Send Orders for Reprints to reprints@benthamscience.net

 Inflammation & Allergy - Drug Targets, 2013, *12*, 308-314

Green Tea Catechins Quench the Fluorescence of Bacteria-Conjugated Alexa Fluor Dyes

Lin Zhao[1,2], Wei Li[1,3], Shu Zhu[1,3], Sheena Tsai[1], Jianhua Li[1], Kevin J. Tracey[1], Ping Wang[1], Saijun Fan[4], Andrew E. Sama[1,3] and Haichao Wang[*,1,3]

[1]*The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA*

[2]*School of Radiation Medicine and Protection, Soochow University Medical College, Suzhou, Jiangsu 215123, China*

[3]*Department of Emergency Medicine, North Shore University Hospital, Manhasset, NY 11030, USA*

[4]*The Radiation Medicine Institute of Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300192, China*

Abstract: Accumulating evidence suggests that Green tea polyphenolic catechins, especially the (-)-epigallocatechin gallate (EGCG), can be cross-linked to many proteins, and confer a wide range of anti-bacterial activities possibly by damaging microbial cytoplasmic lipids and proteins. At the doses that conferred protection against lethal polymicrobial infection (induced by cecal ligation and puncture), EGCG significantly reduced bacterial loads particularly in the liver and lung. To elucidate its bactericidal mechanisms, we determined whether EGCG affected the fluorescence intensities of bacteria-conjugated Alexa Fluor 488 or 594 dyes. When mixed with unconjugated Alexa Fluor 488 or 594 dyes, EGCG or analogs did not affect the fluorescence intensity of these dyes. In a sharp contrast, EGCG and some analogs (e.g., Catechin Gallate, CG), markedly reduced the fluorescence intensity of Gram-positive *Staphylococcus aureus*-conjugated Alexa 594 and Gram-negative *Escherichia coli*-conjugated Alexa 488. Interestingly, co-treatment with ethanol impaired the EGCG-mediated fluorescence quenching of the G^+ *S. aureus*, but not of the G^- *E. coli*-conjugated Alexa Flour dyes. In light of the notion that Alexa Fluor dyes can be quenched by aromatic amino acids, it is plausible that EGCG exerts anti-microbial activities possibly by altering microbial protein conformations and functions. This possibility can now be explored by screening other fluorescence-quenching agents for possible antimicrobial activities.

Keywords: Alexa Fluor 488, Alexa Fluor 594, antimicrobial, EGCG, fluorescence intensity, macrophage, sepsis, tissue bacterial load.

INTRODUCTION

As sequelae of systemic bacterial infections, sepsis and septic shock remain the major cause of increased mortality in the intensive care units, accounting for high health care costs every year [1]. The emergence of antibiotic resistance (AR) has further compromised the successful application of antibacterial drugs in the clinical management of bacterial infections [2-4]. It is thus essential to develop high-efficiency and low-resistance antimicrobial agents to fight against lethal microbial infections.

Brewed from the leaves of *Camellia sinensis*, Green tea has been associated with multiple health benefits against oxidative stress [5], atherosclerosis [5], cancer [6], cardiovascular diseases [7], as well as lethal bacterial infections [8, 9]. These healing properties are attributable to its abundant polyphenolic catechins, such as the epigallocatechin-3-gallate, EGCG, which accounts for up to 50 mg in a single cup of tea [10]. During the last two decades, extensive evidence has emerged to support a wide spectrum of anti-bacterial [11-14], anti-viral [15-17], or anti-fungal properties for EGCG and other polyphenolic catechins [18]. For instance, it is shown that EGCG can render bacteria more sensitive to various anti-bacterial agents [13, 19-21], thereby impairing their resistance to antibiotics. EGCG exerts these anti-bacterial activities partly by damaging bacterial cytoplasmic lipids [22-25], membrane proteins [26], or cytoplasmic enzymes (e.g., gelatinase, protein tyrosine phosphatase, dihydrofolate reductase, or DNA gyrase) [27-29]. Indeed, scanning electron microscopic analysis revealed that EGCG treatment caused alterations in both bacterial cell morphology [30] and outer membrane (e.g., wrinkling with perforations) [31]. As a possible consequence, EGCG impaired the cytoplasmic membrane efflux pump activities, and consequently elevated intracellular antibiotics concentrations, supporting the possible use of EGCG as an adjuvant in antibacterial therapy [19].

Parallel evidence has also indicated that EGCG can be cross-linked to various proteins [9, 16, 32-37], and consequently induces protein conformational alterations [35], suggesting EGCG as a potent protein remodeling agent. For instance, EGCG binds covalently to sulfhydryl groups of membrane proteins, leading to inter-molecular protein cross-linking and accompanying conformational changes [38]. Notably, these protein conformational changes could be monitored by employing novel fluorescence quenching

*Address correspondence to this author at the Department of Emergency Medicine, North Shore University Hospital, 350 Community Drive, Manhasset, NY 11030, USA; Tel: (516)562-2823; E-mail: hwang@nshs.edu

2212-4055/13 $58.00+.00 © 2013 Bentham Science Publishers

techniques [39]. For instance, the Alexa Fluor dyes have been popularly used to fluorescently label various macromolecules because of their relative insensitivity to many chemicals. However, their fluorescence can be quenched by aromatic amino acids (e.g., tyrosine, tryptophan and histidine) in solution-based titrations [40], or intramolecularly when these aromatic residues physically interact with the coupled fluorescence dyes [40]. In the present study, we sought to test the possibility that EGCG may alter the fluorescence of bacteria-conjugated Alexa Fluor dyes, providing a basis for future screening of fluorescence-quenching agents for potential anti-bacterial activities.

