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Does Green Tea Induce Hormesis?

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Abstract

Green tea, and its principal constituent (–)-epigallocatechin-3-gallate (EGCG), are commonly shown to induce biphasic concentration/dose responses in a broad range of cell types, including non-tumor cells, and tumor cell lines. The most active area of research dealt with an assessment of neural cells with application to neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease cell models, often using preconditioning experimental protocols. The general findings demonstrate EGCG-induced hormetic effects resulting in an enhanced acquired resilience within an adaptive and temporally dependent homeodynamic framework. The biphasic dose responses displayed the typical quantitative features of the hormetic dose response with respect to the amplitude and width of the stimulatory response. These findings provide further evidence for the general occurrence of hormetic dose responses with such responses being independent of the biological model, end point, inducing agent, and mechanism. The biphasic nature of these responses has important implications since it suggests optimal dose ranges for end points of public health and therapeutic applications. These findings indicate the need to assess the entire dose-response continuum in order to better define the nature of the dose response, especially in the low-dose zone where such exposures are common in human populations.

Keywords

green tea, EGCG, hormesis, dose response, biphasic dose response, preconditioning

Introduction

Consumption of black and green teas has long been a staple of dietary practices in multiple cultures. Over the past several decades, a growing number of epidemiological investigations have associated the consumption of these products with a broad spectrum of positive health benefits affecting multiple physiological systems,[1-8] reducing the risks of developing various types of cancer, neurodegenerative illness, and cardiovascular disease. For example, epidemiological studies associated tea consumption with a 30% to 60% reduced risk of Parkinson's disease (PD) incidence.[1,8]

Such evidence of potential human benefits has led to an extensive assessment of these products and their principal constituents in a broad spectrum of cellular and whole animal studies. This paper provides an evaluation of the capacity of green tea and its principal constituent (–)-epigallocatechin-3-gallate (EGCG) to induce biphasic[9-11] dose responses for numerous end points in experiment models, with most research involving a broad spectrum of cell types with particular focus on neuronal cells. The findings indicate that hormetic-like biphasic dose responses may be a common feature of green tea/constituent-induced biological effects. These findings are consistent with and extend recent reports of hormetic dose responses for curcumin,[12] ginkgo biloba,[13] and resveratrol.[14] Figure 1 provides a general description of a hormetic dose

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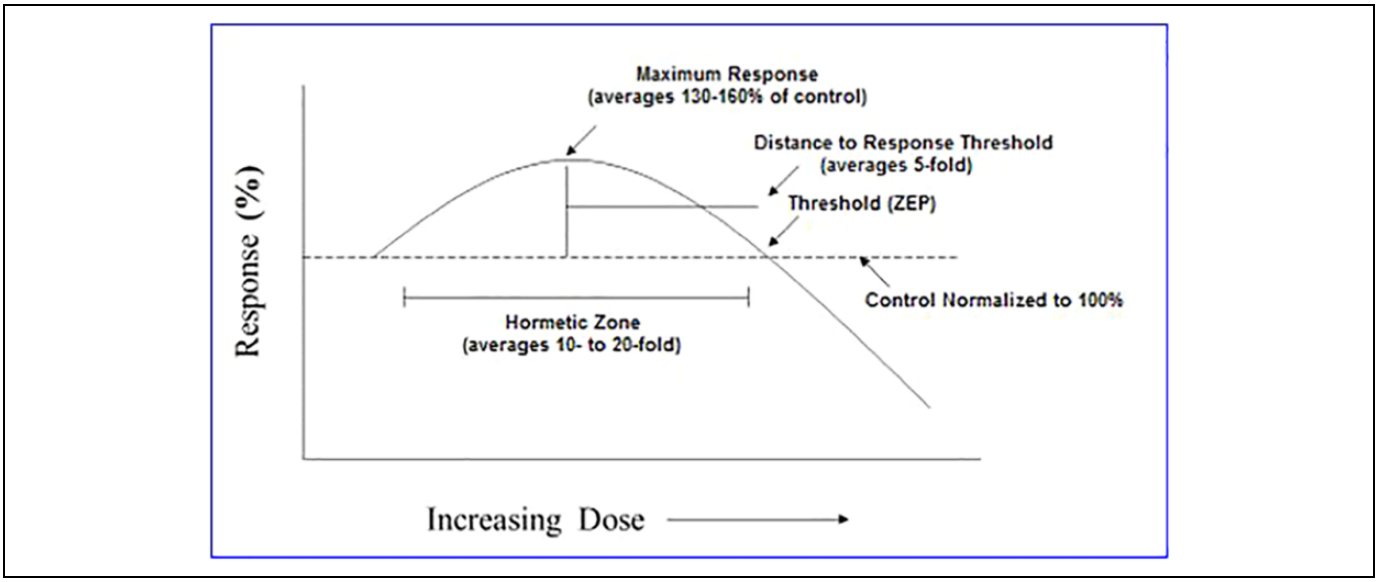

Figure 1. Dose-response curve showing the quantitative features of hormesis.[17]

response, including its quantitative features for amplitude and width of the low-dose stimulatory response. The hormetic dose response can result from a direct stimulation or an overcompensation to an initial disruption in homeostasis. In this case, the hormetic response used would be best studied within a dose-time response context with repeat measurements over time. The hormetic response is also widely studied with a preconditioning experimental protocol,[15,16] which is commonly employed in the green tea/EGCG experimental literature assessed in the present paper.

