International Journal of Cancer Research and Treatment

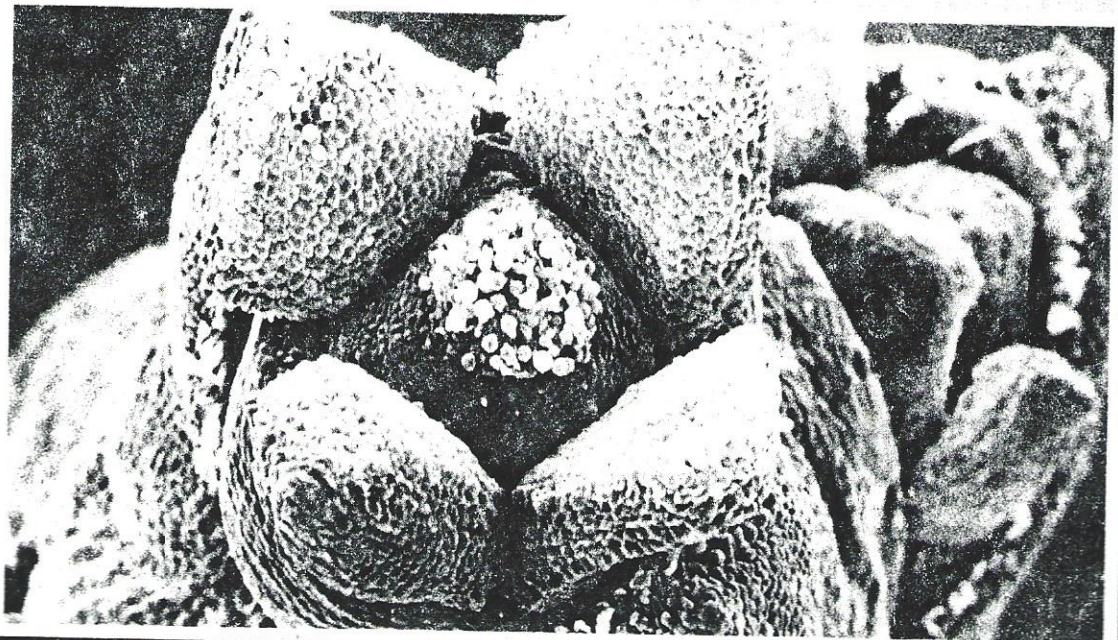
ONCOLOGY

43/S1/86
December 1986

Supplement
for subscribers
free of charge

Mistletoe

Top. Male flower. The male flower consists of four perianth members (in the figure the front segment is cut off to get a better insight).
Bottom. Female flowers. The female flower consists of four perianth members with a simple style in the center. Becker (p. 5). Photo: Ehrbar.



S. Karger
Medical and Scientific
Publishers
Basel · München · Paris
London · New York
New Delhi · Singapore
Tokyo · Sydney

KARGER

ONCOLOGY

Oncology
ISSN 0030-2414
ONCOBS
43(Suppl. 1) 1–70 (1986)

(2)

Mistletoe

Pharmacologically Relevant Components of *Viscum album* L.

For thousands of years, European mistletoe (*Viscum album* L.) has been used for a wide spectrum of therapeutic purposes. Currently, there is an increasing interest in its application to fight cancer. To balance the superstitious and mysticistic literature on mistletoe, this publication is based on the highly scientific results of up-to-date biochemical and pharmacological investigations. It characterizes the biologically active constituents of mistletoe: lectins and their A and B chains, carbohydrates, viscotoxins, and alkaloids. Problems of standardization are discussed as well as the interaction of a mistletoe preparation with tumor cells in vivo. Taken together, the nine papers collected in this special issue can scientifically confirm many assumptions of alternative medicine. They show that mistletoe contains a variety of pharmaceutically relevant components and that their cytotoxic and immunopotentiating activities are of special interest in tumor therapy. Thus this publication is apt to form the basis of a critical and constructive dialogue on mistletoe therapy between advocates of orthodox and alternative medicine.

Contents page V

KARGER

Biologic Properties of the Viscum Album Preparation Iscador®

II. Anti-neoplastic Properties*

*Razvan Rentea, M.D.***, *Robert Hunter, M.D.****, *Edward Lyons, M.D.*****

Abstract: We have investigated the tumor inhibiting properties of Iscador® and found that it can reduce by 72% the growth of a FANFT induced urinary bladder carcinoma which has a high antigenicity: reduces by 42% the growth of a S-108J sarcoma in mice (intermediate antigenicity) and does not affect overall in a statistically significant manner the development of a low antigenicity fibrosarcoma in mice. There is, however, even in this last mentioned system a higher clustering of tumors in the low weight range when the animals are pre-treated with Iscador prior to tumor inoculation.

Introduction

According to Belman(1), Steiner(23) was the first to draw attention to the tumor inhibiting effects of viscum album proteins. This claim has been repeatedly verified in both experimental and clinical studies in Europe made with Iscador, the commercial preparation of viscum album proteins available since the 1930's. Vester(27), working on in vitro models observed a cancerostatic effect of viscum proteins on Hela, Chang liver and S-180 cell cultures. Priemer(15) describes an inhibition effect on RNA synthesis in Yoshida ascites cells from 60 to 95% depending on the type of mistletoe extract used. There is general agreement in the various papers(2,3,10,11,25,28) that independent of the tumor system used the amount of growth inhibition compared to controls was about 50%.

