

Effect of Tea Polyphenols on Anticancer Activity and Cytokines Production

Mi Yae Shon and Sang Hae Nam^{1*}

Dept. of Food and Nutrition, Gyeongsang National University, Jinju 660-701, Korea

¹*Dept. of Food Science, Jinju National University, Jinju 660-758, Korea*

Received September 19, 2007 / Accepted October 22, 2007

Theaflavins (TF) and thearubigins (TR) are constituents of tea pigments which are polyphenols derived from Korean fermentation tea. After TF, TR and [(-) epigallocatechin-3-gallate](EGCG) have been applied to macrophage cell line (RAW264.7) nitric oxide (NO) synthesis and cytokines production were estimated. Cytokines production by enzyme linked immuno-sorbent assay (ELISA) determined. NO production was increased by about 1.5-folds at the dose of 80 µg/ml compared to control and lipopolysaccharide (LPS) stimulation when TF, TR and EGCG were applied to a RAW264.7 cell. Interleukin-6 (IL-6), Tumor necrosis factor (TNF-α) and granulocyte-macrophage colony stimulating factor (GM-CSF) increased depended on concentrations of TF, TR and EGCG. The production of tumor necrosis factor-α increased highly in TR, TF and EGCG group with LPS. These results suggest that TF, TR and EGCG have immune-enhancement effect through the cytokine production. TF, TR and EGCG inhibited cancer cell viability, the anticancer effect of these polyphenols may explain the anti-tumor promotion action and antioxidant activity of these tea constituents.

Key words – Theaflavins (TF), thearubigins (TR), immunomodulator cytokine, anticancer activity, antioxidant activity

Introduction

Tea leaves are rich in catechins, and catechins come in contact with polyphenol oxidase and microorganisms, joining them to one another to form TF and TR during fermenting of tea leaves. Most of the catechin mass of process of fermented is converted to a well defined group of compounds known as TF and TR. Fermented tea contains major amount of these two polyphenols TR and TF which responsible for the distinct color and flavor of fermented tea. TR and TF are major constituents of tea pigments with anti-oxidant, antimutagenic, antiproliferative and antineoplastic activities [22,26]. EGCG is the major polyphenol component of green tea and was reported to inhibit low density lipoprotein (LDL) oxidation in vitro by scavenging oxygen radicals and chelating metal ions which act as catalysts of lipid oxidation [19,25]. Recently, it is increasingly being acknowledged that natural active components and biological activities compatible with beneficial health effects.

Among a variety of mediators released by activated macrophages cells, NO has been identified as a potent molecule that may exert regulatory or cytotoxic effects

when excessively produced. However, the importance of the NO production may be the development of a possible anticancer property provoked by an immunogenic but non-pathogenic particle[1]. TNF-α promote cell-mediated immunity. IL-6 and GM-CSF promote humoral antibody-mediated immune response[6]. Therefore, IL-6, TNF-α and GM-CSF are related to immune reaction, directly or indirectly in the present study. We investigated the effect on production of cytokines and NO in the macrophage cell line, anticancer activity and antioxidant activity for TF, TR and EGCG in the present study.

Materials and Methods

Reagents

The following reagents were used in this study: 2,4,6-tri-pyridyl-2,5,7,8- tetramethylchroman-2-carboxylic acid (TPTZ), sodium nitro-prusside (SNP), N-1-naphthyl-ethylen-diamine, lipopolysaccharide (LPS), avidin-peroxidase, 2'-AZINO-bis (ABTS), EGCG (purity>98%) (Sigma, St. Louis, USA). TNF-α, IL-6 and GM-CSF (Pharmingen, San Diego, USA) levels in macrophage culture medium were determined by enzyme-linked immunosorbent assay. DMEM (Dulbecco's modified Eagle's medium), FBS (fetal bovine serum), 0.05% trypsin-0.02% EDTA and 100 units/ml penicillin-strepto-

*Corresponding author

Tel : +82-55-751-3274, Fax : +82-55-751-3270

E-mail : shnam@jinju.ac.kr

mycin were purchased from GIBCO Co. (Grand Island, NY, USA). RAW264.7 cells and cancer cells (HT-29, A-549, HeLa and HepG2) were purchased from the Korean Cell Line Bank (Seoul, Korea). TF and TR were extracted from Korean microbial fermented tea, which was donated from the Korea Fermented Food Research Institute (KOFRI) and local tea-producing industries.

