Food Sci. Biotechnol. Vol. 15, No. 3, pp. 347 ~ 350 (2006)

Food Science and Biotechnology

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Effects of Single Cell Products of Apple on Stimulating Various Functions of Murine Peritoneal Macrophages

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Abstract The objective of this study was to investigate the possible effects of oral administration of single cell products (SCP) of apple on activating peritoneal macrophages. Apples were processed either for cold-pressed juice or SCP, which were produced by incubating sliced apples with a protopectinase, Sumyzyme MC. Both cold-pressed juice and SCP of apple were administered to C57BL/6 mice for 10 days to compare their efficacy, along with the control group, in stimulating peritoneal macrophages. The viability of macrophages was significantly increased by up to 161% of that of the control following the administration of apple SCP, whereas the viability of macrophages was increased to a lesser extent of up to 143% in the apple juice (AJ) administered group. Administration of apple SCP also induced a significantly higher production of H_2O_2 from macrophages (317% of the control) than that of cold-pressed AJ (210%). Although nitric oxide (NO) production was not increased by the administration of either AJ or SCP, the latter slightly but significantly increased tumor necrosis factor-α (TNF-α) production from macrophages from 560.4 to 579.8 pg/mL. The results of this study suggest that administering SCP is more efficient than administering AJ to stimulate functions of peritoneal macrophages.

Keywords: single cell products of apple, immunostimulating activities, peritoneal macrophages

Introduction

The cell wall of plants is generally composed of three parts: a relatively thin 'primary cell wall', relatively thick 'secondary cell wall' and 'middle lamella' that connects one cell to another cell. The main components of the cell wall are cellulose, hemicellulose, lignin, pectin, and proteins (1). Pectin is a highly polymerized insoluble carbohydrate that is mostly found between cell walls. Pectin is connected through covalent and hydrogen bonds with each other and with other hemicellulose components (2). Protopectin, which is an insoluble pectin compound and the main component of middle lamella, can be hydrolyzed by cell separating enzymes (CSE) (3-5). Thus, CSE act on producing a suspension of loose single cells and are used, on rare occasion, to prepare fruit nectar bases, vegetable purees, and baby and geriatric foods (6-8). The filtering process in conventional apple juice (AJ) production removes much of the dietary fibers such as pectin and cellulose compounds. Therefore, there are advantages in applying CSE over a conventional AJ process to increase the dietary fiber contents, as well as minimize nutritional changes, improve storage stability and decrease the color development in the final products. Since dietary fibers are known to exhibit beneficial functionality such as hypolipidemic and anti-carcinogenic activities in the human body (9-11), oral administration of single cell products (SCP) may also impart beneficial effects. This study was focused on investigating the effects of administering SCP of apple on peritoneal macrophage stimulating activities.

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Received September 15, 2005; accepted March 24, 2006

Materials and Methods

Preparation of single cell products (SCP) of apple *Fuji* apples were purchased from a local market in Sungnam-si, Korea. After thoroughly washing and peeling, the apples were blanched and cut uniformly into 5mm-thick pieces. The sliced apples were added to the equivalent amount of buffer (pH 4.6) and 0.2% Sumyzyme-MC (4,000 U/g, New Nippon Chemical Engineering Co., Anjyo City, Japan), followed by incubation at 40°C for 4 hr in a shaking incubator. Subsequently, a suspension of the macerated apple sample was filtered through a 20-mesh screen. SCP were obtained by centrifuging the suspension at 10,000×g for 15 min. Cold-pressed AJ was prepared by crushing the same amount of sliced samples with a squeezer and filtration.

Animals Six-week-old male C57BL/6 mice (Hanllym Lab Animal, Gyeonggi, Korea) were housed in mesh-bottom, plastic cages in a controlled environment and acclimatized for 1 week. Feed and water were supplied ad libitum. All animal experiments were performed under the guidelines of the Laboratory Animal Experiment Committee of the Korea Food Research Institute.

