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Induction of Apoptosis of Endothelial Cells by *Viscum album*: A Role for Anti-Tumoral Properties of Mistletoe Lectins

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Abstract

Background: *Viscum album* (VA) preparations consist of aqueous extracts of different types of lectins of VA. Mistletoe lectins have both cytotoxic and immunomodulatory properties that support their study for the development for cancer therapy. However, the mechanisms of the anti-tumoral properties in vivo of mistletoe lectins are not fully understood. Because endothelial cells (EC) play a pivotal role in tumor angiogenesis, we tested the hypothesis that VA extracts induce endothelial cell death and apoptosis.
Material and Methods: We investigated the effect of various VA preparations on both human venous endothelial cell (HUVEC) and immortalized human venous endothelial cell line (IVEC) using morphologic assessment of EC, FACScan analysis after propidium iodine and annexin V labeling, and detection of cleavage of poly(A)DP-ribose polymerase (PARP).
Results: All tested VA preparations, except Iscador P, were cytotoxic in IVEC. Apoptosis, assessed by morphologic examination, annexin V labeling, and Western blot analysis for PARP cleavage, was involved in HUVEC cell death induced by VA preparations derived from plants that grow on oak trees (VA Qu FrF).
Conclusions: Results from the present study suggest that VA extract-induced endothelial apoptosis may explain the tumor regression associated with the therapeutic use of VA preparations and support further investigations to develop novel anti-angiogenic compounds based on mistletoe compounds.

Introduction

Viscum album (VA) preparations consist of aqueous extracts from different types of lectins of *Viscum album* or European mistletoe (1,2). Mistletoe lectin (ML) I, II, and III belong to the ribosome-inactivating protein (RIP) family of type II, such as highly toxic ricin and abrin (3). RIP of type II are composed of an N-glycosidase (A chain) and a galactoside-recognizing lectin (B-chain) connected by a disulfide bridge (4). In several in vivo experimental model of tumoral implantation, treatment with VA extracts or purified ML has been associated with tumor regression (5–7). In vitro experimental studies have demonstrated both cytotoxic and immunomodulatory properties of ML that may support its anti-tumoral effect (8,9). Recent evidence suggests that the cytotoxic effect in tumor cells of mistletoe extracts may depend on the induction of apoptosis (10–20). However, the mechanisms underlying VA extract-induced apoptosis have not been fully elucidated (20). Several reported studies on the immunomodulatory effect of mistletoe extracts concern the use of isolated lectins and not the entire preparation used therapeutically, which consist (apart from mistletoe lectins and viscotoxins) of several enzymes, peptides (e.g., viscumamide), amino acids, thiols, amines, polysaccharides, cyclitoles, lipids, phytosterols, triterpines, flavonoids, phenylpropanes, and minerals (2). Scant information is available on the interaction of mistletoe extracts and the endothelial cell (EC) compartment.

Angiogenesis is the development of new blood vessels from preexisting ones. Tumor angiogenesis plays an essential role in tumor progression and metastasis (21). The mechanisms involved in tumor angiogenesis consist of a wide range of phenomena including enhanced division of ECs within the tumor, up-regulation of cell adhesion molecules, and production of angiogenic factors (22,23). Novel anticancer therapy, based on several anti-angiogenesis approaches, has emerged and is undergoing extensive study in Phase I–III trials (24). The anti-angiogenesis effect of ML was examined previously by chorial-lantoic membrane assays in C57BL6 mice inoculated with B16-BL6 melanoma cells and treated with *Viscum album–L. coloratum* agglutinin (25). However, the molecular mechanisms underlying this effect at the level of EC have not been fully elucidated.

The contribution of an EC-mediated effect in the anti-tumoral properties of ML thus needs to be investigated. In this respect, we assessed the interactions between VA extracts and EC. Our in vitro study

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focused on the ability of VA extracts to induce EC death, which in turn may influence tumor angiogenesis. Using primary human venous endothelial cell (HUVEC) and the immortalized human venous endothelial cell line (IVEC), we show that ML induce EC death in a dose- and time-dependent manner. We also demonstrate that VA-mediated EC death involves apoptosis. Together our results provide a new insight into the therapeutic effects of VA extracts.

Material and Methods

VA Extracts

VA Qu FrF and Iscador Qu Spez are aqueous extracts of mistletoe plants growing on oak trees, and Iscador M Spez and Iscador P (Weleda AG, Arlesheim, Switzerland) are aqueous extracts of mistletoe plants growing on apple and pine trees, respectively. Iscador Qu Spez, M Spez and P are fermented extracts. Iscador Qu Spez and M Spez contain standardized concentrations of MLs. These mistletoe preparations also contain several other substances, including amino acids, polysaccharides, and lipids.

