Anti-Cancer Drugs 1997, **8** (suppl 1):S47–S51

Mistletoe therapy for human cancer: the role of the natural killer cells

M. Schink

Verein Filderklinik e.V., Research Department, Im Haberschlai 7, D-70794 Filderstadt, Germany.

Extracts produced from *Viscum album* L. (mistletoe) as well as certain isolated components are able to stimulate different functions of the immune system. The natural killer cells have been suggested as one of the candidates for direct tumour cell destruction. These cells are defined by their ability to mediate non-major histocompatibility complex (MHC) restricted cytotoxicity without prior sensitization against a specific antigen. However, their effectiveness in tumour defence *in vivo* is unclear. In general, natural killer cells are unable to lyse fresh autologous tumour cells *in vitro* unless activated by interleukin-2-preincubation. The results of clinical studies are contradictory, but there is evidence that they may contribute to the prevention of the development of recidives and metastases. In this regard it is interesting that mistletoe extracts are able to stimulate natural killer cell-mediated cytotoxicity *in vitro* directly as well as indirectly in a cytokine-like manner, with the active components being carbohydrates rather than lectins. Clinical application of mistletoe extracts or isolated lectins is reported to induce augmentation of both number and activity of natural killer cells in peripheral blood in a dose-dependent manner; however, non-responders also have been described. In future work it has to be clarified whether a mistletoe-derived modulation of the natural killer system is of benefit in the tumour defence of cancer patients.

Key words: Mistletoe therapy, immune modulation, natural killer cells, cancer, antitumour cytotoxicity.

Introduction

Since the introduction of extracts from *Viscum album* L. (mistletoe) as putative anti-cancer drugs in the 1920s, based on the anthroposophical view of the relation between drug and disease, many investigations were performed to define scientifically acceptable effects of mistletoe therapy on parameters and processes important for tumour defence. These studies showed that mistletoe extracts under certain conditions can act as potent biological response modifiers, able to stimulate nearly all cellular parts of the immune system, at least *in vitro*, including those directly involved in tumour cell destruction [1–4]. Of special interest are observations that extracts or components isolated from mistletoe are also able to elicit cytokine release such as interleukin-1, interleukin-6, tumour necrosis factor-α and possibly interleukin-2 or interferon-γ from immune cells [2,5]. *In vivo*, these intercellular messengers may induce reaction cascades which appear to strengthen the host defence mechanisms of the cancer patient. One of the candidates for a direct or cytokine-mediated indirect stimulation by mistletoe therapy are the natural killer cells. Together with the cytotoxic T lymphocytes and macrophages, these cells build up the defence system responsible for the direct killing of neoplastically degenerated somatic cells.

Correspondence to M. Schink

General characteristics of natural killer cells

Natural killer cells were originally defined by their ability to mediate non-major histocompatibility complex (MHC) restricted cytotoxicity without prior sensitization against a specific antigen [6]. It has been shown also that a subset of T lymphocytes and other leukocytes can exhibit non-MHC restricted cell killing either spontaneously or upon activation [7,8]. However, both mature and precursor natural killer cells are unequivocally distinct from T, B, and myeloid cells and, therefore, represent a distinct leukocyte subset. They are usually identified by their large granular lymphocyte morphology [6], although some natural killer cells can also be of medium size and agranular [9], and by the presence of some characteristic surface markers. Most natural killer cells therefore express the CD56 (neural cell adhesion molecule) cell surface antigen and a specific form of the low affinity receptor for the Fc portion of immunoglobulin G (FcγRIIIA or CD16), while no rearrangement or effective transcription of T-cell receptor or immunoglobulin genes takes place [10,11].

The presence of the FcγRIII enables natural killer cells to bind to cell targets opsonized with immunoglobulin G and lyse them. Actually, the natural killer cell subset is mainly responsible for the phenomenon called antibody-dependent cell-mediated cytotoxicity [12]. Although resting natural killer cells are constitutively provided with natural cytotoxic ability, their activity and target susceptibility can be considerably increased by treatment *in vitro* or *in vivo* with various cytokines, particularly interferon-α, interferon-γ, interleukin-2, interleukin-7 and interleukin-12, with interleukin-2 being the most effective [13]. It was demonstrated that CD56+ and/or CD16+ cells are responsible for the major part of the lymphokine-activated killer activity induced in separated peripheral blood mononuclear cells cultured in the presence of this cytokine [14,15].