Results

Hair Growth

A search of the Web of Science database using the terms green tea and hair growth yields a listing of dozens of patented products that claim to enhance hair growth in humans. That green tea might have the capacity to enhance hair growth has its origins in research concerning androgen-responsive organs, including the prostate and skin, where testosterone is enzymatically converted into 5α-dihydrotestosterone (5α-DHT) via 5α-reductase. The 5α-DHT may enhance the development of acne, male pattern alopecia, and benign prostate hyperplasia. It has been proposed that 5α-reductase inhibition may be of therapeutic value in the treatment of these conditions. In the mid-1980s, 4-azasteroids were found to be competitive inhibitors of 5α-reductase and have clinical applications.[18-20] Such findings led to the discovery that naturally occurring unsaturated fatty acids can also inhibit 5α-reductase, suggesting that such fatty acids may function as endogenous inhibitors of 5α-reductase.[21] In a parallel track, epidemiological studies revealed that prostate cancer, a major cause of death in Western men, was far less frequent among Asian men.[22] Furthermore, after a generation or 2, Asian men who migrated to Europe/North America acquired the same prostate cancer incidence as Western men. These findings suggested that "restraining factors" such as components of the diet may either enhance or retard the progression of prostate cancer.[22] These findings inspired a more detailed consideration of what dietary practices may have contributed to the lower prostate cancer incidence. This line of inquiry led to assessing the role of low fat, high fiber, soya diets, including plant estrogens (eg, lignans and isoflavones phytoestrogens), in prostate cancer. Subsequent research revealed that both isoflavonoids and lignans inhibit 5α-reductase, suggesting that they may affect the low prostate cancer incidence of Asian men.[22]

At this same time, the research group of Shutsung Liao at the University of Chicago, who had first identified azasteroids and other inhibitors of 5α-reductase, broadened their search of such inhibitors to green tea constituents.[23] They found that green tea catechins EGCG and (–)-epicatechin-3-gallate were potent type 1 5α-reductase inhibitors. While their work showed similar findings to those of finasteride (a type 2 5α-reductase inhibitor) and its use to treat benign prostate hyperplasia, these authors did not link green tea constituents to hair growth in this or their subsequent paper published 7 years later.[24] However, Kwon et al made that connection in 2007, being the first to report that EGCG-enhanced human hair growth using dermal papilla cells (DPCs) in vivo and in vitro. Kwon et al stated that "it was reported the EGCG may be useful in the prevention or treatment of androgenetic alopecia by selectively inhibiting 5α-reductase activity (44)."[25] However, no report yet had been published on the effect of EGCG on human hair growth. A careful consideration of the Hiipakka et al paper revealed that it provided no evidence or written statement indicating that EGCG prevents or reverses androgenic alopecia by any means, including 5α-reductase inhibitors.[24] It, therefore, appears that Kwon et al provided the first report that EGCG enhanced hair growth in experimental

systems.[25] Using 3 concentrations (0.01, 0.1, and 0.5 μM), they reported a dose-dependent increase in DPC proliferation using the MTT assay along with similar increases in the phosphorylation of extracellular-signal-regulated kinase and protein kinase B (Akt).

There have been 3 papers published showing hormetic dose responses for EGCG on hair growth parameters. The first, which was published in Chinese,[26] displayed a striking hormetic dose response on the growth of isolated human hair follicles (Figure S1). A hormetic dose response was also reported for human DPCs (Figure S2). However, the dose-response features were considerably different with respect to the concentrations affecting the quantitative features of the stimulatory response, including the maximum stimulatory response. These striking hormetic findings did not result in any follow-up publications for about 8 years, possibly due to the limited indexing of this journal (data not listed in PubMed or Web of Science), making it difficult to identify the paper. However, Shin et al accidentally discovered a hormetic effect of EGCG with human DPCs in 2016.[27] Unaware of the earlier 2008 report of Li et al,[26] they cited the earlier 2003 report of Chung et al[28] confirming the "concentration-dependent dual function" of EGCG, showing a stimulation of human epidermal keratinocyte proliferation (Figure S3). At the optimal concentration of 10 μM, Shin et al reported that EGCG inhibited DHT-induced cellular senescence and reactive oxygen species (ROS) levels.[27] Furthermore, the 10-μM EGCG concentration altered DHT effects on MiRNA (ie, 13 upregulated and 40 downregulated effects; Figure S4).

A third paper showing hormetic effects of EGCG was conducted with mink hair follicles using an ex vivo culture (Figure S5).[29] Mechanistic studies revealed that the stimulation was mediated through soni hedgehog and protein kinase B signaling pathways. More limited dose-response experiments were conducted with DPCs and outer root sheath cells, showing that EGCG enhanced the percent of cells in S phase by about 60% with 0.5 to 1.0 μM, the highest concentrations tested. These findings are fully consistent with the hair follicle experiment which was based on a broader concentration relationship (0.1-5.0 μM). The results of the 3 studies show EGCG affecting multiple hair growth parameters in a hormetic dose-response fashion.

Tumor Cell Stimulation

While EGCG has been generally viewed as displaying anticarcinogen properties based on substantial experiments,[30] the present paper has identified 6 human tumor cell lines in which low concentrations enhanced cell proliferation, with these agents acting in a hormetic dose-response fashion. The tumor cell types include the HELA cell-derived INT-407 tumor line (Figure S6),[31] the human oral squamous carcinoma cell line—SCC-25 (Figure S7),[30] human leiomyoma cell lines (Figure S8),[32] human prostate tumor cell lines Du 145 and HH 870 (Figure S9),[33] and the human ovarian tumor cell line HH 450 (Figure S9).[33] These papers displayed enhanced tumor cell proliferation at low concentrations. However, 3 of these papers strongly emphasized the high dose aspect of the dose-response studies that showed dose-dependent inhibitory responses, emphasizing the protective chemotherapeutic potential of EGCG.[30,32,33] While both papers[30,33] acknowledged the low-dose stimulation in their papers, these findings were not discussed as possible public health or medical issues. In the case of the mesothelioma cell line REN (a tumorigenic P53 mutant, epithelial subtype of malignant mesothelioma), EGCG displayed a hormetic-like biphasic concentration response for apoptotic cell death, enhancing it within the 5- to 10-μM concentration range (Figure S10).[34]

The issue of cancer risk and prevention may well be affected by dose.[35,36] Yet, with respect to human consumption, the concentrations of ingested chemicals in the blood could markedly change throughout the day, dynamically transitioning across possible chemopreventive and tumor-promoting concentration ranges depending on the cell type.

