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Effect of Lentinan in Induction of Apoptosis on Gastric Adenocarcinoma Cells

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Background: Gastric cancer is the second most common cause of cancer death worldwide. Lentinan was shown to induce apoptosis in gastric cancer cells and could be used for the treatment of gastric cancer.
Objectives: In this study, we analyzed anticancer effect of lentinan, a fungal β-glucan, on the gastric adenocarcinoma cell line (AGS).
Materials and Methods: We used the DNA ladder and TUNNEL approaches to evaluate the apoptotic effect of lentinan on the AGS cell.
Results: Evaluation of apoptosis by Apoptotic DNA Ladder in lentinan treated and untreated AGS cells by DNA laddering and fragmentation, and TUNEL tests confirmed that application lentinan caused a significant increase in apoptosis in the AGS cell line.
Conclusions: Treatment of human gastric adenocarcinoma cell line with lentinan can offer a possible approach to counteract the human gastric adenocarcinoma cells, thus can be applied in a combination with the routine gastric cancer therapy drugs.

Keywords: Stomach Neoplasms; Apoptosis; Lentinan

1. Background

Gastric cancer is the second most common causes of cancer related death in the world (1) and is responsible for two third of cancer related death in the developing countries (2). Although surgery is one of the most common ways for gastric cancer treatment, its survival rate is less than 33%. Radiation therapy and chemotherapy as alternatives for surgery in the treatment of gastric cancer are not very promising. Thus there is an urgent need for introducing novel treatment procedures and promising new anti-canceric drugs (1). Recently new treatment approaches for gastric cancer has been proposed (3-6) and among them taking complementary medical therapies while receiving their conventional anti-cancer treatments has attracted many attention (7-12). Shiitake mushroom, *Lentinula edodes* produces lentinan, a β-glucan. The ethanol extract of this mushroom significantly decreased cell proliferation of tumorigenic keratinocyte, whereas it could not change the proliferative response of the non-tumorigenic keratinocyte cell line (13). Two mechanisms have been proposed to be responsible for the anti-cancer effect of this herbal extracts; one is via direct cytotoxic effect and the other is indirectly through immunomodulatory action (14, 15).

2. Objectives

In this study we planned to study the anti-gastric cancer effects of lentinan by evaluation of apoptosis activation in this cancerous cells following treatment by lentinan.

3. Materials and Methods

3.1. Exposure of Gastric Adenocarcinoma Cell Line to Lentinan

AGS (gastric adenocarcinoma cell line) cells obtained from Iranian Pasteur Institute (C131) in RPMI 1640 with 10% FBS and after subculture, 1×10^4 AGS cells seeded to each wells of a 12 well cell culture plates (Falcon, USA) containing 2 mL RPMI 1640 with 10% of FBS and 10% of antibiotic antimycotic solution (Gibco, Glasgow, UK) and after 72 hours supernatant of the wells were removed and the cells washed twice with PBS with 1% FBS, incubated overnight in 2 mm RPMI 1640 supplemented with 1% FBS and 15 mL of different concentration of selenit sodium, lentinan to a final concentration of 10 μg/mL (16) and sterile double distilled water as negative control. After 24 hours, supernatant of the wells were removed, their cells were washed twice with PBS and resuspended by adding Trypsin/EDTA (Gibco, Glasgow, UK) (17, 18). After centrifugation, the pellet cells resuspended in 1 mL of HPSS salt solution and its volume increased to 10 mL with 70% etha-

Implication for health policy/practice/research/medical education:
Results of this study show that lentinan can be used as a complementary treatment in cancer.

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nol. The suspension maintained in -20°C till the time of evaluating experiments (19).

3.2. Evaluation of Apoptosis by Apoptotic DNA ladder

Evaluation of Apoptosis by Apoptotic DNA ladder was done by Apoptotic DNA ladder kit according to its manual (Roche, Germany). Briefly, one of the 15 mL tubes containing AGS treated cells preserved in 70% ethanol was removed from freezer and after thawing, centrifuged at 200g for 10 minutes. Sediment was re suspend in 1 mL culture media containing 1% FBS and centrifuged at 1500g for 5 minutes. The pellet cells, resuspended in 200 mL of PBS and 200 mL of Binding/Lysis Buffer supplied with the Kit was added to the Cell suspension and after incubation, addition of isopropanol, centrifugation and subsequent washing, resulted DNA was dissolved in 200 mL of Kit's elution buffer. Positive control of the kit used as positive control in Gel electrophoresis of DNA. Gel electrophoresis was done in a 2% gel and stained with SYBER Green' I Nucleic Acid Gel Stain.

3.3. Evaluation of Apoptosis by In Situ Cell Death Detection Kit, Fluorescein (TUNEL)

Evaluation of apoptosis by In Situ cell death detection Kit (TUNEL) (Roche, Germany) was done according to the manual of the kit. Briefly, one of the 15 mL tubes containing AGS treated cells preserved in 70% ethanol was removed from freezer and after thawing, centrifuged at 200g for ten minutes. Sediment was resuspend in 1 mL culture media containing 1% FBS and centrifuged at 1500 g for five minutes. The pellet cells, resuspended in PBS to the final concentration of 2×10^7 cells/ml and 100 mL from this cell suspension were transferred into a V-bottom shaped 96 well micro plate. After addition of fixation solution and subsequent incubation, the plate has centrifuged at 300 g for ten minutes and after washing with PBS, the cells were resuspended in 100 mL of permeabilisation solution for two minutes on ice. After washing with PBS, 50 mL of TUNEL reaction mixture was added. For negative control (untreated AGS cells) only 50 mL of labeling solution was added. Non treated, fixed and permeabilized cells incubated with DNaseI recombinant for ten minutes at room temperature was used as positive control. After incubation in darkness, washing and transferring cells into 250 mL of PBS, samples directly analyzed under Olympus x7 fluorescent microscope with WIB filter.

