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Effects of an orally administered mistletoe (type-2 RIP) lectin on growth, body composition, small intestinal structure, and insulin levels in young rats

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The high parenteral toxicity of mistletoe lectin ML-1, a type-2 RIP (ribosome inactivating protein), with LD_{50} values of 5 to 10 μg/kg BW, seriously limits its use as a cytotoxic agent in cancer therapy. As RIP proteins are generally better tolerated when given orally, we investigated the effects of ML-1 on growth, gut and body metabolism and composition by feeding rats diets containing 67 or 200 mg ML-1 kg^{-1} BW for 10 days. Although ML-1 depressed voluntary feed intake and reduced growth rate, none of the rats lost weight during the experiment. ML-1 had no effect on the digestibility of proteins and other components of the diet but because of increased urinary nitrogen losses, the overall N balance and total body N content were reduced, particularly at the higher dose of ML-1. Body fat was also reduced, probably due to the depression of circulating insulin levels. Some organs were affected by oral ML-1: there was hypertrophy of the pancreas and lungs and, most importantly, a dramatic dose-dependent hyperplastic growth of the small intestine, probably due to the avid binding and endocytosis of ML-1 by gut epithelial cells. The plasma level of tumour necrosis factor-α was significantly increased by oral ML-1 and there was a similar, though not significant, rise in Interleukin-1β levels. These similarities in cytokine release by oral and intraperitoneal ML-1 suggest that the oral route may be as effective in tumor suppression as the parenteral. As orally given ML-1 was well tolerated by rats with an extended nontoxic dose range over that of parenteral ML-1, the possible advantages in therapy offered by this route could now be profitably explored. (J. Nutr. Biochem. 9:31–36, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Herbal extracts and teas made of mistletoes have been used for centuries in Germany as folk medicines against various ailments.[1] More recently, preparations and isolated components of mistletoes have been given parenterally in clinical practice as immunomodulating agents for the treatment of some autoimmune diseases and cancer. It is believed that most of this biologic activity is attributable to a number of carbohydrate-binding proteins in mistletoes, which are commonly referred to as mistletoe lectins.[1] In fact, they are type-2 RIP (ribosome-inactivating protein) composed of an *N*-glycosidase A subunit accounting for the ribosome-inactivating activity, and a galactose-specific lectinic B subunit accounting for the carbohydrate-binding activity of the protein. The main and most studied RIP component of mistletoes is ML-1, which is highly toxic, with a reputed LD_{50} value of about 5 μg kg^{-1} on parenteral administration. ML-1 requires the presence of both A and B subunits for full biologic activity, as the individual subunits are only slightly toxic.[2]

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The in vivo tumour-suppressing effects of ML-1 have been attributed to its immunomodulating activity on the host defense system,[3] stimulation of the reticuloendothelial system, and particularly the activation of macrophages.[4] There is in vitro evidence that ML-1 activates human peripheral monocytes or murine macrophages directly for the production of inflammatory monokines, such as tumor necrosis factor, TNF-α, and interleukin-1β, IL-1β.[4] Thus, it is possible that the necrosis of tumor cells induced by ML-1 is not only attributable to its cytotoxicity, but also to its induction of endogenous mediators of the immune system.

A major constraint in the therapeutical use of potentially beneficial type-2 RIP toxins, such as ML-1 and others, is their extreme toxicity when administered parenterally. However, as there are indications that some plant type-2 RIP toxins such as abrin and ricin are less toxic when administered orally,[5,6] it is possible that ML-1 may also be less toxic by this route. Thus, the dose range of ML-1 for potential cancer therapy may be considerably widened by choosing the oral route, providing that its perceived beneficial immunomodulatory effects on the host are comparable and independent of the method of administration. Accordingly, the main objective of this study was to establish in rats a safe threshold dose for the oral administration of purified ML-1, below which there was no overt toxicity and minimal deleterious effects on vital tissues and organs, as measured by nitrogen balance experiments and studies of gut structure and metabolism. In addition, it was intended to perform preliminary studies to establish whether, like the parenterally given ML-1, the orally administered lectin could stimulate the release of cytokines into the blood circulation, which are generally assumed to be essential for the immunomodulatory effects of ML-1.[4] These studies were considered to be a vital prelude to a full comparison of the immunomodulatory and tumor-depressing effects of orally versus parenterally administered ML-1.

