

RESEARCH ARTICLE

Efficacy of Oral Administration of *Lentinula edodes* Mycelia Extract for Breast Cancer Patients Undergoing Postoperative Hormone Therapy

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Abstract

Extract of *Lentinula edodes* mycelia (LEM) is currently utilized as an oral biological response modifier (BRM) medicine for cancer patients. However, its effectiveness for breast cancer patients with postoperative adjuvant hormone therapy has not yet been scientifically verified. In this study, we investigated the influence of LEM on the quality of life (QOL) and immune response in breast cancer patients undergoing postoperative adjuvant hormone therapy. Twenty patients were studied in total. They received only hormone therapy in the first 4 weeks followed by hormone therapy and LEM during the next 8 weeks. Laboratory tests, QOL score and peripheral blood cytokine production levels were evaluated during the study period. No changes in QOL or cytokines were noted after the first 4 weeks. In contrast, during the following combined therapy period, improvements were noted in QOL and cytokine levels. Although a future large-scale investigation is necessary to confirm these results, these data suggest that the concomitant use of LEM with postoperative adjuvant hormone therapy improves the QOL and immune function of patients.

Keywords: *Lentinula edodes* - BRM - breast cancer - hormone therapy - QOL

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Introduction

Lentinula edodes (*L. edodes*) has long been utilized as an edible mushroom in East Asia. It was reported in 1969 that lentinan, a high molecular weight neutral polysaccharide purified from a hot water-extract of *L. edodes* (Chihara et al., 1969), has antitumor activity. Since then, lentinan has been approved as an antitumor injection Biological Response Modifier (BRM) drug in Japan. Subsequently, progress has been made in research on the anti-tumor and immunomodulatory actions of the mycelia of *L. edodes* (Sugano et al., 1982; et al., 1985; Liu et al., 1998; Tanaka et al., 2012). *L. edodes* mycelia extract (L.E.M.) is an oral BRM medicine. Since oral ingestion exerts a low burden on patients and can therefore be easily applied in clinical practice, L.E.M. is widely used as an oral BRM. L.E.M. in combination with chemotherapy has been reported to improve the quality of life (QOL) and immune parameters in gastrointestinal cancer patients (Okuno et al., 2011; Yamaguchi et al., 2011) and advanced cancer patients undergoing immunotherapy (Tanigawa et al., 2012). It has been also reported that L.E.M. improved the quality of life (QOL) and immune parameters in the patients undergoing postoperative adjuvant chemotherapy

for breast cancer (Nagashima et al., 2005), but there have been no research reports on the effects in breast cancer patients receiving long-term postoperative hormone therapy, which constitutes the majority of breast cancer patients. For this reason, the present study was performed in breast cancer patients receiving hormone therapy to evaluate the effects of oral L.E.M. ingestion on the QOL and immune function.

Materials and Methods

Subjects

A total of 20 patients being followed up on hormone therapy after surgery for breast cancer participated in the study (Table 1). Patients were enrolled if they had had breast cancer in Stage 0 (carcinoma in situ, Paget disease), Stage 1 (tumor diameter 2 cm or less, confined to the breast), or Stage 2 (Stage 2a: tumor diameter 2 cm or less but with axillary lymph node metastasis, or tumor diameter 2.1 to 5 cm without axillary lymph node metastasis; Stage 2b: tumor diameter 2.1 to 5 cm with axillary lymph node metastasis, or tumor diameter 5 cm or more but without axillary lymph node metastasis), and completed surgery, chemotherapy, and radiotherapy for the breast cancer at

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least 9 weeks prior to enrollment in this study. The age of the subjects ranged from 40 to 74 years.

The study was conducted at Houju Memorial Hospital, after approval by the ethics committee of the Japanese Society for Complementary and Alternative Medicine and in accordance with the ethical principles that have their origins in the Declaration of Helsinki as well as the clinical study protocol for the present study. Prior to the start of the study, the investigator fully explained to the study subjects about the objectives and methods in the present study as well as other necessary aspects regarding protection of the subjects' human rights. The subjects voluntarily granted their consent to participate in the study in writing.

Drug

L.E.M. was provided by Kobayashi Pharmaceutical Co., Ltd (Osaka, Japan) and was manufactured as follows (Itoh et al., 2009). Briefly, *L. edodes* mycelia were cultivated in a solid medium composed of sugar-cane bagasse and defatted rice bran. The medium containing the mycelia was incubated in hot water, and the soluble fraction was dried. The dried powder was used as L.E.M.. Then, 600 mg of L.E.M. was mixed with starch, trehalose and granulated sugar and then compressed to 3000 mg granulated powder per pack. Each subject consumed three packs a day.