4. Results

4.1. Induction of Apoptosis by Lentinan in AGS Cells

Evaluation of apoptosis by Apoptotic DNA Ladder in lentinan treated and untreated AGS cells showed that DNA laddering and fragmentation in cells treated with lentinan (Figure 1). To confirm that the anti-cancer effects of lentinan is only due to apoptosis, a discrete AGS cell line was treated with the selenite sodium, a necrosis inducing agent, and then cell death induced by selentine sodium was compared to that imposed by lentinan. DNA laddering which is one of the characteristics of apoptosis is obvious in line 5 and necrotic effect of 4 and 2.5 mM of Selenite sodium is seen in lane 2 and 3 of Figure 1. Also, TUNEL test in AGS cells were treated with 10 μg lentinan per mL of RPMI 1640 culture media supplemented with 1% of BSA. This analysis showed a significant increase in apoptosis in comparisons to the untreated AGS cells and Selenite sodium control (the concentrations of selentine sodium applied were 2.5 and 4 mM) (Figure 2) (20).

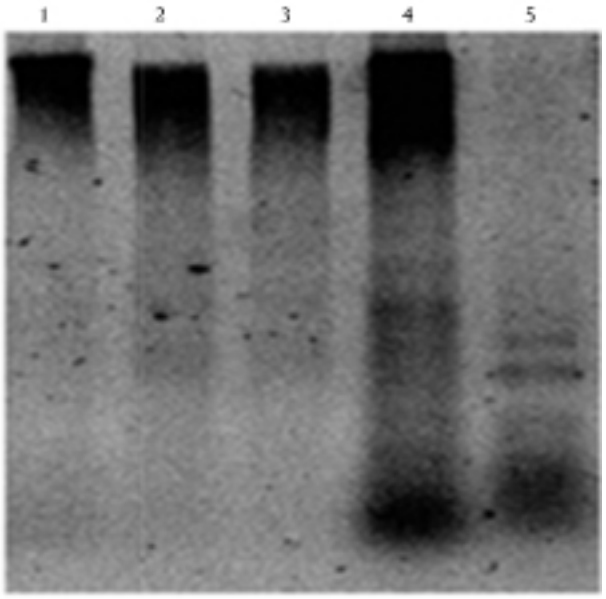
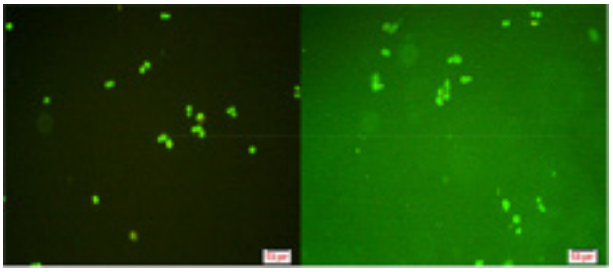


Figure 1. Line 1, untreated AGS cells; line 2 and 3, 4 and 2.5 mM selenite sodium; line 4, control positive of the Kit; line 5, Lentinan in 10 μg/mL concentration. Data from double check experiments.

Figure 2. Induction of Apoptosis by 10 μg/mL of Lentinan



A) A representative TUNEL staining of AGS cells transfected with 10 μg/mL of Lentinan; B) control positive of the test were shown.

5. Discussion

Evaluation of apoptosis by apoptotic DNA ladder in lentinan treated and untreated AGS cells by DNA laddering and fragmentation and TUNEL test confirmed that treatment of AGS cell lines with lentinan in cancer cells significantly increased apoptosis in these cells. There are numerous studies that confirm combination therapy of gastric cancer patients with lentinan and one anticancer drug enhanced survival in patients with advanced gastric cancer (21, 22). Zhao and colleagues showed that application of low concentration of lentinan combined with anti-cancerous drugs has better therapeutic effects on the proliferation of BGC823 cells (23) and Ina et al. in their study showed that Chemo-immunotherapy with lentinan offers a significant advantage over chemotherapy alone in patients gastric cancer (24). Li and colleagues reported that thermotherapy combined with thoracic injection of lentinan showed better effect in patients with lung cancer (25). However there are some controversy about it (26). These reports are inconsistence with our results that lentinan is a useful complementary nutrient in the treatment of gastric cancer and more over results of this study showed that induction of apoptosis is a major pathway in the process of anti-cancerous effects of lentinan.

In this study we used lentinan for treatment of AGS cells and induced apoptosis was evaluated by related apoptotic DNA ladder and TUNNEL. All of these experiments confirmed that treatment of AGS cell lines with lentinan increased apoptosis in these cells.

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Author's Contribution

Conception and design of the study: Reza Najafipour; laboratory work and data analysis and interpretation, Taghi Naserpour Farivar and Pouran Johari.

Financial Disclosure

There is no conflict of interest.

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