Cell Culture Conditions

HUVEC were obtained as previously described (26) from umbilical cords collected from healthy newborns after normal pregnancy and delivery. IVEC (27) were a gift of Professor P. Vicart. The EC were cultured in 0.2% gelatin-coated (Sigma, St. Louis, MO, USA) 75-cm^2 tissue culture flasks (Costar, Cambridge, MA, USA) in M199 medium (Life Technologies, Inc., Grand Island, NY, USA) containing Earle's salts, L-glutamine, and 25 mmol/L HEPES, 100 U/ml penicillin, 100 mg/ml streptomycin, and 0.25 mg/ml fungizone (Life Technologies). HUVEC and IVEC were supplemented with 20% and 10% fetal calf serum (FCS), respectively (Dutscher, Brumath, France). HUVEC were used at second or third passage. For all experiments, cells were grown for 3–4 days in the appropriate medium containing optimal FCS concentration to confluency. All experiments were performed in triplicate.

Assay for Cell Toxicity: Propidium Iodide Uptake

Confluent IVEC and HUVEC were incubated for various periods of time (up to 48 hr) with increasing concentrations of VA preparations (6.25–200 μg/ml) in low serum condition. Cell death was determined by the uptake of propidium iodide (PI) (Sigma) followed by FACScan analysis. At the end of the incubation period, cells were washed twice in PBS, and resuspended in PBS-azide before adding PI (50 μg/ml) to each sample. The uptake of the dye by dead cells was immediately assessed by fluorescence analysis using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA, USA).

Phase Contrast and Cytologic Examinations

Confluent HUVEC were incubated for 48 hr with either M199 with 20% FCS or in low FCS condition with increasing concentrations of VA Qu FrF (50–200 μg/ml). At the end of incubation, cells were observed under a phase contrast microscope for morphologic features. HUVEC were then gently trypsinized, cytocentrifuged, and stained with May Gruenwald Giemsa. Apoptotic EC were defined by cell shrinkage and cytoplasmic and chromatin condensation (28).

Assay for Apoptosis: Propidium Iodide and Annexin V Labeling

HUVEC were incubated for various periods of time (24–48 hr) with increasing concentrations of VA Qu FrF in low serum condition. Annexin V labeling was carried out by staining cells with annexin V-FITC (AnV) (Bender Medsystems, BioWhittaker, France) (2.5 μg/ml) for 30 min on ice. Cells were gently trypsinized, washed with 1× PBS-Azide with 2 mM $CaCl_2$ and resuspended in PBS-Azide before adding PI (50 μg/ml) to each sample. Samples were analyzed for green fluorescence (AnV labeling) and for red fluorescence (PI uptake) using a FACScan flow cytometer. Cells were considered undergoing apoptosis if they were AnV positive and PI negative.

Detection of Cleavage of Poly(A)DP-Ribose Polymerase

HUVEC were incubated for with increasing concentrations of VA Qu FrF (0–50 μg/ml) for 12, 24, 36, and 48 hr in low serum condition. Western blot for the detection of the cleavage of poly(A)DP-ribose polymerase (PARP) was performed as previously described (29). Cells were harvested and lysed in RIPA buffer. After centrifugation at 5000 ×g for 5 min at 5 °C, equal amounts of the lysates were boiled with sample buffer containing SDS and ß-mercaptoethanol, separated on a SDS-PAGE (12.5%) prior to transfer of the proteins on to PVDF membrane. The membrane was blocked with 5% BSA in PBS for 1 hr at room temperature. The membranes were incubated overnight with mouse monoclonal anti-PARP antibody at 4 °C. After washing with PBS containing Tween-20 (0.01%) (PBST) and PBS alone successively, HRP-conjugated sheep anti-mouse antibodies (Amersham France, SA, France) in PBST containing 1% BSA was added and interacted with the membrane overnight at 4 °C. The membrane was washed with PBST and PBS, and the signal detected by means of ECL detection system (Amersham). The density of the bands corresponding to cleaved and uncleaved PAPR was measured using an NIH Image processor.

Results

Viscum album *Extracts from Different Host Trees Induce Variable Cytotoxicity on Immortalized Human Venous Endothelial Cell Line*

As shown in Figure 1, VA Qu FrF induced a potent and dose-dependent mortality of IVEC as assessed by the uptake of PI. Va Qu FrF was the most effective preparation. Iscador Qu Spez and Iscador