Methods and materials

Mistletoe lectin and antibody

ML-1 was isolated from air-dried mistletoe powder; it contained over 95% pure mistletoe lectin of the ML-1 isolectin group as described recently.[7] The preparation was stored as a suspension in 2.67 molar ammonium sulphate. Before use, ML-1 was centrifuged and the pellet was incorporated into the diet. A polyclonal antibody to ML-1 was raised in rabbits by repeated subcutaneous injections of the lectin (0.1 mL; 0.1 g ML-1 mL^{-1} saline).

Animals and diets

All animal management and experimental procedures were performed in strict accordance with the requirements of the UK Animals (Scientific procedures) Act 1986.

Male Hooded-Lister rats (Rowett strain) were weaned at 19 days and given free access to commercial stock diet (Labsure, Manea, UK) and water for 10 days. The rats were then randomly selected into three groups of five animals each, individually caged and restrictedly fed, 6 g rat^{-1} day^{-1}, a lactalbumin-based control diet (LA; 100 g lactalbumin protein kg^{-1} diet; Table 1)[8] for 4 days before the start of the experiment. During the 10-day experiment the control rats (Group 1) continued to receive the LA diet, whereas the rats in Test Groups 2 and 3 were given the LA diet containing a daily dose of 5.4 and 17.5 mg of pure ML-1 respectively, replacing equal amounts of lactalbumin in the diet.

Table 1 Composition of experimental diets

Diet	Control	ML-1 (0.9 mg/g)	ML-1 (3 mg/g)
ML-1	—	0.9	3.0
Lactalbumin	120.0	119.3	117.3
Maize starch	379.6	379.4	379.3
Potato starch	100	100	100
Glucose	150	150	150
Corn oil	150	150	150
Minerals	50	50	50
Vitamins	50	50	50
Silicic acid	0.4	0.4	0.4

Components are given in g kg^{-1} diet. For the composition of 'Minerals' and 'Vitamins' see Ref. 9.

All rats were given 6 g diet g^{-1} (*Table 1*). They were under close observation throughout the experiment, weighed daily, and feces and urine were collected. The rats were blood sampled after 8 days into the experiment and killed on Day 10 under halothane anaesthesia and dissected. The stomach and small and large intestines were washed and, together with other internal organs, freeze-dried, weighed and analyzed. A 2-cm length of the small intestine, 5 cm from the pylorus, was taken from each animal for histology. To establish the effects of ML-1 on the small intestine at an earlier time than 10 days and to measure plasma concentration of TNF-α and IL-1β, two groups of five rats each were first blood-sampled and then fed a 6-g LA diet containing 17.5 mg ML-1 day^{-1} or a 6-g LA diet without ML-1 for 30 hr. At the end, the rats were blood-sampled again, killed, and a 2-cm length of the small intestine, 5 cm from the pylorus was taken from each rat for histology. As positive controls for histology, 10 more rats were fed LA diets containing 18 mg kidney bean phytohaemagglutinin rat^{-1} day^{-1} for the same periods of time to match the ML-1 feeding regime. Five of the rats were killed at 30 hr and another five at 10 days and 2 cm sections of their small intestine were taken and treated the same way as the ML-1 rats.