Mutation and Carcinogen Bioactivation

In chemical carcinogenesis, many carcinogens, such as polycyclic aromatic hydrocarbons, are converted to highly reactive epoxide intermediates that bind tightly to DNA and initiate carcinogenesis. This process is mediated via the aryl hydrocarbon receptor (AhR)-cytochrome P4501A1 (CYP1A1) pathway.[37] CYP1A1 biotransforms both endogenous and exogenous agents to their mutagenic/carcinogenic forms. As a result of the significant role of CYP1A1 in chemically induced carcinogenesis, it has been assessed extensively with respect to whether agents can either activate or suppress this bioactivation process.[38] In detailed experiments, Anger et al reported on the effects of black tea, green tea, white tea, and tea polyphenols on CYP1A1 from rats treated with 3MC (methylcholanthrene).[39] Each type of tea biphasically affected the CYP1A1 activity as measured by EROD activity (Figures S11 and S12). This was unexpected since prior studies reported the inhibition of human and rat hepatic enzyme activity.[40,41] Treatment with EGCG and EGC enhanced enzyme activity about 120% (compared to 100% for controls) at an exposure closely approximated by consumption of a single cup of green tea that used 3 tea bags.[42] These effects suggest that if 3MC-treated rat liver findings could be extrapolated to people, tea consumption may result in a mild activation of CYP1A1 activity rather than its inhibition, hence enhancing carcinogenic effects, but future studies are needed to confirm these theories.

The biphasic dose response for activation was thought to be an example of heteroactivation in which the EGCG binds simultaneously 2 sites. According to Yoon et al, binding at one site causes enzyme inhibition competition with the substrate, while the other site affects activation, possibly via an allosteric mechanism.[43] Allosteric control is the enzyme regulation by binding an effector molecule at a site different from the active site of the enzyme. Of interest to the present assessment is that Anger et al estimated the maximal amplitude of activation for

Table 1. Hormetic Mechanisms for Green Tea and (–)-Epigallocatechin-3-Gallate.

Mechanisms
Nonneuronal and non-tumor cells
1) Bovine mammary epithelial cells improved redox balance and upregulated NF2X2/HMOX-1 pathways; protecting against pro-oxidant stress[45]
2) TPH-1 cell macrophages—EGCG blocked uptake of ricin by cells, preventing toxicity[47]
3) HepG2 cells—preventing Pb toxicity mechanism. Free radical scavenging, heavy metal chelation, prevents lipid membrane oxidation[46]
4) Human epidermal keratinocytes—EGCG enhanced keratinocyte survival and inhibited UV-induced apoptosis by 2 mechanisms: (1) phosphorylating Ser 112 and Ser 136 of Bad protein via the ERK and ARK pathways, respectively, and (2) by increasing the Bcl2 to Bax ratio[28]
5) Retinal pigment epithelial cells (RPE)—The low dose stimulation was potentially harmful; no mechanism was provided; mechanisms were offered for high dose inhibitory response[48]
6) Protection of cells was mediated via activation of Akt signaling and suppression of the P38 JNK pathway[49,50]
PC12 cells
7) EGCG inhibited NFkB nuclear translocation and binding by 6-OHDA in SH-SY5Y cells. EGCG enhanced iron chelation, which prevented nuclear translocation and activation of cell death from NF-kB[51]
8) Protection resulted from maintaining proper mitochondria functioning, preventing apoptosis via the inhibition of caspase 3 activation and down regulated the expression of the pro-apoptosis protein 5Mac in the cytosol[51]
9) EGCG potentiates the neuritogenic effects of BDNF via the involvement of 67-kDa laminin receptor and H_2O_2.[52] This effect required only submicromolar concentrations of EGCG
10) EGCG prevented the down regulation of Shh signaling of corticosterone. EGCG pretreatment reduced Cort-induced down regulation of Gli1 and N-myc, suggesting that EGCG may activate the Shh signaling pathways.[53] Blockage of the Shh pathway by selected pathway inhibitors blocked EGCG-induced protection
SH-SY5Y cells
11) EGCG reversed the occurrence of 6-OHDA-induced decrease of STAT-3 activity.[54] The EGCG increased the phosphorylation of STAT-3.[54] The findings indicate that protective effect of EGCG on neurons against g-OHDA is mediated by STAT-3 activation
Hippocampal neurons
12) The EGCG pretreatment protection against corticosterone-induced toxicity was blocked by the pharmacological inhibitors for ERK1/2 (00126) and P13k/Akt (LY294002).[55] The EGCG pretreatment also reduced the corticosterone-induced activation of receptor-y coactivator 1a (PGC-1a) expression and ATP production[55]
13) EGCG enhances neuronal survival and neuronal differentiation of adult hippocampal precursor cells, which was absent, when P13 k, a protein upstream of Akt, was blocked[56]

green tea polyphenols (GTPP) in situations where the inhibitors were absent.[39] The maximum predicted responses were in the 126% to 175% range. The authors suggested that the process of heteroactivation may be common across all CYP enzymes, affecting a vast range of drug/food interactions. They further suggested that, with respect to CYP1A1, activation of the various teas and their constituents may help explain the lack of epidemiological support for cancer outcomes in some studies.[44] These findings with CYP1A1 at low dose may become of further potential significance if linked to the capacity of EGCG to enhance tumor cell proliferation, perhaps after affecting tumor promotion mechanisms involving clonal expansion.

In a direct comparison between normal and colon cancer cells, EGCG reduced genetic instability as measured by the frequency of micronuclei (MN), nucleoplasmic bridges, and nuclear buds across the entire concentration range tested (5-40 μg/mL) of the human normal colon cell line (NCM 460; Figure S13), but the reverse was seen with the colon adenocarcinoma cell line (Colo205). In the case of the non-tumor cell line (NCM460), low doses of EGCG hormetically increased the expression of MCH1 protein, which is 1 of 7 DNA mismatch repair proteins that act in a coordinated manner to initiate repair of DNA mismatches in humans. In a similar fashion, the EGCG induced a hormetic J-shape for apoptotic cells (Figure S14).