Study design

This was a 12-week, single-arm, open-label study. During the 12-week study period, all subjects received hormone therapy. All subjects first entered a 4-week observation period, followed by an 8-week period of oral L.E.M. ingestion at 1800 mg daily.

Measurements

Blood chemistry tests, hematology tests, urinalysis, and vital sign measurements were performed at Weeks 0, 4, and 12 of the study. The QOL was evaluated using the SF-36 ver.2 (McHorney et al., 1994; Fukuhara et

al., 1998) at Weeks 0, 4, 8, and 12. In addition, for evaluation of the immune function, interferon(IFN) γ and interleukin(IL)-10 produced in the peripheral blood were measured at Weeks 0, 4, and 12. The method for IFN γ and IL-10 measurements was modified from the one previously described by Heriot et al. (2000), and was briefly as follows: RPMI 1640 medium 1000 mL was added to heparinized peripheral blood 250 mL, to which lipopolysaccharide (LPS) was added to a final concentration of 20 μ g/mL. The mixture was then incubated at 37 degrees C in 5%CO₂ for 24 hours. The IFN γ and IL-10 concentrations in the culture supernatant were measured by ELISA (Invitrogen, Carlsbad, California, USA). Until the time of ELISA, the culture supernatant was stored frozen at -80 degrees C.

Statistical analysis

The measurement values were presented as mean \pm SD. Changes in individual parameters at each time point of measurement were evaluated using the paired t-test. SPSS Ver.13 (SPSS Japan Inc, Tokyo, Japan) was used for all statistical analyses, with a two-sided 5% significance level.

Results

QOL score

QOL scores are shown in Table 2. QOL scores were calculated using the norm-based scoring (NBS) system provided by Medical Outcome Trust, Health Assessment Lab, QualityMetric. Among the eight subscales, the mean score at Week 4, the start of L.E.M. ingestion, was lowest for Bodily Pain (BP) at 47.8 \pm 9.0, and highest for Role-Emotional (RE) at 51.7 \pm 10.5. Over the course of the study period, the QOL score did not change during the pre ingestion period during Week 0 to 4, but within 4 weeks of L.E.M. ingestion from Week 4 to Week 8, the total QOL score was found significantly increased, and also a subscale score for Vitality (VT), among other subscales, was found significantly increased. After 8 weeks of L.E.M. ingestion, the VT score was found increased from Week 4 as was seen at Week 8, although the increase was not significant.

Immunological test

Cytokine levels are shown in Table 3. In the whole study population, the cytokine production levels showed no significant changes. Given that individuals with an

Table 1. Subjects Character

| | | |
|---------------------|--------------------|-----------------|
| Age | | 56.6 \pm 11.6 |
| Hormone treatments: | Tamoxifen citrate | 14 |
| | Anastrozole | 4 |
| | Letrozole | 1 |
| | Toremifene citrate | 1 |

*The measurement values were represented as mean \pm SD

Table 2. QOL Score (Norm-based Scoring)

| | Week 4 (at the start of LEM ingestion) | Change of pre ingestion period (week 0)-(week 4) | p value | Change of 4 weeks ingestion (week 8)-(week 4) | p value | Change of 8 weeks ingestion (week12)-(week4) | p value |
|----------------------|--|--|---------|---|---------|--|---------|
| Total | 50.0 \pm 9.7 | 0.4 \pm 9.5 | N.S. | 1.7 \pm 8.6 | p<0.05 | 0.5 \pm 8.5 | N.S. |
| Physical Functioning | 50.9 \pm 7.1 | 0.2 \pm 4.1 | N.S. | 1.1 \pm 4.0 | N.S. | -0.2 \pm 8.8 | N.S. |
| Role-Physical | 50.1 \pm 9.6 | 0.5 \pm 4.3 | N.S. | 0.5 \pm 10.2 | N.S. | -0.6 \pm 7.6 | N.S. |
| Bodily Pain | 47.8 \pm 9.0 | -0.8 \pm 11.5 | N.S. | -0.6 \pm 6.2 | N.S. | -0.8 \pm 7.5 | N.S. |
| General Health | 47.3 \pm 9.2 | 0.9 \pm 6.0 | N.S. | 0.8 \pm 7.3 | N.S. | 0.2 \pm 8.4 | N.S. |
| Vitality | 49.9 \pm 10.7 | -0.5 \pm 7.6 | N.S. | 4.0 \pm 7.2 | p<0.05 | 3.4 \pm 8.6 | N.S. |
| Social Functioning | 50.8 \pm 11.2 | -1.0 \pm 9.9 | N.S. | 2.6 \pm 11.8 | N.S. | -0.4 \pm 10.5 | N.S. |
| Role-Emotional | 51.7 \pm 10.5 | 1.3 \pm 8.5 | N.S. | 2.1 \pm 10.0 | N.S. | -0.5 \pm 7.1 | N.S. |
| Mental Health | 51.4 \pm 10.7 | 0.8 \pm 8.0 | N.S. | 2.9 \pm 9.8 | N.S. | 2.5 \pm 9.3 | N.S. |

