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PERSPECTIVE

Unravelling Green Tea's Mechanisms of Action: More Than Meets the Eye

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After water, tea is the most popular beverage and is consumed by two thirds of the world's population. Three types of tea are available; they vary in the processes of drying and fermentation. Besides black tea, which is the most prevalent form (78%), green tea (20%) and oolong (2%) are produced. The tea plant *Camellia sinensis* is native to South China and was brought to Japan by the Zen priest Eisai. Green tea in particular is an essential part of daily life in China, Japan, and India. Green tea is obtained by steaming freshly harvested tea leaves, thus causing inactivation of enzymes that initiate the fermentation process. This yields a dry and stable product that contains most of the polyphenols and gives green tea its typical color and taste (Bachrach and Wang, 2002). Green tea is rich in polyphenolic compounds, which account for a third of the dry weight of the leaves; the most prominently components are flavonols, commonly known as catechins (Balentine et al., 1997). These include epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate (EGCG). For a long time, green tea was preferred as a beverage because of its attractive flavor and taste. But the scientific community and the popular press have recently pondered that there might be beneficial properties of green tea. Consumption has been associated with anti-inflammatory, antioxidative, antimutagenic, and anticarcinogenic effects (Benelli et al., 2002; Weisburger and Chung, 2002).

In general, plants used for nutrition or for medical therapy contain a series of antioxidants (e.g., among others, vitamin C, vitamin E, β-carotene, polyphenols, or selenium) that act as potent radical scavengers. These compounds are thought to be responsible for many beneficial effects, particularly in inflammatory diseases but also in cancer prevention. The alteration of the redox-state of the cell by antioxidants also triggers fine-tuned changes in signaling pathways and subsequent gene expression (Chen et al., 2002; Pfeilschifter et al., 2003). Tea polyphenols have shown cytostatic properties in several tumor models. In particular, green tea catechins can act at different sites. One facet of the antitumor activities exerted by EGCG seems to be attributable to blocking of the EGF receptor (Liang et al., 1997) and the platelet-derived growth factor receptor (Sachinidis et al., 2000), which can inhibit the growth factor-induced activation of activator protein 1, a transcription factor critically involved in the action of various growth factors and cytokines. Additionally, a recent study has demonstrated an EGCG-dependent inhibition of focal adhesion kinase activity, indicating a negative interference of green tea with cell adhesion and cell movement processes (Liu et al., 2001).

Most interestingly, mice that were infused with either green tea or pure EGCG showed a reduced angiogenic response to vascular endothelial-derived growth factor, a key regulator of angiogenesis, as reflected by a dose-dependent reduction of vessel formation (Cao and Cao, 1999). Protease-dependent degradation of extracellular matrix components is one of the main prerequisites of metastasis. Importantly, in this context, many studies have identified the group of serine and metalloproteinases as downstream targets of antimetastatic activities exerted by green tea polyphenols. Indeed, EGCG was described to inhibit the urokinase-type plasminogen activator, a major activator of the matrix metalloproteinase (MMP) cascade, although the effective EGCG concentration (in the millimolar range) markedly exceeds plasma levels measured in vivo (Cao and Cao, 1999). In contrast to the urokinase-type plasminogen activator, the inhibition of MMP-2 and MMP-9 gelatinases by EGCG, which results in a significant reduction of the invasive behavior of gelatinase-expressing cancer cells, could be observed with concentrations an order of magnitude lower than those reported for the inhibition of urokinase (Benelli et al., 2002). Clinically, high gelatinase expression correlates with high invasive potential and tumor spread and therefore seems to be a negative prognostic factor in many cancers. The proposed mechanisms of MMP inhibition by EGCG are diverse, ranging from noncom-

ABBREVIATIONS: EGCG, epigallocatechin-3-gallate; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor κB; GX, green tea extract; INOS, inducible nitric-oxide synthase; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor; IL, interleukin.

petitive inhibition of enzyme activity caused by a polyphenol-dependent blocking of the catalytic site of MMP-2 and MMP-9 to EGCG-mediated suppression of MMP transcription, as has been described for MMP-2, MMP-9, and membrane type-1-MMP, respectively (Benelli et al., 2002, Maeda-Yamamoto et al., 2003). Mechanistically, the inhibition of MMP-2 and MMP-9 expression by EGCG seems to be mediated by an inhibition of extracellular signal-regulated kinases 1 and 2 and the p38 MAPK, both necessary for transcriptional induction of MMP-9 expression (Eberhardt et al., 2000).

Generation and action of free radicals such as nitric oxide (NO), superoxide (O_2^-), or hydrogen peroxide (H_2O_2) highlight the impact of redox regulation on cellular signal transduction and gene expression and have been reported to prominently regulate matrix production and turnover (Pfeilschifter et al., 2001). The interaction of NO with molecular oxygen (O_2) or O_2^- gives rise to the formation of the potent nitrosating agents N_2O_3 and peroxynitrite ($ONOO^-$), respectively. In addition, *S*-nitrosothiol adducts are formed by the interaction between the N_2O_3 and certain protein thiol groups and evoke signaling by altering protein kinases and phosphatases, G-proteins, ion channels, protein tyrosine kinases, and redox-sensitive transcription factors, such as nuclear factor κB (NFκB), activator protein 1, or CAAT/enhancer binding protein (Pfeilschifter et al., 2003).