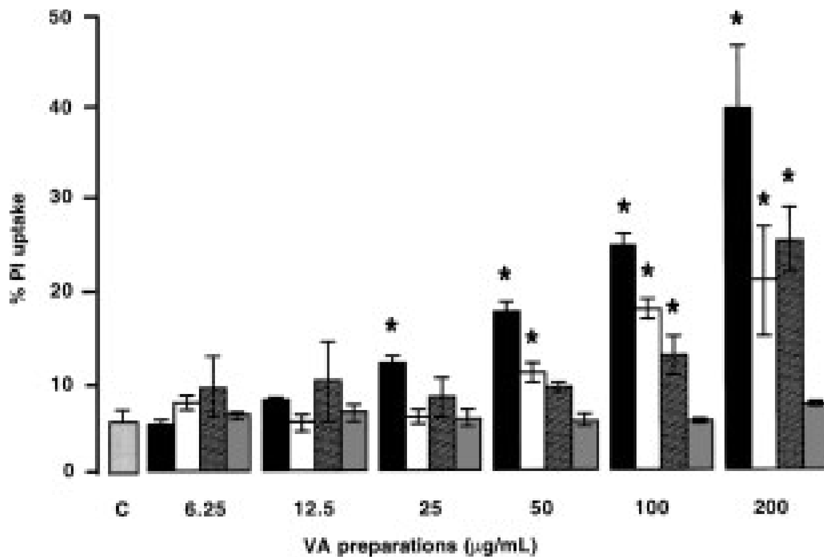

Fig. 1. Cytotoxic effect of VA extracts towards immortalized human venous endothelial cell line. IVEC were grown to confluency in M199 with 10% FCS. Cells were then incubated in low serum condition with increasing concentrations of VA extracts for 24 hr. Cells were then trypsinized, rinsed in PBS, and labeled with PI for FACS analysis. Histograms represent the percentage of dead cells (PI^+) with VA Qu FrF (black bars), Iscador Qu Spez (open bars), Iscador M Spez (hatched bars), and Iscador P (grey bars). $N = 3$ experiments. $*p < 0.05$ compared to control.

M Spez had almost similar cytotoxic properties. IVEC were insensitive to Iscador P at any tested concentration. Because VA Qu FrF was the most effective preparation, further experiments with HUVEC were performed with VA Qu FrF.

Viscum album *Qu FrF Induces Cytotoxicity on Human Venous Endothelial Cells*

Figure 2 depicts the cytotoxic properties of VA Qu FrF toward HUVEC as assessed by the uptake of PI. VA Qu FrF was highly cytotoxic toward HUVEC in a time- and dose-dependent manner. Cytologic studies under phase contrast microscopy of VA Qu FrF-treated HUVEC is illustrated in Figures 3A, 3B, and 3C. In controls, HUVEC grown in monolayer has a cobblestone appearance (Fig. 3A). Cell morphology was unchanged when cells were incubated with low concentrations of VA Qu FrF (12.5 and 25 μg/ml, data not shown). At medium concentration (50 μg/ml: Fig. 3B), HUVEC were elongated, had refractive borders, and exhibited cytoplasmic projections. In addition, numerous cell remnants were present. High concentrations (100–200 μg/ml, Fig. 3C) were highly cytotoxic. The majority of cells were detached and HUVEC were vacuolated with cytoplasmic inclusion and blebs of the plasmic membrane.

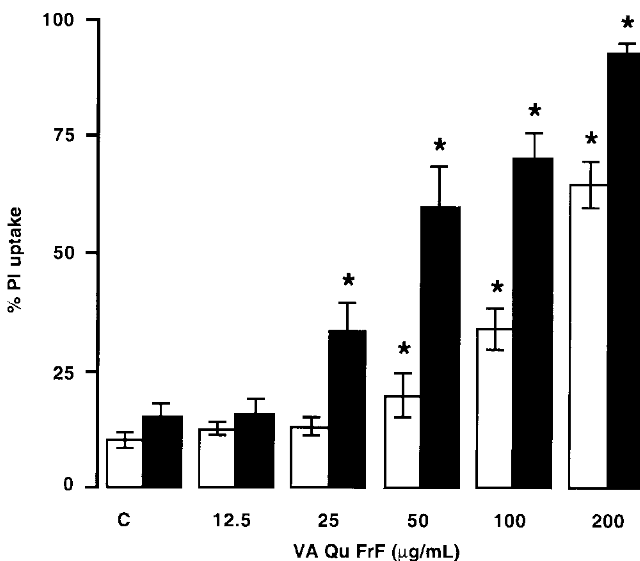

Fig. 2. Cytotoxic effect of VA extracts toward human venous endothelial cells. HUVEC were grown until confluency in M199 with 20% FCS. Cells were incubated in low serum condition with increasing concentrations of VA Qu FrF for 24 h (open bars) or 48 h (black bars). Cells were then trypsinized, rinsed in PBS and labeled with PI for FACS analysis, ($N = 3$ experiments, comparison to control, $* = p < 0.05$).

Viscum album *Extract-Induced Cytotoxicity Involves Apoptosis*

VA extracts induced apoptosis on EC was assessed by three different types of experiments. Cytologic analysis of HUVEC showed typical apoptotic bodies (Fig. 3E). HUVEC grown in 20% FCS presented no cytologic abnormalities and showed extensive mitoses (Fig. 3D). When incubated with high concentrations of VA Qu FrF, apoptotic EC were observed (Fig. 3E). These observations strongly suggest that apoptosis is involved in cytotoxic properties of VA Qu FrF.

Results of FACS analysis are depicted in Figure 4. The percentage of living cells undergoing apoptosis (An^+/PI^-) increased in a time- and dose-dependent manner for VA Qu FrF concentrations above 50 μg/ml. Decreased apoptosis observed for higher concentration (200 μg/ml) is explained by the highly cytotoxic properties of ML at such a concentration with necrosis overtaking apoptosis.