MATERIALS AND METHODS

Animal Model of Polymicrobial Infection (Sepsis)

To evaluate the bactericidal activities of EGCG, we determined its effects on bacterial elimination in an animal model of polymicrobial infection induced by cecal ligation and puncture (CLP) as previously described [9, 41-43]. Briefly, the cecum of BALB/c mice (7-8 weeks, 20-25 g) was ligated at 5.0 mm from the cecal tip, and then punctured once with a 22-gauge needle to allow leakage of bacteria into the peritoneal cavity. EGCG stock solution was prepared in isotonic saline solution at a concentration of 1.0 mg/ml. At 24 and 48 h post CLP, EGCG saline solution (0.1 ml, equivalent to 4.0 mg/kg) was intraperitoneally administered into mice using small (26-gauge) syringe needles. At 52 h post CLP, peritoneal lavage fluid and animal organs were harvested using sterile techniques to quantitate tissue bacterial loads. Briefly, peritoneal lavage was performed with 5 ml of sterile saline using an 18-gauge syringe needle. The liver, spleen, kidneys, and lungs were harvested using sterile techniques, and homogenized at 4°C in 4 volumes of sterile saline. Immediately, the homogenates were serially diluted, and aliquots of several dilutions (50 µl) were plated onto Luria broth agar plates in triplicates (Fisher Scientific, Pittsburgh, PA). Following incubation at 37°C for 24 hours under aerobic conditions, the bacterial Colony-forming units (CFU) were counted manually under transmitted light. The tissue bacterial loads were then calculated, and expressed as CFU per mg of wet tissue. This study was approved and performed in accordance with the guidelines for the care and use of laboratory animals at the Feinstein Institute for Medical Research, Manhasset, New York, USA.

Cell Culture

Murine macrophage-like RAW 264.7 cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA), and cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Grand Island, NY), supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin (Gibco), at 37° C in a humidified 5% CO_2 atmosphere. Alexa Fluor 594-conjugated *Staphylococcus aureus* or Alexa Fluor 488-labeled *Escherichia coli* (Invitrogen, Carlsbad, CA, USA) were re-suspended in 1×PBS, and added to macrophage cultures (at 1.0 µg/ml). Following incubation for 2 h, (-)-Epigallocatechin Gallate (EGCG, 20 µM) was added and incubated for 30 min. Following intensive washings, the intensity of fluorescence was estimated by fluorescence microscopy or flow cytometry, respectively. For flow cytometry, the fluorescence of phagocytosed bacteria was analyzed with a FACSCalibur instrument (Becton Dickinson) equipped with CellQuest software. For each sample, at least 1×10^4 cells were collected and analyzed.

Effects of EGCG on the Fluorescence of Alexa Fluor Dyes

Alexa Fluor 594 and Alexa Fluor 488 carboxylic acids (Invitrogen, Carlsbad, CA, USA) were dissolved in 1 x PBS at the concentration of 250 ng/ml. Bacteria-conjugated Alexa Fluor dyes were re-suspended into 1 x PBS to generate bacterial suspension at the concentration of 40 µg/ml. To determine whether EGCG affects the intensity of Alexa Fluor dyes, the unconjugated or bacteria-conjugated fluorescence dyes were exposed to EGCG (20 µM) or analogs for 30 min. The intensity of the fluorescence was measured using a fluorescence microscope (Carl Zeiss Microimaging) or a Fluorescence Spectrophotometer (F-7000, Hitachi High Technologies America, Inc.).

Statistical Analysis

Tissue bacterial CFU were presented as scatter dot plots (with means). Differences between groups were compared by the Mann-Whitney U test. A P value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Accumulating evidences indicated that Green tea possessed a wide spectrum of anti-microbial [11-14] and anti-viral [16, 17] activities, but the anti-microbial mechanisms remained poorly understood. In the present study, we demonstrated that intraperitoneal administration of EGCG did not significantly reduce bacterial loads in the peritoneal cavity and the blood (data not shown). However, it significantly reduced bacterial CFU in the liver and lung tissue (Fig. **1**), suggesting that EGCG facilitated bacterial elimination in selective organs during systemic polymicrobial infection. The underlying antibacterial mechanisms may be attributable to the possibilities that EGCG facilitates bacterial elimination either directly by interacting and inhibiting their growth, or indirectly by modulating hydrogen peroxide production [44] or macrophage-associated innate immune responses. For patients with severe lung infections (e.g., pneumonia) due to antibiotics-resistant bacteria, other effective therapies are urgently needed. It is possible that EGCG serves as one such potential adjuvant therapy due to its immunomodulatory [45, 9] and antimicrobial activities [11-14, 46].