In leukemias, an inhibition of only about 32% could be achieved(18,19). Pre-treatment with the drug lowers the number of metastases in mice(2,3). When the animals were allowed to go to terminal stages a statistically significant increase in survival time was seen in the treated group(2).

We have used in our studies three different tumors with high, low and respectively intermediate antigenicity, since our previous work(16) showed a strong effect of these proteins on the immune system of the animals and thus a correlation between tumor antigenicity and growth inhibition could be expected. This correlation would also, hopefully, give an additional insight into the tumor inhibiting mechanism of Iscador.

* Part I appeared in *Lab. Invest.* 44:43, 1981
** R. Rentea, Paulina Medical Clinic
*** R. Hunter, Emory University
**** E. Lyons, University of Chicago

Materials and Methods:

1. Animals

a) CD-1, outbred, albino female mice (Charles River Farms, Wilmington, Mass.) They weighed 18-20g, housed 6/cage throughout the experiment, fed ad libidum diet.

b) C-57, BJ mice; outbred females (Jackson, Michigan). They were one year old, 20-25g, also housed 6/cage.

c) Sprague-Dawley female rats (Madison, Wisconsin); aug. 70g at the start of the experiment. Housed 6/cage.

2. Iscador preparation used:

The material was received from Switzerland and handled as described previously(16). Previous experimental work(4,10) has shown that sarcomas respond better to Iscador Pini and carcinomas to Iscador Mali. Thus we used these two combinations throughout.

3. Toxicity and LD 50 were obtained as previously reported(16).

4. Tumor systems used:

a) An autochthonous fibrosarcoma well differentiated, developed in C-57-BJ mice, having a mean appearance rate of 10-15 days and having no known antigenic properties in the host in which it is grown, was used. Approx. 10^6 cells were transplanted subcutaneously into female mice by needle transplantation over the back. Animals were randomized into groups of 6 animals/group. The animals were sacrificed 24 days after tumor transplantation. One group received no drug. This group constituted the controls. The second group received Iscador Pini alternating one day subcutaneously around the tumor and one day intra-peritoneally and a third group was pretreated intra-peritoneally 3 days with Iscador Pini before the tumor bolus was injected. The control group received an equivalent volume of normal saline. Upon sacrificing, the tumors were weighed and analyzed morphologically.

b) A low passage sarcoma – 180 – Japanese line (obtained from Dr. G. Tarnowski, Sloan Kettering, Walker Labs, in Rye, N.Y.) was used. It was developed in the D.C. 1 outbred mice with a very low to no spontaneous regression rate. The mice were inoculated subcutaneously in the flank with a suspension of 10^7 viable tumor cells obtained from the ascites of tumor bearing animals. Mice were randomized into groups of 6 animals/group. Ten days later (respectively sixteen days later in the second group experiment) the animals were sacrificed by cervical dislocation and the tumor

carefully cut out, weighed and processed histologically. In these experiments the thymuses were also weighed and histological confirmation obtained that only thymus was included in the weighed specimen. With the exception of controls all animals were treated with Iscador Mali, 1 injection per day, i.p. The dosage as mentioned above was always a fraction of the LD50 for this particular mouse strain. Thus in experiment 2, (Table 1) group A was treated with 274 mg/kg/day which represents approx. 4% of the LD50. All animals were searched for ascites or evidence of metastases. Tissues processed for histology were fixed in formalin, imbedded in paraffin, and stained with hematoxilin and eosin (H&E). The same procedure applied for all other slides obtained, both in these and the following experiments.

c) As reported previously, Sprague Dawley rats were fed Purina Lab Chow mixed with FANFT: N-4-(5-nitro-2-furyl)-2-thiazolyl-formamide (Saber Labs, Morton Grove, Ill.) The carcinogen contributed 0.35% of the diet. The animals were given water and food ad lib. and they were in a 12 hours light-night cycle. The weights were recorded periodically. The length of FANFT ingestion is summarized in Graph 2. (See last page of this Journal for Graph 2 please.) All groups except F and G were 16 weeks on the carcinogen. Animals in the experimental groups were injected with Iscador Mali i.p. The dose of each group was determined again as a percentage of LD50 (group B: 4%, group C: 5%, group D: 10%, etc.) such that they all fell within the therapeutic dose range but at different ends of the spectrum. Actual mg/kg are summarized in Table 2. The control animals were injected with an equivalent amount of physiological saline solution. The injection site in the abdomen should vary slightly in order to avoid local reactions due to the viscum proteins. The animals were randomized into groups of 6 animals/group and 1 animal was picked at random from each group at 8 weeks for the blast transformation study of thymocytes (reported in Bibliography 16). The other animals were sacrificed at 16 weeks and checked for metastases. The thymuses were excised and weighed, the bladders were dissected out, freed from the adjoining structures (fat, connective tissue, etc.) and ligated at the ureteral neck. They were then inflated with about 0.4cc of formalin and placed in formalin. Two days later they were bisected and examined again. Each half was imbedded in paraffin in 3 sections, perpendicular to the plane of the largest diameter and were cut at equal intervals. The slides were stained with hematoxilin and eosin and examined for hyperplasia and other abnormalities. Hyperplastic areas were graded 1 through 4 as shown in Figure 1. The number of hyperplastic areas multiplied by the grade and divided by the total number of sections looked at in that particular group was designated as the hyperplastic index (H.I.) of that group.