Western blot analysis of PARP cleavage (Fig. 5) confirmed HUVEC apoptosis. It also demonstrated

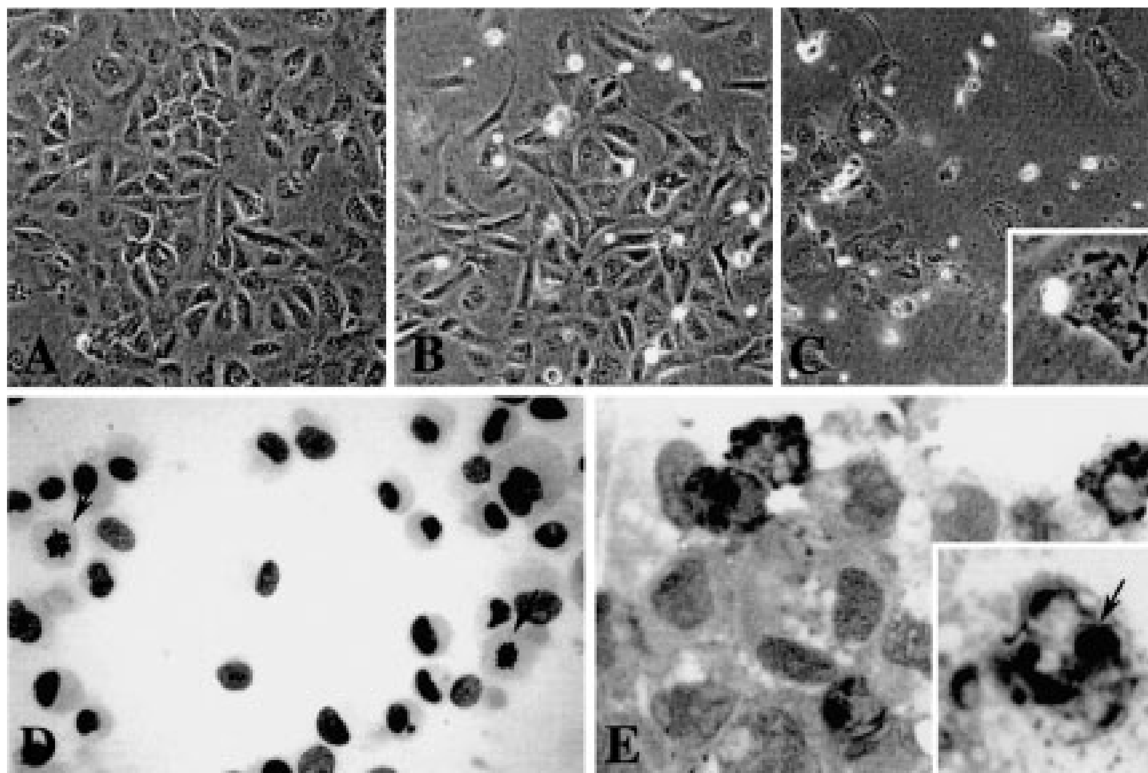

Fig. 3. Phase contrast analysis and cytologic analysis of HUVEC incubated with VA Qu FrF. HUVEC were grown to confluency in M199 with 20% FCS. Cells were incubated in low serum condition without **(A, D)** or with VA Qu FrF at increasing concentration (**B**: 50 μg/ml; **C, E**: 200 μg/ml) for 48 hr. Cellular morphologic features were assessed by phase contrast microscopy. HUVEC were then gently trypsinized, cytocentrifuged, and stained with May Gruenwald Giemsa. In controls, HUVEC grown in monolayer had a cobblestone appearance **(A)** with extensive mitosis (**D,** arrows). At medium concentration (50 μg/ml, **B**), HUVEC were elongated, had refractive borders, and exhibited cytoplasmic projections. At high concentrations (200 μg/ml: **C**), cells were vacuolated with cytoplasmic inclusion and blebs of the plasmic membrane (**C** arrow) and apoptotic bodies (**E** arrow). Panels A–D, original magnification ×400; inset of the panel C, original magnification ×800; panel E, original magnification ×800; and the inset of panel E, original magnification ×1200.

that the cleavage of PARP is involved in VA Qu FrF-induced HUVEC apoptosis.

Discussion

In the present study, we demonstrate that VA album preparations, VA Qu FrF, Iscador Qu Spez and, Iscador M Spez, induce death of immortalized EC in a dose- and time-dependent manner. However, EC are resistant to cytotoxic effects of another ML preparation, Iscador P. Using cytology, annexin V labeling, and Western blot analysis for PARP cleavage, we demonstrate that apoptosis is involved in VA Qu FrF-induced EC death.

