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RESEARCH ARTICLE

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ANTI-INFLAMMATORY AND IMMUNOMODULATORY EFFECTS OF GREEN TEA EXTRACT (GTE)

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ABSTRACT

In recent years, there is growing interest in the fact that immune functions can be modulated by plant polyphenols. Immune system provides us with the specific mechanisms to resist against a particular pathogen or foreign substances and takes part in the preservation of normal cells. In the present study, the effect of green tea extracts (GTE) on modulation of immune responses in RAW 264.7 cell lines was studied. We report here that, the production of proinflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin 1β (IL-1β), and interleukin 6 (IL-6) by RAW 264.7 cells pre-stimulated with lipopolysaccharide (LPS), was inhibited by treatment with GTE. GTE treatment up regulated the secretion of anti-inflammatory cytokine TGF-β. GTE also inhibited lipopolysaccharide (LPS)-induced NO production in RAW 264.7 cells. These results demonstrate the anti-inflammatory and immunomodulatory properties of GTE on cells of innate immune system.

KEYWORDS

Lipopolysaccharide (LPS), Cytokines, Nitric Oxide Green Tea Extracts (GTE).

INTRODUCTION

The immune system is involved in the pathophysiologic mechanisms of several diseases. Modulation of the immune responses to alleviate the diseases has been of interest for many years[1]. Medicinal plants are a rich source of substances which are claimed to induce immunity by the non-specific immunomodulation of granulocytes, macrophages, and natural killer cells and complement functions[2]. Ayurveda, the Indian traditional system of medicine, lays emphasis on promotion of health concept of strengthening host defences against different diseases[3].

Green tea is a product made from the *Camellia sinensis* plant. It can be prepared as a beverage, which can have some health effects. Or an "extract" can be made from the leaves to use as medicine. Green tea contains salubrious polyphenols, in particular catechins, the most abundant of which is epigallocatechin gallate[4]. Green tea also contains carotenoids, tocopherols, ascorbic acid (vitamin C), minerals such as chromium, manganese, selenium or zinc, and certain phytochemical compounds. It is a more potent antioxidant than black tea, although black tea has substances that green tea does not such as theaflavin.

In recent years, the health benefits[5] of consuming green tea, including the prevention of cancer[6] and cardiovascular diseases[7], antiarthritic[8], antibacterial[9], antiangiogenic[10], antioxidative[11], antiviral[12], neuroprotective[13] and cholesterol-lowering effects[14] of green tea and isolated green tea constituents are under investigation.

Human research is still in its infancy and long term human research is required before we determine the appropriate dosage and amount of green tea or green tea extract required in providing these health benefits. Green Tea Extract offers a convenient way to get the benefits of green tea in a highly concentrated green tea pill form. Immunomodulatory and anti-inflammatory effects of GTE are still not very clear. Hence we decided to study the effect of GTE on cells of innate immune system.

In the present study we have studied the effects of GTE on the expression of LPS (Lipopolysaccharide) induced expression of proinflammatory cytokines. Additionally we studied effect of GTE on LPS induced nitric oxide production in RAW 264.7 cell line.

MATERIALS AND METHODS

Chemicals

Trypsin–EDTA, agarose, Griess reagent and LPS (*E. coli* 026:B6) were obtained from Sigma Chemical Company, USA. Fetal calf serum (FCS) DMEM, were obtained from GIBCO BRL. Mouse TNF α, IL-1β, and IL-6 ELISA sets were purchased from BD Pharmingen. All other chemicals were purchased from reputed local manufacturers.

Cell Line

RAW264.7 macrophage cell line originally derived from a tumor induced by Abelson murine leukemia virus in BALB/c mice was used. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 4mMl-glutamine adjusted to contain 2.0 g/l sodium bicarbonate and 4.5 g/l glucose, and 10% fetal bovine serum. The cells adhered and grew to confluent monolayers within 2–3 days. The adherent cells were trypsinized and used for experiments.

RESULTS

GTE inhibits the LPS induced expression of pro-inflammatory cytokines in vitro

Effect of GTE treatment on expression of pro-inflammatory cytokines such as TNF-α, IL-1 β and IL-6 was evaluated by estimating the level of these cytokines in culture supernatants by ELISA.

TNF-α Levels

In vitro treatment of LPS induced TNF α expression in a time dependent manner but treatment of GTE alone did not have any effect on the TNF α expression. When GTE was added in LPS pre treated cells, interestingly level of TNF α was found to be decreased significantly. This result describes the possible anti-inflammatory role of GTE.