Manufacture of microbial fermented tea

Korean fermented tea used in this study is a microbial fermented tea which is inoculated with a wheat bran starter containing the fungus, *Aspergillus wentii* KOFRI 0341, which has potent α -amylase (5.71 U/g), pectinase (910.56 U/g) and polyphenol oxidase (PPO, 4.14 U/g solid) enzymes, isolated from Korean natural fermented tea [24]. Green tea leaves were plucked from the Suncheon city (Jeonnam in Korea) wild tea-producing areas in June, 2005. Fresh tea leaves were withered and then subjected to the rolling process. Rolled leaves were inoculated with microorganism and fermented to aged for 6 months. Fermented teas were dried in an incubator, used for analysis and studied for biological activity.

Analysis of total TF contents

Total TF were determined by the Flavognost method [11]. The percentage of dry matter in the sample was determined by oven-drying. A tea infusion was made with 375 ml of boiling water, added from an overhead boiler into a tare flask, and 9 g of tea. The flask was shaken for 10 min, the infusion filtered through cotton wool, and allowed to cool to room temperature, and then 10 ml were pipette into 10 ml of isobutyl methyl ketone (4-methylpentan-2-one, IBMK : ACS standard from Merck). The mixture was shaken for 10 min and allowed to stand until the layers separated. Two milliliters of the upper layer were pipette into a test tube, followed by 4 ml ethanol and 2 ml Flavognost reagent (2 g diphenylboric acid-2-aminoethyl ester dissolved in 100 ml ethanol). The contents were mixed and color allowed 15 min to develop. The absorbance (A) at 625 nm was read against an IBMK/ethanol (1:1, v/v) blank.

Spectrophotometer measurements of total TR

The method of Roberts and Smith [20] was used to determine total TR. Fifty milliliters of the cool, well-shaken and filtered standard tea infusion from TF analysis were

mixed with 50 ml isobutylmethylketone (IBMK) and gently shaken to avoid formation of an emulsion. The layers were allowed to separate and 4 ml portion of the IBMK layer was taken and made to 25 ml with methanol in a volumetric flask (Solution A). Twenty-five milliliters of the remaining initial IBMK layer were taken in a separate flask and mixed with 25 ml of 2.5% aqueous sodium hydrogen carbonate. The mixture was vigorously shaken before the layers were allowed to separate and the aqueous layer discarded. A 4 ml portion of the washed IBMK layer was made to 25 ml with methanol (Solution B). Two milliliters of a saturated oxalic acid aqueous solution and 6 ml of water were added to a 2 ml portion of the aqueous layer left from the first extraction with IBMK, and diluted to 25 ml with methanol (Solution C). The absorbencies AA, AB, AC of solutions A, B and C at 380 nm were obtained using a CE 393 Cecil Digital grating spectrophotometer with distilled water as the blank. Each fermented tea sample was extracted in triplicate for the determination of the TR fractions and levels. By following the earlier procedures for solvent partitioning of fermentation tea liquor components and based on the fact that mean absorbance of the TR fractions at 380 nm was 0.733, the following equation for estimating total TR was derived: At 380 nm,

$$\% \text{ TR (Total)} = (3750.026.25[2 A_C + A_A - A_B]) / (0.7339 \text{ DM}/100)$$

FRAP assay

This method [3] measures the ability of the antioxidants contained in the TF, TR and EGCG to reduce ferric-tripiridyltriazine (Fe^{3+} -TPTZ) to a ferrous form (Fe^{2+}) which absorbs light at 593 nm. The ferro- and ferric ion from complexes with TPTZ reagent are the main products of this reaction. FRAP level was calculated by plotting a standard curve of absorbance against $\mu\text{mol/l}$ or $\mu\text{mol/g}$ concentration of Fe^{2+} standard solution.

Scavenging of nitric oxide

Nitric oxide (NO) was examined by the Griess reaction and SNP was used as the NO generator [14]. The sample were mixed with an identical volume of 10 mM SNP and incubated at room temperature in the light. After 2.5 hr incubation, Griess reagent was added and the mixture was color-developed for 10 min. The absorbance was measured at 540 nm.