Role of natural killer cells in cancer: experiments with animals and clinical observations

The first indications of an important role for natural killer cells in the immunological surveillance against cancer came from animal studies [16–18]. Treatments such as the application of the alkylating drug cyclophosphamide diminished the natural killing activity and led to increased development of spontaneous and experimental tumour metastases in mice. Adoptive transfer of natural killer cells rich in splenocytes or large granular lymphocytes mediated or restored the antimetastatic ability of the animals.

In humans, some diseases associated with deficient natural killer cell activity, such as Chédiak–Higashi syndrome [19] or X-linked lymphoproliferative syndrome [20], coincide with augmented cancer risk. While the results of the numerous investigations of natural cytotoxicity in cancer patients are contradictory with respect to type and stage of disease, extensively reviewed elsewhere [21–23], several studies involving large numbers of subjects demonstrated a clear relationship between natural killer cell activity and the outcome of metastases or of local recurrence in patients with different primary tumours [24–26]. Despite extensive investigation, it is at present unclear whether an impaired natural cytotoxicity is the result or a cause of the stage of disease, but there is evidence that it may contribute to the prevention of the development of new cancer burdens and the establishment of regional and distant metastases.

Natural killer activity *in vitro*: measurement and target cell susceptibility

Natural killer cells can lyse a variety of target cells *in vitro*, including both normal and tumour-derived cells. However, different cells vary significantly in their susceptibility to natural killer cells. The human erythroleukaemia-derived cell line K562, highly sensitive and lacking detectable amounts of MHC class I and II antigenic determinants [27,28], is the most frequently used target for measuring natural cytotoxicity of human peripheral blood mononuclear cells. The most commonly used cytotoxic assay is the ^{51}Cr-release assay, in which release of the radiolabelled chromium upon lysis of previously labelled target cells is determined [29].

The determination of the lytic capacity of natural killer cells against a target cell line such as K562, while highly sensitive but also highly adjusted to *in vitro* culture conditions, is of limited use for the prediction of the lytic capacity of these cells against tumour cells *in vivo*. Therefore, the cytolysis of fresh autologous tumour cells by the natural killer cells of the same patient has been broadly assessed. It was usually found that autologous tumour cells are normally natural killer cell resistant [6], although some authors reported autologous cell killing capacity in the blood of 10–50% of cancer patients tested, depending on tumour types and metastases [30]. On the other hand, the incubation with interleukin-2 (i.e. induction of lymphokine-activated killer activity) results in an expansion of natural killer cells' *in vitro* antitumour potential for a wider spectrum of targets including fresh tumour cells [31]. This effect may essentially contribute to the antitumour effects observed after adoptive lymphokine-activated killer therapy. Therefore, it can be supposed that immunotherapies when stimulating natural killer cell activity *in vivo* may also stimulate the putative potential of natural killer cells to reduce the patient's risk of recidive or metastasis outcome after surgical tumour rejection. This has to be proved by means of well designed clinical trials.

Effects of mistletoe extracts and isolated components on natural killer activity of peripheral blood mononuclear cells *in vitro*

Since biological response modifiers may influence natural killing either by direct activation of the effector cells or indirectly by the induction of cytokine release with natural killer cell stimulating properties, it is of special interest to first analyse their influence on a definite effector/target system *in vitro*. In the case of mistletoe preparations, several of these investigations have been performed successfully. It was demonstrated that Iscador extracts are able to directly stimulate the killing capacity of CD16+ CD57+ natural killer cells against a variety of target cells when present during the cytotoxicity assay [32,33]. The active component was reported to be a rhamnogalacturonan enhancing the formation of effector/target cell conjugates by a bridging mechanism [34]. The mistletoe preparation *Helixor* did not exhibit any stimulating effect when co-cultivated with the effector/target cell mixture, but an enhancing effect was observed when peripheral blood mononuclear cells are preincubated with that extract in the presence of anti-mistletoe lectin antibodies [35,36]. An oligosaccharide was isolated from the preparation *Helixor*M (mali) which acts as a cytokine inducer, eliciting tumour necrosis factor-α and prostaglandin E_2 release from monocytes and interferon-γ release from T lymphocytes [37]. In contrast to the whole plant extracts, isolated mistletoe lectin I did not induce any stimulation of natural killing in a concentration range of 1–32 ng/ml but acts as an inhibitor when anti-mistletoe lectin antibodies are absent [36].