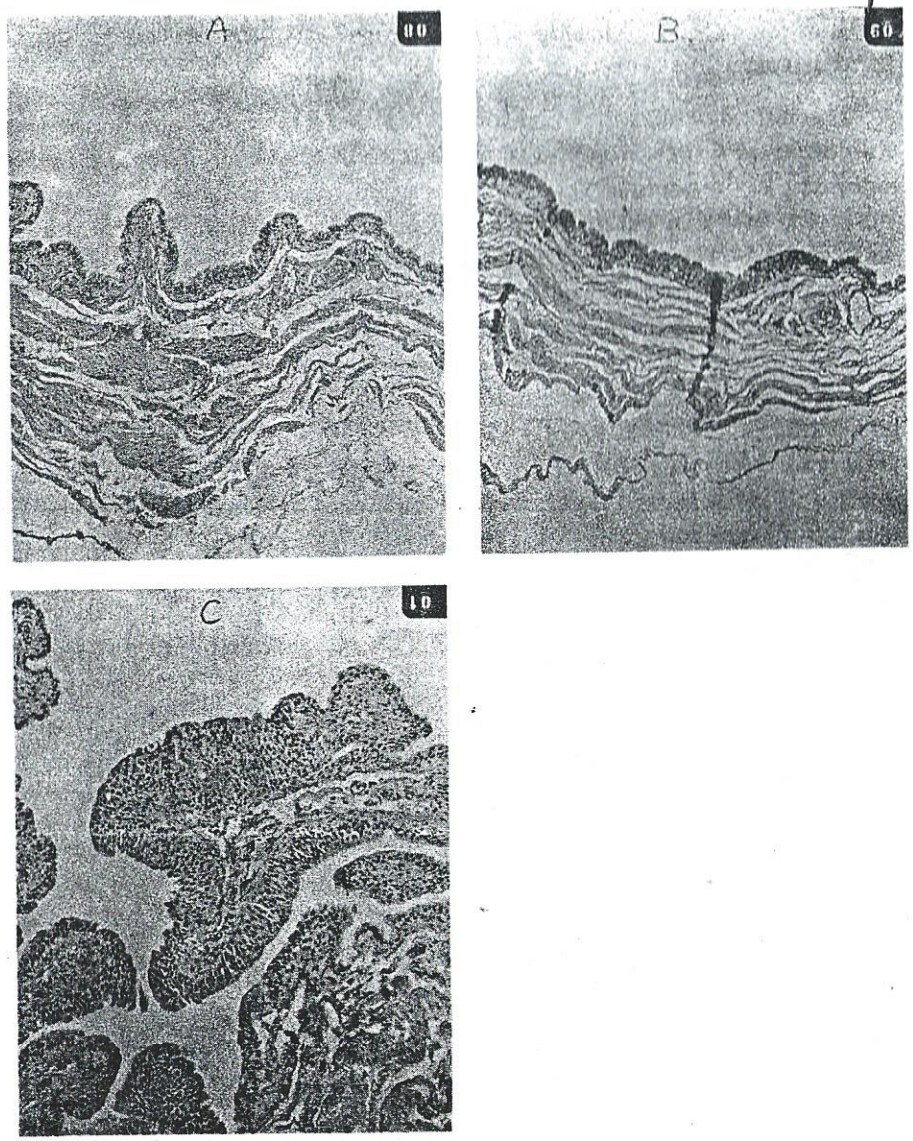


Fig. 1: Typical urinary bladder of rats that had been ingesting FANFT for 16 weeks. (A) Normal urinary bladder epithelium 2-3 cell layer, nuclei are not present close to the surface. (H&E, 100x). (B) +1 hyperplastic area: 3-5 cell layer, nuclei present all the way to the surface (H&E, 100x). Loss of polarity. (C) +4 nodular hyperplasia: loss of polarity, 30-50 cell layer (H&E, 100x).

Oncology 43: 93–97 (1986)

Natural Killer and Antibody-Dependent Cell-Mediated Cytotoxicity Activities and Large Granular Lymphocyte Frequencies in *Viscum album*-Treated Breast Cancer Patients

Tibor Hajto, Christine Lanzrein

Laboratory of Immunology, Lucas Clinic, Arlesheim, Switzerland

Key Words. Natural killer cells · Antibody-dependent cell-mediated cytotoxicity · Large granular lymphocytes · Breast cancer · *Viscum album*

Abstract. 20 breast cancer patients received a single intravenous infusion of Iscador, a mistletoe (*Viscum album* L.) extract. Natural killer (NK) and antibody-dependent cell-mediated cytotoxicity (ADCC) activities as well as the number of large granular lymphocytes (LGL) were investigated in their peripheral blood at various times. Six hours after the start of the infusion, significant decreases and 24 h later, significant increases in NK/ADCC activities and LGL frequencies were observed. These responses followed a kinetic pattern similar to that which has been described by others to occur after treatment with interferon.

