II cell tumours in mice. *Cancer Lett.* 76, 133-137.
- Pryme I.F., Pusztai A. and Bardocz S. (1994b). The initial growth rate of Krebs II ascites cell tumours in mice is slowed down by the inclusion of phytohaemagglutinin in the diet. *Int. J. Oncol.* 5, 1105-1107.
- Pryme I.F., Bardocz S., Grant G., Duguid T.J., Brown D.S. and Pusztai A. (1995). Switching between control and phytohaemagglutinin-containing diets affects growth of Krebs II ascites cells and produces differences in the levels of putrescine, spermidine and spermine. *Cancer Lett.* 93, 233-237.
- Pryme I.F., Bardocz S., Grant G., Duguid T.J., Brown D.S. and Pusztai A. (1996a). Inclusion of the plant lectin phytohaemagglutinin in the diet and its effects on development of murine tumours. In: *Effects of antinutrients on the nutritional value of legume diets*. Vol. 1. Bardocz S., Gelencsér E. and Pusztai A. (eds). COST 98. EC Publication. Luxembourg. pp 34-40.
- Pryme I.F., Bardocz S., Grant G., Duguid T.J., Brown D.S. and Pusztai A. (1996b). The plant lectin PHA as a tool for reducing the progression of tumour growth. In: *Effects of antinutrients on the nutritional value of legume diets*. Vol. 2. Bardocz S. and Pusztai A. (eds). COST 98. EC Publication. Luxembourg. pp. 24-29.
- Pryme I.F., Pusztai A., Grant G. and Bardocz S. (1996c). Phytohaemagglutinin induced gut hyperplasia and the growth of a mouse lymphosarcoma tumour. *J. Exp. Therap. Oncol.* 1, 171-176.
- Pryme I.F., Pusztai A., Grant G. and Bardocz S. (1996d). Dietary phytohaemagglutinin slows down the proliferation of a mouse plasmacytoma (MPC-11) tumour in Balb/c mice. *Cancer Lett.* 103, 151-155.
- Pryme I.F., Bardocz S., Grant G., Ewen S.W.B., Pusztai A. and Pfüller U. (1998a). The plant lectins PHA and ML-1 suppress the growth of a lymphosarcoma tumour in mice. In: *Effects of antinutrients on the nutritional value of legume diets*. Vol. 5. Bardocz S., Pfüller U. and Pusztai A. (eds). COST 98. EC Publication. Luxembourg. pp 215-220.
- Pryme I.F., Pusztai A., Grant G. and Bardocz S. (1998b). The induction of gut hyperplasia by phytohaemagglutinin in the diet and limitation of tumour growth. *Histol. Histopathol.* 13, 575-583.
- Pryme I.F., Pusztai A., Bardocz S. and Ewen S.W.B. (1999a). A combination of dietary protein depletion and PHA-induced gut growth reduce the mass of a murine non-Hodgkin lymphoma. *Cancer Lett.* 139, 145-152.
- Pryme I.F., Bardocz S., Pusztai A. and Ewen S.W.B. (1999b). The growth of an established murine non-Hodgkin lymphoma tumour is limited by switching to a phytohaemagglutinin-containing diet. *Cancer Lett.* 146, 87-91.
- Pryme I.F., Grant G., Pusztai A. and Bardocz S. (1999c). Limiting the availability of polyamines for a developing tumour: an alternative approach to reducing tumour growth. In: *Polyamines in health and nutrition*. Bardocz S. and White A. (eds). Kluwer Academic Publishers. Boston/Dordrecht/London. pp 241-258.
- Pryme I.F., Bardocz S., Pusztai A. and Ewen S.W.B. (2000a). The extent of PHA-induced gut hyperplasia is reduced by a developing murine non-Hodgkin lymphoma tumour. In: *Effects of antinutrients on the nutritional value of legume diets*. Vol. 8. Kroghdal A., Mathiesen S. and Pryme I.F. (eds). COST 98. EC Publication. Luxembourg. pp 65-69.
- Pryme I.F., Pusztai A., Bardocz S. and Ewen S.W.B. (2000b). Dietary phytohaemagglutinin reduces growth of a murine non-Hodgkin lymphoma. In: *Dietary anticarcinogens and antimutagens: Chemical and biological aspects*. Johnson I.T. and Fenwick G.R. (eds). Atheneum Press Ltd. UK. pp 350-354.
- Pryme I.F., Bardocz S., Pusztai A., Ewen S.W.B. and Pfüller U. (2001). Feeding mice the plant lectins PHA and ML-1 decreases tumour mass and mitotic activity, increases lymphocyte infiltration and induces necrosis of a murine non-Hodgkin lymphoma. In: *Effects of antinutrients on the nutritional value of legume diets*. Vol. 9. Pierzynowski S.G., Gee, J.M. and Svendsen J. (eds). COST 98. EC Publication. Luxembourg. pp 48-53.
- Pusztai A. (1991) *Plant lectins*. Cambridge University Press, Cambridge, UK. pp 1-251.
- Pusztai A., Grant G., Williams L.M., Brown D.S. and Bardocz S. (1989). *Phaseolus vulgaris* lectin induces growth and the uptake of polyamines by the rat small intestine *in vivo*. *Med. Sci. Res.* 17, 215-217.
- Pusztai A., Ewen S.W.B., Grant G., Peumans W.J., van Damme E.J.M., Rubio L.A. and Bardocz S. (1990). Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. *Digestion* 46, 308-316.
- Pusztai A., Grant G., Gelencsér E., Ewen S.W.B., Pfüller U., Eifler R. and Bardocz S. (1998). Effects of an orally administered mistletoe (type-2 RIP) lectin on growth, body composition, small intestinal structure, and insulin levels in young rats. *J. Nutr. Biochem.* 9, 31-36.
- Ribereau Gayon G., Jung M.L., Frantz M. and Anton R. (1997). Modulation of cytotoxicity and enhancement of cytokine release induced by *Viscum album* L. extracts or mistletoe lectins. *Anticancer Drugs.* 8 (Suppl 1), S3-8.