Non-Neuro and Non-Tumor Cells

(–)-Epigallocatechin-3-gallate has been shown to act in a hormetic manner for a range of nonneuronal/non-tumor cells, enhancing resilience against various chemical toxins and/or oxidative stress agents/conditions under concurrent and preconditioning experimental exposure protocols. Regardless of the cell model or stressor agent, the EGCG displayed a protective effect at low concentrations, while toxicity typically occurred at high doses.

These studies have addressed a broad spectrum of cells (bovine mammary epithelial cells [Figure S15],[45] HepG2 cells [Figure S16],[46] THP-1-macrophages [Figure S17],[47] retinal pigment epithelial [RPE; Figure S18],[48] INS-1 in rat insulinoma cell lines [Figure S19],[49] and the αTC1-6 pancreatic alpha cell line [Figure S20][50]) with the goal of assessing possible chemopreventive effects of EGCG. (–)-Epigallocatechin-3-gallate was also found to protect the human macrophage cell line THP-1 against the highly toxic agent ricin in a hormetic fashion (Figure S21).[47] While EGCG induced hormesis in all cases, the low-dose positive response for the RPE cells was an undesirable effect since it could lead to enhanced tissue vascularization. However, in all other cases reported, the dose stimulation was considered to be protective. In 2 of the published studies, a preconditioning protocol was included and protection

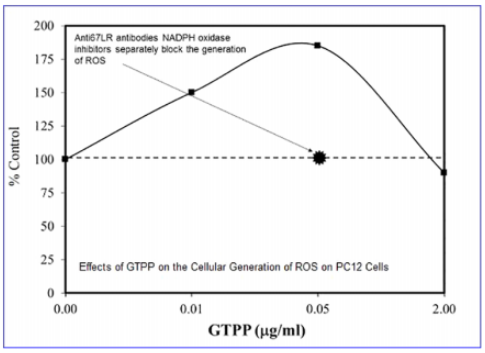

Figure 2. Effects of green tea polyphenols (GTPP) on the cellular generation of reactive oxygen species (ROS) on PC12 cells.[63]

of insulin-producing cells was found.[49,50] Mechanistic features of these studies are summarized in Table 1.

While most of the studies on hormetic effects deal with cell culture studies, a lifetime study concerning the effects of EGCG on the nematode *Caenorhabditis elegans* was recently reported. In this 10-dose study, a striking hormetic effect was shown with the maximum lifespan extension being approximately 15% (Figure S22).[57] The authors linked the EGCG-enhanced longevity to mitohormetic processes involving AMPK/SIRT1/FOXO-dependent redox signaling.

Nerve Cells

(–)-Epigallocatechin-3-gallate experiments using nerve cells showing hormetic dose responses have been reported with 3 types of experimental models: PC12 cells, SH-SY57 cells (Figure S23,[58] Figure S24,[54] and Figure S25),[59] and primary hippocampal cells (Figure S26-S28,[56] Figure S29,[55] and Figure S30)[60] from mice and rats. With respect to PC12 cells, experiments demonstrated that green tea/black tea and/or EGCG alone were effective in both directly stimulating cell proliferation as well as preventing chemically induced neuronal damage by several agents (6-OHDA [Figures S31-S33][57]; paraquat [Figure S34][52]; and L-DOPA [Figure S35][61]) that are known to induce symptoms of PD and AD (Figure S36). In a similar set of experiments, the EGCG prevented corticosterone-induced neuronal damage in both direct toxicities (Figure S37) and preconditioning (Figure S38) experimental frameworks.[62]

In 2012, Gundimeda et al reported that GTPP protected PC12 cells from damage induced by an oxygen-glucose deprivation (OGD) protocol.[63] This protection occurred within a preconditioning protocol with the GTPP administered 2 days prior to the OGD treatment. The GTPP-induced preconditioning activated the 67-kDa laminin receptor (67LR) to which EGCG binds with high affinity (Figure S39). Once activated, the 67LR mediates the generation of ROS via NADPH oxidase activation (Figure 2). The administration of several antioxidants prevented the GTPP-induced preconditioning protective effect. The GTPP protection process also involved activation and translocation of protein kinase C from the cytosol to the mitochondria, as well as several cellular pathway inhibitions, all of which were blocked by the antioxidant treatments. Other experiments using hydrogen peroxide (H_2O_2) or glucose oxidase-generating H_2O_2 supported the above findings with both treatments displaying hormetic dose responses in PC12 (TrkB) cells. Of particular relevance was the inclusion of a dose response with 6 treatments (0.06-6 μg/mL) which displayed a hormetic-like J-shaped concentration response with the optimal concentration of EGCG for preconditioning at 2 μM (Figure S39).

These multiple dose-response relationships display hormetic dose responses using GTPP, EGCG, H_2O_2, and glucose oxidase H_2O_2 metabolic generating systems. These findings support the perspective that GTPP, including EGCG, protects PC12 neurons from ROS stress, as well as enhancing neurite outgrowth in the absence of high-dose oxidative stress. The findings also support the conclusion that GTPP acts via a high-affinity target (ie, 67LR), enhancing/potentiating the capacity of neuritogenic activity mediated by brain-derived neurotrophic factor (BDNF). Within these different hormetic dose-response concentrations of GTPP (0.1 μg/mL) or EGCG (0.5 μM) tend to optimize BDNF neuritogenic activity. These concentrations are far lower than those employed for antioxidant effects as well as in the case of inducing apoptosis in tumor cells, which may be on the order of 10 to 200 μM EGCG.[53] Furthermore, EGCG can also be very quickly auto-oxidized in some cell culture protocols. This process will lead to a rapid lowering of the EGCG into the nanomolar concentration range.