Introduction

For more than 20 years Iscador (a *Viscum album* extract manufactured by Laboratorium Hiscia, Arlesheim, Switzerland; distributed by the Weleda Company in Arlesheim and its affiliates in various countries) has been used in the treatment of cancer. Considerable evidence has been accumulated from both *in vivo* and *in vitro* studies that Iscador has a selective growth inhibitory effect on a variety of tumor cells [19, 21–23, 26, 27, 29], but further investigations are required concerning its antitumor activity in humans. In addition, recent studies have indicated that Iscador may play a role in immunomodulation. *Bloksma* et al. [4] have shown that mistletoe extracts have an adjuvant effect on both the delayed-type hypersensitivity and antibody responses of mice to sheep red blood cells. *Rentea* et al. [25] have found that Iscador treatment of mice and rats resulted in an increase of lymphocytes in thymus cortex. Cortical thymocytes from Iscador-treated mice were 29 times more responsive to concanavalin A than those from untreated mice.

At present, a good cellular correlate of tumor immunity appears to be related to the natural killer (NK) activity and antibody-dependent cell-mediated cytotoxicity (ADCC) in humans [2, 8, 13, 14, 16, 24, 28]. It is generally accepted that the majority of NK cells are within the population of large granular lymphocytes (LGL) [15]. Consequently, our aim was to determine the activity and the frequency of these cell populations after a single intravenous infusion with Iscador in 20 breast cancer patients.

Materials and Methods

Natural and Antibody-Dependent Cytotoxicity Determination by the ^{51}Cr Release Assay

The procedure was slightly modified from that of *Kay and Horwitz* [18]. The mononuclear cells from venous blood were isolated by the Ficoll-Hypaque technique. K_{562} cells (a human myeloid cell line) were used as target cells for NK assay and Chang cells (a human liver cell line) for the ADCC assay. The targets were labelled with 75 µCi ^{51}Cr per 10^6 cells (sodium chromate; specific activity 250–500 Ci/g). The preparation of antibody directed against Chang cells was carried out by repeated injections of a rabbit with 10^6 Chang cells. 10^4 target cells were added to the effector cell suspensions in a total volume of 0.2 ml to yield a final effector to target cell ratio of 40:1, 20:1, 10:1 and 4:1. The maximum amount of ^{51}Cr released by the target cells was determined after treatment with Triton X-100 (0.5%). The assay mixtures were incubated at 37 °C for 4 h in a humidified CO_2 incubator. The supernatant fluid was measured in a gamma counter. The percent-

Oncology 43: suppl. 1, pp. 42–50 (1986)

0030–2414/86/0437–0042 $ 2.75/0

Recent Studies on the Anticancer Activities of Mistletoe (*Viscum album*) and Its Alkaloids[1]

Tasneem A. Khwaja, Cecilia B. Dias, Stephanie Pentecost

Department of Pathology, University of Southern California School of Medicine and Comprehensive Cancer Center, Los Angeles, Calif., USA

Key Words. Anticancer drug evaluation · *Viscum album* · ISCADOR · Alkaloids · Host-parasite relationship

Abstract. Detailed methods for in vitro/in vivo evaluation of anticancer drugs, with special reference to mistletoe extracts, have been reviewed. Mistletoe extracts have been shown to possess significant antitumor activity, in vivo, against murine tumors, Lewis lung carcinoma, colon adenocarcinoma 38 and C3H mammary adenocarcinoma 16/C. Methods for the extraction of biologically active alkaloids from mistletoe and their anticancer activities are presented. The possible origin of alkaloids in mistletoe plants, and their contributions towards a mechanism of anticancer activities of mistletoe axtracts, are proposed.

Introduction

Mistletoe (*Viscum album*) is a common semiparasitic plant which grows on deciduous trees all over the world. Since the early twenties, an aqueous extract of European mistletoe (*V. album*, L.) under the trade name of Iscador has been used for the treatment of human neoplasia [1, 2]. Extracts from the mistletoe plant growing on different trees like apple, pine, oak, etc., have been suggested for the treatment of different types of malignancies. Most of the earlier clinical studies were not very well documented and due to the lack of an acceptable scientific basis of carefully controlled clinical trials, benefits of Iscador use have been controversial [3, 4]. Recently, a number of controlled clinical trials have been started and there are suggestions that Iscador may be beneficial in the postoperative treatment of lung, breast, colon, and cervical carcinomas [2, 5–7]. The anticancer activities of mistletoe have been ascribed to a combination of cytotoxic [8–18] and immunological effects [18–21], and mistletoe treatments, unlike cytotoxic drugs, are not immunosuppressive [2]. In this paper we would like to review methods used for in vitro and in vivo evaluation of anticancer agents with special reference to work on mistletoe extracts and its alkaloidal components. The possible contributions of mistletoe alkaloids towards a mechanism of the anticancer activity of the extract will be discussed.

