Green tea catechin induced phagocytosis can be blocked by catalase and an inhibitor of transient receptor potential melastatin 2 (TRPM2)

Manami Monobe · Kaori Ema ·
Yoshiko Tokuda · Mari Maeda-Yamamoto

Received: 24 February 2013 / Accepted: 15 July 2013 / Published online: 30 July 2013
© Springer Science+Business Media Dordrecht 2013

Abstract The major polyphenols in green tea, (–)-epigallocatechin and (–)-epigallocatechin gallate, have been shown to enhance the phagocytic activity of macrophage-like cells; however, the mechanism involved was not clarified. In this study, we have identified that the catechins induced phagocytosis can be blocked by catalase and an inhibitor of transient receptor potential melastatin 2.

Keywords (–)-Epigallocatechin (EGC) · (–)-Epigallocatechin gallate (EGCG) · Phagocytosis · TRPM2 · Hydrogen peroxide

Introduction

Phagocytosis by macrophages is an important non-specific host-defense mechanism. Previously, we found that pyrogallol-type green tea catechins [(–)-epigallocatechin (EGC); (–)-epigallocatechin gallate (EGCG) etc.]

(Fig. 1) enhanced the phagocytic activity of human macrophage-like cells (Monobe et al. 2010). The increase in phagocytic activity was related to the activation of caspase signaling pathways (Monobe et al. 2010); however, the receptor involved in the activation was not identified. Recently, a 67-kDa laminin receptor (67LR) has been identified as a cell surface receptor for gallate-type catechins (EGCG, ECG etc.) that mediates the anti-cancer effect of EGCG (Tachibana et al. 2004). However, 67LR's activation by EGCG mediates immunosuppressive effects such as anti-allergic activities (Fujimura et al. 2007) and non-immune enhancing effects. Moreover, EGC, a non-gallate type catechin, cannot interact with 67LR (Tachibana et al. 2004). The marked anti-oxidative capacity of catechins does not explain the bioactivity.

Catechins are known to have anti-oxidant activity, but they also have pro-oxidant activity. Catechins react with dissolved oxygen in aqueous solutions, resulting in the generation of hydrogen peroxide (H₂O₂) (Arakawa et al. 2004). This pro-oxidant activity is considered an important part of their anti-cancer, anti-microbial, and apoptosis-inducing properties (Sugisawa and Umegaki 2002; Arakawa et al. 2004; Azam et al. 2004). However, at low levels, H₂O₂ acts as a signaling molecule (Matoba et al. 2000; Vanhoutte, 2001), and activates macrophages through transient receptor potential melastatin 2 (TRPM2) (Kashio et al. 2012). Therefore, we investigated the involvement of H₂O₂ in the enhancement of phagocytic activity by EGC and EGCG.

M. Monobe (✉) · K. Ema · Y. Tokuda
NARO Institute of Vegetable and Tea Science,
2769 Kanaya-Shishidoi, Shimada, Shizuoka 428-8501, Japan
e-mail: monobe@affrc.go.jp

M. Maeda-Yamamoto
NARO National Food Research Institute,
2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

Fig. 1 Green tea catechins with a pyrogallol-type structure

Materials and methods

Phagocytosis assay

The phagocytosis assay was performed as described previously (Monobe et al. 2007). Briefly, to differentiate cells along the monocytic pathway, HL60 cells (ATCC, Rockville, MD, USA), a human acute promyelocytic cell line, were cultured at densities of $1\text{--}5 \times 10^5$ cells/mL in RPMI 1640 medium (Gibco-Invitrogen, Carlsbad, CA, USA) supplemented with 10 % FBS and 120 nM 1,25-dihydroxyvitamin D3 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for more than 1 week. Differentiated HL60 cells (2.5×10^5 cells/225 μ L/well) were seeded into 48-well plates (Falcon 351178; BD Biosciences, Franklin Lakes, NJ, USA). The cells were treated with 25 μ L of EGC (25 μ g/mL; Sigma-Aldrich, St. Louis, MO, USA), EGCG (25 μ g/mL; Sigma-Aldrich), H_2O_2 (12–100 μ M; Wako Pure Chemical Industries), catalase (50 U/mL; Wako Pure Chemical Industries), 2-aminoethoxydiphenyl borate (2-APB, 100 μ M; Enzo Life Sciences, Farmingdale, NY, USA), and/or *N*-(*p*-amylcinnaomyl) anthranilic acid (ACA, 200 μ M; Enzo Life Sciences); 25 μ L of a 1 % suspension of YG-labeled microspheres (Polysciences, Inc., Warrington, PA, USA) was added, and the

solution was incubated at 37 °C for 16 h. The cells were fixed with 2 % formaldehyde for 30 min and resuspended in PBS. The rate of phagocytosis was measured with an EPICSXL-flow cytofluorometer (Beckman Coulter, Fullerton, CA, USA).

Hydrogen peroxide detection

H_2O_2 concentrations in the culture medium were determined using a hydrogen peroxide colorimetric detection kit (Arbor Assays, Ann Arbor, MI, USA) according to the manufacturer's instructions.