Indeed, EGCG can be cross-linked to various proteins [9, 16, 32-34], and consequently change their tertiary structures and biological functions [35], suggesting EGCG as a potent protein remodeling agent. For instance, EGCG binds to HSV-1 virus envelope glycoproteins [16] or influenza haemagglutinin [17], and consequently induces the formation of viral protein complexes. To test the possibility that EGCG facilitated bacterial elimination by conjugating to bacterial proteins, we determined whether EGCG quenched fluorescence of the Alexa Fluor dyes chemically conjugated to various bacteria (e.g., *S. aureus* and *E. coli*) *via* amine-

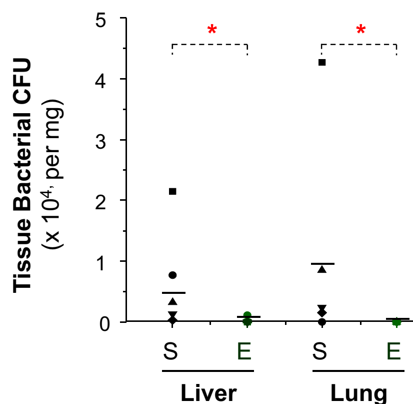

Fig. (1). EGCG facilitated bacterial elimination during polymicrobial sepsis. BALB/c mice were subjected to polymicrobial sepsis by cecal ligation and puncture (CLP), and intraperitoneally administered with EGCG at 24 and 48 h post CLP. At 52 h post CLP, animal organs were harvested *via* sterile techniques, and the number of bacterial colony formation units (CFU) was counted. Each dot represents output (the average CFU of triplicate agar plates) from each animal, and the horizontal lines represent the means of five animals in each experimental group. *, $P < 0.05$ for comparison between EGCG and saline groups.

reactive groups. Indeed, EGCG and an analog, CG, markedly reduced the fluorescence intensity of bacteria-conjugated Alexa Flour 488 or 594 dyes (Fig. **2**). In a sharp contrast, other analogs (e.g., C or G) or antibiotics (e.g., Amphotericin B and Erythromycin, data not shown) did not affect the fluorescence intensity of bacteria-conjugated Alexa Flour dyes (Fig. **2**). Furthermore, these fluorescence quenching properties appeared to attribute to the presence of the galloyl moiety, which was ester-linked with the 3-OH of the Green tea catechins (Fig. **2**).

It is known that the relative concentrations of EGCG (> 800 µg/ml) needed to inhibit the growth of G^- bacteria (e.g., *E. coli, K. pneumoniae, S. typhi, P. mirabilis, P. aeruginosa,* and *S. marcescens*) are relatively higher than the doses (50-100 µg/ml) of EGCG needed to inhibit G^+ bacteria (*S. aureus, S. epidermidis, S. hominis,* and *S. haemolyticus*). This variation may be attributable to the difference in the structure of the G^+ and G^- bacterial cell walls, or the affinities of EGCG to various cell wall components [47]. Interestingly, we found that co-treatment with the absolute ethanol rendered G^+ (but not G^-) bacteria resistant to EGCG-mediated quenching of the bacteria-conjugated Alexa Fluor dyes (Fig. **3**). It was possible that the ethanol-mediated dehydration/condensation of G^+ peptidoglycan hindered the access of EGCG to the peptidoglycan or the inner cytoplasmic membrane proteins, two classes of macromolecules implicated in EGCG binding [48]. Consistently, alternative strategies to block the access of EGCG to targeted bacterial macromolecules (such as by adding exogenous proteins) uniformly impaired EGCG's antibacterial activities [47].

To test the possibility that EGCG directly interacts with the Alexa Fluor dyes to quench the fluorescence, Alexa Fluor 594/488 dyes were separately mixed with EGCG, and