*The measurement values were represented as mean \pm SD. Comparison of each measurement between the two points was tested by the paired t-test

Table 3. Immunological Test

| | Week 0 | LEM ingestion | | | |
|---------------------------------------|-----------------|-----------------|-------------------------|-----------------|-------------------------|
| | | Week 4 | p value (v.s.week 0) | Week 12 | p value (v.s.week 4) |
| IFNγ (pg/ml) | | | | | |
| Total (n=18) | 157 \pm 221 | 151 \pm 220 | N.S. | 168 \pm 232 | N.S. |
| Normal (n=12) | 226 \pm 246 | 218 \pm 245 | N.S. | 229 \pm 266 | N.S. |
| Low (n=6) | 21 \pm 11 | 17 \pm 15 | N.S. | 46 \pm 52 | p<0.05 |
| IL-10 (pg/ml) | | | | | |
| Total (n=18) | 245 \pm 109 | 236 \pm 77 | N.S. | 246 \pm 99 | N.S. |
| Normal (n=12) | 213 \pm 86 | 209 \pm 63 | N.S. | 211 \pm 67 | N.S. |
| Low (n=6) | 308 \pm 131 | 291 \pm 80 | N.S. | 316 \pm 121 | N.S. |
| IFNγ/IL-10 | | | | | |
| Total (n=18) | 1.41 \pm 3.71 | 0.68 \pm 0.79 | N.S. | 0.76 \pm 0.94 | N.S. |
| Normal (n=12) | 2.08 \pm 4.45 | 0.99 \pm 0.81 | N.S. | 1.07 \pm 1.02 | N.S. |
| Low (n=6) | 0.08 \pm 0.06 | 0.06 \pm 0.02 | N.S. | 0.13 \pm 0.05 | p<0.01 |

*The measurement values were represented as mean \pm SD. Comparison of each measurement between the two points was tested by the paired t-test

IFN γ /IL-10 ratio of 0.2 or less are regarded as having decreased immunity (Shibata et al., 2002), a subgroup analysis was conducted with a low-IFN γ /L-10-ratio group (n=6, low group) and defined as those who had an IFN γ /IL-10 ratio of 0.2 or less at Week 4. As a result, in this low group, the IFN γ /L-10 ratio was found significantly increased from 0.06 at Week 4 to 0.13 at Week 12. Also in this subset of patients, the IFN γ production level was found significantly increased from 17 at Week 4 to 46 at Week 12. In contrast, patients who had a normal IFN γ /IL-10 ratio of 0.2 or more at Week 4 (normal group), none of the immune parameters showed changes.

Compliance and clinical finding

Eighteen out of the 20 subjects completed the study. In the blood tests, blood coagulation tests and urinalysis, no clinically significant changes were shown during the study period. No subjects had any serious adverse events. The compliance with oral L.E.M. ingestion during the study period was 75% or higher in all subjects, which met the criteria for data inclusion in analyses. In one case, one subject dropped out of the study at Week 8 because of the subject's request for withdrawal. It was confirmed that no adverse events occurred in this patient during the study participation. Another subject dropped out of the study because of skin allergy symptoms at Week 8. The allergy symptoms developed at week 7 and resolved by Week 8. This subject had had an allergic diathesis with a history of various food allergies to peanuts, mackerel, bell pepper, and other foods, and thus possible influence of meals during the study period, transient change in physical condition, *L. edodes* mycelium, and/or excipients in the test product were considered, but the cause of the symptoms could not be identified. This subject's blood chemistry, hematology, and vital sign values were within the normal range, without changes between before and after the use of L.E.M.. In all other subjects, there were no adverse events during the study period.

Discussion

The present study was performed to investigate the effects of oral L.E.M. ingestion on the QOL and immune function in breast cancer patients receiving postoperative

hormone therapy. In this study, no subjects had any serious adverse events. Therefore, at least in breast cancer patients receiving hormone therapy, the oral ingestion of L.E.M. is likely to be safe.