Under normal circumstances, after drinking 2 cups of green tea in succession, a peak EGCG concentration would be approximately 0.2 μM.[64] Radiolabeled studies with mice orally administered EGCG showed that EGCG is rapidly and widely dispersed, including to the brain.[65] These findings led to the suggestion by Gundimeda et al that "it is possible that the concentration of EGCG (in the brain) will at least be in the nanomolar range of the green tea consumption by humans and it may be sufficient for potentiating the actions of BDNF."[53]

EGCG and Anxiolytic Effects

While EGCG is well known for its antioxidant/ROS scavenging effects, EGCG has also been reported to have modulatory effects on synaptic transmission. For example, Katayama et al reported that EGCG might induce the presynaptic release of acetylcholine in myenteric (gastrointestinal tract) neurons.[66] It can also act as an antagonist of glutamine AMPA receptor-mediated responses.[67] These findings suggest that some of the neuroprotective effects of EGCG may occur via an inhibitory effect on glutamate-mediated excitation on neurons by increasing GABA-mediated inhibition. In fact, a number of flavonoids that are structurally related to EGCG (eg, apigenin, quercetin) may act as ligands of the benzodiazepine $GABA_A$ receptor

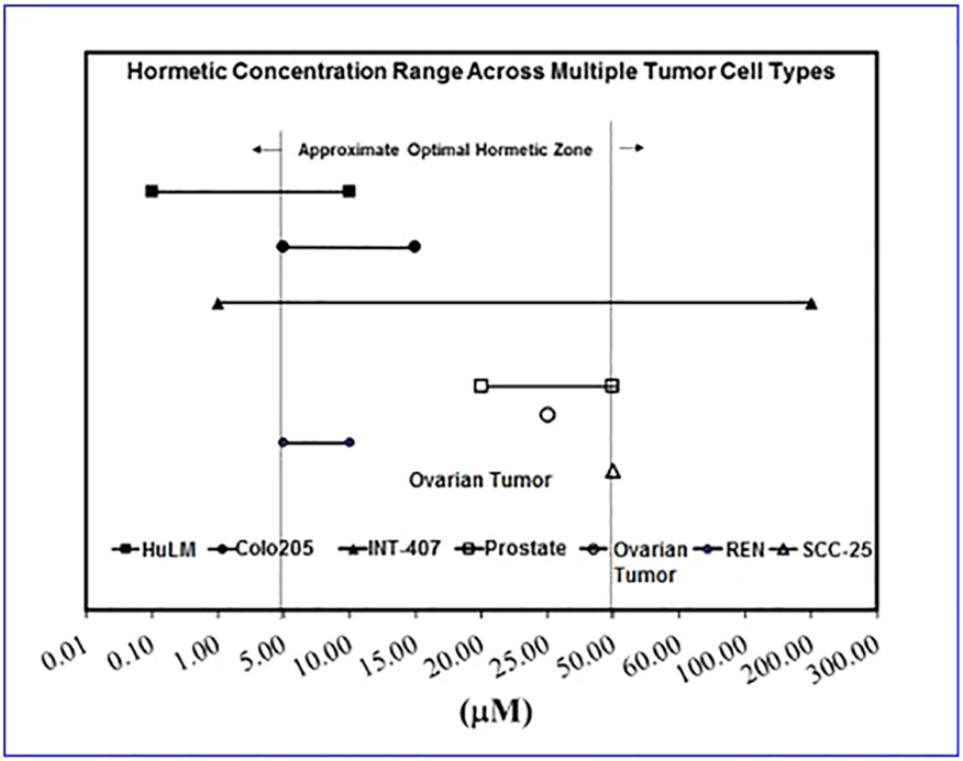

Figure 3. Hormetic concentration range across multiple tumor cell types. Data for each tumor cell response is provided in Figure S8—HuLM; Figure S14—Colo205; Figure S6—INT-407; Figure S9—Prostate and Ovarian; Figure S10—REN-mesothelioma; Figure S7—SCC-25.

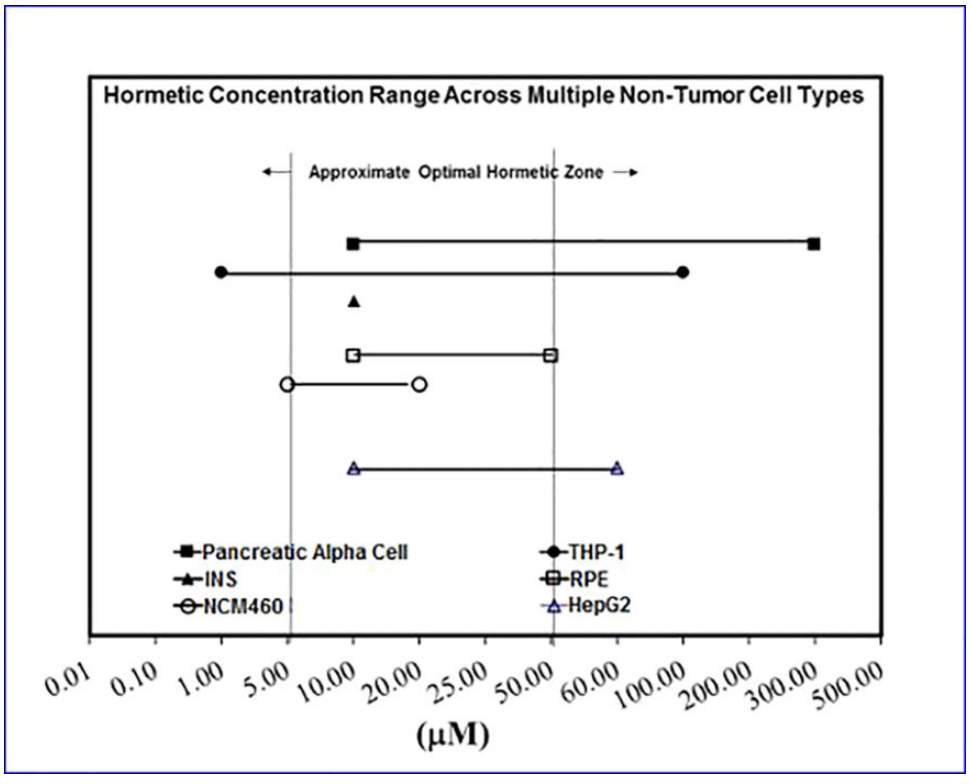

Figure 4. Hormetic concentration range across multiple non-tumor cell types. Data for each cell type are provided in Figure S20—Pancreatic α cell; Figure S21—THP-1; Figure S19—INS; Figure S18—RPE; Figure S13—NCM460; Figure S16—HepG2.

