EFFECT OF KIMCHI PORK SALAMI ON THE PROLIFERATION OF PERITONEAL MACROPHAGES AND TH1/TH2 CYTOKINE RATIO OF BALB/C MICE

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Abstract – This study was conducted to evaluate the effect of kimchi pork salami on the proliferation of peritoneal macrophages and Th1/Th2 cytokine ratio of BALB/C mice. Total 50 mice were allocated in five experimental groups and fed with basal diet (Con), pork salami with 0.5% commercial starter culture (LCS), pork salami with 1.0% commercial starter culture (HCS), pork salami with 0.5% kimchi powder (LKPS) and pork salami with 1.0% kimchi powder (HKPS) for 8 weeks. Proliferation of peritoneal macrophages in HKPS was significantly higher than that of in control (p<0.05). The lymphocyte ratio in LKPS and HKPS was higher than in control, LCS, and HCS (p<0.05). HKPS significantly reduced TNF-a/IL-10 secretion from peritoneal macrophage after stimulation of LPS compare to control and LCS (p< 0.05). Also, IL-2/IL-4 secretion from peritoneal macrophage after stimulation of LPS was significantly reduced by HKPS. This study indicated that supplementation of pork salami with 1% kimchi powder in mice could affects on regulation of immune system.

Key Words – kimchi, pork salami, immune, cytokines, Th1/Th2

I. INTRODUCTION

In these days, scientists have found that fermented meat products contain bioactive substance. Fermented sausages, traditional food of Europe, are made from fermentation of lactic acid bacteria (LAB), which are produced under controlled temperature and relative humidity [2]. Kimchi, traditional fermented food of Korea, is one of the most popular side dish which is manufactured from the addition of various components including green onion, red pepper, ginger, garlic, fermented shrimp or fish and so on. Many scientific studies showed that kimchi is one of the world's healthiest foods which has been shown to have various bioactivity such as anticancer, nutritional benefits, physiological and antioxidant activity due to carotene, vitamin C, chlorophyll, and phenolic compounds [5].

Macrophages are major immune effector cells and inflammatory cells. One major function of macrophages is phagocytosis of cellular and acellular debris during inflammation and healing. Th1/Th2 classification has been useful in relating the overall patterns of cytokine production to clinical outcomes in a variety of pathological states. Hence, a key end-point for optimal immunotherapy is to restore balanced Th1 and Th2 responses, suited to the immune challenges in health and disease [10]. Generally, immune cells response to antigens. Antigen-presenting cells such as macrophages process antigens and present fragments to T-helper cells (Th). Subsequently these, with the help of cytokines, coordinate the activities of varied immune cell types to effectuate an immune reaction [3].

In the present study was conducted to evaluate the supplementation effects of pork salami containing 1% kimchi powder fermented for 14 days on the peritoneal macrophage cell proliferation, white cell counts and Th1/Th2 cell cytokine secretion in BALB/c mice.

II. MATERIALS AND METHODS

Pork salami containing 1% kimchi powder was manufactured, fermented, and aged for 14 days. The proximate compositions of experimental diets were determined according to the methods of AOAC [1]. Female BALB/c mice were obtained from Orient Bio Inc., (Seongnam, Korea). All of the mice (6 weeks old) were maintained at institute of animal resources in Kangwon National University in Korea. The mice were acclimated to

their environment for one week prior to the start of experiments and allocated to five experimental groups (Table 1). Basal diets were consisted with 18.6% crude protein, 6.2% crude fat, 44.2% carbohydrate, 3.5% crude fiber, and 5.3% ash.

Table 1. Design of animal experiments.

Experimental groups	
CON	Mice fed basic diet
CS1.0	Mice fed pork salami with commercial starter culture at 1.0 g/kg, B.W
CS2.0	Mice fed pork salami with commercial starter culture at 2.0 g/kg, B.W
KP1.0	Mice fed pork salami with kimchi powder at 1.0 g/kg, B.W
KP2.0	Mice fed pork salami with kimchi powder at 2.0 g/kg, B.W

Serum triglyceride (TG), total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-C) were measured using an automatic biochemical analyzer (ADVIA 2400; Siemens, USA).

Peritoneal macrophages cell proliferation at the end of each treatments were determined by 3-(4,5-dimethyl-thiazol-2,5-diphenyl)-tetra-zolium bromide (MTT, Sigma-Aldrich Co., USA) assay [6; 8].

To determine white blood cell counts of the excised spleen tissues were fixed in 10% formalin solution for over a week, and ultra-thin sections of spleen tissues were manufactured by ultra-thin sectioning machine. After that, the sections were stained with Harris Hematoxylin & Eosin and observed through microscopes (Olymphus BX 50, Olymphus Optical Lts., Japan). The white blood cell counts of spleen in mice were calculated using the lymphocyte, monocytes, neutrophils, eosinophils and basophils ratio of total white pulp percentage.