Remarkably, plant extracts exert their beneficial effects not only by changing the redox state but also by triggering well-defined signaling cascades that result in a redox-independent regulation of transcriptional activity (Chen et al., 2000). The identification of redox-independent and specific new targets will provide additional progress in the development of plant-derived therapeutics.

In this issue of the journal, Tedeschi et al. (2004) report on the effects of green tea extract (GX) on cytokine-induced iNOS expression in the human epithelial lung and colon cell lines A549/8 and DLD-1. They observed an inhibitory effect of GX on iNOS expression, on iNOS promoter activity, mRNA and protein levels as well as the cytokine-induced formation of nitrite. Band shift experiments document STAT-1, but not NFκB, as the transcription factor responsible for the inhibitory effect of GX. Additional experiments with the antioxidant *N*-acetylcysteine indicate that the down-regulation of iNOS by GX occurs independently of the redox activity of the compound.

Inhibition of pro-inflammatory cytokines and mediators by green tea extracts and EGCG is not new. In RAW264.7 and peritoneal macrophages, EGCG potently reduces lipopolysaccharide-induced TNF-α expression by inhibiting binding of NFκB; in vivo administration of EGCG reduced lethality in endotoxemic BALB/c mice (Yang et al., 1998). Similarly, Suganuma et al. (2000) showed that EGCG inhibited okadaic acid-elicited binding of NFκB in 3T3 fibroblasts, thus leading to a down-regulation of TNF-α expression. Furthermore, in TNF-α transgenic mice, 0.1% GX resulted in down-regulation of TNF-α, IL-1β, and IL-10 expression, clearly indicating an anti-inflammatory action of GX. In A549 lung carcinoma cells, Chen et al. (2002) showed that EGCG inhibits TNF-α–induced promoter activity of the chemokine IL-8 by an interference with the IκB/NFκB pathway. Moreover, EGCG has been reported to inhibit lipopolysaccharide/cytokine-induced iNOS expression in a murine macrophage cell line (Lin and Lin, 1997), human chondrocytes (Ahmed et al., 2002), and mouse peritoneal cells (Chan et al., 1997) by inhibiting NFκB activation. Furthermore, EGCG was recently shown to be a potent inhibitor of interferon-γ–triggered STAT-1α phosphorylation and activation in various human carcinoma cells (Menegazzi et al., 2001).

Although most of the experimental data have focused on the important role of EGCG as the major constituent of green tea, the overall preventive effect of green tea observed in vivo is thought to require the combined actions of several components of tea rather than a single compound (Williams et al., 2000). In general, herbal medicinal drug preparations are complex mixtures of different compounds that often act in a synergistic fashion and exert their full beneficial effects as total extracts (Loew and Kaszkin, 2002). A further potent constituent of green tea is caffeine, which interacts with the polyphenols by improving the antimutagenic activity of regular tea compared with the decaffeinated version (Weisburger et al., 1998). In vitro, tea with caffeine inhibited the growth of several human cancer cell lines, and tumor prevention in humans and rats was improved by regular green tea consumption (Huang et al., 1997). Moreover, green tea extracts are more stable than pure EGCG because of the presence of other antioxidant constituents in the extract. In this context, the study by Tedeschi et al. (2004) in this issue of *Molecular Pharmacology* provides an important demonstration that green tea extract GX in concentrations in the range of the plasma concentration found in humans drinking six to ten cups of green tea per day exerts a specific inhibitory action on interferon-γ-elicited activation of the transcription factor STAT-1α by blocking cytokine-evoked tyrosine phosphorylation. This in turn blocks expression of iNOS and dramatically reduces NO production. Pathological overproduction of NO contributes to vascular dysfunction and organ failure during endotoxemia and in diseases such as inflammatory bowel disease, asthma, or lupus nephritis (Pfeilschifter et al., 2002). Because green tea can be consumed over the long term in large quantities without any (currently known) harmful side effects, and epidemiological data are already available on Chinese and Japanese cohorts (Fujiki et al., 2002), its possible therapeutic potential in inflammatory disease deserves consideration. In addition, the rapid distribution of catechins in most organs observed in mouse models (Suganuma et al., 1999) suggests a possible adjuvant application of green tea that requires further evaluation, hopefully in upcoming human interventional studies.

Despite the many challenges for herbal medicines caused by lack of standardization, frequent contaminations, and the limited reporting of adverse effects (DeSmet, 2002), such medicines have a potential for clinical and research opportunities that should not be neglected. Research progress, particularly studies concerning green tea, provide an interface between alternative medicine and basic pharmacology, especially as related to the molecular basis of action. Progress is being made; we do know at least some of the key active pharmacologic entities of green tea and, importantly, as highlighted by the study of Tedeschi et al. (2004), we are gaining insights regarding their mode of action.

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