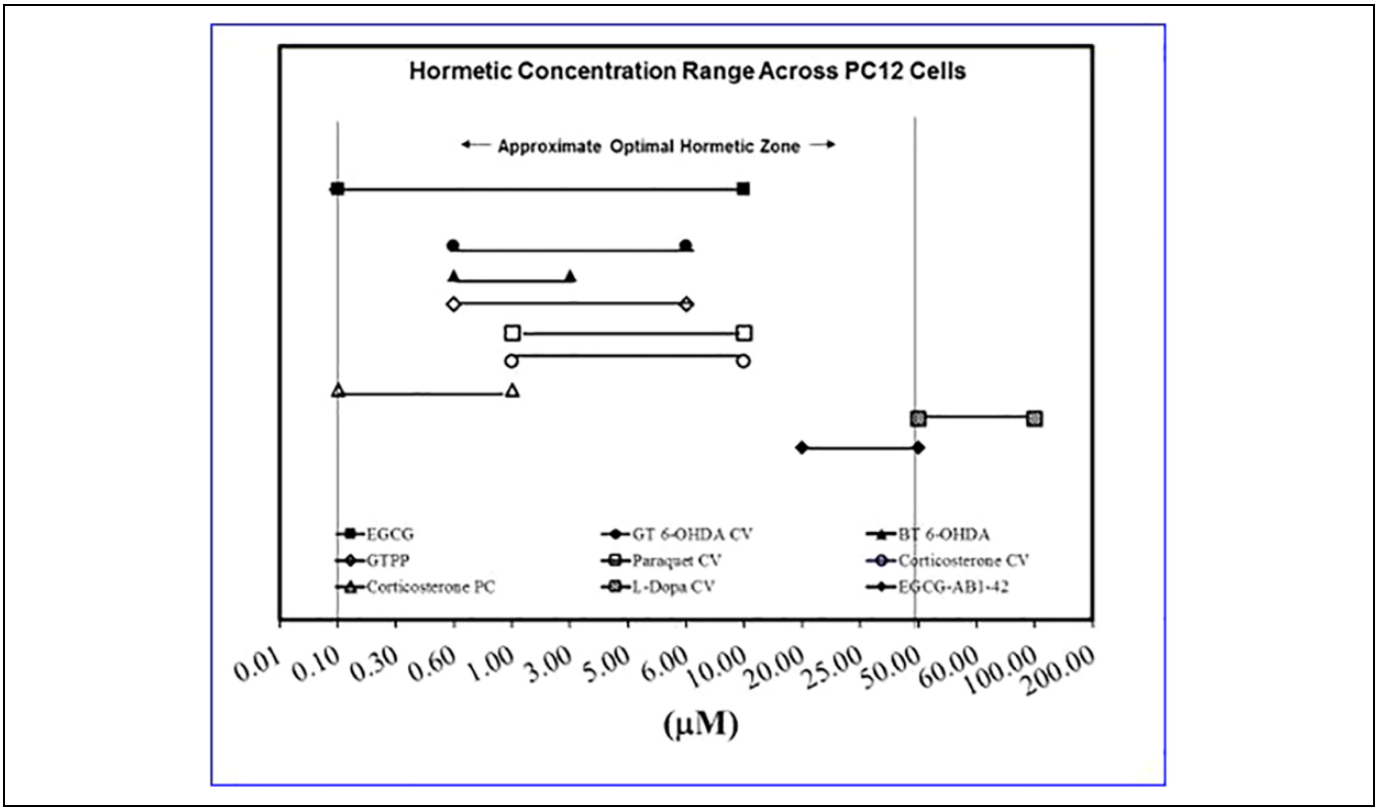

Figure 5. Hormetic concentration range across PC12 cells. Data for each dose response are provided in Figure S37—EGCG; Figure S31—GT 6-OHDA CV and BT 6-OHDA; Figure S39—GTPP; Figure S34—Paraquet CV; Figure S38—Corticosterone CV; Figure S29—Corticosterone PC; Figure S35—L-Dopa CV; Figure S36—EGCG-$AB_{1\text{-}42}$.

site.[68,69] While the sedative activity of some of the agents is due to possible allosteric modulation of the $GABA_A$ receptor, the flavone hispidulin displays the possible allosteric activity of the $GABA_A$ receptor, which is associated with the anticonvulsant activity.[70,71] The anxiolytic effects of flavonoids may, therefore, result from different types of $GABA_A$ receptor activity modulation.

Of particular relevance to the present assessment is that low concentrations of EGCG enhance the potentiation effect of benzodiazepines on the $GABA_A$ receptor.[72] Consistent with this finding is that EGCG reduces stress-mediated effects via modulation of the GABAergic system.[73] Follow up studies by Vignes et al found that EGCG biphasically affected anxiolytic behavior in a similar manner as the benzodiazepine drug chlordiazepoxide (Figure S40).[74] At low doses, the EGCG increased the time spent in the open arms of the plus-maze and decreased step-down latency in the passive avoidance test. Plus maze is an assay evaluating anxiety in animal models and is generally used in neurobiological anxiety research. The EGCG and chlordiazepoxide were fully generalized in substitution studies, suggesting that these 2 agents induced indistinguishable chemical effects in the brain.[74] These findings led the authors to conclude that EGCG may induce anxiolytic activity via an interaction with the $GABA_A$ receptor.