Statistical analysis

All data were derived from at least three experiments. Data are expressed as the means $\pm$ standard deviation (SD). The efficiency of phagocytosis was compared using the paired *t* test. A value of $p < 0.05$ was considered to be significant. *n* represents the number of experiments.

Results and discussion

Previously, we found that pyrogallol-type green tea catechins (EGC, EGCG etc.) (Fig. 1) enhanced the

Fig. 2 EGC (or EGCG)-induced phagocytic activity is inhibited by a catalase. VD3-differentiated HL60 cells were incubated with EGC for 16 h (25 $\mu\text{g/mL}$) (a) or EGCG (25 $\mu\text{g/mL}$) (b) in the absence or presence of a catalase (50 U/mL). Phagocytic activity in the absence of catechins (control) is normalized to 100 %. Values are means ($n = 3$) $\pm$ SD. * $p < 0.01$ versus the control, + $p < 0.01$ versus the absence of catalase. Inserted histograms represent a decrease of fluorescent beads signal enhanced by catechins due to the addition of catalase. Closed histograms represent the control (RPMI), and opened histograms represent the addition of catechins

phagocytic activity of human macrophage-like cells (Monobe et al. 2010), but could not identify the receptor involved in the activation. Recently, it was reported that at low levels, H_2O_2 acts as a signaling molecule (Matoba et al. 2000; Vanhoutte 2001). Therefore, we investigated the involvement of H_2O_2 in the enhancement of phagocytic activity by EGC/EGCG using catalase, an H_2O_2 -degrading enzyme. As shown in Fig. 2, the increase in phagocytic activity was inhibited by the catalase.

Transient receptor potential melastatin 2 is a Ca^{2+} -permeable cation channel activated by adenosine diphosphate ribose and H_2O_2 , and relates to the activation of the innate immune system (Gardella et al. 2000, 2001; Yamamoto et al. 2008; Wehrhahn et al. 2010; Di et al. 2011; Kashio et al. 2012). Therefore, we investigated the involvement of TRPM2 in the enhancement of phagocytic activity by the catechins using the TRPM2 inhibitor, 2-APE and ACA. As shown in Fig. 3, the inhibitors blocked the increase in phagocytic activity. Then, we measured

the H_2O_2 concentration in the macrophage-suspension after the EGC or EGCG was added. As shown in Fig. 4a, 2–6 μM of H_2O_2 was detected after 30 min but no H_2O_2 was detected after 2 h. It has been reported that EGCG in phosphate buffer (pH 7.8) continues producing H_2O_2 for more than 2 h (Arakawa et al. 2004); therefore, we measured the H_2O_2 produced from the catechins in macrophage-free medium supplemented with 10 % FBS. H_2O_2 was detected for at least 2 h (Fig. 4b), and its H_2O_2 level was the same as at 30 min of incubation. Macrophages produce a myeloperoxidase that converts H_2O_2 to H_2O and O_2 . The myeloperoxidase changes EGC and EGCG into polymeric catechins. Therefore, the H_2O_2 produced from EGC/EGCG in macrophage-free medium may disappear within 2 h.

Next, to confirm that H_2O_2 enhances the phagocytic activity, we added it directly. As shown in Fig. 5, the addition of 25 and 50 μM H_2O_2 enhanced the activity. The concentration of H_2O_2 in the macrophage-suspension 30 min after the addition of 25 and 50 μM

Fig. 3 (–)-Epigallocatechin (or EGCG)-induced phagocytic activity is inhibited by 2-aminoethoxydiphenyl borate (2-APB) or *N*-(*p*-amylcinnamoyl) anthranilic acid (ACA). VD3-differentiated HL60 cells were incubated with (a, c) EGC or (b, d) EGCG in the absence or presence of 2-APB (100 μ M) or ACA (200 μ M) for 16 h. Phagocytic activity in the absence of catechins (control) is normalized to 100 %. Values are means ($n = 3$) $\pm$ SD. * $p < 0.05$ versus the control, + $p < 0.05$ versus the absence of 2-APB or ACA

Fig. 4 Concentration of hydrogen peroxide (H_2O_2) in cell medium or cell-free medium. a H_2O_2 concentration in the macrophage-suspension after the addition of EGC (or EGCG). b H_2O_2 concentration in the cell-free medium after the addition of EGC (or EGCG). c H_2O_2 concentration in the macrophage-suspension after the addition of H_2O_2 . Values are means ($n = 3$) $\pm$ SD. + $p < 0.05$ versus before addition of EGC or EGCG, * $p < 0.01$ versus the EGCG at the same time, † $p < 0.01$ versus 60 min after addition of H_2O_2 , § $p < 0.01$ versus 25 μ M H_2O_2 at the same time

Fig. 5 H₂O₂-induced phagocytic activity. VD3-differentiated HL60 cells were incubated with H₂O₂ for 16 h. Phagocytic activity in the absence of H₂O₂ (control) is normalized to 100 %. Values are the means (n = 3) ± SD. *p < 0.01 versus the control

Fig. 6 TRPM2-dependent phagocytic activity in macrophages

H₂O₂ was 2–10 μM (Fig. 4c), the same as that 30 min after the addition of EGC or EGCG (Fig. 4a); however, no H₂O₂ was detected after 1 h.

