RESEARCH COMMUNICATION

Efficacy of Orally Administered Lentinula edodes Mycelia Extract for Advanced Gastrointestinal Cancer Patients Undergoing Cancer Chemotherapy: a Pilot Study

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Abstract

This study investigated the influence of Lentinula edodes mycelia extract (LEM), an oral immunomodulator, on immune function and adverse events from chemotherapy. Subjects comprised 1 gastric and 7 colorectal cancer patients. The first course of treatment was chemotherapy alone and the second was chemotherapy plus concomitant administration of LEM. Adverse events and interferon (IFN)-γ production by CD4+ T, CD8+ T and CD56+ NK/NKT cells were evaluated at the end of each course. Grade 1 or 2 adverse events were observed at the end of the first course for 6 of 8 patients. In comparison, no patients displayed any adverse events at the end of the second course. Tendencies toward improved IFN-γ production by CD4+ T, CD8+ T and CD56+ NK/NKT cells were also seen. These results suggest that concomitant use of LEM with chemotherapy can decrease the incidence of adverse effects from cancer chemotherapy among patients with advanced cancer.

Keywords: Lentinula edodes mycelia extract - cancer chemotherapy - advanced cancer

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Introduction

It is desirable for advanced gastrointestinal cancer patients to continue chemotherapy with fewer adverse effects as long as possible. Oral adjuvant is attractive for them because it has little burden.

Lentinula edodes has long been utilized as an edible mushroom in East Asia. A 1969 report showed that Lentinan, a high molecular weight neutral polysaccharide purified from a hot water-extract of Lentinula edodes (Chihara et al., 1969), has antitumor activity. Since then, Lentinan has been approved as an antitumor injection in Japan. Progress has since been made in research into the antitumor and immunomodulatory actions of the mycelia of Lentinula edodes (Sugano et al., 1982; Sugano et al., 1985; Liu et al., 1998) and Lentinula edodes mycelia extract (LEM) with hot water before germination and after culturing in a medium composed of bagasse and rice bran, is currently utilized as oral adjuvant for cancer patient .LEM has also antitumor and immunomodulatory effects(Kojima et al., 2010; Tanaka, 2011) and LEM in combination with postoperative adjuvant chemotherapy has been reported to improve the quality of life (QOL) (Nagashima,2005). In this study, the influence of LEM on immune function and adverse events resulting from cancer chemotherapy were investigated among advanced cancer patients.

Materials and Methods

Subjects

Subject in this study comprised 8 patients undergoing chemotherapy in the Department of Surgery at Kinki University between 2006 and 2007 (see Table 1). All patients showed a performance status (PS) of 0-2 and were capable of oral ingestion. All study protocols were

Table 1. Clinical and Other Characteristics of the Subjects in this Study

Age	Sex	Primary	Metastasis	Chemotherapy	PS
69	female	colon	lung	irinotecan + UFT	0
71	male	colon	liver	mitomycin C, 5-fluorouracil (HAI) + UFT	0
66	male	rectal	local (gast.) /abdomen. carc.	taxol	0
70	female	colon	liver, lung,	5-fluorouracil + local levofolinate + irinotecan	2
52	female	rectal	lung, liver	5-fluorouracil + levofolinate	0
60	female	rectal	pelvis	UFT	0
60	female	colon	lung, liver	5-fluorouracil + levofolinate	0
54	male	gastric	lymph nodes	5-fluorouracil (p.o.)	0

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approved by the institutional board of the Department of Surgery at Kinki University and were performed in accordance with the ethical principles designated in the Declaration of Helsinki. Prior to the study, subjects were fully informed about the objectives and methods. All subjects voluntarily provided written informed consent to participate in this study.

Drug

LEM was kindly provided by Kobayashi Pharmaceutical (Osaka, Japan), and was prepared as previously reported (Itoh et al., 2009).

Briefly, Lentinula edodes mycelia were cultivated in a solid medium composed of sugar-cane bagasse and defatted rice bran. Medium containing the mycelia was incubated in hot water, and then the soluble fraction was dried and used as LEM.

Study design

This study was conducted as an 8-week single-group open study. During the study period, each subject took two courses of chemotherapy (5-fluorouracil (5-FU), irinotecan, UFT® (uracil and tegafur), levofolinate, mitomycin or taxol). LEM was orally ingested during the second course at a dose of 1800 mg/day continuously for 4 weeks.

Tumor responses and adverse event assessments

All assessments were performed at the end of the first and second courses. Tumor responses and adverse events were evaluated according to the Response Evaluation Criteria for Solid Tumors (RECIST) and the Common Terminology Criteria for Adverse Events (CTCAE) version 2, respectively.

Cytokines and reagents

Recombinant human interleukin (rIL)-12 was provided by the Genetics Institute (Cambridge, MA). Human rIL-18 and human recombinant interferon (IFN)-γ were purchased from MBL Laboratories (Nagoya, Japan) and BD Japan (Tokyo, Japan), respectively. For cell separation, anti-CD56, anti-CD4, and anti-CD8-conjugated MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) were used.

Preparation of peripheral blood lymphocyte (PBL) populations

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll Hypaque gradient centrifugation from venous blood. PBMCs were suspended at 4 × 106 cells/ml in RPMI640 medium supplemented with 10% fetal calf serum, 5 mM Hepes and antibiotics. The suspension was cultured for 30 min in tissue culture dishes to remove adherent cells, yielding PBL populations.

Positive selection of CD56+ NK/NKT cells

CD56+ cells were separated from PBL by magnetic cell sorting using MidiMACS separation columns (Miltenyi Biotec, Bergisch Gladbach, Germany) according to a procedure that has previously been described in detail (Uno et al., 2003). The purity of CD56+ cells was in the range of 70-90% throughout this study. These cells were used as CD56+ NK/NKT cells.