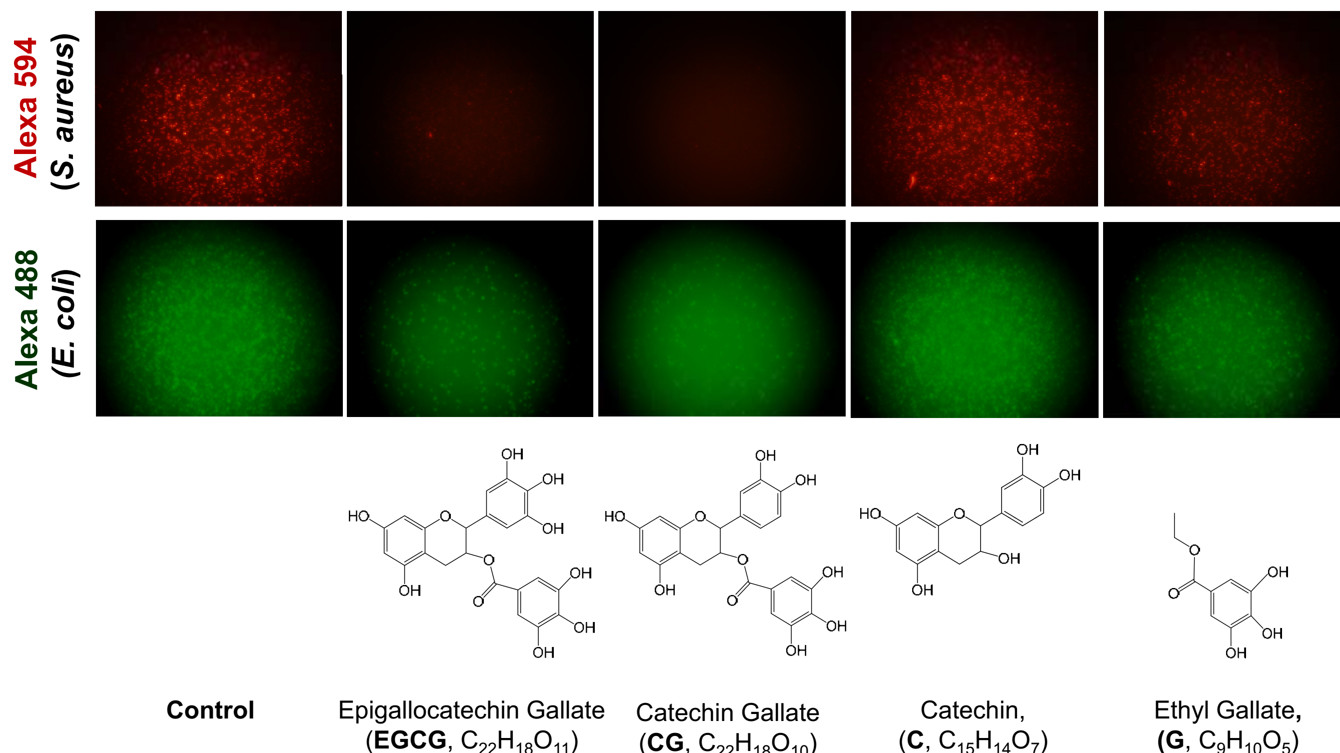

Fig. (2). EGCG reduced the fluorescence intensity of bacteria-conjugated Alexa Fluor dyes. Bacteria-conjugated Alexa Fluor dyes (Alexa 594 – *S. aureus,* Alexa Fluor 488 – *E. coli*) were re-suspended in 1×PBS (1 µg/ml), and seeded onto 24-well culture plates. After incubation for 4 h, EGCG or various analogs (e.g., CG, C, or G) were added to fluorescence-labeled bacteria. Approximately 30 min later, images were acquired with a fluorescence microscope with a digital camera controlled by Meta-Morph software (Universal Imaging; Downingtown, PA).

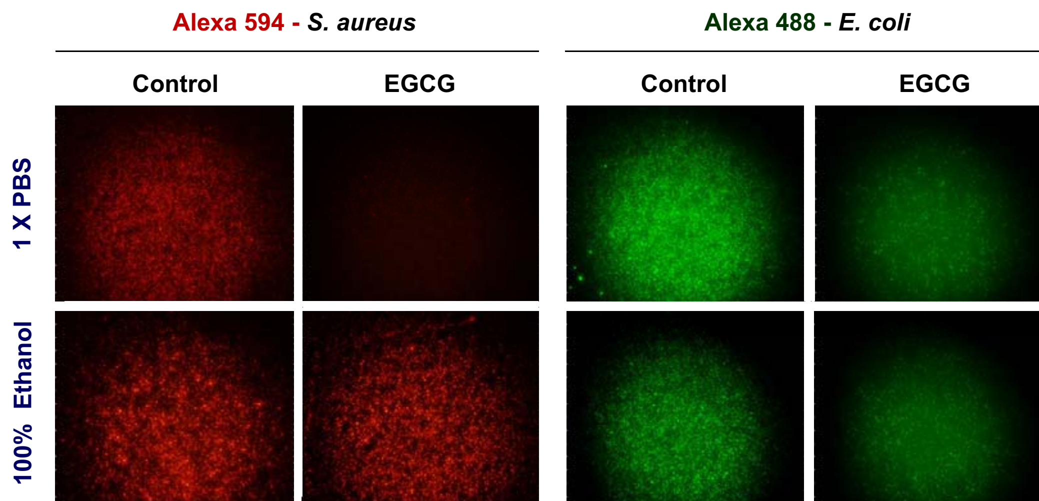

Fig. (3). Ethanol impaired EGCG-mediated quenching of G+ bacteria-conjugated Alexa Fluor dye. Alexa 594 – *S. aureus* or Alexa Fluor 488 – *E. coli* were re-suspended in 1×PBS (1 µg/ml), and added to 24-well culture plates. After incubation for 12 h, the saline solution was carefully removed and replaced with EGCG dissolved either in 1xPBS or 100% ethanol at a final concentration of 20 µM. Approximately 30 min later, images were captured using a fluorescence microscope.

the fluorescence intensities of the mixtures were measured by fluorescence spectrophotometer. Surprisingly, both EGCG and CG failed to reduce the fluorescence intensity of the unconjugated Alexa Fluor 594/488 dyes even after a prolonged incubation (Fig. **4A**). In a sharp contrast, EGCG and CG obviously reduced the fluorescence intensity of bacteria-conjugated Alexa 488 and 594 (Fig. **4B**), confirming that EGCG and CG indeed reduced the fluorescence intensity of bacteria-conjugated Alexa Fluor dyes. In light of the notion that the fluorescence quenching of Alexa Fluor dyes could serve as a sensitive indicator of protein conformational changes [39], we proposed that EGCG and CG induced the fluorescence quenching possibly through cross-linking to bacterial proteins to induce changes in both conformations and biological functions.

Previous studies suggested that EGCG could be internalized into macrophage cytoplasmic vesicles [9],