Analysis

Appropriate dried tissues and carcass samples were combined and ground in a mincer. Lipid was extracted from the ground material (1 g: 100 mL solvent) with chloroform-methanol (2:1, v/v) as before.[9] Nitrogen estimations were done on the defatted carcass material using a Foss Heraeus Macro N automated system. Protein content was calculated as N × 6.25. Immunoreactive plasma insulin was measured by radioimmunoassay[10] using rat insulin as a standard and glucose was determined by an autoanalyzer method[11] as before.[12] Pancreatic insulin was extracted according to the procedure of Melmed et al.[13]

Table 2 Nutritional performance of rats fed diets containing ML-1 and control diets (mg rat^{-1} day^{-1})

	Control	ML-1		SD
		5.4	17.5	
Lectin intake (mg)	—	53.7	175	
Food intake (g) (over 10 days)	60.0	59.0	60.0	
Initial body weight (g)	82.2	84.7	81.1	2.5
Final body weight (g)	96.5—c	91.7—b	83.2—a	2.0
Gain (g)	14.5—c	7.0—b	2.1—a	1.7

Values with different letters in a horizontal row—a, b, c—are significantly different ($P < 0.05$).

Table 3 Nitrogen accretion and body composition of rats fed ML-1 diets (mg rat^{-1} day^{-1})

	Control	ML-1 5.4	ML-1 17.5	SD
Dry body weight (g)	27.9—c	26.6—b	23.0—a	0.8
Lipid (g)	5.5—c	4.6—b	4.0—a	0.6
Nitrogen (g)	2.70—b	2.78—b	2.40—a	0.11
Faeces (g)	3.22—a	ND	3.32—a	0.53
Faecal nitrogen (mg)	137.0—a	ND	135.0—a	21.0
Urine nitrogen (mg)	156.0—a	ND	446.0—b	21.0
Nitrogen balance (mg)	684—b	ND	398—a	30
Apparent N digestibility (%)	85.9—a	ND	86.2—a	2.1
Dry matter digestibility (%)	94.7—a	ND	94.5—a	0.9

Values with different letters in a horizontal row—a, b, c—are significantly different ($P < 0.05$).

Histology

Sections of the small intestine were fixed in 4% buffered (pH 7) paraformaldehyde. The formalin-fixed sections were embedded in paraffin wax, sectioned at 3 μm, and after dewaxing with xylene stained with haematoxylin-eosin for measurement of crypt and villus sizes. Ten properly oriented jejunal crypts were selected at random from each animal and their length measured. Results were calculated as means (±SD) of five rats per treatment group. The sections were also used for immunohistology using an anti-ML-1 rabbit polyclonal antibody and a previously described streptavidin-biotin complex (sABC) peroxidase technique[12] to detect the presence of ML-1 on epithelial cells. Briefly, the endogenous peroxidase was first blocked by 1.5% (v/v) H_2O_2 in methanol, after which the sections were trypsinized for 15 min, reacted with the specific anti-ML-1 antibody, followed by incubation with swine anti-rabbit immunoglobulin G (E0353, Dako) and then by sABC with horseradish peroxidase (K0377, Dako). The enzyme was visualized with 3,3′-diaminobenzidine, darkened by $CuSO_4$ and counterstained with haematoxylin.

Measurement of cytokines of TNF-α and IL-1β

These measurements using a commercial rat TNF-α ELISA kit and a mouse IL-1β ELISA kit (Genzyme Corp. Cambridge, MA USA) were performed on heparinized plasma samples from rats after feeding them diets containing 17.5 mg ML-1 diet or control diet for 30 hr. The results were compared with those of pre-experimental plasma samples from the same rats.

Statistics

One-way ANOVA was performed on the data using the Minitab statistical software package (Minitab, New York, NY USA) and multiple comparisons were done by the Tukey test using the Instat statistical package (Graphpad Software Inc, San Diego, CA USA). Results were expressed as arithmetic means with their pooled SD.

Table 4 Serum and pancreas insulin concentrations of rats given a daily dose of 17.5 mg mistletoe lectin

Source	Insulin level (ng ml^{-1}) Control	ML-1	SD
Serum; 0 time	1.66—a	1.40—a	0.25
Serum; 8 days	1.85—a	1.12—b	0.28
Pancreas (μg) 10 days	63.4—a	50.9—b	5.0

Values with different letters in a horizontal row are significantly different ($P < 0.05$).