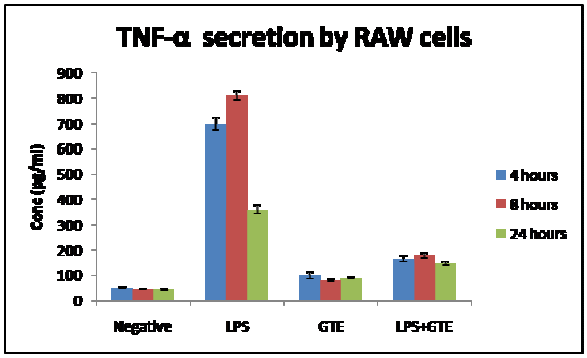


Figure 1

TNF-α levels: RAW cells were treated with LPS (1µg/ml) and GTE and expression of TNFα was monitored after 4, 8, and 24 h of treatment. In another set of experiments cells were treated with LPS and after 6 h GTE was added to the culture. Then level of TNF-α expression was monitored in the culture supernatant by ELISA. The data points indicate mean±SE for four replicates in each group. Representative data from one of the three similar experiments are shown.

IL-1β Levels

IL-1β levels were measured by ELISA and same as TNF-α expression, it was found that GTE treatment abolished the LPS induced IL-1β levels in the culture supernatants in a time dependent manner but treatment of only GTE could not induce the expression of IL-1β *in vitro*

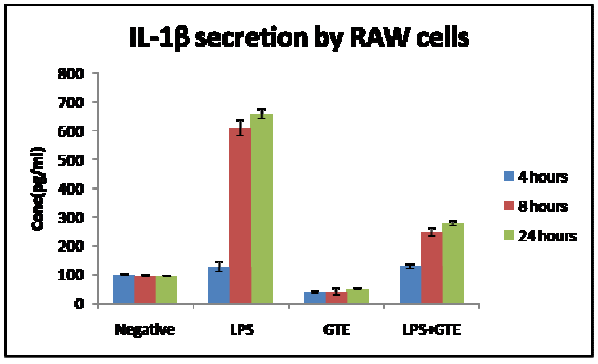


Figure 2: IL-1β levels

RAW cells were treated with LPS (1µg/ml) and GTE and expression of IL-1β was monitored after 4, 8, and 24 h of treatment. In another set of experiments cells were treated with LPS and after 8 h, GTE was added to the culture.

Level of IL-1β release in the culture supernatant was measured by ELISA. The data points indicate mean±SE for four replicates in each group. Representative data from one of the three similar experiments are shown

IL-6 Levels

IL-6 is also a pro-inflammatory cytokine, but many groups have earlier reported that it may have anti inflammatory role also. Here I am reporting the inhibition of IL-6 expression induced by LPS; by GTE *in vitro.*

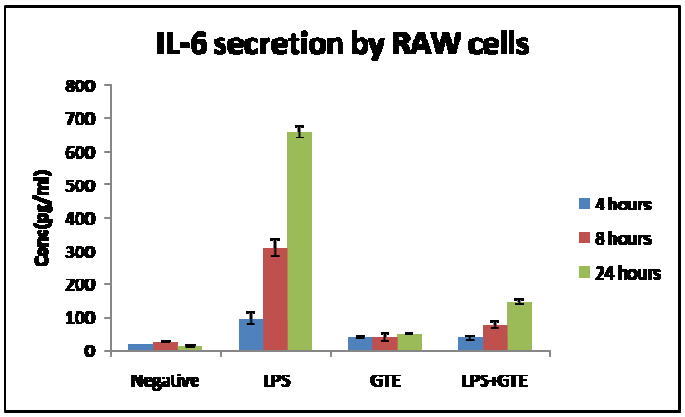


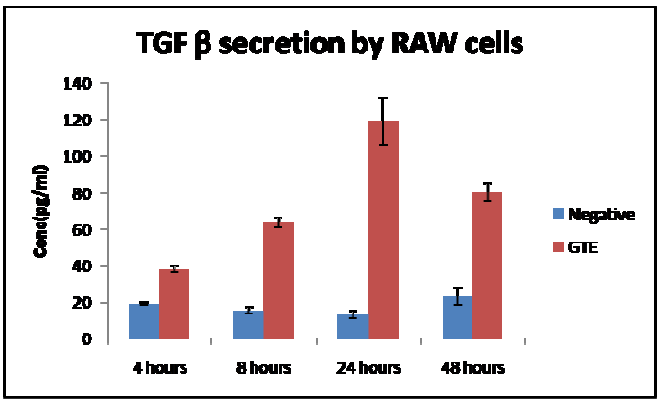
Figure 3

IL-6 levels: RAW cells were treated with LPS (1µg/ml) and GTE and expression of IL-6 was monitored after 4, 8, and 24 h of treatment. In another set of experiments cells were treated with LPS and after 4 h, GTE was added to the culture. Level of IL-6 release in the culture supernatant was measured by ELISA. The data points indicate mean±SE for four replicates in each group. Representative data from one of the three similar experiments are shown.