Hülsen *et al.* [38–40] also investigated the influence of Iscador and *Helixor* mistletoe preparations on isolated peripheral blood mononuclear cells by preincubation for 20 h and subsequent addition of target cells without removal of the plant extract. They found stimulations but also non-responses, and even inhibitory effects, on the *in vitro* natural killer activity against K562. The effect of the different mistletoe preparations tested was also variable and strongly dependent on the respective donor. The authors described the trend of a better reactivity of cancer patients in comparison to

healthy donors as well as a more favourable situation for female than for male cancer patients. How representative an individual reaction pattern of the peripheral blood mononuclear cells from a certain patient is for the *in vivo* situation is unclear and has to be elucidated in suitable clinical trials.

Influence of mistletoe (lectin) therapy on natural killer cells *in vivo*

A possibly therapeutically relevant impact of mistletoe administration on the natural killer cells *in vivo* was demonstrated for the first time by Hajto *et al.* [41,42]. They determined the number of large granular lymphocytes in peripheral blood and the *in vitro* cytotoxicity of the isolated natural killer cells of cancer patients treated by a single intravenous infusion of the mistletoe preparation Iscador. A significant increase of both parameters 24–48 h after the treatment was found. Thereafter, the count and activity of the natural killer cells declined to the baseline levels. An optimal dose for that plant preparation was postulated [43], and the regular application of this dose over a period of 7 months led to a significant increase in the large granular lymphocyte count in peripheral blood of 14 cancer patients [44].

Similar effects were observed after subcutaneous administration of isolated galactoside-specific mistletoe lectin I to rabbits and mice [42,43]. Again, dose dependence was demonstrated, with 0.8–1 ng lectin/kg body weight being the most effective dose. The other lectins (mistletoe lectin II and III) from mistletoe were also found to stimulate the natural killer cells *in vivo* [42], but their dose-dependence has not yet been assessed. It has to be stressed that these lectins do not inevitably show the same behaviour as mistletoe lectin I and so up to now their real effectiveness is unclear. In the mouse model, higher doses of mistletoe lectin I may also be effective and, for example, Joshi *et al.* [45] demonstrated that 10 ng mistletoe lectin I/kg body weight, if applied intraperitoneally and injected several times on alternate days, results in a significant increase of natural killer cell counts in the spleen and an enhancement of natural killer cell mediated cytotoxicity of the murine spleen cells after 12 days of lectin treatment.

Beuth *et al.* [46,47] applied isolated galactoside-specific lectin from mistletoe to humans for the first time. After repeated subcutaneous injections of 0.5–1 ng lectin/kg body weight to cancer patients for 4–5 weeks, a significant increase in the peripheral blood natural killer cell count could often be achieved; however, the occurrence of non-responders was also observed. These patients did not react immunologically to the lectin therapy and suffered from a progressive tumour growth even under the treatment. Similar data were presented for patients treated with the whole plant extract *Eurixor* [48]. For the latter, the producer's statement about the lectin content of the preparation was used for its application at a dose corresponding to the isolated component.

As mentioned above, sugar components but not lectins from mistletoe extracts are able to support natural killer cell killing activity *in vitro*. Therefore, it is likely that mistletoe lectin I therapy acts indirectly through the induction of cytokine release, favourable for augmentation of natural killer cell count and activity. Not only the mistletoe lectin(s) are able to stimulate the natural immune system *in vivo*, however, as Kuttan and Kuttan [49] described a significant rise in natural killer cell activity of murine spleen cells 48–72 h after administration of a viscotoxin-like 5 kD peptide without any lectin properties isolated from the plant extract Iscador. Also, the antibody-dependent cell-mediated cytotoxicity was elevated with a maximum increase detectable 72 h after the application of the peptide. Whether the natural killer cell-promoting abilities of the mistletoe carbohydrates are of therapeutic relevance is at present unclear but, with respect to a modulation of the natural immune system, it seems that the whole plant extract still may hold some advantages over isolated components such as mistletoe lectin I.