Table 5 Effects of dietary ML-1 (mg rat^{-1} day^{-1}) on organ weights of rats

Tissue weights (mg	Control	ML-1 5.4	ML-1 17.5	SD
Small intestine	2368—a	2962—b	3964—c	187
Pancreas	472—a	523—b	605—c	31
Liver	3885—a	4291—ab	3739—a	167
Kidneys	691—a	800—b	789—b	38
Lungs	476—a	426—a	619—b	54

No significant differences in the weights of stomach, caecum, colon, spleen, thymus, heart, and skeletal muscle (coleus, plantaris and gastrocnemius) were found.
Values in a horizontal row with different letters—a, b, c—are significantly different ($P < 0.05$).

Results

Nutritional data

Daily exposure to ML-1 in the diet was moderately well tolerated by the rats, particularly by the animals in Group 2 that received 5.4 mg ML-1 d^{-1}. However, as preliminary feeding studies indicated that the voluntary diet intake was depressed by the daily dose of 17.5 mg ML-1 to 6 g diet day^{-1} in Group 3, for meaningful comparison of the diets, rats in Groups 1 and 2 were also only given a 6-g diet day^{-1}.

Inclusion of ML-1 reduced growth rate in a dose-dependent manner (*Table 2*) although none of the rats lost weight over the 10-day experiment. The dry body weight of rats given the low and the high dose of ML-1 was reduced by 7 and 20% respectively compared with that in control animals (*Table 3*). Body N content was also significantly reduced in rats given the higher ML-1 dose, but not because of effects on protein or dry matter digestibility since faecal weight and its N content were unaffected by ML-1. However, urinary N excretion was highly elevated in rats given ML-1 and consequently the overall N balance of rats given ML-1 in the diet was less than 70% of that in control rats (*Table 3*). The lipid content of the body of rats given ML-1

Table 6 Crypt length and number of midvillus enterocyte apoptotic figures in the small intestine of rats fed diets containing 200 mg ML-1 kg^{-1} BW after 30 hr and 10 days of feeding

	Control (10 days)	ML-1 30 hr	ML-1 10 days	SD
Crypt length (μm)	104—a	170—b	258—c	4
Apoptotic cells 100^{-1} villus	4—a	27—b	78—c	2

For comparison, the crypt length in rats given phytohaemagglutinin at the same dietary concentration was 175 and 258 μm after 30 hr and 10 days, respectively, whereas the number of apoptotic figures was 10 per 100 villi.
Values in a horizontal row with different letters—a, b, c—are significantly different ($P < 0.05$).