Viability of macrophages After 1 week of acclimatization, C57BL/6 mice were randomly divided into three groups: control (water), AJ, and SCP fed groups. Each group was further divided into subgroups of 7 mice each. The control group mice were orally administered 0.25 mL of water once a day while the AJ mice were orally administered the same amount of cold-pressed AJ. The SCP group was administered SCP that were processed from the same amount of apples as the juice products with water added to give a concentration of 52.1 mg/0.25 mL. On day 10, the mice were killed by ether anesthesia and peritoneal macrophages were collected. The collected cells

were washed twice with RPMI 1640 and then cultured in RPMI 1640 containing 10% FBS, 1 mM glutamine, 100 units of penicillin, and 0.1 mg/mL of streptomycin. Macrophages obtained from either control, AJ or SCP fed group were plated at a concentration of 5×10^4 cells per well in a 96-well tissue culture plate, and then incubated for 2 hr at 37°C in a 5% CO^2 incubator followed by two washings with warm media and incubation for an additional 24 hr. After incubation, 50 μL of MTT (1 mg/mL) was added to the wells which were incubated for another 2 hr at 37°C followed by the addition of 150 μL of DMSO and reading at 540 nm to measure the percentage viability of the macrophages of the control, AJ, and SCP groups.

Nitric oxide (NO) assay The amount of stable nitrite, the end product of NO generation by macrophages, was determined by a colorimetric assay. Following 24 hr incubation of macrophages, 100 mL of culture supernatant from each well was transferred to another 96-well tissue culture plate. Then, the Griess reagent (100 μL, 1% sulfanilamide in 30% glacial acetic acid and 0.1% napthylenediamine dihydrochloride in 60% glacial acetic acid) was added. For the positive control, 20 μL of lipopolysaccharide (LPS) (1 μg/mL) was added to the macrophages followed by incubation and Griess reagent addition. The mixture was incubated at room temperature for 10 min. The absorbance at 540 nm was read and the nitrite concentration was determined by extrapolating the sodium nitrite standard curve.

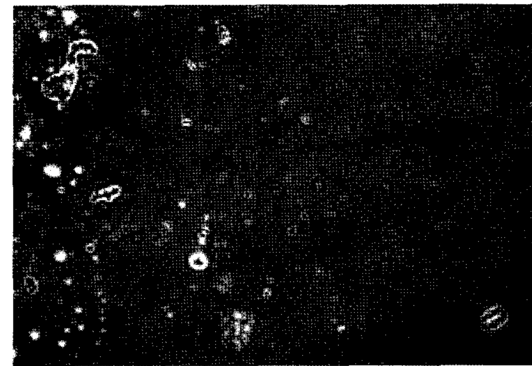
Tumor necrosis factor-α (TNF-α) and H_2O_2 assay The TNF-α and H_2O_2 levels in the 24-hr incubated macrophage culture media of control, LPS-treated, AJ, and SCP fed groups were quantified using ELISA kits (Assay Design Inc., Ann Arbor, MI, USA) according to the manufacturer's instructions.

Statistical analysis The Statistical Analysis System (SAS) software ver. 6.11 was used for the data analysis. All analyses were determined by Duncan's multiple range test at $p<0.05$.

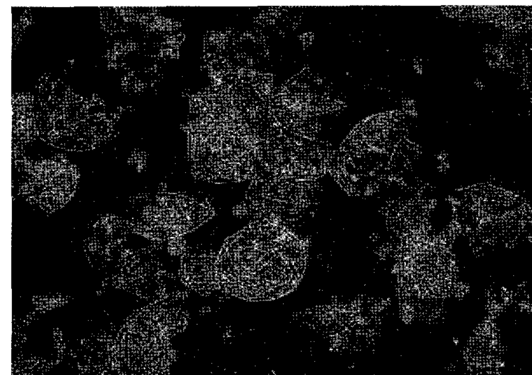
Results and Discussion

The biggest stumbling block in CSE processing for commercial application is its low yields. The yields of cold-pressed AJ and SCP in this study were 58.8 and 12.3%, respectively. When photomicrographs of cold-pressed AJ (A) and SCP (B) of apples were compared, a high proportion of intact cell walls and contents were observed in the latter but not in the former (Fig. 1). This result indicated that treatment of apples with Sumyzyme-MC properly hydrolyzed the protopectin, thus separating cells from each other and liberating some of the cell wall components.