Discussion

The present assessment provides a concentration-response comparison across multiple tumor cell types, multiple non-tumor cell types, PC-12 cells treated with green tea, black tea and EGCG, various chemical toxins (6-OHDA, paraquat, L-Dopa, and corticosterone), and SH-SY5 (treated with rotenone and 6-OHDA; Figures 3-6). In general, there was a tendency for the hormetic stimulation zone to be in the 0.1 to 50 μM range. While a comparison of optimal concentration-response ranges would be of some theoretical and practical interest, there is a considerable lack of methodological standardization for a number of procedures, precluding confident estimates. For example, there was a wide range of cells used per well across studies. A study by Elbling et al revealed that changing the ratio of the cell number to working well volume could markedly affect end point outcomes, changing from toxicity to chemoprevention.[75] These findings and other variables across experimental protocols and cell types suggest caution with respect to offering general conclusions about optimal hormetic concentration ranges.

In order to effectively assess the possible occurrence of hormetic dose-response relationships, it is necessary that the study design be sufficiently robust with respect to the number of doses/concentrations along with some consideration given to the concentration/dose interval strategy. Of the 40 cases

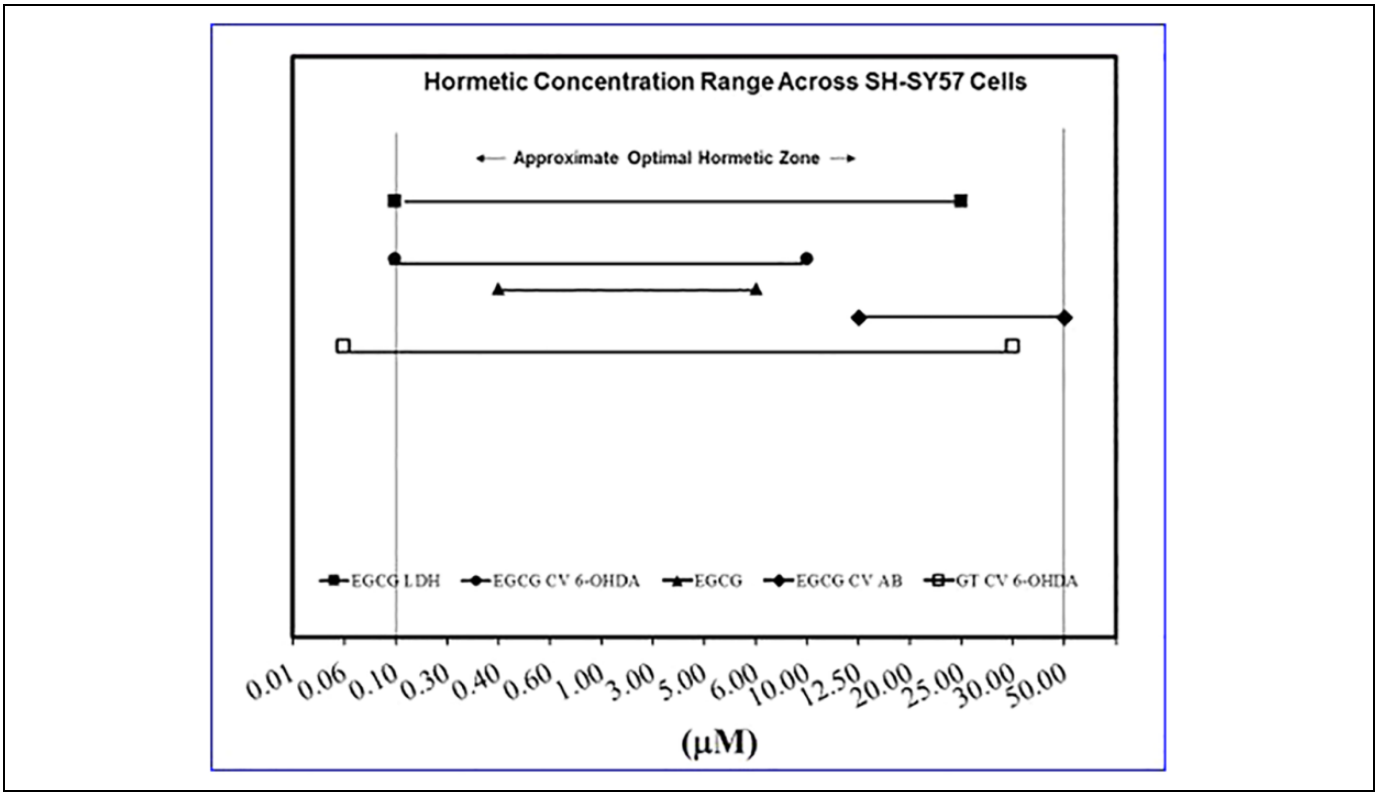

Figure 6. Hormetic concentration range across SH-SY57 cells. Data for each dose response are provided in Figure S23—EGCG LDH; Figure S24—EGCG CV 6-OHDA; Figure S25—EGCG and EGCG CV AB; Figure S32—GT CV 6-OHDA.

showing hormetic dose responses, about 95% had 4 or more concentrations/doses, with nearly half of the studies having 6 or more concentrations/doses. An assessment of the concentration/dose-related features of the hormetic concentration/dose responses reported in the present paper yielded a 150% median maximum response with a range of 115% to 375%. These findings are consistent with the vast body of literature on hormetic dose responses that show that the maximum dose responses tend to be in the 130% to 160% response range.[9,76,77] The dose range width of the stimulatory response had a median value of 12.5-fold. Despite this rather modest median value, the range of stimulatory dose responses was very broad, from less than 2-fold to 1000-fold. The high variation seen for the stimulatory width range has been commonly reported in the hormetic dose-response literature. Thus, the quantitative features of the hormetic dose response for GTPP are consistent with the general qualitative pattern of responses reported in an extensive hormetic dose-response literature.