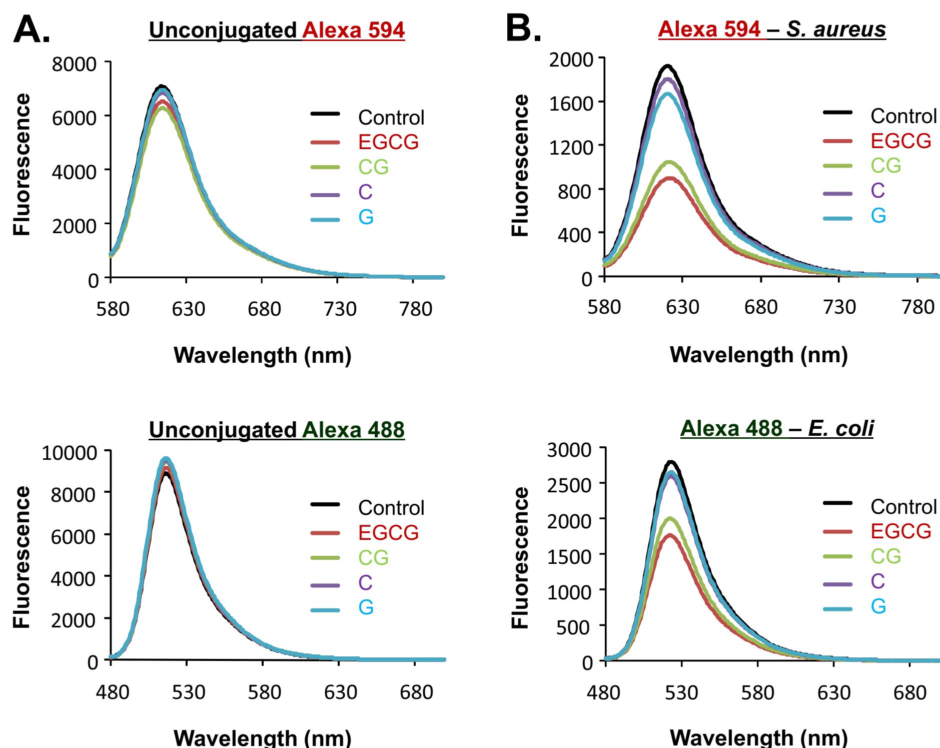

Fig. (4). EGCG quenched the fluorescence of bacteria-conjugated, but not the unconjugated, Alexa Flour dyes. The unconjugated (0.25 µg/ml, **A**) or bacteria-conjugated (40 µg/ml, **B**) Alexa Fluor dyes (594 or 488) were mixed with EGCG or analogs (CG, C, or G) for 30 min, and the fluorescence emission intensity of the mixture was measured using a Fluorescence Spectrophotometer.

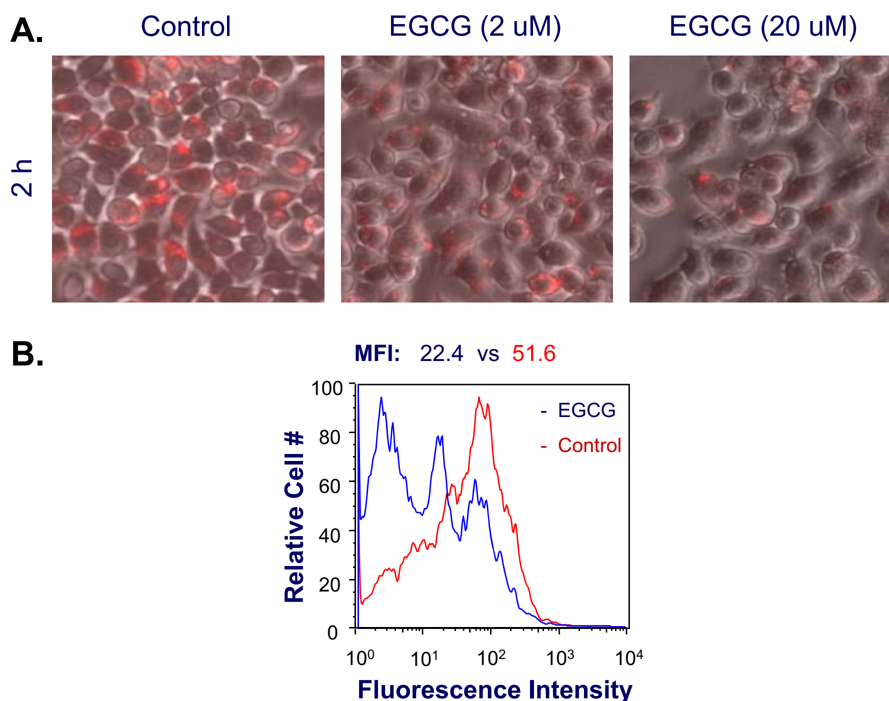

Fig. (5). EGCG reduced the fluorescence of bacteria-conjugated Alexa Flour dye *in vivo*. Bacteria-conjugated Alexa Flour 594 was added to macrophage cultures in the absence or presence of EGCG for 2 h. Following extensive washings to remove extracellular bioparticles, the fluorescence intensity were evaluated by microscopic (**A**) or flow cytometric analysis (**B**). The mean fluorescence intensities (MFI) of control and EGCG-treated macrophages were provided.

thereby inhibiting intracellular survival of various microbes (e.g., *Listeria monocytogenes* or *Mycobacterium tuberculosis*) in macrophage cultures [49, 50]. It was thus important to determine whether EGCG could similarly reduce the fluorescence intensity of ingested bacteria-Alexa Flour dyes in macrophage cultures. As predicted, the fluorescence-labeled bacteria were rapidly phagocytosed by macrophages within 2 hours (Fig. **5A**). Interestingly, EGCG treatment similarly reduced the fluorescence intensity of bacteria-conjugated Alexa Fluor 594 within macrophages (Fig. **5A**). This reduction of the fluorescence intensity was confirmed by flow cytometry analysis of macrophage cultures: EGCG similarly reduced the mean fluorescence intensity from 51.6 in control to 22.4 in EGCG-treated macrophages (Fig. **5B**).