A variation in the sensitivity to VA extract-induced apoptosis depending on the cell type investigated, and on the preparation used has been previously reported (10,14,17,18,20). We previously observed that B cell lines were refractory to VA Qu FrF-induced apoptosis (20). Iscador P is devoid of any cytotoxic properties toward B and T lymphoblastoid and monocytic cell lines (J.-P. Duong Van Huyen, unpublished data). Furthermore, it has recently been reported that VA extracts have proliferative properties on several tumor cell lines (30). These conflicting results lead us to investigate the interaction between EC and VA preparations of different origin in view of the crucial role of EC in vascular and cancer biology. Immortalized EC were sensitive to VA Qu FrF, Iscador Qu Spez, and M Spez, and fully resistant to the Iscador P preparation. The mechanisms underlying the variable sensitivity of a given preparation are not fully understood. Because VA preparations contain a standardized concentration of MLs with clearly demonstrated properties, these results suggest that effects of MLs may be modulated, either positively or negatively, by other components present in VA extracts. Thus, apart from the importance for understanding the molecular mechanisms of action of purified MLs, there is a need for an evaluation of the properties of the entire unfractionated VA extracts that are administered to cancer patients for nonspecific immunostimulation (31).

ML-induced apoptosis has been well demonstrated in various cell types (10,13,14,18,20). The present work demonstrates that apoptosis is involved in VA extract-induced EC death. The mechanisms of

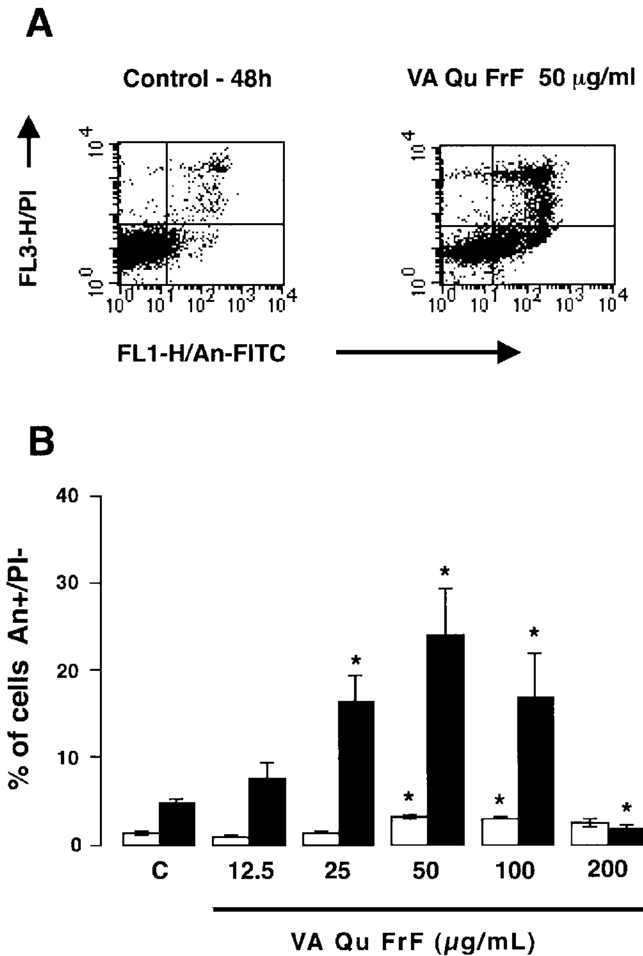

Fig. 4. VA Qu FrF induced-apoptosis of HUVEC as assessed by annexin V labeling. HUVEC were grown to confluency in M199 with 20% FCS. Cells were then incubated in low serum condition with increasing concentrations of VA Qu FrF for either 24 (open histograms) or 48 hr (black histograms). Cells were then trypsinized, rinsed twice in PBS, and labeled with PI and AnV for FACS analysis. Representative dot-plots of control **(left)** and 50 μg/ml **(right)** are presented in **(A)**. Histograms **(B)** represent the percentage of apoptotic cells (AnV^+/PI^-) ($N = 3$ experiments, compared to control. $*p < 0.05$).

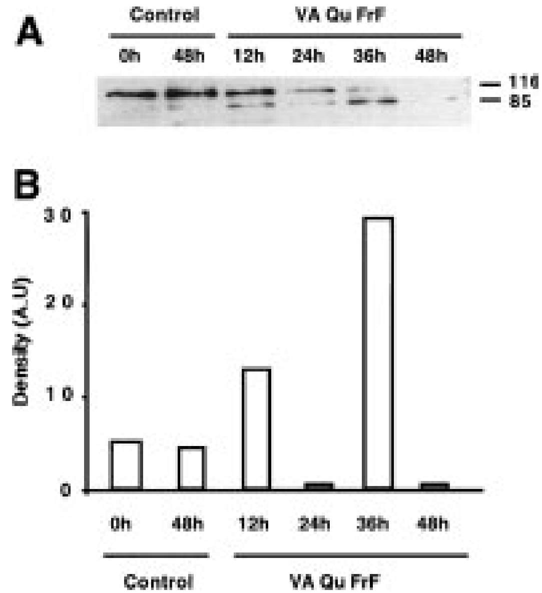

Fig. 5. VA Qu FrF induced-apoptosis of HUVEC involves cleavage of PARP. HUVEC were grown to confluency in M199 with 20% FCS. Cells were then incubated in low serum condition without or with VA Qu FrF (50 μg/ml) for 12, 24, 36, and 48 hr. PARP cleavage was assessed by Western blot **(A)** (non-cleaved PARP and cleaved PARP are 116 and 85 KDa, respectively). **(B)** Histograms of the intensity of cleavage of PARP deduced from densitometry scanning of the electrophoretic profiles.