Since Sakai and Okushima (11) first reported the enzymatic solubilization of pectin from citrus peel protopectin, many efforts have been made to apply enzyme treatments to the production of pectins from fruit peels. However, few studies have been performed to investigate the health beneficial activity of these products. When SCP of apple and cold-pressed AJ were orally administered to C57BL/6 mice for 10 days, the viability of peritoneal macrophages obtained from both groups were significantly increased to 161 and 143% of the control group, respectively (Fig. 2). Macrophages have been known to play an important role in the host protection against a wide range of tumors and microorganisms. Our results suggested that cell wall components in SCP probably enhanced the viability of the macrophages further, even though other compounds in apples such as flavonoids and



(A)



(B)

Fig. 1. Photomicrographs of apple juice (A) and single cell products of apple (B) treated with cell separating enzyme (Sumyzyme MC) (×100).

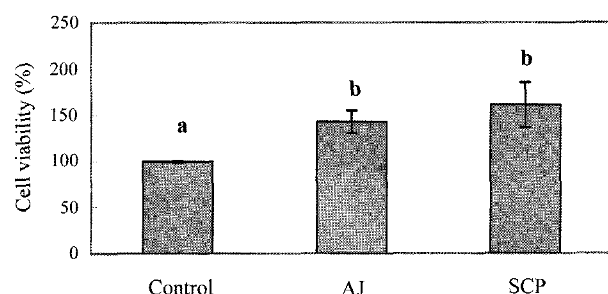


Fig. 2. Effects of administration of single cell products of apple on the viability of peritoneal macrophages of C57BL/6 mice. Control: water fed, AJ: apple juice fed, and SCP: single cell products of apple fed groups. Mean values with different superscripts are significantly different ($p<0.05$).

carotenoids seemed mainly responsible for such increases.

The effects of orally administered apple SCP on NO and H_2O_2 production were examined. When macrophages are activated, their production of reactive oxygen substances (ROS), such as H_2O_2 and NO, is also accelerated in general. These ROS have been identified as important messengers involved in the destruction of tumor cells by activated macrophages (13). Hydrogen peroxide production of macrophage was significantly increased from 28.1 ng/mL in the control to 59.2 and 89.1 ng/mL in AJ and SCP fed groups, respectively (Fig. 3). It is generally known that polysaccharides, including LPS used as a positive control in this study, enhance the production of H_2O_2 from macrophages (14, 15). As observed in this study, cell wall polysaccharides in the SCP group further increased production of H_2O_2 from peritoneal macrophages. However, oral administration of either SCP or AJ caused no significant increase in NO production from macrophages (Fig. 4).

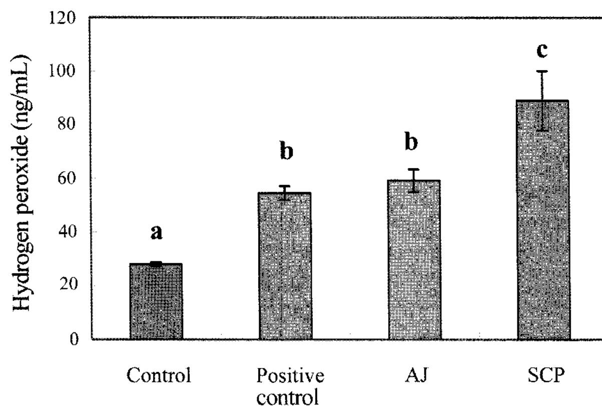


Fig. 3. Effects of administration of single cell products of apple on the production of hydrogen peroxide from macrophages of C57BL/6 mice. Control: water fed, Positive Control: LPS treated, AJ: apple juice fed, and SCP: single cell products of apple fed groups. Mean values with different superscripts are significantly different ($p<0.05$).