Conclusions

The results stated above clearly indicate that mistletoe preparations, as well as some isolated components, may be of benefit in modulating the natural immune system of cancer patients. On the other hand, the role of the natural killer cells in cancer defence has not yet been clarified. Apart from obvious differences with respect to type and staging of tumour disease, several questions arise that have to be clarified in future studies: are the results of *in vitro* natural killer activity tests transferable to the *in vivo* situation (i.e. is there a relationship between modulation of killing of certain cell lines and autologous tumour cells) and is there a correlation between modulation of natural killer cell activity and progress of disease (i.e. recidive outcome or behaviour of metastases)? The answers to these questions will have to determine whether or not screening of the natural killer cell modulating activity of mistletoe therapy is useful for the prolongation of survival time and the improvement of quality of life.

Some of these questions are the subject of a clinical study being performed at present at the communal hospital Filderklinik, Filderstadt, Germany, promoted by the German Bundesministerium für Bildung und Wissenschaft, Forschung und Technologie (BMBF) [50].

References

1. Berg PA, Stein G: Mistletoe extracts: effective modulators of the natural and specific immune system? [in German]. *Der informierte Arzt-Gazette Médicale* 1994, **15**: 769–776.
2. Beuth J, Steuer MK, Lenartz D, Pulverer G: Mistletoe lectins: therapeutically relevant immunomodulators in oncology [in German]. *Z Allg Med* 1996, **72**: 24–27.

