Journal of Food Science and Agricultural Technology

International peer-reviewed scientific online journal

Published online: http://jfat.mfu.ac.th

Original Research Article

Immuno-regulatory activities of non-starch polysaccharide extracted from rice during grain development

Praemai Jungrussameepanich[1], Tzou Chi Huang[2] and Prisana Suwannaporn[1]*

[1]Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand

[2]Department of Biological Science and Technology, National Pingtung University of Science and Technology, Neipu 912, Taiwan

ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form 18 December 2014
Accepted 28 January 2015

Keywords:
Grain development
Immune
Macrophage
NSP
Rice

ABSTRACT

Rice in an immature ripening growth phase is a source of vitamin, bioactive compounds and dietary fiber such as non-starch polysaccharides (NSP). NSP from plant has been shown to have immuno-stimulatory activities. In this study, rice at 13th days after anthesis was extracted for the water soluble NSP by enzymatic gravimetric method. The NSP was isolated and purified using gel filtration column. The immuno-regulatory activity was tested on murine macrophage cell line (RAW264.7) including, morphological changes of actin cytoskeleton and expression of costimulatory molecules. The results showed that NSP did not induce cytotoxic effect. In addition, the changing of morphology of macrophage cells was detected after treated with NSP. NSP from rice could enhance the expression of costimulatory molecules (CD80 and CD86) and major histocompatibility complex molecules II (MHCII) in dose dependently manner. These results indicated that NSP from the immature rice was non-toxic and had effect on the immuno-regulatory activity on macrophage cells.

* *Corresponding author*:
Email: prisana.s@ku.ac.th

Published by School of Agro-Industry, Mae Fah Luang University

ISSN: 2408-1736

INTRODUCTION

Rice is staple food in Thailand and others country in Asia. Consumption of rice mostly eating cooked rice that from mature rice grains due to mature grains has high carbohydrate which is the source of energy. Moreover, rice grains were consumed in the stage of immature grains especially ripening growth phase called milky stage. Rice grains in the milky stage were made to be a beverage called rice milk. It is a source of vitamin, mineral, bioactive compounds and dietary fiber such as non-starch polysaccharides. (Lin and Lai, 2011)

Immune system has physiological functions to defense against infectious microbiology and foreign substances. The immune system can be divided into two functional including innate immune system and adaptive immune system. Innate immune system is the early line of defense against infectious agents and it consists of cellular and biochemical defense mechanisms that are response rapidly to infections. Adaptive immune system has an ability to remembers and respond more vigorously to repeated exposures to the same infection agents. (Abbas *et al.* 2012; London, 2010)

Macrophages are important for support the homeostasis and host defense against intracellular parasitic bacteria, pathogenic protozoa and fungi, as well as tumors. Not only initiate innate immune responses but macrophages also effect to cells that contribute to the resolution of these responses. Activated macrophages are mentioned to be the important monocytes of the host defense against tumor growth (Kim *et al.*, 2004*).* Macrophages play an important role to protect the host by engulfing and killing microbes, present antigens to lymphocytes and release biologically active compounds which regulate the activity of other cells. (Leiro *et al.*, 2007) Moreover, macrophage cells can initiate the adaptive immune response to pathogens by presenting antigen to CD4+ T cells via class II MHC antigen (Beutler, 2004).

The T cell immune response need signals from co-stimulatory which delivered through one or more receptors on the surface of T cells. T cell-stimulatory pathway is initiated when the CD28 binds to the B7 ligands, B7-1 (CD80) and B7-2 (CD86), on the antigen-presenting cells. CD80 and CD86 are expressed on the surface of antigen-presenting cells with the interaction to cytotoxic T lymphocyte antigen-4 expressed on activated T cells and mediate critical T cell inhibitory signals (Vasu *et al.*, 2003). When activated B cells express CD80 and CD86, they received or exchanged the signals from T cells (O'Neill *et al.*, 2007).

Some types of T lymphocytes are required to interact with other cells in immune system such as dendritic cells, macrophages, and B lymphocytes. Other type of T lymphocytes must be interacting with infected host cell. T cell antigen receptors are designed to specific which could see antigen displayed by host cell surface molecules not antigens on microbes or free antigen in circulation fluids. To perform that, major histocompatibility complex (MHC) molecules are function to display the host cell-associated antigens for recognition. MHC molecules are proteins which are expressed on the surfaces of host cells. Class I molecules are expressed on all nucleated cells, while class II molecules are expressed on dendritic cells, B lymphocytes, macrophages and some type of cells (Abbas *et al*., 2012).

