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Effect of *Lentinus edodes* polysaccharide on oxidative stress, immunity activity and oral ulceration of rats stimulated by phenol

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ABSTRACT

Oral ulceration in 30 rats was induced by phenol. Rats were randomly divided into the following three experimental groups (each group contained 10 rats): group 1, untreated oral ulceration rats (control group); group 2, polysaccharides-treated oral ulceration rats (low-dose polysaccharides group); group 3, polysaccharides-treated oral ulceration rats (high-dose polysaccharides group). Low-dose and high-dose groups were treated with 0.3 ml of polysaccharides solution by orally. Control group were treated with the same volume of physiological saline. The treatments lasted for 18 days. Then, sera and oral mucosa from ten rats with oral ulceration, and 20 rats with *Lentinus edodes* polysaccharide (LEP) treatment were analysed. The results showed that LEP administration significantly raised activities of serum antioxidant enzymes and decreased levels of serum, mucosal interleukin-2 (IL-2) and tumor necrosis factor alpha (TNF-α) in rats with oral ulceration. These results suggest that LEP may play a part in ameliorating oral ulceration.

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1. Introduction

An oral ulcer (from Latin ulcus) is the name for the appearance of an open sore inside the mouth caused by a break in the mucous membrane or the epithelium on the lips or surrounding the mouth. The types of oral ulcers are diverse, with a multitude of associated causes including: physical or chemical trauma, infection from microorganisms or viruses, medical conditions or medications, cancerous and sometimes nonspecific processes. The histopathological changes in the pre-ulcerative stage include infiltration of the epithelium by mononuclear (lymphocytic) cells. There is a cell-mediated immune response that involves T-cells with generation of tumor necrosis factor alpha (TNF-α) from these and other leucocytes (macrophages and mast cells) (Jurge, Kuffer, Scully, & Porter, 2006). TNF-α is a major inflammatory cytokine that has a chemotactic effect on neutrophils (Royer-Pokora et al., 1995) so driving acute inflammation and expression of major histocompatibility (MHC) complexes. This results in the targeting of epithelial cells for attack by cytotoxic (CD8+) T-cells. Other cytokines that may also be implicated include interleukin-2 (IL-2), or interleukins IL-1β and IL-6 (Kim et al., 2003).

During the past 30 years, many polysaccharides and polysaccharide–protein complexes have been isolated from mushrooms, fungi, yeast, algae, lichens, and plants. The biological activities of these polysaccharides have attracted more attention recently in the biochemical and medical fields because of their immunomodulatory and antitumor effects (Ajith & Janardhanan, 2003; Santos-Neves et al., 2008; Wong, Wong, Chiu, & Cheung, 2007; Zhang, Cheung, Chiu, Wong, & Ooi, 2006). Recently, lentinan, schizophyllan, and krestin have been accepted as immunoceuticals in several oriental countries (Borchers, Stern, Hackman, Keen, & Gershwin, 1999 and Liu, Ooi, & Fung, 1999). Augmentations of NK, cytotoxic T lymphocytes (CTL) and delayed-type hypersensitivity (DTH) responses against tumor antigen were observed after administration of Lentinan (Li et al., 2008; Vetvicka et al., 2007; Miyakoshi, Aoki, & Mizukoshi, 1984). Administration of lentinan, schizophyllan, and PSK was known to inhibit the growth of various transplantable tumors in experimental animals and increase the survival rate (Liu et al., 2007). *Lentinus edodes* (shiitake) comprise the groups of edible fungi with important medicinal properties and biotechnological and environmental applications. Pharmacological function of polysaccharides isolated from *L. edodes* have been reported in china (Shen, Su, Xu, & Bi, 2007; Zhong, Lin, Wang, & Liu, 2007). A recent research investigated the effect of the LEP on oral ulceration of rats (Mi, Li, & Ou, 2005). In this study, we evaluated the effect of LEP on the healing rates of the rats with oral ulceration.

2. Methods

2.1. Materials

LEP was prepared according to the method described by Chen, Zhong, Zeng and Ge (2008).