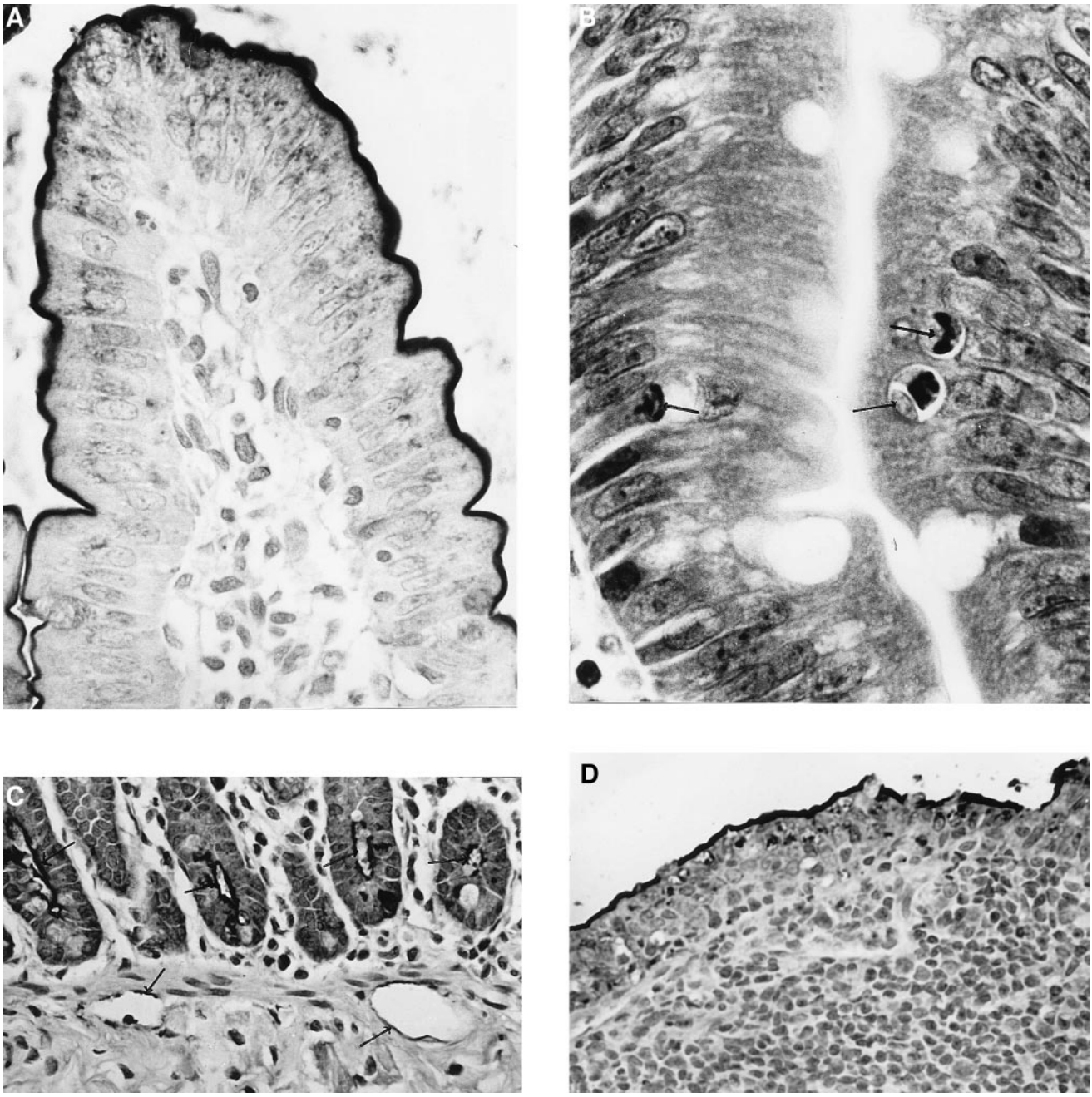

Figure 1 Binding of ML-1 to epithelial cells and apoptotic figures on small intestinal villi in rats given ML-1 in their diet: (A) Binding and endocytosis of ML-1 to jejunal apical and midvillus enterocytes (×63); (B) apoptotic figures in sections stained with haematoxylin and eosin of jejunal midvillus epithelial cells (see arrows, ×63); (C) binding of ML-1 to jejunal crypt enterocytes and walls of subepithelial blood vessels (see arrows, ×40); and (D) binding of ML-1 to Peyer's patch M cells of the rat small intestine. Sections A; C, and D were first reacted with an anti-ML-1 antibody and stained with sABC technology.

was similarly reduced in a dose-dependent manner; this was probably attributable to the depression of both serum and pancreatic insulin levels. These changes became significant at the higher dietary dose of ML-1 (*Table 4*).

Tissue weights

Both absolute and relative weights (weight 100 g^{-1} BW) of some of the organs and tissues of the rats were affected in a dose-dependent way by dietary exposure to ML-1 (*Table 5*). Thus, the absolute weights of the small intestine, pancreas, lungs and kidneys and the relative weight of the liver (calculated from values in *Tables 3* and *5*) were significantly increased in comparison with control rats. However, no significant differences in the weights of stomach, caecum, colon, spleen, thymus, heart, and skeletal muscle (soleus, plantaris and gastrocnemius) were found.

Table 7 Changes in plasma concentration of TNF-α and IL-1β in rats given ML-1 (200 mg kg^{-1} rat BW) and control diets for 30 h

	Pre-experimental	30 hr	
	(LA + ML-1)	LA	ML-1
TNF-α (pg mL^{-1} plasma)	8.6 ± 14.5	16.3 ± 7.3	810 ± 157*
IL-1β (pg mL^{-1} plasma)	276 ± 265	898 ± 543	2984 ± 2211

The results are means (±SD).
*$P < 0.001$.