The polysaccharides which have immune enhancing properties not only improve host defense against pathogens but they also enhance the actions of adaptive immunity. Immune-enhancing of polysaccharides could control the modulation levels of lymphocytes, macrophages, cytokines and antibodies. (Ren *et al.*, 2014) Polysaccharide from botanical sources, such as mushrooms, algae, lichens and higher plant, were mentions to the biomedical activities due to their therapeutic properties. Macrophage stimulation and modulation of complements systems are the cause of immunostimulatory, anti-tumor, bactericidal and others therapeutic effects of botanical polysaccharides. Polysaccharide derived from higher plant have been showed to enhance the functions of macrophage such as increase the production of ROS and NO, enhance cytokines and chemokines secretions enhance the activation of phagocytic activity and increase the activity against tumor cells and microbes (Schepetkin and Quinn, 2006). The health benefits of polysaccharide from plants might be the reason why this research interested in immuno-stimulatory activities of rice grain during development. The aim of this research is to find the immuno-regulatory activities of polysaccharide extracted from rice during grain development which are important to stimulate the activities of adaptive immune system especially T cell and B cell.

MATERIALS AND METHODS

Extraction of water soluble non-starch polysaccharide

Rice grain (Pathumthani 1 variety) was collected from Pathum Thani Rice Research Center (Rice Department, Ministry of Agriculture, Thailand) at 13th DAA (ripening stage) and stored at -18°C before extraction. Samples were ground and defatted with hexane in ratio ground rice: hexane 1:5 (w/v) for 1 hour at room temperature with continuous stirring. The water soluble NSP from defatted samples were extracted using enzymatic-gravimetric method according to Bunzel *et al.* (2001). Starch was removed by heat-stable α-amylase and glucomylase. Protein was removed by alkaline protease. The supernatant was kept for further isolation of water soluble NSP. Ethanol 95% (v/v) was added and leaved for precipitation. After centrifugation, the residue was washed with 78% (v/v) ethanol, 95% (v/v) ethanol and acetone. Finally, the residue was dissolved in double distilled water and freeze-dried to obtain water soluble NSP (RNSP).

Macrophage cells culture

Murine macrophage cells, RAW264.7, was grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) supplemented with 10% (v/v) fetal bovine serum (FBS), and 0.5% streptomycin and penicillin. Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2.

Effect of RNSP macrophage cells morphology

Macrophage cells (RAW264.7) was cultured on coverslips in a 12-well culture plate for 12 h. Cells were treated with RNSP at 200 μg/mL. Samples were incubated at 37°C for 24 h. LPS (25 ng/mL) was used as positive control and non-treated sample was used as negative control. The cells were stained with antibodies phalloidin FITC-labeled (Santa Cruz Biotechnology, USA). The macrophage cells morphology was observed by fluorescence microscope (Olympus BX51, Olympus Corporation, Tokyo, Japan).

Expression of co-stimulatory molecules

RAW264.7 at a density of 10^6 cells/mL was seeded in 12-wells plate

and incubated with RNSP (0, 50, 100, 150 and 200 µg/mL) at 37°C for 24 h. LPS (25 ng/mL) was used as positive control and non-treated sample was used as negative control. Then, cells were stained with anti-CD80, anti-CD86, or major histocompatibility complex (MHC) class II (SouthernBiotech, USA). Cells were collected for determined cell surface expression by flow cytometer (FACScan, Becton Dickinson Biosciences, USA). The results were expressed as percentage of negative control.

Statistical analysis

Statistical significance was determined with a one-way analysis of variance (ANOVA). The design of experiment is completely randomized design (CRD). The data reported are average of triplicate determinations (SPSS program version 19.0 for Windows). The differences between means were tested using the Duncan's new multiple range tests (DMRT) at $P<0.05$.