These results suggest that the H₂O₂ produced from EGC and EGCG may contribute to the activation of macrophages through TRPM2 (Fig. 6). EGCG at less than 100 μM does not cause critical oxidative damage (8-OHdG) in HL-60 cells (Furukawa et al. 2003; Oikawa et al. 2003). Further study is needed to clarify the mechanisms of macrophage activation by EGC or EGCG; however, this finding may be important for

understanding the immune regulatory properties of catechins.

Acknowledgments This work was supported by a grant for project research (Development of fundamental technology for analysis and evaluation of functional agricultural products and functional foods), Ministry of Agriculture, Fishery and Forestry, Japan.

References

- Arakawa H, Maeda M, Okubo S, Shimamura T (2004) Role of hydrogen peroxide in bactericidal action of catechin. *Biol Pharm Bull* 27:277–281
- Azam S, Hadi N, Khan NU, Hadi SM (2004) Prooxidant property of green tea polyphenols epicatechin and epigallocatechin-3-gallate: implications for anticancer properties. *Toxicol In Vitro* 18:555–561
- Di A, Gao XP, Qian F, Kawamura T, Han J, Hecquet C, Ye RD, Vogel SM, Malik AB (2011) The redox-sensitive cation channel TRPM2 modulates phagocyte ROS production and inflammation. *Nat Immunol* 13:29–34
- Fujimura Y, Umeda D, Yano S, Maeda-Yamamoto M, Yamada K, Tachibana H (2007) The 67 kDa laminin receptor as a primary determinant of anti-allergic effects of O-methylated EGCG. *Biochem Biophys Res Commun* 364:79–85
- Furukawa A, Oikawa S, Murata M, Hiraku Y, Kawanishi S (2003) (–)-Epigallocatechin gallate causes oxidative damage to isolated and cellular DNA. *Biochem Pharmacol* 66:1769–1778
- Gardella S, Andrei C, Poggi A, Zocchi MR, Rubartelli A (2000) Control of interleukin-18 secretion by dendritic cells: role of calcium influxes. *FEBS Lett* 481:245–248
- Gardella S, Andrei C, Lotti LV, Poggi A, Torrisi MR, Zocchi MR, Rubartelli A (2001) CD8(+) T lymphocytes induce polarized exocytosis of secretory lysosomes by dendritic cells with release of interleukin-1β and cathepsin D. *Blood* 98:2152–2159
- Kashio M, Sokabe T, Shintaku K, Uematsu T, Fukuta N, Kobayashi N, Mori Y, Tominaga M (2012) Redox signal-mediated sensitization of transient receptor potential melastatin 2 (TRPM2) to temperature affects macrophage functions. *Proc Natl Acad Sci U S A* 109:6745–6750
- Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, Takeshita A (2000) Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J Clin Invest* 106:1521–1530
- Monobe M, Ema K, Kato F, Hirokane H, Maeda-Yamamoto M (2007) Technique for screening immune-enhancing polysaccharides in food using 1,25-dihydroxyvitamin D3-differentiated HL60 cells. *J Agric Food Chem* 55:2543–2547
- Monobe M, Ema K, Tokuda Y, Maeda-Yamamoto M (2010) Enhancement of phagocytic activity of macrophage-like cells by pyrogallol-type green tea polyphenols through caspase signaling pathways. *Cytotechnology* 62:201–203
- Oikawa S, Furukawa A, Asada H, Hirakawa K, Kawanishi S (2003) Catechins induce oxidative damage to cellular and

- isolated DNA through the generation of reactive oxygen species. *Free Radic Res* 37:881–890
- Sugisawa A, Umegaki K (2002) Physiological concentrations of (–)-epigallocatechin-3-O-gallate (EGCg) prevent chromosomal damage induced by reactive oxygen species in WIL2-NS cells. *J Nutr* 132:1836–1839
- Tachibana H, Koga K, Fujimura Y, Yamada K (2004) A receptor for green tea polyphenol EGCG. *Nat Struct Mol Biol* 11:380–381
- Vanhoutte PM (2001) Endothelium-derived free radicals: for worse and for better. *J Clin Invest* 107:23–25
- Wehrhahn J, Kraft R, Harteneck C, Hauschildt S (2010) Transient receptor potential melastatin 2 is required for lipopolysaccharide-induced cytokine production in human monocytes. *J Immunol* 184:2386–2393
- Yamamoto S, Shimizu S, Kiyonaka S, Takahashi N, Wajima T, Hara Y, Negoro T, Hiroi T, Kiuchi Y, Okada T, Kaneko S, Lange I, Fleig A, Penner R, Nishi M, Takeshima H, Mori Y (2008) TRPM2-mediated Ca^{2+} influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. *Nat Med* 14:738–747