Available online at www.sciencedirect.com
ScienceDirect
Cancer Letters 264 (2008) 218–228
CANCER Letters
www.elsevier.com/locate/canlet

Molecular mechanisms of mistletoe plant extract-induced apoptosis in acute lymphoblastic leukemia in vivo and in vitro

Georg Seifert [a,*], Patrick Jesse [a], Alfred Laengler [c], Tobias Reindl [a], Maria Lüth [a], Stephan Lobitz [a], Günter Henze [a], Aram Prokop [a,1], Holger N. Lode [b,1]

[a] *Department of Pediatric Oncology/Hematology, Otto-Heubner-Center for Pediatric and Adolescent Medicine (OHC), Charité, Universitätsmedizin Berlin, Germany*
[b] *Department of Pediatrics and BMT, Experimental Oncology, Otto-Heubner-Center for Pediatric and Adolescent Medicine (OHC), Charité, Universitätsmedizin Berlin, Germany*
[c] *Department of Paediatrics, Gemeinschaftskrankenhaus Herdecke, Germany*

Received 12 November 2007; received in revised form 16 January 2008; accepted 18 January 2008

Abstract

Viscum album (Mistletoe) is one of the most widely used alternative cancer therapies. Aqueous mistletoe extracts (MT) contain the three mistletoe lectins I, II and III as one predominant group of biologically active agents. Although MT is widely used, there is a lack of scientifically sound preclinical and clinical data. In this paper, we describe for the first time the in vivo efficacy and mechanism of action of MT in lymphoblastic leukemia. For this purpose, we first investigated both the cytotoxic effect and the mechanism of action of two standardized aqueous MTs (MT obtained from fir trees (MT-A); MT obtained from pine trees (MT-P)) in a human acute lymphoblastic leukemia (ALL) cell line (NALM-6). MT-A, MT-P and ML-I inhibited cell proliferation as determined by Casy® Count analysis at very low concentrations with MT-P being the most cytotoxic extract. DNA-fragmentation assays indicated that dose-dependent induction of apoptosis was the main mechanism of cell death. Finally, we evaluated the efficacy of MT-A and MT-P in an in vivo SCID-model of pre-B ALL (NALM-6). Both MTs significantly improved survival (up to 55.4 days) at all tested concentrations in contrast to controls (34.6 days) without side effects.

Keywords: Mistletoe; ALL; Leukemia; Apoptosis; SCID; NALM-6; Helixor; Alternative; Phytotherapy

1. Introduction

Cytotoxic substances derived from plants (e.g. vinca alkaloids or paclitaxel (Taxol)) are often used in oncology as highly potent drugs and/or serve as model for synthetic compounds [1]. Extracts from *Viscum album* I (MT) belong to the most frequently used complementary cancer treatments in Europe and have been used for more than 80 years on a

* Corresponding author. Address: Charité – Universitätsmedizin Berlin, Otto-Heubner Centrum für Kinder – und Jugendmedizin, Klinik für Pädiatrie mit Schwerpunkt Onkologie/Hämatologie, Augustenburger Platz 1, 13353 Berlin, Germany. Tel.: +49 30 450 666087; fax: +49 30 450 566946.
E-mail address: georg.seifert@charite.de (G. Seifert).
[1] These authors contributed equally to this work.

0304-3835/$ - see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2008.01.036

more or less empirical basis. Indications for the use of MT are the reduction of treatment-associated side effects during chemotherapy and as adjuvant therapy [2]. However, no in vivo data or data from controlled studies on the use of MT in leukemia have been published to date. One reason for the use of whole MT is an assumed synergistic therapeutic effect of the different components contained in the extract. This hypothesis is so far only partly substantiated [3]. However, analysis of the extracts reveals a number of pharmacologically interesting components which are known to possess synergistic effects – both with each other and with conventional antineoplastic drugs [3–5].

The best investigated components of aqueous mistletoe extracts are the mistletoe lectins I, II and III. However, most of the published studies have been with ML-I. The cytotoxic effect produced by ML-I is brought about by its receptor-mediated uptake into the target cell and subsequent cleavage into A and B chains. Inactivation of the 60S ribosomal subunit by the *N*-glycosylated ML-I-A chain then leads to inhibition of ribosomal protein synthesis. The ML-I-B chain is responsible for the receptor-mediated uptake. The apoptosis inducing action of mistletoe lectin I as type II ribosome inactivating protein results in the inhibition of cytosolic protein biosynthesis with subsequent activation of an only partially identified mitochondrial apoptosis pathway involving receptor independent activation of effector caspases [6–13]. The precise mechanisms by which mistletoe extracts induce apoptosis are however still only rudimentarily understood. The three known MT lectins differ in their monosaccharide specificity as well as in their molecular weight. The A chain of the mistletoe lectins is largely conserved in all three isoforms [14] while the B chain is lectin-specific [15]. In spite of the close kinship of the lectins there are marked differences in their cytotoxic activity. In an assay with the human leukemia cell line Molt-4, for example, the cytotoxicity of ML-III was found to be 10 times greater than that of ML-I [16,17]. Marked differences in the systemic stimulation of cytokine release have also been shown. In addition to ML-I, II and III the clinically used preparations contain numerous other biologically active substances such as viscotoxins, polysaccharides, triterpenes, lipids, amines, phytosterols, flavonoids, phenylpropanes and several enzymes. Various authors have reported a growth-inhibiting action of MT and isolated ML-I both in vitro and in vivo in individual tumors. However, some of the results are still contradictory [18–21].

There are no in vivo data to date on the experimental significance of MT in acute leukemia. The aim of our investigations was to examine the therapeutic efficacy of mistletoe extracts in ALL and identify the relevant mechanisms of action. Since the preparations used in clinical practice are usually whole extracts of mistletoe, and since we still know too little about the main active components and the differences between the components depending on the host tree, we decided to use one mistletoe lectin rich preparation (MT-P (HELIXOR®-P)) and one mistletoe lectin poor preparation (MT-A (HELIXOR®-A)) for our investigations.

2. Materials and methods

2.1. NALM-6 cells

Human ALL cell lines NALM-6 were obtained from the DSMZ (Bonn, Germany) and was maintained by serial passages in RPMI-1640 medium (GIBCO Laboratories, Grand Island, NY, USA) containing 25 mM Hepes buffer, 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin and 100 M glutamine. The cells were grown in a humidified atmosphere of 5% CO_2 and air and expanded every other day. Prior to injection into SCID mice, cells were washed once and then resuspended in filter-sterilized phosphate-buffered saline (PBS). Mice received 10^6 cells via the dorsal tail vein.

2.2. Transplantation of ALL (NALM-6) into SCID mice (CB-17 SCID/SCID)

Ten-week-old male SCID mice (CB-17 SCID/SCID) weighing 20 g were obtained from Charles River WIGA (Sulzfeld, Germany). They were housed and maintained in a specific pathogen-free (SPF) facility. They were maintained under pathogen-free conditions in the animal facility of the Max Delbrück Center of Molecular Medicine (Berlin, Germany). They were fed autoclaved standard diet purchased from Sniff (Soest, Germany) and acidified drinking water ad libitum.

2.3. Assessment of toxicity of aqueous mistletoe extract in SCID mice

SCID mice (CB-17 SCID/SCID) were treated by intraperitoneal injection of MT-P and A at different doses every 4 days a week. Control mice were injected with an equivalent volume of PBS. The mice were carefully monitored for symptoms of toxicity, and weighted twice each week. The mean weight of each group of mice was calculated and used as a parameter of toxicity.