In conclusion, here we demonstrated that EGCG facilitated bacterial elimination in selective organs (e.g., the liver and lung) in an animal model of lethal polymicrobial infection. Furthermore, we found that EGCG effectively quenched the fluorescence of bacteria-conjugated, but not the unconjugated, Alexa Fluor dyes. It is plausible that EGCG may be cross-linked to bacterial proteins to induce their conformational and functional changes. These conformational alterations may allow the physical proximity and chemical interaction between the Alexa Fluor dyes with aromatic amino acid residues (e.g., tyrosine, tryptophan, or histidine) of bacterial proteins, leading to quenching of the fluorescence signals (Fig. **6**). Thus, our experimental data suggest that Green tea catechins exert anti-microbial activities possibly by altering microbial protein conformations and functions. Given the extensive evidence in supporting the antibacterial activities of EGCG, it is important to screen other fluorescence-quenching agents for possible antimicrobial activities in future studies. Further

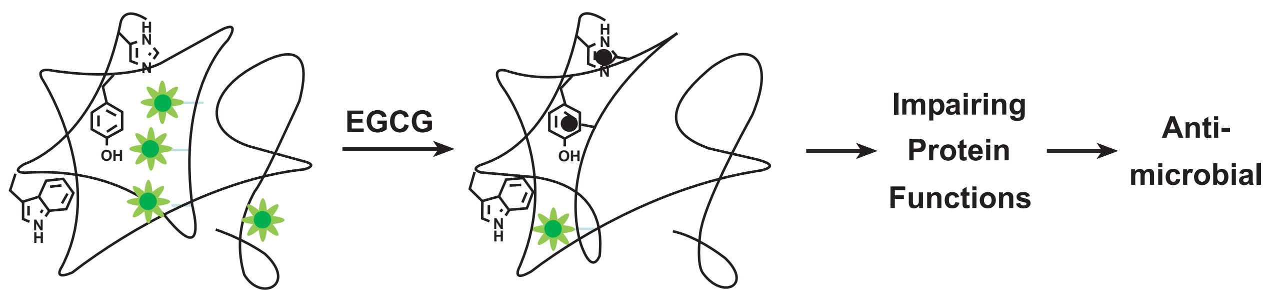

Fig. (6). Proposed model for EGCG-mediated anti-microbial activities. EGCG may be cross-linked to various microbial proteins to induce possible conformational changes, which allows physical proximity and chemical interaction between the Alexa Fluor dyes with aromatic amino acid residues, leading to quenching of the fluorescence signals. It is now possible that Green tea catechins exert anti-microbial activities partly by altering microbial protein conformations and functions.

investigation in this area may prompt the development of novel antibiotics to conquer lethal microbial infections.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENTS

We thank Dr. Kai Yang (The Laboratory of Molecular Imaging & Nanomedicine, NIH/NIBIB) for help with the fluorescence spectrophotometer experiments. This work was supported by the National Center of Complementary and Alternative Medicine (NCCAM, R01AT05076) and the National Institute of General Medical Sciences (NIGMS, R01GM063075).

REFERENCES

[1] Angus, D. C.; Linde-Zwirble, W. T.; Lidicker, J.; Clermont, G.; Carcillo, J.; Pinsky, M. R. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit. Care Med.,* **2001**, *29,* 1303-1310.

[2] Sipahi, O. R. Economics of antibiotic resistance. *Expert Rev. Anti. Infect. Ther.,* **2008**, *6,* 523-539.

[3] Volles, D. F.; Branan, T. N. Antibiotics in the intensive care unit: focus on agents for resistant pathogens. *Emerg. Med. Clin. North Am.,* **2008**, *26,* 813-34, x.

[4] Duke, T.; Michael, A. Increase in sepsis due to multi-resistant enteric gram-negative bacilli in Papua New Guinea. *Lancet,* **1999**, *353,* 2210-2211.

[5] Frei, B.; Higdon, J. V. Antioxidant activity of tea polyphenols *in vivo*: evidence from animal studies. *J. Nutr.,* **2003**, *133,* 3275S-3284S.

[6] Crespy, V.; Williamson, G. A review of the health effects of green tea catechins in *in vivo* animal models. *J. Nutr.,* **2004**, *134,* 3431S-3440S.

[7] Vita, J. A. Tea consumption and cardiovascular disease: effects on endothelial function. *J. Nutr.,* **2003**, *133,* 3293S-3297S.

[8] Li, W.; Ashok, M.; Li, J.; Yang, H.; Sama, A. E.; Wang, H. A Major Ingredient of Green Tea Rescues Mice from Lethal Sepsis Partly by Inhibiting HMGB1. *PLoS ONE,* **2007**, *2,* e1153.

[9] Li, W.; Zhu, S.; Li, J.; Assa, A.; Jundoria, A.; Xu, J.; Fan, S.; Eissa, N. T.; Tracey, K. J.; Sama, A. E.; Wang, H. EGCG stimulates autophagy and reduces cytoplasmic HMGB1 levels in endotoxin-stimulated macrophages. *Biochem. Pharmacol.,* **2011**, *81,* 1152-1163.

[10] Graham, H. N. Green tea composition, consumption, and polyphenol chemistry. *Prev. Med.,* **1992**, *21,* 334-350.

[11] Gordon, N. C.; Wareham, D. W. Antimicrobial activity of the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) against clinical isolates of Stenotrophomonas maltophilia. *Int. J. Antimicrob. Agents,* **2010**, *36,* 129-131.

[12] Song, J. M.; Lee, K. H.; Seong, B. L. Antiviral effect of catechins in green tea on influenza virus. *Antiviral Res.,* **2005**, *68,* 66-74.