Determination of cell viability

Anticancer activity was measured using the SRB assay [23]. All samples were sterilized by filtration through a 0.2 μm filter prior to analysis in the cell test. The test cells used in this assay were A-549, HT-29, HeLa and HepG2 cells. Cells were maintained as adherent cell cultures in DMEM medium supplemented with 10% FBS and 10 units of penicillin and 10 $\mu\text{g}/\text{ml}$ streptomycin at 37°C in a humidified incubator containing 5% CO_2 . Cancer cells were transferred into 96 well plates and incubated for 24 hr prior to the addition of test samples. Samples were added and incubated for 48 hr. The effects of the samples on the growth of human cancer cell lines were evaluated for their cytotoxic activity.

Determination of nitrite

RAW264.7 cells were cultured in DMEM medium supplemented with 10% FBS and 10 units of penicillin and 10 $\mu\text{g}/\text{ml}$ streptomycin at 37°C in a humidified incubator containing 5% CO_2 . The 100 μl of test extract was then added and serially diluted to give final concentrations of 80, 40, 20 and 10 $\mu\text{g}/\text{ml}$. Cells were then stimulated with 200 U/ml of 10 $\mu\text{g}/\text{ml}$ LPS for another 24 hr. The presence of nitrite, a stable oxidized product of NO, was determined in cell culture media by Griess reagent (1% sulfonamide and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% H_3PO_4). The 100 μl of cell culture supernatant was removed and combined with 100 μl of Griess reagent in a 96-well plate followed by spectrophotometric measurement at 550 nm using a microplate reader (HT Biotec Ins, USA). Nitrite concentration in the supernatants was determined by comparison with a sodium nitrite standard curve [27].

Measurement of cytokine

IL-6, TNF- α and GM-CSF concentrations in supernatants from macrophage cultures were determined by enzyme-linked immunosorbent assay (ELISA) using antibody from Pharmingen, according to manufacturer's instruction [12]. Cells were incubated with LPS (10 $\mu\text{g}/\text{ml}$) in the presence of different concentrations of test samples for 24 hr. The supernatants were collected and stored at -80°C before analysis. The amount of IL-6, TNF- α and GM-CSF was measured by an ELISA. The ELISA was determined by coating 96-well plates with each of 100 μl aliquots of anti-mouse IL-6, TNF- α and GM-CSF monoclonal antibodies. Before use and between subsequent steps in the assay,

coated plates were washed with phosphate-buffered saline (PBS) containing 0.05% tween-20. Recombinant cytokines were diluted and used to establish the standard curve. Assay plates were exposed to biotinylated anti-mouse IL-6, TNF- α and GM-CSF, and avidine peroxidase, and ABTS substrate solution containing 30% H_2O_2 . The plates were read at 405 nm.

Statistical analysis of data

The experiments shown are a summary of the data from at least three experiments and are presented as the mean \pm SD. Statistical significance of the data was evaluated by the ANOVA. The value of $p < 0.05$ was accepted as statistically significant.

Results

The TF and TR contents of the fermentation tea samples are showed in Table 1. Contents of TF and TR on the Korean microbial fermented tea (TF: 74.4 $\mu\text{M}/\text{g}$, TR: 37.2%) were analyzed to be higher than reported black tea aged tea by oxidase of green tea leaf (TF: 28.3 $\mu\text{M}/\text{g}$, TR: 16.9% from paper of Martin O. P. et al. [15]).

Table 2 shows differences in total antioxidant capacity measured by the FRAP method on TF, TR and EGCG. The FRAP value was found within the range 10-300 μM . Table 3 shows results for the NO scavenging effect of TF, TR and EGCG. At a concentration of 80 $\mu\text{g}/\text{ml}$, the NO scavenging activity are was TF(78.00 \pm 2.85%), TR(51.00 \pm 2.36%) and EGCG(88.00 \pm 3.71%).