Among breast cancer patients, postoperative adjuvant chemotherapy has been described to decrease the QOL (Hatam N et al., 2011; Kornblith AB et al., 2011; Ohsumi S et al., 2012), while postoperative hormone therapy has been described to relatively maintain the QOL (Ochayon et al., 2010; van Nes et al., 2012). In the present study as well, the QOL scores before the start of oral L.E.M. ingestion were close to the reference scores, thereby indicating relatively maintained QOL in the subjects. Among the QOL subscales, however, the scores for three subscales of Bodily Pain (BP), General Health Perception (GH), and Vitality (VT) before the start of oral L.E.M. ingestion were below the reference scores. Of these three subscales, the VT score was found significantly increased at 4 weeks of oral L.E.M. ingestion, and increase in the VT score was maintained at 8 weeks of oral L.E.M. ingestion, although no longer significant. The total QOL score was also found significantly increased at 4 weeks of oral L.E.M. ingestion. It has been reported that oral ingestion of L.E.M. has protective effects against impairment of QOL in breast cancer patients receiving postoperative adjuvant chemotherapy (Nagashima et al., 2005). Also in patients receiving hormone therapy, this study demonstrated improved QOL following oral L.E.M. ingestion. This study's findings indicate that L.E.M. has the potential to improve the QOL in a broad range of postoperative breast cancer patients.

For evaluation of immune function in this study, cytokine production levels in peripheral blood following stimulation with LPS were quantitatively measured. LPS stimulation of peripheral blood causes a series of immune responses starting with involvement of monocytes and dendritic cells, and the resultant production of cytokines is regarded to reflect an integral part of the immune responses. In fact, it has been documented that following LPS stimulation of peripheral blood, the amount of IFN γ production decreases in association with cancer progression and the amount of IL-10 production increases in association with cancer progression (Heriot, 2000). It has been also documented that the IFN γ /IL-10 ratio decreases in patients with advanced cancer (Shibata et al., 2002; Tanigawa et al., 2012). The low-IFN γ /IL-10-ratio group, defined as those who had an IFN γ /IL-10 ratio of 0.2 or less before the start of oral L.E.M. ingestion, showed significant increases in the IFN γ /IL-10 ratio as well as the IFN γ level after oral ingestion of L.E.M. In breast cancer patients receiving postoperative adjuvant chemotherapy, oral ingestion of L.E.M. has been reported to restore the suppression of host immune responses by chemotherapy (Nagashima et al., 2005). Taken together with the results for hormone therapy of this study, the currently available findings indicate that L.E.M. has the potential to improve the immunity in postoperative breast cancer patients. Also, based on the fact that patients who had a normal IFN γ /IL-10 ratio of 0.2 or more before the start of oral L.E.M. ingestion showed no changes in immune parameters, it is speculated that oral L.E.M. ingestion does not boost the

immunity in general, but only in the immunosuppressed. A study in tumor-bearing mice reported that L.E.M. led to a decrease in tumor inducing regulatory T (Treg) cells and resolution of immunosuppression (Tanaka et al., 2011; 2012). In addition, a study in patients receiving immunotherapy reported that L.E.M. inhibited the increase of Treg cells (Tanigawa et al., 2012). It has been reported that IL-10 related Treg cells are associated with poor prognosis for breast cancer patients (Sisirak et al., 2013). In light of these findings, there is a possibility in the present study that L.E.M. influenced certain immunosuppressive factors such as Treg cells and, which should be clarified in future studies.

In summary, oral L.E.M. ingestion is safe and can be effective in improving the QOL and immune function in breast cancer patients receiving hormone therapy. Nevertheless, this implication needs to be confirmed in further larger-scale studies.