Methods

In vitro Evaluation

The most economical way to evaluate the anticancer effects of an agent is to study its effects on the growth of tumor cells in a minimum essential medium (MEM) containing 10% fetal calf serum. The drug-exposed cells (in duplicates) are incubated in a humidified CO_2 incubator at 37 °C for 2–4 days, depending upon the population-doubling time of the tumor cells. At the end of the incubation period the cells are counted and the degree of cell growth inhibition is calculated from a comparison with untreated controlled cells grown under identical conditions. The type of cell lines used have varied from laboratory to laboratory depending upon individual needs. The National Cancer Institute (NCI) in the United States recommends the use of KB cells (a human nasopharyngeal carcinoma) for the evaluation of anticancer drugs in vitro [22]. The cell growth inhibition is determined by estimating the protein contents (Lowry's method) of the drug-treated and untreated controls. NCI has also recommended the use of suspension culture of mouse

[1] Work supported in part by a grant from the Jerome Foundation.

Oncology 43: suppl. 1, pp. 51–65 (1986)

0030-2414/86/0437-0051 $ 2.75/0

Immunomodulatory Effects of Iscador: A *Viscum album* Preparation

Tibor Hajto

Laboratory of Immunology, Lucas Clinic, Arlesheim, Switzerland

Key Words. *Viscum album* · Breast cancer · Mitogen response · Phytohemagglutinin · Concanavalin A · Granulocytes · Phagocytic activity · Natural killer cells · Antibody-dependent cell-mediated cytotoxicity · Large granular lymphocytes · α-Interferon

Abstract. The immunomodulatory effects of Iscador (a mistletoe, *Viscum album* extract) were investigated. After a single intravenous infusion of Iscador several immunological parameters in the peripheral blood of breast cancer patients were examined. Parallel with neutrophilia, and with an increase of juvenile neutrophils, a significant enhancement of phagocytic activity of granulocytes was shown. After short decreases during the first 24 h, significant increases in natural killer (NK) and antibody-dependent cell-mediated cytotoxicity (ADCC) activities as well as augmented levels of large granular lymphocytes were observed. These NK/ADCC responses followed a kinetic pattern similar to that after treatment with α-interferon described by others. Further significant increases in mitogenic responses to phytohemagglutinin and concanavalin A were observed.

Introduction

In this clinic cancer patients have been treated with Iscador for more than 20 years. Parallel with its growth inhibitory effect on a variety of tumor cells [1–9] much evidence has accumulated to suggest that Iscador may play a role in immunomodulation.

More than 60 years ago, it was considered possible [10] that Iscador had an immunotherapeutic importance for cancer, but the first significant impulse for the recent immunological research was given 15 years ago by Nienhaus and Leroi [12], and Nienhaus et al. [11]. They demonstrated that repeated intraperitoneal or intravenous injections of Iscador in mice induced an enlargement of the thymus. Later, Rentea et al. [13] confirmed and extended these results. They demonstrated in CD-1 mice that the thymic trophic influence was maintained over a long period of time following repeated intraperitoneal injections of Iscador and that this resulted from an increased proliferation of cortical thymocytes. The quality of this proliferative activity differed from the reactions observed in autoimmune disease and following direct antigen injection. Thymocytes of Iscador-treated animals were significantly more responsive to concanavalin A (Con A) than those of untreated controls. Bloksma et al. [14, 15] and Kwaja et al. [16] have found that *Viscum album* preparations may be a good adjuvant for delayed hypersensitivity response to sheep red blood cells (SRBC) in mice. Rentea et al. [13] and Bloksma et al. [14, 15] have demonstrated that Iscador augmented the amount of antibody produced by animals injected with SRBC. Bloksma et al. [15] have investigated the effect of Iscador on nonspecific inflammation with the foot pad swelling test in mice. Iscador induced a dose-dependent swelling. Zschiesche [17] and Bloksma et al. [15] have studied, with colloidal carbon clearance technique, the effect of Iscador on phagocytic activity in mice. After intravenous or intraperitoneal injection of *Viscum album* preparations a significant enhancement was observed relative to the phagocytosis in control animals. Spreafico [Istituto di Ricerche Farmacologiche 'Mario Negri', Milano, unpubl. obs.] investigated the nonspecific cytotoxicity of peritoneal

Oncology 43: suppl. 1, pp. 66–70 (1986)

0030–2414/86/0437–0066 $ 2.75/0

Pleura Carcinosis

Cytomorphological Findings with the Mistletoe Preparation Iscador and Other Pharmaceuticals

(With 1 color plate)

Georg Salzer

Ludwig Boltzmann Institute for Clinical Oncology (Directors: Prof. Dr. H. Denck and Prof. Dr. G. Alth), Vienna–Lainz, Austria

Key Words. Iscador · Pleura carcinosis · Reaction · Cytomorphology

Abstract. Over 160 cases of pleura carcinosis were treated systematically by local intrapleural instillation of Iscador, and the results were documented by cytomorphological examination of the punctate. The results show that, under these conditions, Iscador has two distinct activities, i.e. cytotoxic activity and immunostimulating activity.

Introduction

It may seem surprising that a clinical study should appear in the edition of a journal dedicated to fundamental research on the constituents of mistletoe. It is, however, my belief that the simple cytomorphological observations in vivo, described in this work, can provide a foundation for the results obtained in vitro.

In recent decades a most diverse range of medicaments has been applied in the local treatment of pleura carcinosis [2], which is one of the most common terminal complications of many malignomas, especially mammary carcinoma. At the Ludwig Boltzmann Institute for Clinical Oncology in the hospital at Vienna-Lainz, in collaboration with head physician Dr. D. Böck of the Pathological-Bacteriological Institute at the Lainz hospital, the mistletoe preparation, Iscador (Weleda AG, Arlesheim, Switzerland), has been used since 1976 for intrapleural treatment. The following is an account of cytomorphological observations from 168 cases that have so far been treated and precisely documented.