3. Matthiessen PF, Tröger W: The mistletoe therapy of cancer [in German]. In: *Kombinierte Tumortherapie*. Edited by Wrba H. Stuttgart: Hippokrates-Verlag; 1995:271–290.
4. Pfüller U: Immunomodulation by ingredients of mistletoe [in German]. In: *Grundlagen der Misteltherapie*. Edited by Scheer R, Becker H, Berg PA. Stuttgart: Hippokrates-Verlag; 1996:170–182.
5. Hajto T, Hostanska K, Frei K, Rordorf C, Gabius HJ: Increased secretion of tumor necrosis factor α, interleukin 1, and interleukin 6 by human mononuclear cells exposed to β-galactoside-specific lectin from clinically applied mistletoe extract. *Cancer Res* 1990, **50**: 3322–3326.
6. Trinchieri G: Biology of natural killer cells. *Adv Immunol* 1989, **47**: 187–375.
7. Lanier LL, Kipps TJ, Philipps JH: Functional properties of a unique subset of cytotoxic CD3+ T-lymphocytes that express Fc receptors for IgG (CD16/leu-11 antigen). *J Exp Med* 1985, **162**: 2089–2106.
8. Adams DO, Hamilton TA: Phagocytic cells. Cytotoxic activities of macrophages. In: *Inflammation. Basic Principles and Clinical Correlates*. Edited by Galin JI, Goldstein IM, Snyderman R. New York: Raven, 1988:471–492.
9. Inveraldi L, Witson JC, Fuad SA, Winkler-Pickett RT, Ortaldo JR, Balch FH: CD3 negative 'small agranular lymphocytes' are natural killer cells. *J Immunol* 1991, **146**: 4948–4952.
10. Ritz J, Schmidt RE, Michon J, Hercend T, Schlossman SF: Characterization of functional surface structures on human natural killer cells. *Adv Immunol* 1988, **42**: 181–211.
11. Whiteside TL, Herberman RB: Characteristics of natural killer cells and lymphokine-activated killer cells. *Immunol Allergy Clin North Am* 1990, **10**: 663–704.
12. Kay HD, Bonnard GD, West WH, Herberman RB: A functional comparison of human Fc-receptor-bearing lymphocytes active in natural cytotoxicity and antibody-dependent cellular cytotoxicity. *J Immunol* 1977, **118**: 2058–2066.
13. Baxevanis CN, Papamichail M: Characterization of the anti-tumor immune response in human cancers and strategies for immunotherapy. *Crit Rev Oncol Hematol* 1994, **16**: 157–179.
14. Philipps JH, Lanier LL: Dissection of the lymphokine activated killer phenomenon: relative contribution of peripheral blood natural killer cells and T lymphocytes to cytolysis. *J Exp Med* 1986, **164**: 814–825.
15. Baxevanis CN, Reclos GJ, Gritzapis AD, Dedoussis GVZ, Papamichail M: Elevated PGE_2 production by monocytes is responsible for depressed levels of natural killer (LAK) cell function in patients with breast cancer. *Cancer* 1993, **72**: 491–501.
16. Hanna N, Burton RC: Definite evidence that natural killer (NK) cells inhibit experimental tumor metastases *in vitro*. *J Immunol* 1981, **127**: 1754–1758.
17. Gorelick E, Wiltrout RH, Okumua K, Habu S, Herberman RB: Role of NK cells in the control of metastatic spread and growth of tumor cells in mice. *Int J Cancer* 1982, **30**: 107–112.
18. Barlozzari T, Leonhardt J, Wiltrout RH, Herberman RB, Reynolds CW: Direct evidence for the role of LGL in the inhibition of experimental tumor metastases. *J Immunol* 1985, **134**: 2783–2789.
19. Roder JC, Halitosis T, Klein M, *et al.*: A new immunodeficiency disorder in humans involving NK cells. *Nature* 1980, **284**: 553–555.
20. Sullivan JL, Bryon KS, Brewster FE, Purtilo DT: Deficient natural killer cell activity in X-linked lymphoproliferative syndrome. *Science* 1980, **210**: 543–545.
21. Pross HF: The involvement of natural killer cells in human malignant disease. In: *Immunobiology of Natural Killer Cells, Vol. II*. Edited by Lotzová E, Herberman RB. Boca Raton, FL: CRC Press, 1986:11–27.
22. Pross HF, Lotzová E: Role of natural killer cells in cancer. *Nat Immunol* 1993, **12**: 279–292.
23. Brittenden J, Heys SD, Ross J, Eremin O: Natural killer cells and cancer. *Cancer* 1996, **77**: 1226–1243.
24. Schantz SP, Savage HE, Racz T, Taylor DL, Sachs PG: Natural killer cells and metastases from pharyngeal carcinoma. *Am J Surg* 1989, **158**: 361–366.
25. Schantz SP, Ordonez NG: Quantification of natural killer cell function and risk of metastatic poorly differentiated head and neck cancer. *Nat Immunol Cell Growth Regul* 1993, **10**: 278–288.
26. Tartter PA, Steinberg B, Barron DM, Martinelli G: The prognostic significance of natural killer cytotoxicity in patients with colorectal cancer. *Arch Surg* 1987, **122**: 1264–1268.
27. Ortaldo JR, Oldham RK, Cannon GC, Herberman RB: Specificity of natural cytotoxic reactivity of normal human lymphocytes against a myeloid leukemia cell line. *J Natl Cancer Inst* 1977, **59**: 77–82.
28. Jondal M, Pross HF: Surface markers on human B- and T-lymphocytes. VI. Cytotoxicity against cell lines as functional markers for lymphocyte subpopulations. *Int J Cancer* 1975; **15**: 596–605.
29. Pross HF, Callewaert D, Rubin P: Assays for NK cell cytotoxicity: their values and pitfalls. In: *Immunology of Natural Killer Cells*. Edited by Lotzová E, Herbermann RB. Boca Raton, FL: CRC Press, 1986: pp 1–20.
30. Uchida A, Kariya Y, Okamoto N, Kihara T, Inoue N, Sugie K: Biological significance of autologous tumor killing in human cancer patients and its modulation by biological therapy. In: *Chemoimmunoprevention of Cancer*. Edited by Pastorino U, Hong WK. Stuttgart: Thieme-Verlag; 1991: pp 9–13.
31. Naume B, Espevik T: Effects of IL-7 and IL-2 on highly enriched CD56+ natural killer cells. *J Immunol* 1991, **147**: 2208–2214.
32. Hamprecht K, Handgretinger R, Voetsch W, Anderer FA: Mediation of human NK-activity by components in extracts of *Viscum album*. *Int J Immunopharmacol* 1987, **9**: 199–209.
33. Zhu HG, Zollner TM, Klein-Franke A, Anderer FA: Enhancement of MHC-unrestricted cytotoxic activity of human CD56+ CD3– natural killer (NK) cells and CD3+ T cells by rhamnogalacturonan: target cell specificity and activity against NK-insensitive targets. *J Cancer Res Clin Oncol* 1994, **120**: 383–388.
34. Mueller EA, Anderer FA: Chemical specificity of effector cell/tumor cell bridging by a *Viscum album* rhamnogalacturonan enhancing cytotoxicity of human NK cells. *Immunopharmacology* 1990, **19**: 69–77.
35. Klett CY, Anderer FA: Activation of natural killer cell cytotoxicity of human blood monocytes by a low molecular weight component from *Viscum album* extract. *Arzneim-Forsch/Drug Res* 1989, **39**: 1580–1585.
36. Schultze J: *Zelluläre Immunreaktionen bei mit einem Mistelextrakt therapierten Patienten*. Thesis, University of Tübingen, Medical Faculty, 1992
37. Mueller EA, Anderer FA: A *Viscum album* oligosaccharide activating human natural cytotoxicity is an interferon γ inducer. *Cancer Immunol Immunother* 1990, **32**: 221–227.
38. Hülsen H, Kron R, Mechelke F: Influence of *Viscum album* preparations on the natural killer cell-mediated cytotoxicity of peripheral blood. *Naturwissenschaften* 1989, **76**: 530–531.
39. Hülsen H, Jilg S, Mechelke F: Influence of mistletoe preparations on the *in vitro* activity of the natural killer cells from cancer patients [in German]. *Therapeutikon* 1992, **6**: 585–588.
40. Hülsen H, Born U: Influence of mistletoe preparations on the *in vitro* activity of the natural killer cells from cancer patients (part 2) [in German]. *Therapeutikon* 1993, **7**: 434–439.
41. Hajto T, Lanzrein C: Natural killer and antibody-dependent cell-mediated cytotoxicity activities and large granular lymphocyte frequencies in *Viscum album*-treated breast cancer patients. *Oncology* 1986, **43**: 93–97.
42. Hajto T, Hostanska K, Gabius HJ: Modulatory potency of the β-galactoside-specific lectin from mistletoe extract (Iscador) on the

