Significantly Higher Levels of Activated NK cells with Supplemental Mistletoe Therapy in Patients with Breast Cancer and Chemotherapy in a Prospective Randomised, Double-blind Clinical Study

L. Auerbach, V. Dostal, I. Václavik-Fleck, E. Kubista, A. Rosenberger, S. Rieger, W. Tröger, J.M. Schierholz

Summary

In a prospective randomised, double-blind clinical pilot study breast cancer patients (stage I/II) were treated additionally with mistletoe extract (Helixor®) or placebo during chemotherapy with CMF (cyclophosphamide, methotrexate, 5-fluorouracil) and radiation treatment (Sandwich scheme) at the Vienna University Clinic (AKH), Department of Special Gynaecology. Prognostic and immunological parameters such as the quality of life were studied. Additionally, the feasibility of a double-blind study with mistletoe extract was examined.

In total, 23 patients with histologically verified breast cancer T1-2 N0-1 M0 were recruited. 20 patients fulfilled the in- and exclusion criteria and were treated according to the protocol with 6 treatment cycles of an adjuvant chemotherapy with CMF over a time period of 6 months. The analysis showed that a large proportion of the patients as well as physicians were unblinded during the course of the study. The patients in the control group showed a significant decrease of activated NK cells (p = 0.001; n = 11) with chemotherapy. This reduction was not observed in the group treated with Helixor® (n = 9). The activated NK cells remained approximately at the same level and were significantly higher than in the control group (p = 0.005).

Keywords

Viscum album, mistletoe, chemotherapy, cyclophosphamide, breast cancer patients, randomised double-blind clinical study

Introduction

In Europe mistletoe therapy is the most frequently applied unconventional supplemental therapy in tumour diseases. Following the basic introduction by Rudolf Steiner, the founder of anthroposophy, in 1920 a scientific basis for the different mechanisms of efficacy and effects has been found in the mean time:

1. Cytotoxicity via induction of tumour cell apoptosis
2. Stimulation of immunocompetent cell populations
3. DNA-stabilisation in peripheral blood lymphocytes
4. Improvement of quality of life in tumour patients

The cytotoxic efficacy of the extracts is assigned to the viscotoxins and mistletoe lectins, whereas the immunomodulating properties derive from lectins and viscotoxins as well as from poly- and oligosaccharides, and other components (Büssing, 2000).
In the last 20 years several clinical studies have been conducted with mistletoe therapy, the methodological quality of which frequently did not correspond to generally accepted clinical standards (Kienle et al. 2003). The clinical “gold standard” according to Evidence-Based Medicine (EBM) Level 1, the prospective randomised, double-blind study type, which is very controversial for mistletoe therapy, was examined with respect to feasibility in this study. Additionally, immunological parameters, as well as quality of life under chemotherapy, were tested.

**Materials and Methods**

**Methods**

The prospective randomised, double-blind clinical pilot study was carried out at the University Clinic for Gynaecology, Department of Special Gynaecology, AKH Vienna. The conduct of the study corresponded to the requirements for GCP and was approved by the ethics committee of the Vienna AKH. Written informed consent was obligatory for patients participating in the study. The complete study procedure was continuously monitored. The biometrical evaluation as well as randomisation, data verification, and data analysis was carried out by the Institute of Medical Data Processing at the University of Tübingen.

**Study goals:**

- Verification of the feasibility of double-blind randomisation (by means of a CRF)
- Influence of mistletoe therapy with Helixor® A on the sister chromatid exchange rate (SCE) in PBMC during an adjuvant chemotherapy
- Influence of different cellular immune system parameters, especially activated CD69+ NK cells
- Tolerance of mistletoe therapy during chemotherapy
- Quality of life

**Inclusion criteria:**

- Patients with surgically treated breast cancer classified as $T_{1-2}N_{0-1}M_{0}$, where a CMF sandwich therapy was indicated
- Patients with a Karnofsky index of $\geq 60$
- Patients with sufficient kidney function, defined as serum creatinine $\leq 1.5$ mg %
- Patients with sufficient liver function, defined as SGOT and SGPT $\leq 2x$ upper normal value
- Patients with sufficient bone marrow function, defined as leukocytes $\geq 3,000/\text{ml}$, thrombocytes $\geq 100,000/\text{ml}$
- Legal capability of patients
- Patients having given informed consent
Exclusion criteria:

- Participation in another clinical or surveillance study within the previous 4 weeks
- Concomitant participation in other tests or studies
- Proved therapy with other mistletoe preparations within the previous 6 months
- Concomitant therapy with other immunostimulating agents during the 6 CMF cycles
- Pregnancy and lactation
- Patients treated with antidepressants
- Patients with secondary tumours
- Patients with lymphoma or leukaemia
- Patients with auto-immune diseases
- Patients with severe acute infections
- Patients with severe concurrent diseases in the field of internal medicine

Patients

23 patients with breast cancer were recruited; 20 patients complied with the in- and exclusion criteria, and 16 patients completed the study. The patients were randomised into 2 groups: one group received sodium chloride 0.9 % (placebo, n = 12, s.c. three times a week) according to the protocol, and the other group received mistletoe total extract Helixor® A (n = 11) in increasing concentrations of 1, 5, 10, 20, 30, 50 up to 100 mg (s.c., three times a week).

The patients were treated with standard CMF chemotherapy (cyclophosphamide 600 mg/m², methotrexate 40 mg/m², 5-fluorouracil 600 mg/m² i.v. on day 1 and day 8). 13 patients (with breast conservation surgical therapy) from both groups additionally received radiotherapy (radiation 25 x 2 Gy) after the 3rd CMF cycle (Sandwich scheme) (control n = 7, active substance n = 6) (for treatment schedule, see Figure 1). Of the 23 recruited patients, 3 had to be excluded from the mistletoe group after screening; one patient did not accept the blinding, and 2 did not meet the inclusion criteria. Also, one patient in the control group was excluded from the study due to a thromboembolism during chemotherapy and the study.

Two patients discontinued the study during the 4th cycle, one in the control group (insufficient compliance) and one in the Helixor® group (increased axillary lymph nodes). Another patient from the Helixor® group had to be excluded due to insufficient compliance. This resulted in 3 drop-outs after screening and 4 drop-outs during the course of the study (2 patients per treatment group) (Fig. 2).

Therefore, 20 patients were evaluated after 3 cycles of CMF, and 16 patients after the complete cycles and the observation period of 12 months.
Treatment
Day 1 and 8 of a cycle (6 cycles/4 weeks)

- Cyclophosphamide 600 mg/m² as 0.5 – 1 hr infusion i.v.
- Methotrexate 40 mg/m² as short infusion i.v.
- 5- Fluorouracil 600 mg/m² as 0.5 – 1 hr infusion i.v.

Radiation with 50 Gy after the 3rd CMF-cycle (Sandwich scheme)

<table>
<thead>
<tr>
<th>1.1 Control group</th>
<th>1.1 Treatment group</th>
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<tr>
<td>For all patients in addition to CMF therapy the placebo preparation (physiol. NaCl solution) is intended.</td>
<td>For all patients in addition to CMF therapy the test preparation Helixor® A (fir mistletoe) is intended.</td>
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Total observation time 12 months

**Figure 1:** Treatment Schedule

**Figure 2:** Patient Flowchart

**Materials**

Test ampoules: the test medication for the active substance and placebo groups were labelled with identically named preparations. 1 ml Helixor® A test ampoules were manufactured according to GMP standards (HELIXOR Heilmittel GmbH & Co. KG, Rosenfeld, Germany). The protein content was 63 mg/ml (Bradford) and the mistletoe lectin content was 124 ng/ml, mainly mistletoe lectin III. The placebo ampoules contained sterile sodium chloride solution (0.9 %, manufacturer: HELIXOR Heilmittel GmbH & Co. KG, Rosenfeld, Germany).
Laboratory

All tests were conducted prior to study start and before each CMF cycle.

- **Laboratory routine** (AKH Vienna): Hb, leukocytes, thrombocytes, creatinine, protein, SGOT, SGPT, γ-GT, LDH, CRP
- **Determination of SCE rate**: (Prof. Dr. Büssing, Herdecke; Prof. Dr. Rüdiger, Vienna): blood sampling for SCE determinations were carried out additionally respectively on the 2nd day of the running CMF cycle. A detailed description of the method is found in Büssing et al. (1995).

Statistical methods

All collected data were recorded twice, matched and checked for plausibility. The data entry was carried out at the Institute of Medical Data Processing, University Hospital Tübingen with the MS-ACCESS 97 software package. The evaluation was done with SAS 8.0. Differences between the active substance and control groups with respect to the SCE rate and quality of life were tested using the t-Test. Group comparisons with respect to the share of activated NK cells were carried out by Wilcoxon’s rank sum test for each treatment cycle.

Determination of Quality of Life

The results of EORTC quality of life questionnaires (QLQ-C30) and the Karnofsky Performance Index were determined during screening and during the ongoing follow-up prior to every CMF cycle. Additionally, the patients evaluated their daily well-being on a linear analogue scale.