Technique

After aspiration of the effusion, with addition of 1 ml sodium citrate to prevent coagulation, and submission of a sample for cytological examination, 10 ml of the remaining discharge was mixed with 1 ml of 5% Iscador, then instilled into the pleural cavity. Punctures were repeated at 1-week intervals until discharge was absent, and samples were taken at every puncture for cytological examination. Dessication was complete after 1–9 (on average 3) punctures, followed by instillation of Iscador [1, 2].

Preparation of Material for Cytological Examination

The sample of exudate (10–15 ml) was centrifuged at 3000 rpm for about 3 min. Using a loop, two smears were prepared from the sediment, dried in the air, then stained by the May-Grünwald-Giemsa procedure.

General Cytological Observations

In most cases, the 1st untreated punctate contained abundant tumor cells, a normal number of lymphocytes, and no eosinophilic granulocytes [3, 4]. In addition, as in all pleura exudates, a varying number of pleura epithelial cells were found. In subsequent, treated punctates, the number of tumor cells decreased markedly, or tumor cells were totally absent. At the same time, degenerate tumor cells and detritic cell ghosts were observed. This was often accompanied by a strong increase in the number of lymphocytes and eosinophilic granulocytes. A rosette arrangement of lymphocytes around tumor cells is not uncommon, with lymphocytes occasionally penetrating the associations of tumor cells.

The cytological observations were evaluated by using the grading scheme of Papanicolaou (O–V). Cell counting, as performed in hematology, is not possible with smear preparations. An in-house

Table I. Evaluation of the cell content of punctates

Superabundant	5
Abundant	4
Moderately abundant	3
Sparsely distributed	2
Sporadic	1
Absent	0

Oncology 43: suppl. 1, pp. 23–34 (1986)

0030–2414/86/0437–0023 $ 2.75 0

Mistletoe Lectins and Their A and B Chains

Hartmut Franz

Staatliches Institut für Immunpräparate und Nährmedien, Berlin, DDR

Key Words. *Viscum album* · Lectins · Biological activity · Protein chains · Glycoconjugates

Abstract. Mistletoe lectins are of high biological activity. The mistletoe lectin I (ML I) is a naturally occurring conjugate of an enzyme (A chain) and a lectin (B chain). Its cytotoxicity is caused by inhibiting the protein synthesis on the ribosomal level. Prominent properties of the A chain are mitogenicity and inhibition of the protein synthesis in cell-free systems. The A chain is also a candidate for the construction of immunotoxins. The B chain as well as the intact lectins activate macrophages and release lymphokines from lymphocytes. They both inhibit the allergen-induced histamine release from leukocytes and the collagen-induced serotonin release from platelets. It cannot be excluded that the combination of selectively cytotoxic and immunopotentiating properties of mistletoe lectins and their chains are decisive for the therapeutic effects of mistletoe preparations.

Introduction

Lectins are generally accepted to be proteins or glycoproteins with specific binding sites for sugars which are not antibodies or enzymes [19, 25]. The name 'lectin' is derived from Latin (legere = to choose, to pick out). Lectins occur in a variety of viruses, bacteria, fungi, plants, animals and in man. During the last 20 years lectins have been of increasing interest but their biological functions are only partially known. Many lectins are able to agglutinate cells. They are not necessarily the product of cells belonging to the immune system of vertebrates. Therefore they must be strictly distinguished from antibodies. On the other hand it cannot be excluded that there exists a developmental connection between lectins, enzymes and antibodies [9, 18]. A few lectins (the so-called toxic lectins) consist of A and B chains and belong to the most toxic glycoproteins. Interestingly the first lectin prepared by Stillmark in 1888 was the highly toxic ricin, which is very similar to the lectins from mistletoe. Since ricin a great number of plant lectins have been detected mainly by testing of their hemagglutinating activity. Thus Krüpe [26] and Bird [2] found that extracts of mistletoe (*Viscum album*) agglutinate erythrocytes. Pardoe et al. [39] found a hemagglutinating, galactose-specific lectin in the pseudoberries of *V. album*, which also reacts with (agglutinates) tumor cells (Burkitt-EB 2 lymphoma). The results of the further investigations of the mistletoe lectins are reviewed in this paper.

Preparation and Characterization of Mistletoe Lectins

Nomenclature

The most common method for the isolation of lectins is affinity chromatography, which means the binding of the lectin from a crude aqueous extract to a solid phase bearing the corresponding sugar (glycoconjugate), and in a second step the elution of the lectin under nondenaturating conditions whether by use of suitable sugars or by more or less acid buffer solutions. In order to enrich mistletoe lectin(s), affinity chromatography was firstly used by Luther et al. [30], who took human B erythrocytes as solid phase. The lectin was liberated by heating the agglutinate (56 °C, 15 min). Lutsik [33] utilized agar gel. We used insolubilized human immunoglobulin G [10, 11] for the same purpose (immobilized by heat denaturation or by crosslinking with glutaraldehyde or by binding to

Oncology 43: suppl. 1, pp. 8–15 (1986)

0030–2414/86/0437–0008 $ 2.75/0

Structure and Properties of Polysaccharides from *Viscum album* (L.)