[13] Stapleton, P. D.; Shah, S.; Anderson, J. C.; Hara, Y.; Hamilton-Miller, J. M.; Taylor, P. W. Modulation of beta-lactam resistance in Staphylococcus aureus by catechins and gallates. *Int. J. Antimicrob. Agents,* **2004**, *23,* 462-467.

[14] Steinmann, J.; Buer, J.; Pietschmann, T.; Steinmann, E. Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. *Br. J. Pharmacol.,* **2013**, *168,* 1059-1073.

[15] Zhao, M.; Jiang, J.; Zheng, R.; Pearl, H.; Dickinson, D.; Fu, B.; Hsu, S. A proprietary topical preparation containing EGCG-stearate and glycerin with inhibitory effects on herpes simplex virus: case study. *Inflamm. Allergy Drug Targets,* **2012**, *11,* 364-368.

[16] Isaacs, C. E.; Wen, G. Y.; Xu, W.; Jia, J. H.; Rohan, L.; Corbo, C.; Di, M., V; Jenkins, E. C., Jr.; Hillier, S. Epigallocatechin gallate inactivates clinical isolates of herpes simplex virus. *Antimicrob. Agents Chemother.,* **2008**, *52,* 962-970.

[17] Nakayama, M.; Suzuki, K.; Toda, M.; Okubo, S.; Hara, Y.; Shimamura, T. Inhibition of the infectivity of influenza virus by tea polyphenols. *Antiviral Res.,* **1993**, *21,* 289-299.

[18] Park, B. J.; Park, J. C.; Taguchi, H.; Fukushima, K.; Hyon, S. H.; Takatori, K. Antifungal susceptibility of epigallocatechin 3-O-gallate (EGCg) on clinical isolates of pathogenic yeasts. *Biochem. Biophys. Res. Commun.,* **2006**, *347,* 401-405.

[19] Sudano, R. A.; Blanco, A. R.; Giuliano, F.; Rusciano, D.; Enea, V. Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells. *Antimicrob. Agents Chemother.,* **2004**, *48,* 1968-1973.

[20] Hu, Z. Q.; Zhao, W. H.; Hara, Y.; Shimamura, T. Epigallocatechin gallate synergy with ampicillin/sulbactam against 28 clinical isolates of methicillin-resistant Staphylococcus aureus. *J. Antimicrob. Chemother.,* **2001**, *48,* 361-364.

[21] Zhao, W. H.; Hu, Z. Q.; Hara, Y.; Shimamura, T. Inhibition of penicillinase by epigallocatechin gallate resulting in restoration of antibacterial activity of penicillin against penicillinase-producing Staphylococcus aureus. *Antimicrob. Agents Chemother.,* **2002**, *46,* 2266-2268.

[22] Sun, Y.; Hung, W. C.; Chen, F. Y.; Lee, C. C.; Huang, H. W. Interaction of tea catechin (-)-epigallocatechin gallate with lipid bilayers. *Biophys. J.,* **2009**, *96,* 1026-1035.

[23] Kamihira, M.; Nakazawa, H.; Kira, A.; Mizutani, Y.; Nakamura, M.; Nakayama, T. Interaction of tea catechins with lipid bilayers investigated by a quartz-crystal microbalance analysis. *Biosci. Biotechnol. Biochem.,* **2008**, *72,* 1372-1375.

[24] Kajiya, K.; Hojo, H.; Suzuki, M.; Nanjo, F.; Kumazawa, S.; Nakayama, T. Relationship between antibacterial activity of (+)-catechin derivatives and their interaction with a model membrane. *J. Agric. Food Chem.,* **2004**, *52,* 1514-1519.

[25] Ikigai, H.; Nakae, T.; Hara, Y.; Shimamura, T. Bactericidal catechins damage the lipid bilayer. *Biochim. Biophys. Acta,* **1993**, *1147,* 132-136.

[26] Maeyama, R.; Kwon, I. K.; Mizunoe, Y.; Anderson, J. M.; Tanaka, M.; Matsuda, T. Novel bactericidal surface: Catechin-loaded surface-erodible polymer prevents biofilm formation. *J. Biomed. Mater. Res. A.,* **2005**, *75,* 146-155.

[27] Blanco, A. R.; La Terra, M. S.; Babini, G.; Garbisa, S.; Enea, V.; Rusciano, D. (-) Epigallocatechin-3-gallate inhibits gelatinase activity of some bacterial isolates from ocular infection, and limits their invasion through gelatine. *Biochim. Biophys. Acta,* **2003**, *1620,* 273-281.

[28] Okamoto, M.; Leung, K. P.; Ansai, T.; Sugimoto, A.; Maeda, N. Inhibitory effects of green tea catechins on protein tyrosine phosphatase in Prevotella intermedia. *Oral Microbiol. Immunol.,* **2003**, *18,* 192-195.

[29] Gradisar, H.; Pristovsek, P.; Plaper, A.; Jerala, R. Green tea catechins inhibit bacterial DNA gyrase by interaction with its ATP binding site. *J. Med. Chem.,* **2007**, *50,* 264-271.

[30] Si, W.; Gong, J.; Tsao, R.; Kalab, M.; Yang, R.; Yin, Y. Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. *J. Chromatogr. A.,* **2006**, *1125,* 204-210.

[31] Cho, Y. S.; Oh, J. J.; Oh, K. H. Synergistic anti-bacterial and proteomic effects of epigallocatechin gallate on clinical isolates of imipenem-resistant Klebsiella pneumoniae. *Phytomedicine,* **2011**, *18,* 941-946.