In vitro treatment of GTE enhances the expression of anti-inflammatory cytokine TGF-β

Pro-inflammatory response is subsided by release of anti-inflammatory cytokines such as IL-10 and TGF-β. In the present study, the effect of GTE on the expression of TGF-β was studied.

It was found that GTE treatment led to the induction of expression of TGF-β in a time dependent manner where it reaches maximum level by 24 h. This result goes well with earlier results and establishes GTE as an anti-inflammatory product.



In vitro treatment of macrophages with green tea extracts (GTE) inhibits LPS induced release of nitric oxide

Inflammatory responses are coupled with generation of reactive nitrogen species (RNI) and reactive oxygen species (ROS). LPS induces the production of nitric oxide (NO). GTE treatment of RAW macrophages alone did not induce the NO release but when GTE was added to LPS treated cells, it inhibited the NO production induced by the LPS treatment. These results show that GTE inhibits generation of NO which also plays an important role in anti-inflammatory response.

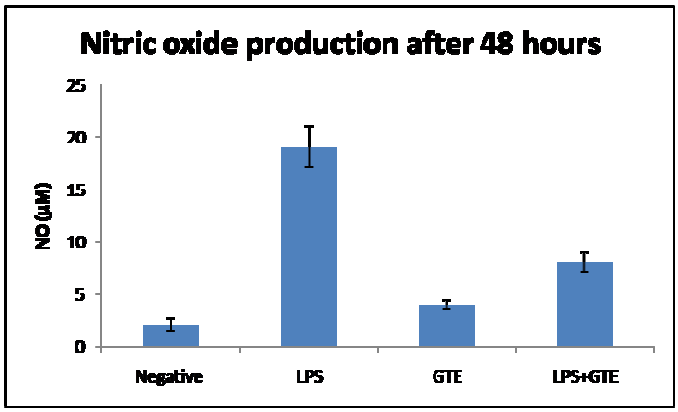


Figure 5

Modulation of LPS induced nitric oxide release by GTE. Cells were first treated with LPS for 8 h and then treated with GTE for 48 h. NO was estimated in the culture supernatants by Griess reagent. The data points indicate mean±SE for four replicates in each group. Representative data from one of the three similar experiments are shown.

DISCUSSION

Inflammation is the reaction of living tissues to injury, infection or irritation[15,16]. Inflammation causes migration of phagocytic cells like neutrophils, macrophages etc. Phagocytes produce an oxidative burst at the site of microbial invasion which helps in the eradication of foreign materials[17,18]. Additionally inflammatory responses are coupled with production of pro-inflammatory cytokines also. The uncontrolled release of reactive oxygen species is assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis. Administration of agents, which control the inflammatory responses in inflamed areas, might be a remedy in these cases.

Anti-inflammatory response is mediated by the release of anti-inflammatory cytokines such as IL-10 and TGF-β. These cytokines help in abolishing the inflammatory response, and weaken the intensity harm that may be caused due to prolonged inflammation[19]. The action of these anti-inflammatory cytokines is antagonistic to the pro-inflammatory cytokines. They are also involved in quenching of intracellular ROS[20,21] and RNI.

Our results demonstrated that GTE treatment of macrophages itself did not have any effect over up or down regulation of expression of TNF-α, IL-1β and IL-6 but when GTE was added to LPS treated cells, it inhibited the expression of all of these cytokines. Furthermore, GTE treatment up regulated the expression of anti-inflammatory cytokine TGF-β. Thus, albeit the mechanism of this inhibition is yet to be worked out, it's reasonably evident that GTE imparts anti-inflammatory effects on macrophages.

GTE treatment also inhibited the LPS induced production of NO, so this may be a probable mechanism for the inhibition of pro-inflammatory stimuli. But the mechanism underlying such effects needs to be deciphered. Knowledge of the detail mechanism, will pave

the way for the use of GTE as anti-inflammatory agent and may be helpful in the cure of several immunological disorders such as allergy, auto-immune diseases etc.