E. Jordan, H. Wagner

Institut für Pharmazeutische Biologie der Universität München, BRD

Key Words. *Viscum album* · Mistletoe · Polysaccharides · Arabinogalactan · Galacturonan · Immunological activity

Abstract. Polysaccharides are possibly involved in the pharmacological effects of *Viscum album* (mistletoe) extracts, which are used in cancer therapy. Therefore the water-soluble polysaccharides of the fresh plant and the fermented proprietary preparation Iscador® were isolated and characterized inter alia by methylation analysis, partial hydrolysis and C-13-NMR spectroscopy. The main polysaccharide of the green parts of *Viscum* is a highly esterified galacturonan whereas in *Viscum* 'berries' a complex arabinogalactan is predominant. Both types of these constituents were found in Iscador but with definite changes in molecular weight and structure. An interaction between the arabinogalactan and the galactose-specific lectin (ML I) in *Viscum* could be demonstrated. In three immunological tests (granulocyte, chemiluminescence, carbon clearance test) the polysaccharides failed to increase phagocytic activity of granulocytes and macrophages.

Introduction

Amongst the high molecular weight compounds that occur in mistletoe, the polysaccharides must be considered as possible active principles, since it is known that a number of polysaccharides can unspecifically affect the immune defence system. Polysaccharides with immunostimulant activity are found in fungi, algae and lichens, and in various higher plants (Eupatorium, Echinacea, Acanthopanax, Chamomilla, Sabal and others) [for review, see ref. 1, 17]. Fungal polysaccharides of the lentinan or schizophyllan type display immune-induced tumor toxicity, which is why these preparations are used for adjuvant tumor therapy in Japan. Structural analysis and investigation of the immunological properties of mistletoe polysaccharides can therefore contribute to an understanding of the determinants of action and activity in cancer therapy.

Existing Studies

As early as 1961, a polysaccharide was isolated from the 'berries' of *Viscum album* (L.) which inhibited tumor growth when injected intraperitoneally into rodents [2]. In a clinical experiment, this high molecular weight substance was shown to be suitable for the treatment of neutropenias, which had arisen from leukemia itself, or had resulted from the extensive chemotherapy of malignant diseases [3]. Bloksma et al. [4] investigated a polysaccharide fraction from *V. album*, in comparison with the expressed sap and the proprietary pharmaceutical Iscador for its ability to stimulate humoral and cellular immunity. Under suitable experimental conditions the polysaccharide fraction was shown to possess adjuvant properties, as shown by a delayed hypersensitivity test. At very high doses of 120 mg/kg, it was also active in the carbon clearance test.

Apart from some investigations by Mangenot et al. [5], Müller [2] and Krzaczek [6] on the sugar composition of these polysaccharides, no work on their structural elucidation has been published.

New Investigations

We began our investigation with the isolation of polysaccharides from stems and leaves of mistletoe, which are by far the most predominant component of

Oncology 43: suppl. 1, pp. 35–41 (1986)

0030-2414/86/0437-0035 $ 2.75/0

Comparison of the Effects of Fermented and Unfermented Mistletoe Preparations on Cultured Tumor Cells

Gilles Ribéreau-Gayon, Marie-Louise Jung, Dominique Di Scala, Jean-Paul Beck

Institut de Physiologie et de Chimie biologique, Université Louis Pasteur, Strasbourg, France

Key Words. Mistletoe preparations · Mistletoe lectins · Hepatoma tissue culture · Human leukemia cells

Abstract. The bacterially fermented mistletoe preparation Iscador, used in cancer therapy for 30 years, and the recently prepared unfermented preparation, have been tested on rat hepatoma tissue culture (HTC) cells and human leukemia Molt 4 cells. As observed by phase-contrast microscopy, treatment of HTC cells with fermented or unfermented Iscador, at a concentration corresponding to 1 mg of fresh plant per milliliter culture, led to rapid lysis of cellular membranes. At a lower concentration, 0.1 mg/ml, unfermented Iscador led to the formation of polynucleated cells. On Molt 4 cells, fermented Iscador also produced cytolysis but after a longer time of action. Unfermented Iscador showed a much stronger cytotoxic effect on these cells than on HTC cells. Fermented Iscador was slightly more potent than unfermented Iscador in inhibiting the growth of HTC cells, but on Molt 4 cells fermented Iscador was less active than unfermented Iscador. DNA synthesis, measured by [^{3}H]thymidine incorporation in HTC and Molt 4 cells, was inhibited by fermented and unfermented Iscador with the same type of differences of action as on cell growth. Fermented Iscador contained a low amount of lectins, approximately 100 ng/ml, while unfermented Iscador contained about 10 times more. A purified mistletoe lectin produced effects on HTC and Molt 4 cells similar to those of unfermented preparations. HTC cells were 100 times less sensitive to this lectin than Molt 4 cells. These results are discussed in relation to the known biological effects of lectins.