Mistletoe lectin and tumour growth

- Schink M. (1997). Mistletoe therapy for human cancer: The role of the natural killer cells. *Anti-Cancer Drugs* 8, S47-S51.
- Schumacher U., Feldhaus S. and Mengs U. (2000). Recombinant mistletoe lectin (rML) is successful in treating human ovarian cancer cells transplanted into severe combined immunodeficient (SCID) mice. *Cancer Lett.* 150, 171-175.
- Seiler N., Delcros J.G. and Moulinoux J.P. (1996). Polyamine transport in mammalian cells. An update. *Int. J. Biochem. Cell. Biol.* 28, 843-861.
- Stein G. and Berg P. (1998). Modulation of cellular and humoral immune responses during exposure of healthy individuals to an aqueous mistletoe extract. *Eur. J. Med. Res.* 3, 307-314.
- Stein G., Henn W., von Laue H. and Berg P. (1998). Modulation of the cellular and humoral immune responses of tumor patients by mistletoe therapy. *Eur. J. Med. Res.* 3, 194-202.
- Stein G.M., Pfüller U., Schietzel M. and Büssing A. (2000a). Toxic proteins from European mistletoe (*Viscum album* L.): Increase of intracellular IL-4 but decrease of IFN-gamma in apoptotic cells. *Anticancer Res.* 20, 1673-1678.
- Stein G.M., Pfüller U., Schietzel M. and Büssing A. (2000b). Intracellular expression of IL-4 and inhibition of IFN-gamma by extracts from European mistletoe is related to induction of apoptosis. *Anticancer Res.* 20, 2987-2994.
- Vervecken W., Kleff S., Pfüller U. and Büssing A. (2000). Induction of apoptosis by mistletoe lectin 1 and its subunits. No evidence for cytotoxic effects caused by isolated A- and B-chains. *Int. J. Biochem. Cell Biol.* 32, 317-326.
- Wenzel-Seifert K., Lentzen H. and Seifert R. (1997). In U-937 promonocytes, mistletoe lectin 1 increases basal $[Ca^{2+}]_i$, enhances histamine H-1 and complement C5a-receptor mediated rises in $[Ca^{2+}]_i$, and induces cell death. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 355, 190-197.
- Yoon T.J., Yoo Y.C., Choi O.B., Do M.S., Kang T.B., Lee K.H., Azuma I. and Kim J.B. (1995). Inhibitory effect of Korean mistletoe (*Viscum album coloratum*) extract on tumor angiogenesis and of hematogenous and non-hematogenous tumor cells in mice. *Cancer Lett.* 97, 83-91.
- Yoon T.J., Yoo Y.C., Kang T.B., Baek Y.J., Huh C.S., Song S.K., Lee K.H., Azuma I. and Kim J.B. (1998). Prophylactic effect of Korean mistletoe (*Viscum album coloratum*) extract on tumour metastasis is mediated by enhancement of NK cell activity. *Int. J. Immunopharmacol.* 20, 163-172.
- Yoon T.J., Yoo Y.C., Kang T.B., Shimazaki K., Song S.K., Lee K.H., Kim S.H., Park C.H., Azuma I. and Kim J.B. (1999). Lectins isolated from Korean mistletoe (*Viscum album coloratum*) induce apoptosis in tumour cells. *Cancer Lett.* 136, 33-40.

Accepted September 6, 2001