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Research paper

Forest adjuvant anti-cancer therapy to enhance natural cytotoxicity in urban women with breast cancer: A preliminary prospective interventional study

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ABSTRACT

Introduction: Studies have shown both significantly diminished natural cytotoxicity and immunosuppression in breast cancer patients after standard anti-cancer treatments. Therefore, an integrative approach employing adjuvant therapy in addition to current treatments is required to enhance immunoactivation. This preliminary prospective interventional study aimed to assess the feasibility of forest therapy as an adjuvant to enhance natural cytotoxicity.

Methods: This was a feasibility study of 11 volunteer women aged 25–60 years with stage III breast cancer. All subjects were exposed to daily forest therapy for 14 days whilst living in accommodation in a forest. Interventions included a relaxing daily 2-h morning walk (3 miles), free time tailored to subjects interest, group interaction and prepared meals based on nutritional standards. Outcome measures included natural killer (NK) cell populations and levels of perforin and granzyme B.

Results: Data from all participants were analysed. The mean volume of NK cells increased from 319.4 μL in the city to 444.6 μL in the forest after forest therapy ($p < 0.01$). The mean level of perforin increased from 216.9 pg/mL in the city to 344.9 pg/mL in the forest and then further increased to 463.2 pg/mL after subjects returned to the city ($p < 0.02$). The mean level of granzyme B increased from 4.4 pg/mL in the city to 11.2 pg/mL in the forest and then further increased to 20.2 pg/mL after subjects returned to the city ($p < 0.02$).

Conclusions: This study demonstrates the potential of forest therapy as an adjuvant anti-cancer therapy after standard treatments. A definitive trial with a control group should now be performed with larger sample sizes and long-term follow-up periods to confirm the feasibility and potential therapeutic effectiveness of this approach.

1. Introduction

Breast cancer, the most frequently diagnosed cancer in women worldwide, has been reported to account for 23% of all new cases of cancer in women since the beginning of the millennium [1]. The incidence rate of breast cancer has been increasing due to recent increases in screening activity and intensity, especially in economically developing countries [2]. Conversely, breast cancer mortality rates have been decreasing due to the early application of standard anti-cancer treatments such as surgery, radiotherapy, or chemotherapy with early detection strategies including mammography [1,3].

Despite the advanced treatment strategies for breast cancer, various studies [4–9] have shown that immunosuppression is common after standard anti-cancer treatments, and that natural killer (NK) cell activity is particularly suppressed. NK cells are cytolytic effector lymphocytes of the innate immune system that are especially critical for immune surveillance of tumours [10–12]. As it has been observed that peripheral blood natural cytotoxicity is diminished significantly in breast cancer patients, stimulation of NK cell activity after standard anti-cancer treatments has been considered important for breast cancer patients [10,11]. In addition, the activation of the NK cells that produce anti-cancer proteins such as perforin and granzymes when targeting cancer cells has been found to play a critical role in the host anti-cancer defence mechanisms [12]. Therefore, to minimise

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immunosuppression and enhance NK cell activity, an adjuvant anti-cancer therapy after standard treatments is needed.

Recent studies [13–15] conducted in Asian countries have reported that spending time in a forest environment and engaging in activities in the forest such as forest walking or forest bathing trips enhances NK cell activity to induce increased levels of anti-cancer proteins in healthy subjects.

The beneficial effect of this natural environment on NK activity is considered to be related to the enhanced psychoneuroimmunological effects brought about by the reduction in psychological stress experienced in the forest [16–19]. In addition, increased exposure to volatile chemical substances derived from trees such as phytoncides in the forest is considered to lead to stimulation of the proliferation of NK cells [13,18,20]. However, there have been no trials conducted to investigate the use of forest therapy as an adjuvant therapy to improve natural immunoactivation for breast cancer patients.

In this study, we aimed to evaluate the feasibility of forest therapy as an adjuvant anti-cancer therapy by measuring the NK cell population, and levels of two NK cell-released intracellular cytolytic molecules, in breast cancer patients who had undergone standard anti-cancer therapies.