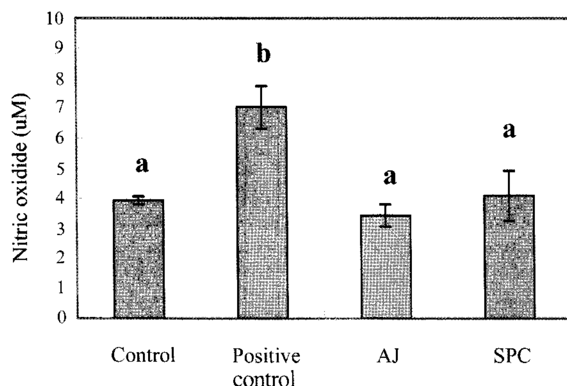


Fig. 4. Effects of administration of single cell products of apple on the production of nitric oxide from macrophages of C57BL/6 mice. Control: water fed, Positive Control: LPS treated, AJ: apple juice fed, and SCP: single cell products of apple fed groups. Mean values with different superscripts are significantly different ($p<0.05$).

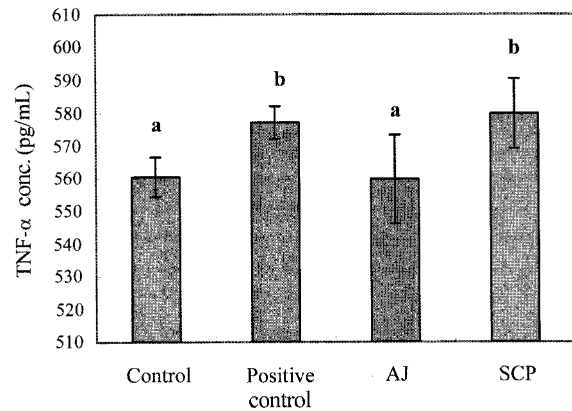


Fig. 5. Effects of administration of single cell products of apple on the production of TNF-α from macrophages of C57BL/6 mice. Control: water fed, Positive Control: LPS treated, AJ: apple juice fed, and SCP: single cell products of apple fed groups. Mean values with different superscripts are significantly different ($p<0.05$).

TNF-α is a cytokine produced mainly by activated mononuclear phagocytes that function to stimulate the recruitment of neutrophils and monocytes to sites of infection and to activate these cells to eradicate microbes (16, 17). Although oral administration of AJ didn't change the level of TNF-α production from the macrophages, a slight but significant increase from 560.4 to 579.8 pg/mL was observed in the SCP administered group (Fig. 5).

Epidemiological data showed that high consumption of vegetables and fruits are consistently associated with a low risk of cancer and cardiovascular disease (18-20). Some key compounds that are found richly in fruits and vegetables, such as vitamins, polyphenols, and pectins are responsible for such health-promoting activities. Polyphenols are the major phytochemicals in fruits. Many studies have discovered that polyphenols have antioxidative and immunomodulatory activities (21-25). As observed in this study, administering AJ significantly stimulated the macrophages. Although an identification of the responsible compound for such macrophage stimulating activities is beyond the scope of this study, we can assume that consumption of apple SCP, particularly cell wall polysaccharides, stimulates macrophage activities more effectively than mere AJ administration.

References

1. Brett CT, Waldron KW. Physiology and Biochemistry of Plant Cell Walls. Chapman & Hall, London, UK. p.45 (1996)
2. Tolbert NE. The Biochemistry of Plants. Vol. 1, Academic Press, New York, NY, USA. pp.101-116 (1980)
3. Nakamura T, Hours RA, Sakai T. Enzymatic maceration of vegetables with protopectinase. J. Food Sci. 60: 468-472 (1995)
4. Toyama N. A cell separating enzyme as a complementary enzyme to cellulase and its applications in processing of vegetables. J. Ferment. Technol. 43: 683-689 (1965)
5. Toyama N, Owatashi H. Extraction of green tea components from manufactured tea leaves using cellulase and cell separating enzyme. J. Ferment. Technol. 44: 830-834 (1966)
6. Zetelaki-Horvath K, Gatai K. Disintegration of vegetable tissues by endo-polygalacturonase. Acta Aliment.Hung. 6: 227-240 (1977)
7. Zetelaki-Horvath K, Gatai K. Application of endo-polygalacturonase to vegetables and fruits. Acta Aliment. Hung. 6: 353-377 (1977)