host defense system *in vivo* in rabbits and patients. *Cancer Res* 1989, **49**: 4803–4808.

43. Hajto T, Hostanska K, Gabius HJ: Cytokines as lectin-induced mediators in mistletoe therapy [in German]. *Therapeutikon* 1990, **4**: 136–145.
44. Hajto T, Hostanska K, Fornalski M, Kirsch A: Antitumoural activity of the immunomodulatory effective β-galactoside-specific mistletoe lectin during the clinical application of mistletoe extracts (*Iscador*) [in German]. *Dtsch Zeitschr Onkol* 1991, **23**: 1–6.
45. Joshi SS, Komanduri KC, Gabius S, Gabius HJ: Immunotherapeutic effects of purified mistletoe lectin (ML-I) on murine large cell lymphoma. In: *Lectins and Cancer*. Edited by Gabius HJ, Gabius S. Berlin: Springer-Verlag, 1991:206–216.
46. Beuth J, Ko HL, Tunggal L, Geisel J, Jeljaszewicz J, Steuer MK, Pulverer G: Mistletoe lectin as an immuno modulator in oncology [in German]. *Z All Med* 1994, **70**: 159–164.
47. Beuth J, Stoffel B, Ko HL, Buss G, Tunggal L, Pulverer G: Immunostimulating activity of different doses of mistletoe lectin-1 in patients with breast cancer [in German]. *Arzneim Forsch/Drug Res* 1995, **45**: 505–507.
48. Beuth J, Ko HL, Tunggal L, Geisel J, Pulverer G: Comparative studies on the immunoactive potency of galactoside-specific lectin from pure mistletoe against a standardized extract [in German]. *Arzneim Forsch/Drug Res* 1993, **43**: 166–169.
49. Kuttan G, Kuttan R: Immunomodulatory activity of a peptide isolated from *Viscum album* extract (NSC 635 089). *Immunol Invest* 1992, **21**: 285–296.
50. Goyert A, Schink M: Comparison of the effect of natural killer cells on tumour and immunomodulation in peripheral blood with clinical progress during mistletoe therapy [in German]. In: *Informationsbrief Unkonventionelle Medizinische Richtungen und Unkonventionelle Methoden der Krebsbekämp-fung, Ausgabe Nr. 2*. Herdecke, Germany: Working Groups of the UMR and UMK; 1996:6–8.