RESULTS AND DISCUSSION

Effect of RNSP on macrophage cells morphology

The activation of macrophage RAW264.7 cells after treated with RNSP was observed under fluorescence microscope. Figure 1 shows the effect of RNSP on macrophage cell morphology, it can be show that the morphological of RAW264.7 cells were slightly change and becomes larger and spreader after treated with RNSP. Moreover, the changing of RNSP treated-cell and LPS treated-cell were found to be similar. LPS, a component of the outer membrane of gram-negative bacteria, is the potent activators of macrophages and monocytes which is the cause of cells morphological changes (Leung *et al.*, 2006). The activation of macrophage is the cause of immunomodulation, wound-healing, anti-tumor and destruction of invading pathogens. Moreover, activated macrophage also produced the cytokines and chemokines which are involved in immune response especially to non-specific host defense (Schepetkin and Quinn, 2006).

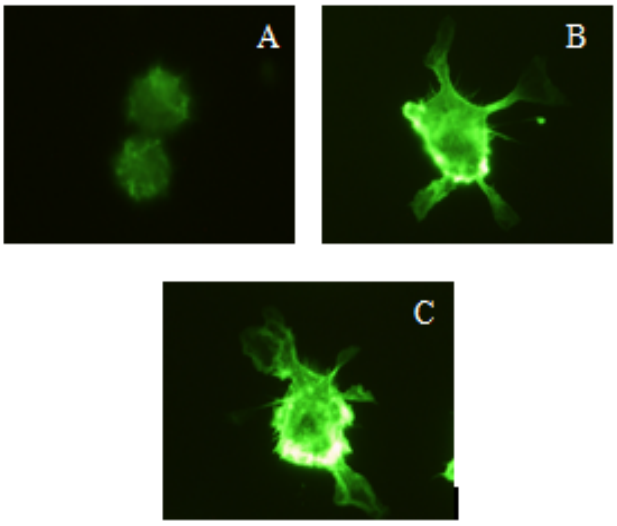

Figure 1 Macrophage morphological changes after treated with for 24 h with RNSP observed under fluorescence microscope. Macrophage cell (A), macrophage cell treated with LPS 25 ng/mL (B) and treated with RNSP 200 µg/mL (C)

Effect of RNSP on the surface expression of co-stimulatory molecules

The surface expression of co-stimulatory molecules, CD80 and CD86, from macrophage cells were determined and were showed in Figure 2 and Figure 3. It was found that, RNSP dose dependently increased the expression of co-stimulatory molecules both CD80 and CD86 as LPS did and increase with the increasing of RNSP concentration (50-200 µg/mL).

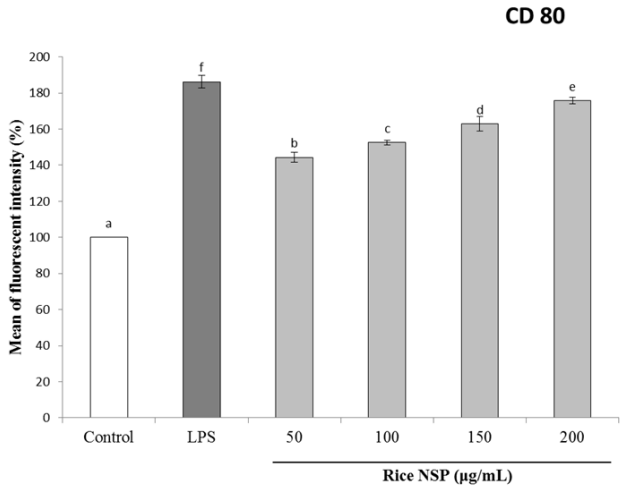

Figure 2 Effect of RNSP on the expression of surface molecules, CD80, by murine macrophage cells (RAW264.7). Cells were cultured for 24 h with RNSP (50-200 µg/ml) or with LPS (25 ng/ml)

These results suggested that RNSP induce the expression of co-stimulatory molecules which are involved in modulating the activation of T cell (Kim *et al.*, 2013). CD80 and CD86 have and important function to provide co-stimulatory signals for cytokine production and T cell proliferation (Vasu *et al.*, 2003). The macrophages cell after the phagocytosis of pathogenic microorganisms, they will present the antigen of the pathogens to corresponding helper T cell via the expression of co-stimulatory molecules and they will be an important antigen-presenting cell regulate the innate and adaptive immune responses during infection of pathogenic microorganisms (Li *et al.*, 2014).