apoptosis induced by RIP II is not well understood as yet. Apoptotic properties of both A and B ricin chains have been reported with contradictory results (11–15). Recent studies have clearly demonstrated that apoptosis mediated by ML-I requires lectin internalization and that was independent of a surface receptor-mediated pathway (12,32). These results suggest that the mechanisms of VA extract-induced apoptosis may depend on the cell type investigated. Concerning lymphoblastoid cells, using a Fas-resistant HuT78.B1 T cell line, we recently emphasized that the Fas pathway is not involved in ML-induced apoptosis (20) as previously reported by others. We have also shown that VA Qu FrF induced a dramatic decrease in the amount of anti-apoptotic proteins Bcl-2 and Bcl-X proteins in T lymphocytes (20), a finding consistent with the release of cytochrome C from mitochondria induced by ML I (10). The mechanisms of EC apoptosis induced by VA Qu FrF have not been previously evaluated. HUVEC apoptosis was associated with the cleavage of PARP that suggest that caspase activation is involved in this process as it has been recently reported in lymphoblastoid T-cell lines (10,16,32). Further experiments are underway to determine which caspase are crucial in VA extract-induced EC apoptosis.

In vitro VA extract-induced EC apoptosis raises the question of its pathophysiologic relevance and its clinical implications and benefits in regulating neoplasia and inflammation. In addition to its gatekeeping role between blood and tissue, the endothelium plays a pivotal role in several biological processes. EC actively participate in the regulation of blood flow and coagulation, in initiation and enhancement of inflammation, and in angiogenesis that is fundamental to reproduction, development, and repair (33). Tumoral angiogenesis is a critical step in the neoplasm progression (21). Neovascularization permits tumors to grow and metastasize. Clinical applications of research on angiogenesis have emerged for the use in diagnosis, prognosis, or therapy of neoplasia (24,34). Several studies have clearly demonstrated that EC apoptosis is implicated in the physiologic inhibition of angiogenesis (22). Anti-angiogenic peptides (angiostatin and endostatin) induce EC apoptosis (35,36). Mauceri et al. (37) demonstrated a dramatic EC cytotoxicity and

tumor regression with a combination of radiation therapy and angiostatin. The present work clearly demonstrates the induction of EC apoptosis by VA extracts may provide the basis for the use of VA preparations as anti-angiogenic compounds. This is in agreement with the work of Yoon et al. (38), who observed that VA *coloratum* extracts suppressed tumor growth in vivo and inhibited the number of blood vessels oriented toward the tumor mass. In addition, in vitro experiments showed that VA coloratum extracts inhibited the proliferation of rat EC (38).

Our results also raise the question of the safety of VA extracts. The treatment with high doses of ricin, another member of the RIP II family, is associated with vascular leak syndrome, thrombotic microangiopathy, increased EC monolayer permeability, and EC death (39–44). EC activation and apoptosis have been clearly implicated in the pathogenesis of thrombotic microangiopathy and hemolytic and uremic syndromes (45–47). Although these side effects have never been reported to our knowledge with VA extracts, the therapeutic safety of VA extracts should be further investigated.

In conclusion, the present study demonstrates that VA extracts induce EC apoptosis that may interfere with tumoral angiogenesis and thus may support some of the anti-tumoral properties of VA extracts. Further experiments that establish the significance of inhibition of EC proliferation by VA extract in therapeutics are currently being performed.