This study reveals that green tea and several of its principal constituents, especially EGCG, induce a wide range of hormetic dose responses, generally within an in vitro biological context, for a wide range of cell types within both direct stimulation and preconditioning experimental protocols. These responses occurred for end points such as hair growth stimulation, protecting insulin-producing cells, enhancing neurogenesis, and building resilience in multiple cell types. Of potential public health and medical interest is that EGCG induced neuronal protection in model systems such as PC12 cells,[51-54,61-63] SY-SY5Y cells,[58,78] and primary hippocampal neurons.[55,56,60] These studies consistently demonstrated that GTPP and EGCG were effective in preventing neuronal damage from chemical agents with well-known capacities to induce PD-like symptoms. Low-dose EGCG treatment provided significant protection within a hormetic dose-response framework using various preconditioning experimental protocols. (–)-Epigallocatechin-3-gallate was also effective in preventing similar damage from high levels of corticosterone.[62] Similar protection was reported with SH-SY5Y cells using a preconditioning protocol with 6-OHDA.[62]

These findings are consistent with and extend the findings of Calabrese et al on hormetic approaches to the treatment of PD.[79] They reported evidence for hormetic dose responses for 50 agents that prevent PD-related effects in 1 or more experimental models, including PC-12, SH-SY5Y, and MN9 cells, using the model chemical inducing agents 6-OHDA, MPT, rotenone, and paraquat. The hormetic dose-response features noted in the above report of Calabrese et al[79] closely paralleled the findings reported for EGCG.[80]

There has also been interest in whether dietary agents such as polyphenols (eg, GTTP) have the capacity to affect brain plasticity. In vitro and preclinical researches have suggested the potential for these agents to modulate neuroplasticity.[81-83] Multiple groups have explored neuroplasticity via the generation of neurons in the hippocampus of the adult and how agents

in the diet such as EGCG may affect adult neurogenesis. Such research has assessed whether dietary components could blunt the harmful effects of neurodegenerative diseases on hippocampal neurogenesis and learning and memory.[84,85] Within this context, EGCG attracted considerable interest since it not only is effective in enhancing resilience in neuronal cells, but it can also cross the blood–brain barrier.[65,86,87]

H_2O_2 is a broadly documented second messenger[88] and a relatively stable molecule. Low concentrations of H_2O_2 often induce prosurvival pathways in multiple cell types, including neuronal cells. The principal prosurvival activities include kinase-driven oxidation of cysteines located within the active sights of numerous phosphatases and affect the regulation of multiple transcription factors, including P53, NFkB, AP-1, and Nrf2. Of particular importance is the concentration of H_2O_2 and its specific site of generation that mediates its second messenger activity.[89] In practical terms, the targets of H_2O_2 activity need to be in the same molecular vicinity as the H_2O_2 production site. When extracellular H_2O_2 is applied to experimental systems (the cell cultures) in the range of 0.1 to 5.0 μM, these typically result in intracellular H_2O_2 levels of about an order of magnitude lower (0.01-0.5 μM).[89] This is an intracellular concentration range that still can directly stimulate cell division and a complex set of cell adaptations. In fact, Keap 1 intermolecular disulfide formation via cysteine is the molecular switch for the activation of Nrf2 via low concentrations of H_2O_2.[90] However, when higher concentrations of H_2O_2 are applied, they lead to the induction of apoptosis and death.[91] Of direct relevance to the mechanistic assessment of EGCG is that all polyphenols in growth media generate H_2O_2. Since most polyphenols (including EGCG) interact with cell membranes,[92-94] H_2O_2 molecules typically leave the extracellular environment, partially diffusing into the cells. Based on these processes, Erlank et al proposed that low concentrations of polyphenols, including EGCG, generate H_2O_2 at very low concentrations in vivo at the level of arteries and capillaries, inducing cellular adaptation to oxidative stress.[89]

Of particular interest are widespread reports of low concentrations of H_2O_2 (0.1-5.0 μM-cell culture) inducing cell proliferation and activation of Nrf2.[89] Yet, Plauth et al emphasized that when cells are stressed slightly, they focus their limited resources on cellular repair, while decreasing cellular proliferation and nucleotide synthesis.[95] The concentration of resveratrol cited for this cell proliferation response reduction in NHEK cells was 50 μM. This suggests lower concentrations may activate cell proliferation, whereas higher concentrations inhibit these activations. Data from Plauth et al study with NHEK cells shows strong inhibition of several cell proliferation parameters by 50-μM resveratrol.[95] Significant suppression of PPAT, the first step in purine nucleotide biosynthesis, occurred at 50 μM. Such findings suggest that the hormetic optimal for cell proliferation occurs at low concentrations, while at modestly higher concentrations, a constellation of cellular resilience genes are activated at the expense of proliferation. That is, the findings of Plauth et al offer the potential to differentiate a cell proliferation hormetic effect from a hormetic-induced resiliency effect over a narrow concentration range.[95] Whether these findings with resveratrol can be applied to EGCG or other agents is not known.

Of potential health relevance is that the concentration used in several studies showed hormetic effects within the concentration range seen in humans. This suggests that an optimal dosing scheme could be developed for public health and therapeutic applications. These findings are in accordance with the hormetic effects obtained by the studies that investigated the real-life exposure scenario to combination of stimuli that focus on 2 main characteristics: low doses and chronic exposure.[96-99] Finally, the present findings of a possible wide spectrum of beneficial effects suggest that epidemiological literature, which has consistently shown beneficial effects of the consumption of green tea, may be due to its hormetic effects.[39] The present findings should, therefore, be useful in hypothesis generation and testing and study design consideration for both experimental and epidemiological studies of green tea and its principal constituents.

Innovation

Many epidemiological studies have associated the consumption of green tea with a plethora of positive health benefits. This study documents that green tea and its principal constituent EGCG widely induce hormetic dose responses, an issue of clinical significance because it provides an opportunity to develop an optimal dosing scheme for public health and therapeutic applications.

Materials and Methods

PubMed, Web of Science, and Google Scholar databases were searched for articles using the terms hormesis or hormetic, biphasic dose-response, *U*-shaped dose response, adaptive response, and preconditioning in combination with green tea or EGCG. All relevant articles were iteratively evaluated for references cited and for all papers citing these papers. All research groups publishing on green tea/EGCG dose responses were assessed for possible relevant publications in the above databases. Green tea/EGCG hormesis phenomenon for specific end points are presented and discussed in Results and Discussion sections.

Declaration of Conflicting Interests

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Supplemental Material

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