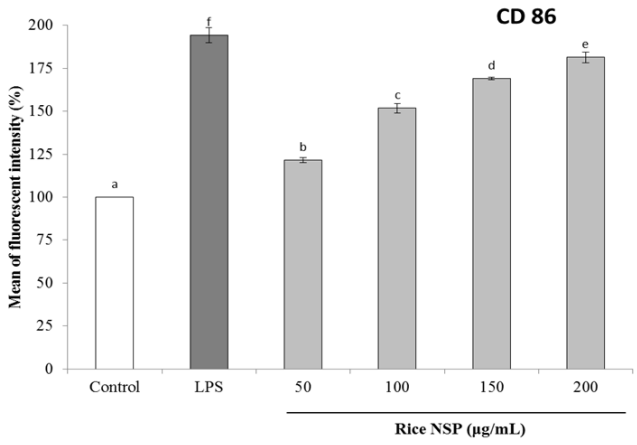

Figure 3 Effect of RNSP on the expression of surface molecules, CD86, by murine macrophage cells (RAW264.7). Cells were cultured for 24 h with RNSP (50-200 µg/mL) or with LPS (25 ng/mL)

Effect of RNSP on the surface expression of MHC-II

After treated the macrophage cells with RNSP, the up-regulation of MHC-II was observed as showed in Figure 4. The surface expression of MHC-II from macrophage cells was significantly increased in dose-dependently manner by RNSP (50-200 µg/mL). The up-regulation of MHC-II implicated in antigen presenting and T cell activation. (Meng *et al.*, 2014) Mature dendritic cells are characterized by the high levels of MHC-II during activation. Therefore, mature dendritic cells gain a high ability to activate T cells. (Park *et al.*, 2003) The up-regulation of MHC-II could involve in the helping of interaction between T cells and antigen presenting cells for the activities of anti-tumors. (Kim *et al.*, 2004)

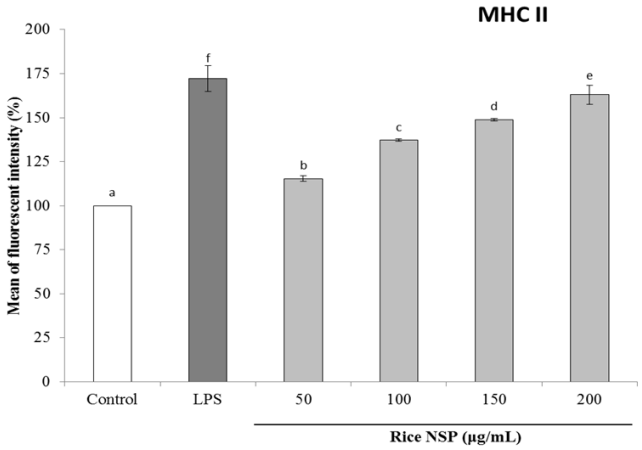

Figure 4 Effect of RNSP on the expression of surface molecules, MHC-II, by murine macrophage cells (RAW264.7). Cells were cultured for 24 h with RNSP (50-200 µg/mL) or with LPS (25 ng/mL).

RAW264.7 cells were also found to increase the co-stimulatory molecules and also MHC-II in a dose-dependent manner after treatment with polysaccharide from *Cordyceps sinensis* fungus, traditional Chinese medicines (3-15 µg/mL). Moreover, this type of polysaccharide also increases the surface expression of co-stimulatory CD80 and CD86 and MHC-II antigen by dendritic cells which are important to up regulate the immune activity including initiate antitumor T cell responses (Meng *et al.*, 2014).

CONCLUSION

In conclusion, these results indicated that NSP from the immature rice was non-toxic and induced the macrophage activation. Moreover, it also enhanced the expression of costimulatory molecules, CD80 and 86, and major histocompatibility complex (MHC) molecules II in murine macrophage cellls. Taken together, NSP from the immature rice had effect on the immuno-regulatory activity on macrophage cells which is related to stimulating host immunity.

ACKNOWLEDGEMENTS

This work was supported by Thailand Research Fund-Research and Researchers for Industries (TRF-RRI) and The Graduate School of Kasetsart University. And also thanks to The National Pingtung University of Science and Technology (NPUST), Taiwan for providing the excellent equipment and facilities.

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