Introduction

The fermented mistletoe preparation Iscador inhibits the development of experimental tumor cells (Ehrlich ascites carcinoma, Sarcoma 180 and Lewis lung carcinoma), so that the survival time of treated animals is equal or higher than that of mice treated with 5-fluorouracil, a well-known antitumoral agent [1]. Moreover, fermented Iscador stimulates humoral and cellular immunity in mice [2, 3]. A marked increase in the weight of the thymus, corresponding to a higher proliferation rate of cortical thymocytes, is also observable and this effect is reversible [4, 5]. In cancer patients, fermented Iscador stimulates the activity of natural killer cells [6].

Recently, unfermented mistletoe preparations became available with the technical possibilities of sterile filtration. No biochemical study of the transformation of the mistletoe substances during the bacterial fermentation has yet been made. It is known that the cytotoxic lectins are rapidly degraded [7], but the metabolic products have not been identified. In a first attempt to establish the characteristics of fermented and unfermented preparations, we compared their effects on two tumor cell lines in culture, rat hepatoma tissue culture (HTC) and Molt 4 cells.

Materials and Methods

Iscador Preparations and Mistletoe Lectins

The fermented and unfermented mistletoe preparations Iscador were obtained from the Hiscia Institute, Arlesheim, Switzerland. The term fermented Iscador refers to the fermented preparation obtained from oak mistletoe (Iscador Quercus). The unfermented preparation was also obtained from oak mistletoe. In brief, fermented Iscador was prepared by grinding mistletoe, followed by an addition of water (1 ml/g of mistletoe) and a suspension of *Lacto-*

Int. J. Immunopharmac., Vol. 9, No. 2, pp. 199–209, 1987.
Printed in Great Britain.

0192–0561/87 $3.00+.00
International Society for Immunopharmacology.

MEDIATION OF HUMAN NK-ACTIVITY BY COMPONENTS IN EXTRACTS OF *VISCUM ALBUM*

K. HAMPRECHT, R. HANDGRETINGER, W. VOETSCH and F. A. ANDERER

Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Tübingen, F.R.G.

(*Received* 28 *February* 1986 *and in final form* 22 *September* 1986)

Abstract — *Viscum album* extracts (Iscador®) were investigated for their potency to influence NK cytotoxicity *in vitro*. *In vitro* short term cytotoxicity assays (4 h) with human peripheral mononuclear cells (PMNC) and human K 562 tumor cells showed a drastic enhancement of NK cytotoxicity in the presence of *V. album* extracts. The presence of the *V. album* components during tumor cell lysis was essential since pre-incubation of PMNC with *V. album* extract followed by thorough washing did not lead to enhancement of NK cytotoxicity. One responding effector cell was identified as a member of the large granular lymphocyte (LGL) family carrying both Leu 7 and Leu 11 surface markers. Furthermore, monocytes depleted of LGL, but not differentiated macrophages, showed a weak enhancement of their cytolytic activity in the presence of *V. album* extract. Fractionation of *V. album* extracts revealed two active fractions one (C1) with about 3–4000 D and the other (C2) < 1000 D. Both components enhanced NK cytotoxicity of LGL (Leu 7$^+$, Leu 11$^+$) as well as of monocytes showing enhancing effects also against moderately NK-sensitive tumor cell lines.

The ability to mediate spontaneous cytotoxicity against a wide variety of tumor cells is a function predominantly expressed by natural killer (NK) cells and monocytes and is found in a wide range of mammalian species. NK cells have been identified as a cell type with large granular lymphocyte (LGL) morphology (Timonen, Saksela, Ranki & Häyry, 1979). The ability to lyse tumor cells spontaneously might play an important role in immune surveillance and offers a potent alternative in immunological protection in all those instances in which T cells do not appear to play an effector role. The major physiological response modifiers of the spontaneous cytotoxicity of NK cells as well as of monocytes, already characterized, are interferons and other lymphokines (Einhorn, Blomgren & Strander, 1978; Trinchieri & Santolini, 1978; Herberman, Ortaldo & Bonnard, 1979; Adams & Hamilton, 1984; Kimber & Moore, 1984; Koff, Fogler, Gutterman & Fidler, 1985). In situations of pathological imbalance of the regulation of spontaneous cytotoxicity other biological response modifiers, i.e. of plant origin, might be of interest. The most promising sources available to screen for such activities are plant extracts with some relevant clinical history.

We investigated preparations of *Viscum album* (mistletoe) which are occasionally used in human adjuvant cancer therapy in Europe (Leroi, 1977; Salzer, 1978; Koch & Voss, 1980; Salzer, 1981). *V. album* extracts were reported to contain some tumor-inhibitory activity (Selawry, Schwartz & Haar, 1959). We now demonstrate that *V. album* extracts strongly increase the cytolytic activity of a LGL subpopulation and to a lower degree cells of a monocyte population when tested in cytotoxicity assays against human tumor cell lines. Furthermore, we identified two components in *V. album* extracts, one with a molecular weight of about 3–4000 D and the other smaller than 1000 D which strongly mediated the spontaneous cytotoxicity of human NK cells against different target cell lines.

EXPERIMENTAL PROCEDURES

Materials

V. album *preparations*. Commercially available preparations (batch 5021 C; Weleda, Schwaebisch Gmuend, FRG) with the trade name Iscador (*Viscum album mali*; 5% solution based on the weight of the fresh plant) were used in all experiments. Iscador is a