To assess the anticancer activity properties of polyphenols TR, TF and EGCG, we used cancer cell line, A-549, HT-29, HeLa and HepG2 cells. In Tables 2 and 3, anticancer activity was observed in A-549, HT-29, HeLa and HepG2 cells. Treatment with polyphenols TR, TF and EGCG caused a dose-dependent reduction in cell numbers of cancer cells. EGCG was more effective in inhibiting the growth of these cancer cell lines than TF and TR at 80 $\mu\text{g}/$

Table 1. TF and TR contents of Korean fermented tea

Contents	
TF ($\mu\text{mol}/\text{g}$)	74.40 \pm 4.8 ^b
TR (%)	37.19 \pm 3.5 ^a

The results are expressed as mean \pm SD of triplicate assays. ^{a,b}Means with the different letters are significantly different from corresponding controls at the 0.05 level of significance.

Table 2. Effect of TF, TR and EGCG on ferric reducing ability (FRAP value, μM)

($\mu\text{g}/\text{ml}$)	Control	TF	TR	EGCG
0	3.23 \pm 1.05 ^a			
10		24.51 \pm 2.77 ^c	9.34 \pm 1.52 ^b	34.61 \pm 2.45 ^c
20		56.38 \pm 4.93 ^d	29.14 \pm 2.67 ^c	76.78 \pm 3.21 ^e
40		110.18 \pm 17.39 ^f	76.42 \pm 5.87 ^c	147.13 \pm 11.24 ^g
80		269.17 \pm 12.59 ^h	121.11 \pm 9.99 ^f	312.23 \pm 11.27 ⁱ

The results are expressed as mean \pm SD of triplicate assays. ^{a-i}Means with the different letters are significantly different from corresponding controls at the 0.05 level of significance.

Table 3. NO-scavenging activity of TF, TR and EGCG (%)

($\mu\text{g}/\text{ml}$)	TF	TR	EGCG
10	7.20 \pm 3.951 ^b	4.21 \pm 0.11 ^a	25.50 \pm 2.75 ^d
20	25.64 \pm 1.91 ^d	13.64 \pm 0.92 ^c	55.47 \pm 1.34 ^f
40	41.90 \pm 5.69 ^e	27.35 \pm 1.12 ^d	74.90 \pm 2.61 ^g
80	78.00 \pm 2.85 ^g	51.00 \pm 2.36 ^{df}	88.00 \pm 3.71 ^h

The results are expressed as mean \pm SD of triplicate assays. ^{a-h}Means with the different letters are significantly different from corresponding controls at the 0.05 level of significance.

ml. The observed inhibition rate of TR, TF and EGCG were 74.2%, 76.6% and 81.1% for HeLa at 80 $\mu\text{g}/\text{ml}$, respectively, whereas those for TR, TF and EGCG were 68.1%, 69.7%, 89.7%, 74.7%, 70.7% and 82.4% for HT-29 and HepG2 at 80 $\mu\text{g}/\text{ml}$, respectively (Table 4,5).

As shown in Fig. 1 and 2, TF, TR and EGCG alone could stimulate the production of NO, IL-6, TNF- α and GM-CSF (unstimulated cells, 3.44 \pm 0.02 μM for NO, 0.09 \pm 0.03 ng/ml for TNF- α , 0.6 \pm 0.002 ng/ml for GM-CSF and 0.28 \pm 0.14 ng/ml for IL-6, TF alone (80 $\mu\text{g}/\text{ml}$), 37.53 \pm 0.02 μM for NO, 5.77 \pm 0.012 ng/ml for TNF- α and 4.54 \pm 0.11 ng/ml for IL-6; TR alone (80 $\mu\text{g}/\text{ml}$), 33.86 \pm 0.14 μM for NO, 4.49 \pm 0.02 ng/ml for TNF- α and 2.50 \pm 0.16 ng/ml for IL-6; EGCG alone (80 $\mu\text{g}/\text{ml}$), 39.70 \pm 0.09 μM for NO, 7.60 ng/ml for TNF- α and 5.95 \pm 0.11 ng/ml for IL-6). These productions were higher than LPS alone. The amount of TNF- α was significantly higher in the TF, TR and EGCG with LPS only - treated cells than LPS - treated cells (about 2~3.5 fold for TNF- α).