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References

1. Olsnes S, Stirpe F, Sandvig K, Pihl A. (1982) Isolation and characterization of viscumin, a toxic lectin from Viscum album L (mistletoe). *J. Biol. Chem.* **257**: 13263–13270.
2. Franz H, Ziska P, Kindt A. (1981) Isolation and properties of three lectins from mistletoe (Viscum album L.). *Biochem. J.* **195**: 481–484.
3. Barbieri L, Battelli MG, Stirpe F. (1993) Ribosome-inactivating proteins from plants. *Biochim. Biophys. Acta* **1154**: 237–282.
4. Eschenburg S, Krauspenhaar R, Mikhailov A, Stoeva S, Betzel C, Voelter W. (1998) Primary structure and molecular modeling of mistletoe lectin I from Viscum album. *Biochem. Biophys. Res. Commun.* **247**: 367–372.
5. Yoon TJ, Yoo YC, Choi OB, et al. (1995) Inhibitory effect of Korean mistletoe (Viscum album coloratum) extract on tumor angiogenesis and metastasis of haematogenous and non-haematogenous tumor cells in mice. *Cancer Lett.* **97**: 83–91.
6. Lenartz D, Andermahr J, Plum G, Menzel J, Beuth J. (1998) Efficiency of treatment with galactoside-specific lectin from mistletoe against rat glioma. *Anticancer Res.* **18**: 1011–1014.
7. Burger AM, Mengs U, Schuler JB, Fiebig HH. (2001) Anticancer activity of an aqueous mistletoe extract (AME) in syngeneic murine tumor models. *Anticancer Res.* **21**: 1965–1968.
8. Hajto T, Hostanska K, Frei K, Rordorf C, Gabius HJ. (1990) Increased secretion of tumor necrosis factors alpha, interleukin 1, and interleukin 6 by human mononuclear cells exposed to beta-galactoside-specific lectin from clinically applied mistletoe extract. *Cancer Res.* **50**: 3322–3326.
9. Kuttan G, Kuttan R. (1992) Immunological mechanism of action of the tumor reducing peptide from mistletoe extract (NSC 635089) cellular proliferation. *Cancer Lett.* **66**: 123–130.
10. Bantel H, Engels IH, Voelter W, Schulze-Osthoff K, Wesselborg S. (1999) Mistletoe lectin activates caspase-8/FLICE independently of death receptor signaling and enhances anticancer drug-induced apoptosis. *Cancer Res.* **59**: 2083–2090.
11. Bussing A, Suzart K, Bergmann J, Pfuller U, Schietzel M, Schweizer K. (1996) Induction of apoptosis in human lymphocytes treated with Viscum album L. is mediated by the mistletoe lectins. *Cancer Lett.* **99**: 59–72.
12. Bussing A, Suzart K, Schweizer K. (1997) Differences in the apoptosis-inducing properties of Viscum album L. extracts. *Anticancer Drugs* **8**(suppl 1): S9–S14.
13. Bussing A, Schietzel M. (1999) Apoptosis-inducing properties of Viscum album L. extracts from different host trees, correlate with their content of toxic mistletoe lectins. *Anticancer Res.* **19**: 23–28.
14. Hostanska K, Hajto T, Weber K, et al. (1996) A natural immunity-activating plant lectin, Viscum album agglutinin-I, induces apoptosis in human lymphocytes, monocytes, monocytic THP-1 cells and murine thymocytes. *Nat. Immun.* **15**: 295–311.
15. Langer M, Mockel B, Eck J, Zinke H, Lentzen H. (1999) Site-specific mutagenesis of mistletoe lectin: the role of RIP activity in apoptosis. *Biochem. Biophys. Res. Commun.* **264**: 944–948.
16. Lyu SY, Park WB, Choi KH, Kim WH. (2001) Involvement of caspase-3 in apoptosis induced by Viscum album var. coloratum agglutinin in HL-60 cells. *Biosci. Biotechnol. Biochem.* **65**: 534–541.
17. Ribereau-Gayon G, Jung ML, Frantz M, 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–S8.
18. Mockel B, Schwarz T, Zinke H, Eck J, Langer M, Lentzen H. (1997) Effects of mistletoe lectin I on human blood cell lines and peripheral blood cells. Cytotoxicity, apoptosis and induction of cytokines. *Arzneimittelforschung* **47**: 1145–1151.
19. Yoon TJ, Yoo YC, Kang TB, et al. (1999) Lectins isolated from Korean mistletoe (Viscum album coloratum) induce apoptosis in tumor cells. *Cancer Lett* **136**: 33–40.
20. Duong Van Huyen, J. P., Sooryanarayana, S. Delignat, M. F. Bloch, M. D. Kazatchkine, and S. V. Kaveri. 2001. Variable sensitivity of lymphoblastoid cells to apoptosis induced by Viscum album Qu FrF, a therapeutic preparation of mistletoe lectin. *Chemotherapy* **47**: 366–376.
21. Folkman J. (1995) Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat. Med.* **1**: 27–31.
22. Carmeliet P. (2000) Mechanisms of angiogenesis and arteriogenesis. *Nat. Med.* **6**: 389–395.
23. Keshet E, Ben-Sasson SA. (1999) Anticancer drug targets: approaching angiogenesis. *J. Clin. Invest.* **104**: 1497–1501.
24. Folkman J, Browder T, Palmblad J. (2001) Angiogenesis research: guidelines for translation to clinical application. *Thromb. Haemost.* **86**: 23–33.
25. Park WB, Lyu SY, Kim JH, et al. (2001) Inhibition of tumor growth and metastasis by Korean mistletoe lectin is associated with apoptosis and antiangiogenesis. *Cancer Biother. Radiopharm.* **16**: 439–447.
26. Xu C, Poirier B, Duong Van Huyen JP, et al. (1998) Modulation of endothelial cell function by normal polyspecific human intravenous immunoglobulins: a possible mechanism of action in vascular diseases. *Am. J. Pathol.* **153**: 1257–1266.
27. Vicart P, Testut P, Schwartz B, Llorens-Cortes C, Perdomo JJ, Paulin D. (1993) Cell adhesion markers are expressed by a