Measurement of Th1/Th2 of TNF-a, IL-2, IL-4 and IL-10 concentration in culture supernatants was measured by using enzyme linked immunosorbent assay (ELISA) kits.

Data were analyzed by using the General Linear Model procedure of the SAS software (SAS Institute Inc., Cary, NC, USA) followed by Duncan's multiple range test. Data are expressed as means±SE. Differences were considered significant at the level of $p<0.05$.

III. RESULTS AND DISCUSSION

The body weight gain, feed intake and feed efficiency ratio were ranged 0.04~0.05 g/day, 2.68~3.01 g/day and 0.01~0.02, respectively (Table 2) This result indicates that negative supplementation of kimchi pork salami had no effect on physiological body condition of BALB/c mice.

Table 2. Effect of pork salamis with kimchi powder on the body weight gain, feed intake and feed efficiency ratio of BALB/c mice

[1)]Groups	Body weight gain (g/day)	Feed intake (g/day)	FER
CON	0.05±0.004[a]	2.86±0.105[a]	0.02±0.001[a]
LCS	0.04±0.004[ab]	2.76±0.030[a]	0.02±0.001[ab]
HCS	0.04±0.004[b]	3.01±0.065[a]	0.01±0.001[b]
LKPS	0.04±0.004[ab]	2.68±0.265[a]	0.02±0.002[ab]
HKPS	0.05±0.007[ab]	2.88±0.155[a]	0.02±0.002[a]

[a-b]Means±SE within same column with different superscript letters differ significantly at $p<0.05$.
[1)] Refer to Table 1.

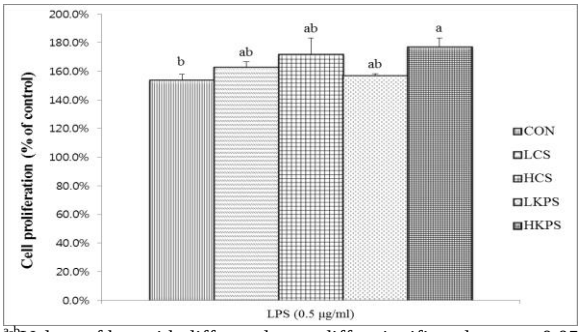
Serum lipid profile of mice fed LKPS and HKPS

Triglyceride (TG), total cholesterol (TC), HDL-cholesterol and LDL-cholesterol of experimental groups showed no significant differences (data not shown). This result suggests that the supplementation of LKPS and HKPS in mice had no effect on lipid profile.

Figure 1 shows the effect of kimchi powder salami on cell proliferation of peritoneal macrophages in BALB/c mice. The cell proliferation of peritoneal macrophages after 48 h of activation by LPS was significantly increased and showed approximately 77% in HKPS but there are no significant differences among control, LCS, HCS and LKPS. Proliferation of the peritoneal macrophage in HKPS was significantly higher than control. Kim *et al.* [4] indicated that administration of fermentation-polysaccharide was higher macrophage activities than control activated by LPS. Macrophages, antigen-presenting cell, are cells for cell mediated immunity and humoral immunity, and are important factor on the innate

immune system [9]. So, this result may show that supplementation of kimchi pork salami could modulate immune system because macrophages are major immune effector cells.

Figure 1. Effects of pork salamis with kimchi powder on the cell proliferation of peritoneal macrophage extracted from BALB/c mice.



[a-b]Values of bar with different letters differ significantly at $p < 0.05$.
[1)] Refer to Table 1

The white blood cell counts of splenocytes in all treatments are shown in Table 3. The lymphocytes, known as play an important role in immune system, were higher in LKPS and HKPS than in control, LCS and HCS ($p< 0.05$). Also, monocytes were significantly higher in LKPS and HKPS than in control, LCS and HCS. On the other hand, neutrophils and eosinophils of BALB/c mice were significantly lower in LKPS and HKPS than in control, LCS and HCS. There was no significant difference in basophils.