Discussion

More duration time of fermentation with microorganisms led to raise in TF and total TR contents. Polyphenols TR, TF and EGCG may be beneficial to human health by resulting in a lower cancer risk. To measure the antioxidant

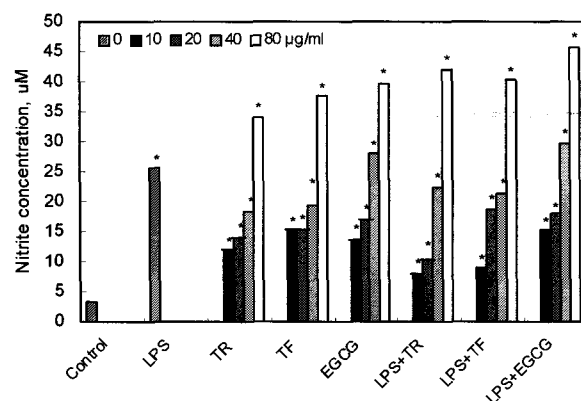


Fig. 1. Effect of TR, TF and EGCG on the production of nitrite in a macrophage cell line. RAW264.7 (5×10^4 cells/well) were cultured either in medium alone or in medium that contained LPS (10 $\mu\text{g}/\text{ml}$) or TR, TF and EGCG for the production of nitric oxide. After 48 hr of culture, the amounts of nitrite production were measured by the Griess method as described under Materials and Methods. * $p < 0.05$: Significant difference between untreated control and other experimental groups. The results are expressed as mean \pm SD of triplicate assays.

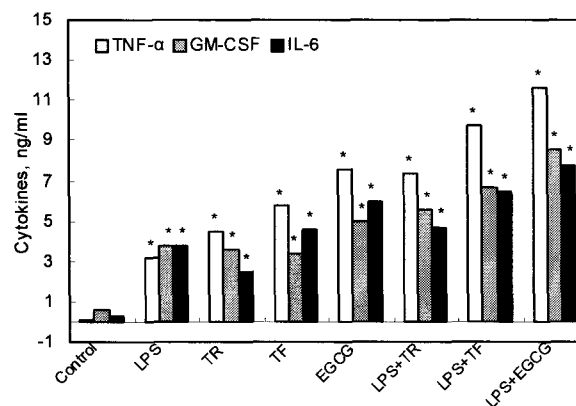


Fig. 2. RAW264.7 cells (5×10^4 cells/well) were stimulated with LPS (10 $\mu\text{g}/\text{ml}$) or TR, TF and EGCG (80 $\mu\text{g}/\text{ml}$) for 24 hr and the supernatants were assayed for various cytokines. Cytokine levels in culture supernatants were determined as described under Materials and Methods. * $p < 0.05$: Significant difference between untreated control and other experimental groups. The results are expressed as mean \pm SD of triplicate assays.

capacity of TR, TF and EGCG was employed FRAP assays that utilize the same single electron transfer mechanism. This method are generally employed to evaluate plant material [17]. Plant extracts contain different phenolic compounds. A correlation was found for the total phenolic content and the FRAP assays and this is in correspondence with Schlesier et al.[21]. Although values of FRAP were

Table 4. Inhibition of cancer cell lines growth by TF, TR and EGCG

Concen-trations ($\mu\text{g/ml}$)	Inhibition (%)					
	A-549			HT-29		
	TR	TF	EGCG	TR	TF	wwEGCG
Control	0	0	0	0	0	0
10	20.88 \pm 7.24 ^a	15.14 \pm 9.56 ^a	38.71 \pm 4.79 ^b	44.66 \pm 5.41 ^b	30.90 \pm 8.05 ^a	54.42 \pm 3.79 ^c
20	38.71 \pm 2.43 ^b	40.16 \pm 3.76 ^b	48.32 \pm 4.93 ^{bc}	55.07 \pm 1.67 ^{cd}	53.73 \pm 0.92 ^c	68.77 \pm 4.18 ^e
40	55.19 \pm 5.59 ^{cd}	56.43 \pm 8.47 ^{cd}	66.82 \pm 3.40 ^{ef}	61.84 \pm 2.16 ^{ce}	63.03 \pm 2.02 ^{de}	82.62 \pm 6.64 ^f
80	67.60 \pm 2.09 ^{ef}	62.69 \pm 6.33 ^{de}	74.57 \pm 1.98 ^f	68.08 \pm 4.18 ^e	69.65 \pm 6.90 ^e	89.67 \pm 2.54 ^f

The results are expressed as mean \pm SD of triplicate assays. ^{a-f}Means with the different letters are significantly different from corresponding controls at the 0.05 level of significance.