stable human endothelial cell line transformed by the SV40 large T antigen under vimentin promoter control. *J. Cell Physiol.* **157**: 41–51.
28. Hebert MJ, Gullans SR, Mackenzie HS, Brady HR. (1998) Apoptosis of endothelial cells is associated with paracrine induction of adhesion molecules: evidence for an interleukin-1beta-dependent paracrine loop. *Am. J. Pathol.* **152**: 523–532.
29. Prasad NK, Papoff G, Zeuner A, et al. (1998) Therapeutic preparations of normal polyspecific IgG (IVIg) induce apoptosis in human lymphocytes and monocytes: a novel mechanism of action of IVIg involving the Fas apoptotic pathway. *J. Immunol.* **161**: 3781–3790.
30. Gabius HJ, Darro F, Remmelink M, et al. (2001) Evidence for stimulation of tumor proliferation in cell lines and histotypic cultures by clinically relevant low doses of the galactoside-binding mistletoe lectin, a component of proprietary extracts. *Cancer Invest.* **19**: 114–126.
31. Stein GM, Schietzel M, Bussing A. (1998) Mistletoe in immunology and the clinic (short review). *Anticancer Res.* **18**: 3247–3249.
32. Lavastre V, Pelletier M, Saller R, Hostanska K, Girard D. (2002) Mechanisms involved in spontaneous and Viscum album agglutinin-I-induced human neutrophil apoptosis: Viscum album agglutinin-I accelerates the loss of antiapoptotic Mcl-1 expression and the degradation of cytoskeletal paxillin and vimentin proteins via caspases. *J. Immunol.* **168**: 1419–1427.
33. Cines DB, Pollak ES, Buck CA, et al. (1998) Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* **91**: 3527–3561.
34. Thompson WD, Li WW, Maragoudakis M. (2000) The clinical manipulation of angiogenesis: pathology, side-effects, surprises, and opportunities with novel human therapies. *J. Pathol.* **190**: 330–337.
35. Dhanabal M, Ramchandran R, Waterman MJ, et al. (1999) Endostatin induces endothelial cell apoptosis. *J. Biol. Chem.* **274**: 11721–11726.
36. Lucas R, Holmgren L, Garcia I, et al. (1998) Multiple forms of angiostatin induce apoptosis in endothelial cells. *Blood* **92**: 4730–4741.
37. Mauceri HJ, Hanna NN, Beckett MA, et al. (1998) Combined effects of angiostatin and ionizing radiation in antitumour therapy. *Nature* **394**: 287–291.
38. Yoon TJ, Yoo YC, Kang TB, et al. (1998) Prophylactic effect of Korean mistletoe (Viscum album coloratum) extract on tumor metastasis is mediated by enhancement of NK cell activity. *Int. J. Immunopharmacol.* **20**: 163–172.
39. Vitetta ES, Stone M, Amlot P, et al. (1991) Phase I immunotoxin trial in patients with B-cell lymphoma. *Cancer Res.* **51**: 4052–4058.
40. Schindler J, Sausville E, Messmann R, Uhr JW, Vitetta ES. (2001) The toxicity of deglycosylated ricin A chain-containing immunotoxins in patients with non-Hodgkin's lymphoma is exacerbated by prior radiotherapy: a retrospective analysis of patients in five clinical trials. *Clin. Cancer Res.* **7**: 255–258.
41. Taylor CM, Williams JM, Lote CJ, et al. (1999) A laboratory model of toxin-induced hemolytic uremic syndrome. *Kidney Int.* **55**: 1367–1374.
42. Soler-Rodriguez AM, Ghetie MA, Oppenheimer-Marks N, Uhr JW, Vitetta ES. (1993) Ricin A-chain and ricin A-chain immunotoxins rapidly damage human endothelial cells: implications for vascular leak syndrome. *Exp. Cell Res.* **206**: 227–234.
43. Hughes JN, Lindsay CD, Griffiths GD. (1996) Morphology of ricin and abrin exposed endothelial cells is consistent with apoptotic cell death. *Hum. Exp. Toxicol.* **15**: 443–451.
44. Lindstrom AL, Erlandsen SL, Kersey JH, Pennell CA. (1997). An in vitro model for toxin-mediated vascular leak syndrome: ricin toxin A chain increases the permeability of human endothelial cell monolayers. *Blood* **90**: 2323–2334.
45. Pijpers AH, van Setten PA, van den Heuvel LP, et al. (2001) Verocytotoxin-induced apoptosis of human microvascular endothelial cells. *J. Am. Soc. Nephrol.* **12**: 767–778.
46. Laurence J, Mitra D. (1997) Apoptosis of microvascular endothelial cells in the pathophysiology of thrombotic thrombocytopenic purpura/sporadic hemolytic uremic syndrome. *Semin. Hematol.* **34**: 98–105.
47. Bombeli T, Schwartz BR, Harlan JM. (1999) Endothelial cells undergoing apoptosis become proadhesive for nonactivated platelets. *Blood* **93**: 3831–3838.