Figure 2. White cells in the splenocytes of BALB/c mice

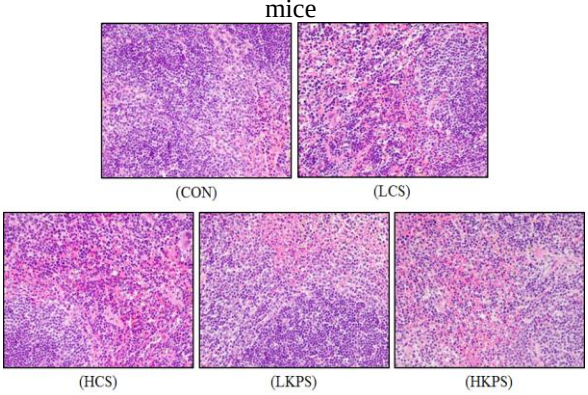


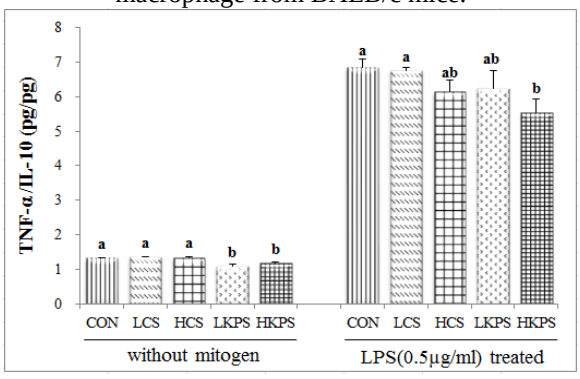
Table 3. White cell counts of BALB/c mice after fed experimental diets (%)

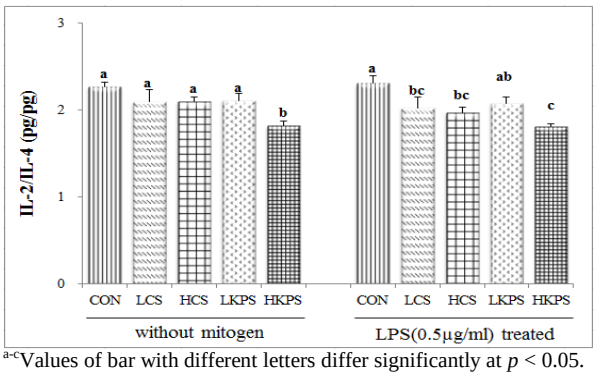
[1)]Groups	Lympho-cytes	Mono-cytes	Neutro-phils	Eosin-ophils	Baso-phils
CON	76.00± 0.577[b]	6.67± 0.333[b]	18.00± 1.528[a]	2.00± 0.000[abc]	0.00± 0.000[a]
LCS	78.33± 0.333[b]	7.67± 0.667[b]	18.00± 1.000[a]	3.33± 0.667[a]	0.00± 0.000[a]
HCS	74.67± 1.764[b]	9.33± 0.333[a]	19.00± 1.000[a]	3.00± 0.577[ab]	0.00± 0.000[a]
LKPS	85.00± 3.464[a]	10.00± 0.000[a]	13.00± 0.577[b]	1.00± 0.000[c]	0.00± 0.000[a]
HKPS	85.00± 0.577[a]	10.67± 0.667[a]	14.00± 1.528[b]	1.67± 0.333[bc]	0.00± 0.000[a]

[a-b]Means±SE within same column with different superscript letters differ significantly at $p<0.05$.
[1)] Refer to Table 1

TNF-a/IL-10 and IL-2/IL-4 ratios of experimental groups are shown in Figure 3. TNF-a/IL-10 ratio of LKPS and HKPS without LPS was significantly decreased than that of control at 1.09 pg/pg and 1.18 pg/pg. TNF-a/IL-10 ratio of HKPS treated with LPS was significantly decreased than that of control and LCS at 5.55 pg/pg. Liu and Lin [7] also suggested that TNF-a/IL-10 cytokine secretion ratios in the presence of LPS were remarkably decreased by ethanol extract from strawberry and mulberry juices in dose-dependent manners. IL-2/IL-4 ratio of HKPS without LPS was significantly decreased at 1.83 pg/pg, and IL-2/IL-4 ratio of HKPS treated with LPS significantly decreased at 1.81 pg/pg. As a result, these results show that supplementation of high concentration kimchi pork salami in mice decrease Th1/Th2 imbalance, and have anti-inflammatory effect.

Figure 3. Supplementation effect of kimchi pork salami on the Th1/Th2 ratio of peritoneal macrophage from BALB/c mice.





[a-c]Values of bar with different letters differ significantly at $p < 0.05$. The limit of detection of ELISA kits used in this study was <15.6 pg/ml. The lipopolysaccharide (LPS) concentration was 0.5 ug/ml.

[1)] Refer to Table 1

IV. CONCLUSION

Proliferation of peritoneal macrophages in mice fed 1% kimchi pork salami was significantly increased compare to control. Also, supplementation of high concentration kimchi pork salami decreased Th1/Th2 ratio of BALB/c mice. Further research need to be done to determine the effect of supplementation of 1% kimchi pork salami on *Enterobacteria* to identify if KPS1 has beneficial effect on gut health or not.

ACKNOWLEDGEMENTS

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