2. Methods

2.1. Participants

Women with breast cancer living in a metropolitan area who were not exposure to a forest environment since being diagnosed with breast cancer were invited to participate in the study through the Korean Breast Cancer Society. No formal sample size calculation was performed because this was a preliminary feasibility study. Eligibility criteria included stage I to III breast cancer; city residence; completion of standard treatments including surgery, radiotherapy, and/or chemotherapy. Twelve female volunteers who met the eligibility criteria were interviewed and screened by research medical doctors at Korea University Hospital. Exclusion criteria included; current smoker, pregnant, current alcohol user, diabetes mellitus, presence of metastasises, infection, serious physical disability, or a mental disorder. One woman withdrew from the study at the beginning of the stay in the forest (day 1) owing to a family matter. The remaining 11 women (aged 25–60 years) completed the forest therapy and all measurements. The study protocol was approved by the Institutional Review Board of Korea University Hospital (AN11043-002), and all participants ($n = 11$) provided written informed consent.

2.2. Forest therapy

All participants travelled by shuttle bus in the summer season to the designated forest, named Saneum Natural Recreational Forest, located in the National Park in Gyeonggi Province in South Korea and close to the metropolitan area (a distance of 40 miles from Seoul). All subjects stayed at log cabins in the forest for 14 days and underwent daily forest therapy. In forest therapy, all subjects spent 2 h every morning (9:30 am–11:30 am) walking outside (3 miles). The participants additionally had the option to spend their free time in the afternoon inside or outside in the forest engaged in reading, writing, sewing, chess, photography, or drawing, depending on the participants' interests. All subjects socially interacted with each other in the forest during the study. All participants were provided daily meals and snacks designed based on general nutritional standards by a nutritionist from the Nutrition Department at Korea University Anam Hospital. Research medical doctors supervised the medical conditions of participants in the forest and provided any medical support when required. No physical or psychological side effects or complications from the forest therapy were reported by any subject during individual interviews with the researchers and allocated medical doctors.

2.3. Measurement parameters

The outcome measures to assess the results of the forest therapy were the NK cell population and the levels of perforin and granzyme. Blood samples were collected by research nurses at the hospital and in the forest in the morning using collection tubes containing an anticoagulant. The samples were sent to the laboratory of the Department of Bio-pathological Diagnosis at Korea University Hospital within 4 h after blood collection. Three sets of blood samples were collected. The baseline blood sampling was conducted at the hospital on the day of the subjects' departure to the forest (day 1). The second blood samples were collected in the forest on the last day of the subjects' 2-week stay in the forest (day 14). The final, follow-up blood sampling was conducted at the hospital a week after the subjects' returned home from the forest (day 21). To measure the NK cell population, flow cytometric analysis was performed using the Cytomics FC 500 flow cytometer (Beckman Coulter, Inc., Brea, CA, USA) according to the manufacturer's protocol. 100 μL of the blood sample was mixed with 10 μL of $CD45^+$-ECD monoclonal antibody and 20 μL of CD3-FITC/CD56-PC5 monoclonal antibody. All the fluorochrome-conjugated antibody reagents and cell fixing and lysing solutions (VersaLyse Lysing Solution) were purchased from Beckman Coulter.

Levels of perforin and granzyme B were measured in serum and plasma samples using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Abcam Biotechnology Co., Ltd., Cambridge, UK) according to the manufacturer's protocol.

2.4. Statistical Analysis

Outcome variables were summarised as means ± standard deviation (SD). All data were confirmed to be normally distributed using the Shapiro–Wilk test. The comparisons of within-group differences of the outcome measures were analysed using paired *t*-tests. SPSS statistical software (Version 12.0, SPSS Inc., Chicago, IL, USA) was used for the analyses. Two-sided tests and a significance level of 0.05 were used for all statistical analyses.

3. Results

All subjects ($n = 11$) who completed the study including the designated forest therapy had similar measurements with respect

Table 1
Characteristics of the subjects at baseline (Day 1).

Characteristics*	Subjects ($N = 11$)
Age, yr	56 ± 5.12
Marital status, no. (%)	
Married	11 (100)
Single or divorced	0 (0)
Type of surgery, no. (%)	
Modified radical mastectomy	11 (100)
Parietal mastecotmy	0 (0)
Postoperative Treatment, no. (%)	
Chemotherapy	11 (100)
Radiotherapy	11 (100)
Stage of breast cancer	
Stage II	0 (0)
Stage III	11 (100)
Stage IV	0 (0)