REFERENCES

1. Ting LM, Kim AC, Cattamanchi A, Ernst JD, *Mycobacterium tuberculosis* inhibits IFN-γ transcriptional responses without inhibiting activation of STAT1, J Immunol, 163:3898–906, (1999).
2. Mukherjee K, Parashuraman S, Krishnamurthy G, Majumdar J, Yadav A, Kumar R, et al, Diverting intracellular trafficking of *Salmonella* to the lysosome through activation of the late endocyticRab7 by intracellular delivery ofmuramyl dipeptide, J Cell Sci, 115:3693–701, (2002).
3. Kedzierska K, Ellery P, Mak J, Lewin SR, Crowe SM, Jaworowski A, HIV-1 down-modulates γ signaling chain of FcγR in human macrophages: a possible mechanism for inhibition of phagocytosis, J Immunol, 168:2895–903, (2002).
4. Sabu MC, Priya TT, Ramadasan K, Ikuo N, Beneficial effects of green tea: A literature review. Chinese Medicine, 5:13, (2010).
5. McKay DL, Blumberg JB, The role of tea in human health: An update. J Am Coll Nutr 21:1-13, (2002).
6. Kavanagh KT, Hafer LJ, Kim DW, Mann KK, Sherr DH, Rogers AE, Sonenshein GE, Green tea extracts decrease carcinogen-induced mammary tumor burden in rats and rate of breast cancer cell proliferation in culture. J Cell Biochem, 82:387-398, (2001).
7. Sueoka N, Suganuma M, Sueoka E, Okabe S, Matsuyama S, Imai K,Nakachi K, Fujiki H, A new function of green tea: prevention of life style related diseases. Ann N Y Acad Sci, 928:274-280, (2001).
8. Haqqi TM, Anthony DD, Gupta S, Ahmad N, Lee MS, Kumar GK, Mukhtar H, Prevention of collagen-induced arthritis in mice by a polyphenolic fraction from green tea. Proc Natl Acad Sci USA, 96:4524-4529, (1999).
9. Sudano Roccaro A, Blanco AR, 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, 48:1968-1973, (2004).
10. Sartippour MR, Shao ZM, Heber D, Beatty P, Zhang L, Liu C, Ellis L, Liu W,Go VL, Brooks MN, Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. J Nutr, 132:2307-2311, (2002).
11. Osada K, Takahashi M, Hoshina S, Nakamura M, Nakamura S, Sugano M, Tea catechins inhibit cholesterol oxidation accompanying oxidation of low density lipoprotein in vitro. Comp Biochem Physiol Part C Toxicol Pharmacol, 128:153-164 (2001).
12. Weber JM, Ruzindana-Umunyana A, Imbeault L, Sircar S, Inhibition of adenovirus infection and adenain by green tea catechins. Antiviral Res, 58:167-17, (2003).
13. Weinreb O, Mandel S, Amit T, Youdim MBH, Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. J Nutr Biochem, 15:506-516 (2004).
14. Raederstorff DG, Schlachter MF, Elste V, Weber P. Effect of EGCG on lipid absorption and plasma lipid levels in rats. J Nutr Biochem, 14:326-332, (2003)
15. Toda M, Okubo S, Ohnishi R, Shimamura T, Antibacterial and bactericidal activities of Japanese green tea. Nippon Saikingaku Zasshi, 44:669-672 (1989).

16. Miliani FL, Nielsen OH, Andersen PS, Girardin SE, Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation Clinical & Experimental Immunology 147(2):227-235 (2007).
17. Yee YK, Koo MWL, Szeto ML. Chinese tea consumption and lower risk of Helicobacter infection. J Gastroenterol Hepatol, 17:552-555, (2002).
18. Takabayashi F, Harada N, Yamada M, Murohisa B, Oguni I, Inhibitory effect of green tea catechins in combination with sucralfate on Helicobacter pylori infection in Mongolian gerbils. J Gastroenterol, 39:61-63, (2004).
19. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD, Diehl AM, Leptin regulates proinflammatory immune responses. FASEB J, 12(1):57-65 (1998).
20. Dona M, Dell'Aica I, Calabrese F, Benelli R, Morini M, Albini A, Garbisa S, Neutrophil restraint by green tea: inhibition of inflammation, associated angiogenesis, and pulmonary fibrosis. J Immunol, 170:4335-4341(2003).
21. Koo MWL, Cho CH, Pharmacological effects of green tea on the gastrointestinal system. Eur J Pharmacol, 500:177-185,(2004)