Histology

In agreement with the large increases in small intestinal weight, ML-1 induced a highly significant increase in the size of small intestinal crypts, which was comparable to that occurring with kidney bean phytohaemagglutinin[5] included as a positive control (*Table 6*). This increase was already significant after 30 hr, but became more substantial after feeding for 10 days. ML-1 was avidly bound by membranes of both villus and crypt epithelial cells, with extensive endocytosis occurring (*Figure 1*). It induced frequent apoptosis of enterocytes with about 78 apoptotic figures per 100 villi at midvillus after 10 days, in comparison with four apoptotic cells in the LA control rats (*Figure 1*). However, there was no apoptosis in the small intestinal crypts of either test or control animals. Although ML-1 was also avidly bound by Peyer's patch M cells, its internalization in this dome region was apparently slight (*Figure 1*).

Plasma levels of TNF-α and IL-1β

Feeding rats diets containing ML-1 for 30 hr significantly increased their plasma concentration of TNF-α. Although there was a similar increasing trend with IL-1β the results did not reach full significance (*Table 7*).

Discussion

One of the main findings of this work was that, in contrast to the high parenteral toxicity of ML-1, with LD_{50} values of 5 μg kg^{-1} BW, rats tolerated oral doses of up to 200 mg ML-1 kg^{-1} BW without displaying overt toxicity symptoms. However, ML-1 in the diet dose-dependently reduced voluntary feed intake and decreased, but did not entirely arrest rat growth over 10 days. This is in dramatic contrast with the drastic reduction in body weight as early as 2 days after NMRI albino mice (Naval Medical Research Institute, USA) received an the intraperitoneal injection of as little as 1 μg ML-1 kg^{-1} BW.[14] Although fecal output of rats was not affected by oral ML-1, systemic catabolism was increased leading to high urinary urea output, reduced body N accretion, lipid depletion, and other changes in body composition usually regarded as antinutrient effects.

ML-1 had only minimal effects on the liver, thymus, and skeletal muscle, which is again in stark contrast to the drastic effect of low intraperitoneal doses of ML-1 (0.6 μg kg^{-1} BW) on the liver and many of its enzymes.[14] It also contrasts with the effects of dietary kidney bean lectin, PHA, on these tissues.[5,8] However, the effects of ML-1 on the gut and pancreas were similar to those of PHA and other dietary lectins, with hyperplastic growth of the small intestine, pancreatic hypertrophy, and reduced serum insulin levels.[5,8] Thus, despite causing some antinutritive effects and also substantially increasing apoptosis of villus enterocytes, oral ML-1 was much less toxic for rats than parenteral ML-1 for mice, with the relative toxic dose levels differing by at least four orders of magnitude.

In the present work the immunomodulating effects of orally administered ML-1 were not studied detail. However, the results of preliminary studies indicated that, similar to the effects of intraperitoneally injected ML-1, plasma levels of TNF-α were also significantly increased in rats that were given ML-1 orally. Similar trend was observed with the levels of IL-1β although the increase did not reach full significance (*Table 7*). Thus, the release of cytokines that is thought to be an essential step in immunomodulation and tumor depression caused by parenterally administered mistletoe lectin preparations can also apparently occur with high doses of ML-1 given orally. Moreover, a structurally and functionally similar type-2 RIP, abrin, isolated from *Abrus precatorius*, has previously been successfully used previously as an oral immunomodulating therapeutic agent in the treatment of AIDS and depression of tumor growth.[6] This gives us grounds to believe that the oral administration of ML-1 may be similarly successful in clinical cancer therapy, particularly as the oral route is generally regarded more convenient than the parenteral for the safe administration of toxic RIP lectins. Moreover, it was shown in the present work that orally administered ML-1 was as potent growth factor for the small intestine as phytohaemagglutinin, PHA. It is therefore possible that the growth depressant effects of ML-1 on tumors are not only attributable to its cytoxicity, but also to its stimulation of gut growth competing with the tumor for and starving it of essential polyamines and nutrients in a way similar to that of PHA for transplanted lymphosarcomas in mice.[15–17]