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Powdered *L. edodes* (100 g) were homogenized and extracted three times in a blender with 2 L of distilled water for 3 h at room temperature and then at 100 °C for 3 h. The whole extract was filtered and centrifuged at 12,000 rpm for 30 min at 4 °C. The supernatant was concentrated to 80 ml and precipitated by the addition of ethanol in 1:4 ratio (v/v) at room temperature (RT). After overnight precipitate, the sample was centrifuged as described above, and the precipitation was dissolved in 100 ml of distilled water. This process was repeated three times. The precipitate was then washed with sevage reagent (isoamyl alcohol and chloroform in 1:4 ratio) (Staub, 1965) and freeze dried, giving the crude polysaccharide from *L. edodes*.

2.2. Subjects

Thirty wistar rats were used. They were approximately 90 days old and, upon arrival, weighed between 201 and 250 g. They were initially housed in group cages and housed on dry wood-shavings bedding with ad lib food and water. Two weeks after arrival, at the beginning of the experiment proper, the rats were weighed and moved to individual plastic cages. Food and water were always freely available in the cages, except as noted in the procedures. The procedures employed received prior approval in accordance with the laws and regulations controlling experiments on live animals in China.

2.3. Animal treatment

Oral ulceration of rat was induced by the method described by Jiang, Fu, Tian, Wang, and Liu (2003). Polysaccharides were dissolved in distilled water and were fed by gavage to mice once a day. Rats were randomly divided into the following three experimental groups (each group contained 10 rats): group 1, untreated oral ulceration rats (control group); group 2, polysaccharides-treated oral ulceration rats (low-dose polysaccharides group); group 3, polysaccharides-treated oral ulceration rats (high-dose polysaccharides group). Low-dose and high-dose groups were treated with 0.3 ml of polysaccharides solution by orally. Control group were treated with the same volume of physiological saline. The treatments lasted for 18 days. Twenty-four hours later after the last drug administration, the animals were euthanized. Oral ulceration area was accurately measured. Blood samples were collected and centrifuged at 3000 rpm/min at 4 °C for 10 min to obtain serum. Oral mucosa was excised from the animal and stored at −20 °C. Oral mucosa was centrifuged and supernatants were then subjected to the measurement of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities, and malonaldehyde (MDA) levels. The healing rate (%) of the oral ulceration was calculated by the following equation: the healing rate $(\%) = 100 \times (A - B)/A$, where A is the initial average area of the oral ulceration and B is the final average area of the oral ulceration.

2.4. Analysis

SOD, GSH-Px activities and MDA level were assessed using commercially available kits (Stressgen Biotechnologies, Victoria, BC, Canada) and according to the kits' manual. One unit of enzyme activity was defined as the amount of protein needed to decrease the reference rate to 50% of maximum inhibition.

IL-1β and TNF-α were measured by standard ELISA techniques using commercially available BD OptEIA ELISA kits (BD Biosciences, San Diego, CA), as described previously (Stapp, Sjoelund, Lassiter, Feldhoff, & Feldhoff, 2005).

3. Results

3.1. Effect of polysaccharides on serum antioxidant enzymes activities and MDA level

We studied the effects of the LEP on serum antioxidant enzymes of oral ulceration rats (Table 1). The results show that administration of LEP for 18 days significantly decreased serum MDA production and increased antioxidant enzymes activities in oral ulceration rats ($P < .05$, $P < .01$) compared to those of untreated control group. Moreover, the serum antioxidant enzymes activities in high-dose LEP group were stronger than those in low-dose LEP group. The effectiveness of the LEP in maintaining antioxidant enzyme activities paralleled the effects of the compound on MDA production.

3.2. Effect of polysaccharides on serum IL-1β and TNF-α level

We studied the effects of the LEP on serum IL-1β and TNF-α level of oral ulceration rats (Table 2). The results show that administration of LEP for 18 days significantly increased serum IL-1β and TNF-α level in oral ulceration rats ($P < .05$, $P < .01$) compared to those of untreated control group. Moreover, the serum IL-1β and TNF-α level in high-dose LEP group were stronger than those in low-dose LEP group.