Table 5. Inhibition of cancer cell lines growth by TF, TR and EGCG

Concen-trations ($\mu\text{g/ml}$)	Inhibition (%)					
	HepG2			HeLa		
	TR	TF	EGCG	TR	TF	EGCG
Control	0	0	0	0	0	0
10	9.13 \pm 4.74 ^a	6.95 \pm 1.36 ^a	36.00 \pm 8.05 ^b	27.98 \pm 6.90 ^b	16.38 \pm 11.30 ^a	44.93 \pm 2.99 ^c
20	59.56 \pm 8.62 ^c	44.89 \pm 8.37 ^b	62.61 \pm 3.93 ^{cd}	48.96 \pm 8.23 ^c	41.71 \pm 2.84 ^c	60.30 \pm 2.41 ^d
40	63.39 \pm 12.76 ^{cd}	58.86 \pm 3.66 ^c	72.76 \pm 1.51 ^{de}	63.58 \pm 4.25 ^d	61.50 \pm 8.76 ^d	66.79 \pm 2.01 ^d
80	74.71 \pm 3.15 ^{de}	70.73 \pm 1.30 ^{ce}	82.36 \pm 3.68 ^e	74.23 \pm 1.26 ^{ef}	76.62 \pm 1.71 ^{ef}	81.16 \pm 1.61 ^f

The results are expressed as mean \pm SD of triplicate assays. ^{a-f}Means with the different letters are significantly different from corresponding controls at the 0.05 level of significance.

not significantly different, all studied samples showed high ferric reducing ability. TR, TF and EGCG quenched RNS effectively. Therefore, in cell free study, NO scavenging activity correlate with total phenolics content, which suggested that TR, TF and EGCG have the critical ability for the NO scavenging activity. The specific structural feature of phenolics needed for NO scavenging has been investigated previously, and it has been found that the presence of a catechol group in a flavonoid is essentially required for excellent NO scavenging ability [8].

As for inhibitory effect of TF, TR and EGCG on cell proliferation were found to be active against the cancer cell lines and showed a concentration-dependent affectivity. When exposed in vitro TF, TR and EGCG, inflammatory response can develop the capacity to kill tumor cells, and these stages are called fully activated nuclear factor- κ B (NF- κ B). Once activated, NF- κ B initiates the transcription of numerous genes coding for proteins involved in inflammatory and immune responses, including TNF- α and iNOS [9]. NO was produced in high amounts by iNOS in activated macrophages. Therefore, the tumoricidal activity induced by TF, TR and EGCG appeared to be mediated by the production of NO. The inhibitory effects of EGCG appeared to be higher than those of TF and TR because of

the purity of EGCG (98%) was higher than TF (80%) and TR (80%). Among tea components, polyphenols EGCG, TF and TR are generally considered to be the effective components, and a commonly discussed mechanism is the antioxidative activity of these polyphenols. These inhibitory effects were due to antioxidative effects because tea component-treated cells were affected at the doses used in this study. Meantime, tea polyphenols can spare, β -carotene and vitamins C and E, contributing to the overall antioxidant protection mechanism of the cell.

In this study, TF, TR and EGCG (10~80 $\mu\text{g/ml}$) increased NO, IL-6 and GM-CSF production, TNF- α production tend to increased highly in EGCG treatment. NO has been identified as a major effector molecule produced by macrophages and is involved in the regulation of apoptosis and in host defenses against microorganisms and tumor cells [5]. NO is related to cytotoxic function of macrophages against a variety of tumors and microorganisms [13,10,16], we examined the effects of TF, TR and EGCG on NO production using RAW264.7 macrophages cells. As shown in Fig. 1, the treatment of the cells with TF, TR and EGCG induced an increase in the production of NO by in dose-dependant manners, whereas NO scavenging activity was increased in cell free system. To the best our knowl-