Results

With respect to consent and recruitment, such a study can be referred to as feasible. Eight out of 9 patients treated with mistletoe (89 %) and 5 out of 11 control patients (46 %) recognised the treatment medication despite the blinding – probably due to local reactions which either did or did not occur. The investigator recognised the treatment medication in 16 of 20 patients (80 %). The aim of the blinding, to limit the development of deliberate or unintentional bias, could not be attained with such high rates of unblinding.

During the course of chemotherapy the SCE rate in peripheral lymphocytes increased in both groups as expected, in the mistletoe group to a lesser extent than in the control group; the positive differences in favour of the mistletoe therapy (Fig. 3) however, were not significant. Additionally it was determined that the amount of activated NK cells (CD56+/CD69+/CD45) remained almost stable over time up to the 6th cycle in the mistletoe group, whereas the patients with the placebo application (control) showed a significant decrease in the amount of activated NK cells (p = 0.001) (Fig. 4). In the mistletoe group the amount of activated NK cells were significantly higher from the 4th CMF cycle on than in the control group (p = 0.005).
With respect to the other laboratory and immune system parameters, as well as the quality of life, no differences between the active substance and the control groups could be found. The tolerance of the mistletoe therapy during chemotherapy was good. In 3 cases a harmless redness with a diameter of over 5 cm was found to have appeared at the point of subcutaneous injection, and there were 2 cases of headache. Other side effects did not occur. In contrast to the placebo group the mistletoe group did not show any chemotherapy-induced leukopenia.

Discussion

The present study resulted in the following:

- No problems with administering mistletoe therapy along with chemotherapy
- Problems with the double-blind design due to a relatively fast unblinding by patients as well as investigators
- Fewer applications of concomitant therapeutics under chemotherapy (all leukopenia cases in the placebo group)
- Stabilising of activated NK cells under supplementary mistletoe therapy

It is known that the amount and activity of natural killer cells correlates with reduced metastases in breast cancer (Carson et al., 2001; Clausen et al., 2003; Hülsen et al., 1989; Konjevic et al., 1995; White et al., 1982; Wiltschke et al., 1995; Yacyshyn et al., 1995; Zielinski et al., 1989). In this respect, proof that the decrease of activated NK cells due to chemotherapy can be inhibited by concomitant mistletoe therapy could be of great clinical relevance. In the present study, a statement with respect to a reduced rate of metastasis or a higher rate of survival due to supplemental mistletoe therapy could not be made because of the observation time of 12 months alone. However, the stabilising effect on the activated NK cells could possibly provide a suitable indirect surrogate parameter in future clinical trials for replicable target criteria within breast cancer treatment.

As the quality of life in the total group during the adjuvant chemotherapy with CMF was only affected marginally, a significant improvement could not be observed under mistletoe therapy by means of the EORTC questionnaire. According to experience, the positive influence of mistletoe therapy on quality of life becomes more marked in advanced tumour stages. Due to the almost complete unblinding of patients with subcutaneous mistletoe application, it is questionable whether a valid blinding can be achieved in future studies over longer periods of time.

The in vitro test results of Büssing (Büssing et al., 1995, 1996), showing that mistletoe preparations (Helixor® A) can significantly lower the cyclophosphamide-induced increase in the SCE rate as a measure of mutagenic effects in peripheral blood lymphocytes (immune protective effect), could not be verified in vivo in this pilot study. Although the increase in the SCE rate turned out to be less in the mistletoe group, the difference was not significant and was not proved because of the low patient number. Nevertheless the fact that, in contrast to the control group, there were no incidences of leukopenia in the mistletoe group could result from the immunoprotective effect of Helixor® A.
**Fig. 3**: Reduction of the SCE rate with concomitant mistletoe therapy (ns = difference to control not significant)

Mean SCE Rate
Breast Cancer Stage I-II (CMF +/- Helixor)

*Title y-axis (Titel y-Achse):*
Pre-post changes – mean course

*Title x-axis (Titel x-Achse):*
CMF chemotherapy cycle

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**Fig. 4:** Significantly higher amounts of activated NK cells in the mistletoe group compared to control (p = 0.005) starting at the 4th cycle: Stabilisation of the activated NK cells in % with mistletoe therapy, in contrast significant decrease in the control group (p = 0.001)

Activated NK cells (CD56+/CD69+/CD45) 
Breast Cancer Stage I-II (CMF +/- Helixor)

*Title y-axis (Titel y-Achse):*
Amount of activated cells (%)

*Title x-axis (Titel x-Achse):*
CMF chemotherapy cycle

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References


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