3.3. Effect of polysaccharides on IL-1β and TNF-α level in oral mucosa

We studied the effects of the LEP on IL-1β and TNF-α level in oral mucosa of rats (Table 3). The results show that administration of LEP for 18 days significantly increased IL-1β and TNF-α level in oral mucosa of rats ($P < .05$, $P < .01$) compared to those of untreated control group. Moreover, the IL-1β and TNF-α level in oral mucosa of rats of high-dose LEP group were stronger than those in low-dose LEP group.

Table 1
Effect of polysaccharides on serum antioxidant enzymes and MDA level

Group	SOD (U/ml)	GSH-Px (U/ml)	MDA (μmol/ml)
Control group	227.51 ± 11.27	19.51 ± 1.61	6.17 ± 0.42
LEP (I)	324.21 ± 20.73[a]	37.54 ± 2.71[a]	4.05 ± 0.31[a]
LEP (II)	390.04 ± 25.91[a]	48.20 ± 3.15[a]	3.03 ± 0.16s[a]

[a] $P < .01$, compared with control group.

Table 2
Effect of polysaccharides on serum IL-1β and TNF-α level

Group	IL-1β (pg/L)	TNF-α (pg/L)
Control group	158.29 ± 9.03	123.25 ± 10.11
LEP (I)	110.23 ± 4.09[a]	84.18 ± 3.02[a]
LEP (II)	77.12 ± 1.87[a]	66.15 ± 2.35[a]

[a] $P < .01$, compared with control group.

Table 3
Effect of polysaccharides on IL-1β and TNF-α level in oral mucosa

Group	IL-1β (pg/L)	TNF-α (pg/L)
Control group	194.13 ± 11.74	128.35 ± 8.11
LEP (I)	147.26 ± 8.07[a]	98.27 ± 6.03[a]
LEP (II)	80.21 ± 4.18[a]	57.22 ± 2.02[a]

[a] $P < .01$, compared with control group.

Table 4
Effect of polysaccharides on oral ulceration healing rate

Group	Average diameter (mm)		Healing rate (%)
	1 d	18 d	
Control group	2.32 ± 0.14	2.21 ± 0.11	4.7
LEP (I)	2.21 ± 0.10	1.13 ± 0.06[a]	48.9
LEP (II)	2.33 ± 0.18	0.52 ± 0.04[a]	77.8

[a] $P < .01$, compared with control group.

3.4. Effect of polysaccharides on oral ulceration healing rate

The observation of increased oral ulceration healing rate of experimental rats after administration of LEP was confirmed by assessing the final average area of the oral ulceration (Table 4). The final average diameter of the oral ulceration in experimental rats was significantly decreased ($P < .01$) in the two polysaccharides groups (1.13 ± 0.06, 0.52 ± 0.04) compared with that of the control group (2.21 ± 0.11). Compared with control group, the significantly increased oral ulceration healing rate of experimental rats after treatment with LEP (Table 4) were found. Moreover, the oral ulceration healing rate in experimental rats of high-dose LEP group were stronger than those in low-dose LEP group.

4. Discussion

Oral ulceration is characterised by multiple, recurrent, small, round, or ovoid ulcers with circumscribed margins, erythematous haloes, and yellow or grey floors that present first in childhood or adolescence (Ilan, Droguett, & Roy, 1997; Ilan et al., 2000). Oral ulceration is common in china, affecting up to 25–50% of adults. Most ulcers are small (<5 mm in diameter) and heal in 7–10 days. Larger ulcers need a longer time to healing. The cause of oral ulceration is still unknown.