[32] Tachibana, H.; Koga, K.; Fujimura, Y.; Yamada, K. A receptor for green tea polyphenol EGCG. *Nat. Struct. Mol. Biol.,* **2004**, *11,* 380-381.

[33] Ehrnhoefer, D. E.; Bieschke, J.; Boeddrich, A.; Herbst, M.; Masino, L.; Lurz, R.; Engemann, S.; Pastore, A.; Wanker, E. E. EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. *Nat. Struct. Mol. Biol.,* **2008**, *15,* 558-566.

[34] Bieschke, J.; Russ, J.; Friedrich, R. P.; Ehrnhoefer, D. E.; Wobst, H.; Neugebauer, K.; Wanker, E. E. EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity. *Proc. Natl. Acad. Sci. U. S. A.,* **2010**, *107,* 7710-7715.

[35] Chandra, G. K.; Tripathy, D. R.; Dasgupta, S.; Roy, A. Interaction of (-)-epigallocatechin gallate with lysozyme-conjugated silver nanoparticles. *Appl. Spectrosc.,* **2012**, *66,* 744-749.

[36] Zhu, J.; Declercq, J.; Creemers, J. W.; Chen, C.; Cui, Y.; Van de Ven, W. J.; Vermorken, A. J. Limitations of inhibitory activities of polyphenols on furin-mediated substrate processing. *Curr. Med. Chem.,* **2012**, *19,* 3641-3650.

[37] Weng, Z.; Greenhaw, J.; Salminen, W. F.; Shi, Q. Mechanisms for epigallocatechin gallate induced inhibition of drug metabolizing enzymes in rat liver microsomes. *Toxicol. Lett.,* **2012**, *214,* 328-338.

[38] Chen, R.; Wang, J. B.; Zhang, X. Q.; Ren, J.; Zeng, C. M. Green tea polyphenol epigallocatechin-3-gallate (EGCG) induced intermolecular cross-linking of membrane proteins. *Arch. Biochem. Biophys.,* **2011**, *507,* 343-349.

[39] Lindhoud, S.; Westphal, A. H.; Visser, A. J.; Borst, J. W.; van Mierlo, C. P. Fluorescence of Alexa fluor dye tracks protein folding. *PLoS One,* **2012**, *7,* e46838.

[40] Chen, H.; Ahsan, S. S.; Santiago-Berrios, M. B.; Abruna, H. D.; Webb, W. W. Mechanisms of quenching of Alexa fluorophores by natural amino acids. *J. Am. Chem. Soc.,* **2010**, *132,* 7244-7245.

[41] Li, W.; Zhu, S.; Li, J.; Huang, Y.; Zhou, R.; Fan, X.; Yang, H.; Gong, X.; Eissa, N. T.; Jahnen-Dechent, W.; Wang, P.; Tracey K.J.; Sama, A. E.; Wang, H. A hepatic protein, fetuin-A, occupies a protective role in lethal systemic inflammation. *PLoS ONE,* **2011**, *6,* e16945.

[42] Li, W.; Zhu, S.; Zhang, Y.; Li, J.; Sama, A. E.; Wang, P.; Wang, H. Use of animal model of sepsis to evaluate novel herbal therapies. *J. Vis. Exp.,* **2012**, 3926.

[43] Li, W.; Li, J.; Sama, A. E.; Wang, H. Carbenoxolone blocks endotoxin-induced PKR activation and HMGB1 release. *Mol. Med.,* **2013**, *19*, 203-211.

[44] Arakawa, H.; Maeda, M.; Okubo, S.; Shimamura, T. Role of hydrogen peroxide in bactericidal action of catechin. *Biol. Pharm. Bull.,* **2004**, *27,* 277-281.

[45] Chirumbolo, S. The role of quercetin, flavonols and flavones in modulating inflammatory cell function. *Inflamm. Allergy Drug Targets,* **2010**, *9,* 263-285.

[46] Yamamoto, Y.; Matsunaga, K.; Friedman, H. Protective effects of green tea catechins on alveolar macrophages against bacterial infections. *Biofactors,* **2004**, *21,* 119-121.

[47] Yoda, Y.; Hu, Z. Q.; Zhao, W. H.; Shimamura, T. Different susceptibilities of Staphylococcus and Gram-negative rods to epigallocatechin gallate. *J. Infect. Chemother.,* **2004**, *10,* 55-58.

[48] Zhao, W. H.; Hu, Z. Q.; Okubo, S.; Hara, Y.; Shimamura, T. Mechanism of synergy between epigallocatechin gallate and beta-lactams against methicillin-resistant Staphylococcus aureus. *Antimicrob. Agents Chemother.,* **2001**, *45,* 1737-1742.

[49] Kohda, C.; Yanagawa, Y.; Shimamura, T. Epigallocatechin gallate inhibits intracellular survival of Listeria monocytogenes in macrophages. *Biochem. Biophys. Res. Commun.,* **2008**, *365,* 310-315.

[50] Matsunaga, K.; Klein, T. W.; Friedman, H.; Yamamoto, Y. Legionella pneumophila replication in macrophages inhibited by selective immunomodulatory effects on cytokine formation by epigallocatechin gallate, a major form of tea catechins. *Infect. Immun.,* **2001**, *69,* 3947-3953.

Received: June 12, 2013 **Revised: August 7, 2013** **Accepted: August 8, 2013**