edge, a dose response of TF, TR and EGCG on NO production may be explained by stimulation by biosynthetic mechanisms to low levels in cell system. Based on these finding the present data suggest that TF, TR and EGCG may have different effects on the function of normal and tumor cell lines and cell free and in cell system. IL-6 and GM-CSF is regulatory cytokines lead to a Th-2 type humoral immunity [18], while production of TNF- α lead to a Th-1 type cellular response. Many cancer vaccines elicit strong cellular immune responses leading to the production of Th-1 type cytokines such as TNF- α [7]. TNF- α can act on monocytes and macrophages in an autocrine manner to enhance various function responses, such as cytotoxicity to tumor cells, and induce the expression of a number of other immunoregulatory and inflammatory mediators [2]. TNF- α also modulates immune response by triggering the production of number of other regulatory cytokines such as IL-6 and GM-CSF [13]. TNF- α secretion by TF, TR and EGCG was highly stimulated in combination with LPS. These results suggest that TF, TR and EGCG can provide synergistic induction on TNF- α and that TF, TR and EGCG might have a beneficial effect in the treatment of various diseases like asthma and cancer through the immune-enhancement.

Also, more detailed work is required on the protective effects against cancer cell and immuno-modulating effects to know their exact mechanism of action.

Acknowledgement

This study was supported by Technology Development Program of the Ministry of Agriculture and Forestry, Republic of Korea.

Reference

- Andras, H., C. Ildiko, B. Tamas and K. C. Laszlo. 2005. The cytotoxic antitumor effect of MTH-68/H, a live attenuated Newcastle disease virus is mediated by the induction of nitric oxide synthesis in rat peritoneal macrophages *in vitro*. *Cancer Lett.* **20**, 1-11.
- Baugh, J. A. and R. Bucala. 2001. Mechanisms for modulating TNF alpha in immune and inflammatory disease. *Curr. Opin. Drug. Discov. Devel.* **4**, 635-650.
- Benzie, I. F. F. and J. J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as measurement of "antioxidant power": The Frap assay. *Anal. Biochem.* **239**, 70-76.
- Brannellec, D. and S. Chouaib. 1991. TNF: antitumoral agent at the border lines of immunity and inflammation. *Oathologie* **39**, 230-239.
- Brune, B. 2003. Nitric oxide: NO apoptosis or turning it ON? *Cell Death Differ.* **10**, 864-869.
- Carter, L. L. and R. W. Dutton. 1996. Type 1 and type 2: a fundamental dichotomy for all T-cell subsets. *Curr. Opin. In. Immu.* **8**, 336-342.
- Dalgleish, A. G. 2000. Cancer vaccines. *British J. Cancer* **82**, 1619-1624.
- De la Puerta, R., M. E. M. Domingue, V. Rui-Gutierrez, J. A. Flavill and J. R. S., Hoult. 2001. Effects of virgin olive oil phenolics on scavenging of reactive nitrogen species and upon nitrenergic neurotransmission. *Life Sci.* **69**, 1213-1222.
- Feihl, F., M. Oddo, B. Waeber, L. Liaudet. 2004. Inhibitors of nitrogenoxide species production in animal models of inflammation and future directions for therapy in inflammatory disorders. *Curr. Med. Chem. Anti-Inflammatory Anti-Allergy Agents* **3**, 239-259.
- Hibbs, J. B. Jr., R. R. Taintor, Z. Vavrin and E. M. Rachlin. 1980. Nitric oxide: a cytotoxic activated macrophage effector molecule. *Biochem. Biophys. Res. Commun.* **157**, 87-94.
- Hilton P. J. and R. T. Ellis. 1972. Estimation of market value of Central African tea by theaflavin analysis. *J. Agric. Food Chem.* **50**, 1833-1839.
- Jeong, H. J. 2003. Immune-enhancement effect of the herbal combination Allergina. *Clinica. Chimica. Acta.* **337**, 77-84.
- Keller, R. and R. Keist. 1989. Abilities of activated macrophages to manifest tumoricidal activity and to generate reactive nitrogen intermediates: a comparative study in vitro and ex vivo. *Biochem. Biophys. Res. Commun.* **157**, 87-94.
- Marcocci, L., J. J. Maguire, M. T. Droy-Lefaix and L. Packer. 1994. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. *Biochem. Biophys. Res. Commun.* **201**, 748-755.
- Martin, O. P., O. Okinda, M. Richard and M. Mutuku. 2004. Changes in thearubigin fractions and theaflavin levels due to variations in processing conditions and their influence on black tea liquor brightness and total colour. *Food Chem.* **85**, 163-173.
- Nathan, C. F. 1987. Secretory products of macrophages. *J. Clin. Invest.* **79**, 319-326.
- Ozgen, M., R. N. Reese, A. Z. Tulio, J. C. Scheerens and A. R. Miller. 2006. Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) methods. *J. Agric. Food Chem.* **54**, 1151-1157.
- Parronchi, P., D. Macchia, M. P. Piccinni, P. Biswas, C. Simonelli, E. Maggi, M. Ricci, A. A. Ansari and S. Romagnani. 1991. Allergen and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. In: proceedings of the National Academy of Sciences of the United States of