Inflammatory reactions induced by phenol are a significant source of reactive oxygen species (ROS). ROS, e.g., H_2O_2 and the superoxide radical (O_2^-), play a causative role in oral ulceration. Inflammatory injury is associated with increased generation of ROS, among other mediators (Gilroy & Perretti, 2005). Among the ROS, H_2O_2 can remain for a long duration inside the cell and often performs the role of a second messenger for various physiological stimuli such as angiotensin (Ushio-Fukai, Alexander, Akers, & Griendling, 1998), inflammatory cytokines, growth factors (Bae et al., 1997), and transforming factors (Lafon et al., 1996). To control the destructive potential of ROS, cells have developed several defence mechanisms, including enzyme systems such as superoxide dismutases, catalase, glutathione peroxidase, and gastric peroxidases (Brawn & Fridovich, 1980; Halliwell & Gutteridge, 1981). Treatment with phenol induced the expected actions in the oral mucosa of rats, and those included severe histological damages (necrosis, ulceration), and a significant decrease in the activities of serum antioxidant enzymes and the concentrations of the serum and mucosa IL-1β and TNF-α. Mushrooms play an important role in food and medicine (Molitoris, 1994), so many researchers paid much attention on them. One (1 → 3)-β-d-glucan, named *Lentinan* as an antitumor polysaccharide, has been isolated from the fruiting body of *L. edodes* by Maeda and Chihara (1973) and Maeda, Chihara, and Ishimura (1974). Antioxidant activities of LEP have been reported (Chen et al., 2008; Choi, Lee, Chun, Lee, & Lee, 2006). In this work we selected LEP as a reference anti-ulcer drug because it has been shown a much better anti-ulcer effect (Ngai, & Ng, 2003). It can been observed that the activities of antioxidant enzymes in rats of oral ulceration were significantly enhanced with LEP treatment ($P < .01$). This indicates that excessive accumulation of free radicals in oral mucosa tissue may be a potential cause of oral ulceration. Moreover, LEP is capable of acting as an antioxidant, and thus can reduce mucosal damage mediated by oxygen free radicals.

Many researchers view the causes of aphthous ulcers as a common end product of many different disease processes, each which is mediated by the immune system.[2] Aphthous ulcers are thought to form when the body becomes aware of and attacks chemicals which it does not recognize. The presence of the unrecognized molecules garners a reaction by the lymphocytes, which trigger a reaction that causes the damage of a mouth ulcer. Repeat episodes of mouth ulcers can be indicative of an immunodeficiency, signalling low levels of immunoglobulin in the mucous membrane of the mouth. Vitamin C deficiencies may lead to scurvy which impairs wound healing, which can contribute to ulcer formation (Cheney & Rudrud, 1974). Similarly deficiencies in vitamin B12, iron, zinc (Challacombe, Barkhan, & Lehner, 1977) and folic acid has been linked to oral ulceration. Tetracycline suspension is a common antibiotic prescribed for mouth ulcers. Some doctors may also prescribe a local anesthetic, such as lidocaine, for cases of multiple or severe mouth ulcers. But, perhaps, it may be possible to set up a system or periodic table where plants and other natural remedies could, according to their properties, be arranged to produce specific formulae that provide well-being for a given pathology. Some researchs (Aksungur et al., 2004; Sannomiya et al., 2005) confirm a high healing rate, without side effects, in patients treated with plants and other natural remedies. This suggests that plants and other natural remedies are a superior therapeutic tool.

In general, the immunostimulatory effects of lentinan are attributed to macrophages and T-cells. Several studies reported changes in cytokine production of macrophages or monocytes in mice and in humans, e.g., enhanced production of TNF-α or IL-1 after in vivo lentinan administration (Lull, Wichers, & Savelkoul, 2005 and Hou, Zhang, Xiong, Li, & Yang, 2008). Lentinan treatment also enhances production of NO and the cytotoxic activity of macrophages (Ohno et al., 2001) and (Hou & Chen, 2008). Lentinan also influences the frequency of T-cell populations as well as the effector function of T-cells (Shin et al., 2003). Previous studies have shown some effects of lentinan on specific immune functions, and our present work show that administration of LEP can dose-dependently significantly increase serum and oral mucosa IL-1β, TNF-α levels in rats of oral ulceration. At last, it can be found that healing rate of oral ulceration have been significantly enhanced with LEP treatment. This is consistent with the original report by who found that LEP was effective to oral ulceration in animal experiment (Gao, Hirakawa, Min, Nakamura, & Hattori, 2006).

In conclusion, the present work has suggested that the LEP can ameliorate phenol-induced oral ulceration, and that, in view of the fact that phenol induces ulceration by an antioxidant action, and that LEP contain relatively high amounts of anti-oxidant substances, it is possible that the mechanism of the protective action of LEP is partly via an oxidant action, however, other mechanisms cannot be excluded.

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