8. Zetelaki-Horvath K, Urbanyi G. Determination of particle size of vegetable tissue by a sedimentation technique after enzymatic disintegration. Acta Aliment. Hung. 7: 69-78 (1978)
9. Guillon F, Champ M. Structural and physical properties of dietary fibres, and consequences of processing on human physiology. Food Res. I. Stud. 33: 233-245 (2000)
10. Harris PJ, Ferguson LR. Dietary fibres may protect or enhance carcinogenesis. Mutat. Res. 443: 93-110 (1999)
11. Chai YM, Kim MJ, Lim BK, Lee YJ, Rhee IK, Lee IS, Rhee SJ. Effects of dietary fiber manufactured from *Quercus mongolica* on imprpvement of antioxidative defense system in rats fed high cholesterol diet. Food Sci. Biotechnol. 13: 772-776 (2004)
12. Sakai T, Okushima M. Microbial production of pectin from citrus peel. Appl. Environ. Microb. 39: 908-912 (1980)
13. Lowenstein CJ, Dinerman JL, Snyder SH. Nitric oxide: A physiologic messenger. Ann. Intern. Med. 120: 227-237 (1994)
14. Son EH, Moon EY, Rhee DK, Pyo S. Stimulation of various functions in murine peritoneal macrophages by high mannuronic acid-containing alginate (HMA) exposure *in vivo*. Int. Immunopharmacol. 1: 147-154 (2001)
15. Stuelp-Campelo PM, de Oliveira BM, Leão AMAC, Carbonero ER, Gorin PAJ, Iacomini M. Effect of soluble α-D-glucan from the lichenized fungus *Ramalina celastri* on macrophage activity. Int. Immunopharmacol. 2: 691-698 (2002)
16. Duerksen-Hughes PJ, Day D, Laster SM, Zachariades NA, Aquino L, Gooding LR. Both tumor necrosis factor and nitric oxide participate in lysis of simian virus 40-transformed cells by activated macrophages. J. Immunol. 149: 2114-2122 (1992)
17. Stuehr DJ, Nathan, CF. Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. J. Exp. Med. 169: 1543-1555 (1989)
18. World Cancer Research Fund/American Institute of Cancer Research, Food, Nutrition, and the Prevention of Cancer: a Global Perspective, Washington DC, USA. (1997)
19. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer prevention: a review. J. Am. Diet Assoc. 96: 1027-1039 (1996)
20. Morton LW, Abu-Amsha CR, Puddey IB, Croft KD. Chemistry and biological effects of dietary phenolic compounds: relevance to cardiovascular disease. Clin. Exp. Pharmacol. P. 27: 152-159 (2000)
21. Visioli F, Borsani L, Galli C. Diet and prevention of coronary heart disease: the potential role of phytochemicals. Cardiovasc. Res. 47: 419-425 (2000)
22. RiceEvans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Bio. Med. 20: 933-956 (1996)
23. Wang H, Cao GH, Prior RL. Oxygen radical absorbing capacity of anthocyanins. J. Agric. Food Chem. 45: 304-309 (1997)
24. Middleton EJ. Effect of plant flavonoids on immune and inflammatory cell function. Adv. Exp. Med. Biol. 439: 175-182 (1998)
25. Kim HK, Choi MG, Kwon JH, Kim KH. Physiological activities of *Brassica oleracea* var. *capita* extracts as affected by varieties and solvents. Food Sci. Biotechnol. 13: 367-371 (2004)