- America 88, 4538-4542.
19. Riemersma, R. A., C. A. Rice-Evans., R. M. Tyrrell., M. N. Clifford and M. E. Lean. 2001. Tea flavonoids and cardiovascular health. *J. Med.* 94, 277-282.
 20. Roberts, E. A. H. and R. F. Smith. 1961. Spectrophotometer measurements of theaflavins and thearubigins in black tea liquors in assessments of quality in teas. *The analyst* 86, 94-98.
 21. Schlesier, K., M. Harwat, V. Bohm and R. Bitsch. 2002. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *J. Agric. Food Chem.* 50, 81-86.
 22. Shiraki, M., Y. Hara, T. Osawa, H. Kumon, T. Nakauma and S. Kawaskishi. 1994. Antioxidative and antimutagenic effects of theaflavins from black tea. *Mut. Res.* 323, 29-34.
 23. Skehan, P., R. Storeng, D. Scudiero, A. Monks, L. McMahon, D. Vistica, T. Warren, H. Bokesch, S. Kenney and M. R. Boyd. 1990. New colorimetric cytotoxicity assay for anticancer drug screening. *J. National Cancer Institute* 82, 1107-1112.
 24. Shon, M. Y., S. K. Park and S. H. Nam. 2007. Antioxidant activity of theaflavin and thearubigin separated from Korean microbially fermented tea. *J. Food Sci. Nutr.* 12, 7-10.
 25. Terao, J., M. Piskula and Q. Yao. 1994. Protective effects by epicatechin, epicatechin gallate and quercetin on lipid peroxidation in phospholipid bilayers. *Arch. Biochem. Biophys.* 308, 278-284.
 26. Xie, B., H. Shi, Q. Chen and C. T. Ho. 1993. Antioxidant properties of fractions and polyphenol constituents from green, oolong and black teas. *Proc. Natl. Sci. Council Rep. China.* 17, 77-84.
 27. Yee, S. T., Y. R. Jeong, M. H. Ha, S. H. Kim, M. M. Byun and S. K. Jo. 2000. Induction of nitric oxide and TNF- α by herbal plant extract in mouse macrophage. *J. Korean Soc. Food Sci. Nutr.* 29, 342-348.

초록 : 차 폴리페놀화합물의 사이토카인 생성 및 항암능에 대한 영향

손미에 · 남상해^{1*}

(경상대학교 식품영양학과, ¹진주산업대학교 식품과학과)

국산 미생물 발효차의 폴리페놀 색소성분들인 테아플라빈(TF)과 테아루비긴(TR) 및 EGCG를 macrophage cell line (RAW264.7)에 적용하여 nitric oxide 합성 및 사이토카인 생성을 평가하였다. 사이토카인 생성은 TF, TR 및 EGCG를 RAW264.5 cell에 적용하였을 때, 80 $\mu\text{g/ml}$ 농도에서 대조군과 LPS 촉진 처리에 비하여 nitric oxide 생성은 약 1.5배 증가하였다. IL-6, TNF- α 및 GM-CSF는 TF, TR 및 EGCG 농도에 의존적으로 증가하였다. TNF- α 생성은 크게 증가하였으며, 이는 TF, TR 및 EGCG가 사이토카인 생성을 통하여 면역증강 효과를 가질 것으로 나타났다. TF, TR 및 EGCG는 총 페놀 함량에 비례하여 항산화능을 나타내었으며, 암세포 증식을 유의적으로 억제하였다. 이들 폴리페놀물질의 억제효과는 그 성분들의 항암촉진작용 및 항산화활성에 의한 것으로 판단된다.