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References

- Chihara G, Maeda Y, Hamuro J, et al (1969). Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) sing. *Nature*, **222**, 687-8.
- Fukuhara S, Bito S (1998). Translation, adaptation, and validation of the SF-36 health survey for use in Japan. *J Clin Epidemiol*, **51**, 1037-44.
- Hatam N, Ahmadloo N, Ahmad Kia, et al (2011). Quality of life and toxicity in breast cancer patients using adjuvant TAC (docetaxel, doxorubicin, cyclophosphamide), in comparison with FAC (doxorubicin, cyclophosphamide, 5-fluorouracil). *Arch Gynecol Obstet*, **284**, 215-20.
- Heriot AG (2000). Reduction in cytokine production in colorectal cancer patients: association with stage and reversal by resection. *Br J Cancer*, **82**, 1009-12.
- Itoh A, Isoda K, Kondoh M, et al (2009). Hepatoprotective effect of syringic acid and vanillic acid on concanavalin a-induced liver injury. *Biol Pharm Bull*, **32**, 1215-9.
- Kornblith AB, Lan L, Archer L, et al (2011). Quality of life of older patients with early-stage breast cancer receiving adjuvant chemotherapy: a companion study to cancer and leukemia group B 49907. *J Clin Oncol*, **29**, 1022-8.
- Liu M, Li J, Kong F, Lin J, Gao Y (1998). Induction of immunomodulating cytokines by a new polysaccharide-peptide complex from culture mycelia of *Lentinus edodes*. *Immunopharmacology*, **40**, 187-98.
- McHorney CA, Ware JE Jr, Lu JF et al (1994). The MOS 36-item Short-Form Health Survey (SF-36): III. Tests of data quality, scaling assumptions, and reliability across diverse patient groups. *Med Care*, **32**, 40-66.
- Nagashima Y, Sanpei N, Yamamoto S, et al (2005). Evaluation of host immunity and side effects in breast cancer patients treated with adjuvant chemotherapy (FEC therapy). *Gan To Kagaku Ryoho*, **32**, 1550-2.
- Ochayon L, Zelker R, Kaduri L, et al (2010). Relationship between severity of symptoms and quality of life in patients with breast cancer receiving adjuvant hormonal therapy. *Oncol Nurs Forum*, **37**, 349-58.
- Ohsumi S, Shimosuma K, Ohashi Y, et al (2012). Subjective and objective assessment of edema during adjuvant chemotherapy for breast cancer using taxane-containing regimens in a randomized controlled trial: the national surgical adjuvant study of breast cancer 02. *Oncology*, **82**, 131-8.
- Okuno K, Uno K (2011). Efficacy of orally administered *Lentinula edodes* mycelia extract for advanced gastrointestinal cancer patients undergoing cancer chemotherapy: a pilot study. *Asian Pac J Cancer Prev*, **12**, 1671-4.
- Shibata M, Nezu T, Kanou H, et al (2002). Decreased production of interleukin-12 and type 2 immune responses are marked in cachectic patients with colorectal and gastric cancer. *J Clin Gastroenterol*, **34**, 416-20.
- Sisirak V, Faget J, Vey N, et al (2013). Plasmacytoid dendritic cells deficient in IFN α production promote the amplification of FOXP3 regulatory T cells and are associated with poor prognosis in breast cancer patients. *Oncoimmunology*, **2**, 22338.
- Sugano N, Hibino Y, Choji Y, et al (1982). Anticarcinogenic actions of water-soluble and alcohol-insoluble fractions from culture medium of *Lentinus edodes* mycelia. *Cancer Lett*, **17**, 109-14.
- Sugano N, Choji Y, Hibino Y, et al (1985). Anticarcinogenic action of an alcohol-insoluble fraction (LAP1) from culture medium of *Lentinus edodes* mycelia. *Cancer Lett*, **27**, 1-6.
- Tanaka K, Ishikawa S, Matsui Y, et al (2011). Oral ingestion of *Lentinula edodes* mycelia extract inhibits B16 melanoma growth via mitigation of regulatory T cell-mediated immunosuppression. *Cancer Sci*, **102**, 516-21.
- Tanaka K, Ishikawa S, Matsui Y, et al (2012). Combining a peptide vaccine with oral ingestion of *Lentinula edodes* mycelia extract enhances anti-tumor activity in B16 melanoma-bearing mice. *Cancer Immunol Immunother*, **61**, 2143-52.
- Tanigawa K, Ito Y, Sakai M, et al (2012). Evaluation of quality of life and immune function in cancer patients receiving combined immunotherapy and oral administration of *Lentinula edodes* mycelia extract. *Gan To Kagaku Ryoho*, **39**, 1779-81.
- van Nes JG, Fontein DB, Hille ET, et al (2012). Quality of life in relation to tamoxifen or exemestane treatment in postmenopausal breast cancer patients: a Tamoxifen Exemestane Adjuvant Multinational (TEAM) Trial side study. *Breast Cancer Res Treat*, **134**, 267-76.
- Yamaguchi Y, Miyahara E, Hihara J (2011). Efficacy and safety of orally administered *Lentinula edodes* mycelia extract for patients undergoing cancer chemotherapy: a pilot study. *Am J Chin Med*, **39**, 451-9.