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References

1 Franz, H. (1989). Viscaceae lectins. In *Advances of Lectin Research* (H. Franz, ed.), 2:28–59, Springer, Heidelberg
2 Gossrau, R. and Franz, H. (1990). Histochemical response of mice to mistletoe lectin I (ML I). *Histochemistry* **94,** 531–537
3 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
4 Mannel, D.N., Becker, H., Gundt, A., Kist, A., and Franz, H. (1991). Induction of tumor necrosis factor expression by a lectin from *Viscum album. Cancer Immunol. Immunother.* **33,** 177–182
5 Pusztai, A., (1991). *Plant Lectins.* Cambridge University Press, Cambridge, UK
6 Tung, T. C. (1991). Orally administrable anti-metastatic lectin compositions and methods. *United States Patent*, Patent Number: 5,053,386
7 Eifler, R., Pfüller, K., Göckeritz, W., and Pfüller, U. (1994). Improved procedures for isolation of mistletoe lectins and their

subunits: lectin pattern of the European mistletoe. In *Lectins: Biology, Biochemistry, Clinical Biochemistry* (J. Basu, M. Kundu, and P. Chakrabarty, eds.), 9:144–151, Wiley Eastern Limited, New Delhi, India

8 Bardocz, S., Grant, G., Pusztai, A., Franklin, M.F., and Carvalho, A. de F.F.U. (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

9 Grant, G., Dorward, P.M., and Pusztai, A. (1993). Pancreatic enlargement is evident in rats fed diets containing raw soybeans (*Glycine max*) or cowpeas (*Vigna unguiculata*) for 800 days but not those fed diets based on kidney beans (*Phaseolus vulgaris*) or lupinseed (*Lupinus angustifolius*). *J. Nutr.* **123,** 2207–2215

10 MacRae, J.C., Bruce, L.A., Hovell, F.B. de B., Hart, I.C., Inkster, J., Walker, A., and Atkinson, T. (1991). Influence of protein nutrition on the response of growing lambs to exogenous bovine growth hormone. *J. Endocrinol.* **130,** 53–61

11 Marks, V. (1959). An improved glucose-oxidase method for determining blood, c.s.f. and urine glucose levels. *Clin. Chim. Acta* **4,** 395–400

12 Pusztai, A., Grant, G., Bardocz, S., Baintner, K., Gelencsér, E., and Ewen, S.W.B. (1997). Both free and complexed trypsin inhibitors stimulate pancreatic secretion and change duodenal enzyme levels. *Amer. J. Physiol.* **272,** G340–G350

13 Melmed, R.N., Turner, R.C., and Holt, S.J. (1973). Intermediate cells of the pancreas II. The effects of dietary soyabean trypsin inhibitor on acinar-β cell structure and function in the rat. *J. Cell Sci.* **13,** 279–295

14 Gossrau, R. and Franz, H. (1990). Histochemical response of mice to mistletoe lectin I (ML I). *Histochemistry* **94,** 531–537

15 Pryme, I.F., Pusztai, A., and Bardócz, S. (1994). A diet containing the lectin phytohaemagglutinin (PHA) slows down the proliferation of Krebs II cell tumours in mice. *Cancer Letters* **76,** 133–136

16 Pryme, I.F., Pusztai, A., Grant, G., and Bardocz, S. (1996). Dietary phytohaemagglutinin slows down the proliferation of a mouse plasmacytoma (MPC-11) tumour in Balb/c mice. *Cancer Letters* **103,** 151–155

17 Pryme, I.F., Pusztai, A., Grant, G., and Bardocz, S. (1996). Phytohemagglutinin-induced gut hyperplasia and the growth of a mouse lymphosarcoma tumor. *J. Exper. Therapeut. and Oncol.* **1,** 171–176