Positive selection of CD8+ and CD4+ T cells

A PBL population was labeled with anti-CD56-conjugated microbeads and cleared of CD56+ cells by two passages through MidiMACSLS+ columns (Miltenyi Biotec, Bergisch Gladbach, Germany). Contamination by CD56+ cells in the resulting CD56- population was <1%. CD56-CD8+ cells were positively separated by exposure to a magnetic field, as described previously((Uno et al., 2003)). Purity ofCD56-CD8+ cells was 95-99%. The eluted population (CD56-CD8- cells) was treated with anti-CD4-conjugated microbeads and exposed to a magnetic field. CD56-CD4+CD8- cells were positively separated (purity, 95-99%). These cells were used as CD4+ and CD8+ T-cell populations after the removal of residual macrophages by adherence to plastic.

Stimulation with rIL-12 or rIL-18

CD4+ and CD8+ T-cell populations (2×10^5 cells/well) and CD56+ cell-enriched populations (1×10^5 cells/well) suspended in complete culture medium were distributed into each well of 96-well plates at volume of 0.2 ml/well. Cells were cultured with 1000 pg/ml of rIL-12, 100 ng/ml rIL-18 or rIL-12+rIL-18 in 5% CO2 at 37°C for 20 h. Supernatants were harvested by centrifugation and stored at -80°C until use.

Measurement of IFN-γ

Concentrations of IFN-γ were measured by enzyme-linked immunosorbent assay according to a previously described procedure (Uno et al., 2003).

Statistical analysis

The measurement values are presented as mean ± standard error of the mean. Comparisons of each measurement between the two courses were analyzed using Student's paired t-test. SPSS version 13 (SPSS Japan, Tokyo, Japan) was used for all statistical analyses using a two-sided significance level of ≤5%.

Results

Adverse events

Adverse events that occurred during chemotherapy were judged using the criteria of CTCAE version 2. No adverse events assessed as grade 3 or worse occurred with any treatment. Nausea was observed in the first course (4/8 grade 1, 2/8 grade 2), along with abdominal pain (1/8 grade 1) but no adverse events were seen in the second course.

IFN-γ production by CD4+ T, CD8+ T and CD56+ NK/NKT cells

CD4+ and CD8+ T cells did not produce IFN-γ with stimulation using IL12 or IL18 alone, while IFN-γ production by CD4+ T cells stimulated using IL12+IL18 (from 471 pg/ml to 627 pg/ml), CD8+ T cells stimulated using IL12+IL18 (from 1493 pg/ml to 1735 pg/ml) and CD56+ NK/NKT cells stimulated using IL18 (from 8298 pg/ml to 9000 pg/ml) tended to increase ($p<0.1$).

Clinical findings

All patients were evaluated for response to chemotherapy with and without LEM. No patients showed marked response (complete or partial response). However, no patients deteriorated during the treatment period. All patents were assessed as showing stable disease (SD) on computed tomography (data not shown).

Other parameters

No clinically significant differences were detected in any of the other parameters investigated.

Discussion

In this study, adverse events up to grade 2 were observed in the first course. However, no adverse events were observed in the second course. Accordingly, LEM ingestion under the conditions of this study appears to have been useful for decreasing the incidence of adverse effects caused by cancer chemotherapy.

With 5-FU, irinotecan, UFT, levofolinate, mitomycin C and taxol mono- or combination cancer chemotherapies, mild to moderate nausea and pain or other side effects are common (Gonzalez Baron et al., 1993; Naitoh et al., 1997; Takiuchi et al., 1998; Malet-Martino et al., 2002; Glimelius, 2005; Casado et al., 2008). In terms of effectiveness for patients undergoing chemotherapy, LEM has been reported to improve quality of life among breast cancer patients treated with adjuvant chemotherapy (Nagashima et al., 2005). However, no reports have clarified the effects of LEM on adverse events from chemotherapy. This is the first report on LEM to confirm effects against adverse events caused by chemotherapy. LEM has been reported to contain phenolic compounds, syringic and vanillic acid, with highly antioxidant activity (Itoh et al., 2009). Antioxidants can reportedly reduce the side effects of anti-cancer agents(Conklin, 2000), so the LEM constituents syringic and vanillic acid might have contributed to the suppression of adverse events in this study. However, LEM does not have high levels of syringic and vanillic acid contents, so components of LEM other than antioxidants seem likely to be involved in attenuating chemotherapy side effects.

In this study, grade 1 nausea and abdominal pain and grade 2 nausea were encountered, and no suspension of chemotherapy was needed in the first course. Further evaluation of LEM effects for grade 3 or worse side effects in cancer patients with chemotherapy is required.

IFN-γ production by CD4+, CD8+ T and CD56+ NK/NKT cells tended to be increased by LEM in this study. LEM reportedly acts to recover NK cell-activity suppressed by cancer chemotherapy (Nagashima et al., 2005) and β-glucan in LEM-activated antigen-presenting cells (APCs)(Kojima et al., 2010), but this is the first report the CD4+ and CD8+ T-cell activation effects of LEM in cancer patient.

Tumor-bearing patients reportedly exhibit high IL-12 or IL-18 responsiveness in T cells, and this responsiveness is induced depending on the presence of functional sets of APCs (Uno et al., 2003), so LEM might enhance CD4+ and CD8+ T-cell responsiveness by activating APCs. However, limited data were available from this study and further investigations are necessary.

In summary, ingestion of LEM decreased adverse events in a preliminary study. Ingestion of LEM appears effective for patients with advanced cancer undergoing chemotherapy. Larger-scale controlled studies are thus warranted.

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