

Editorial

Is green tea good for HIV-1 infection?

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HIV-1 infection ultimately results in impaired specific immune function by virtue of the initial binding of the HIV-1 virion envelope glycoprotein 120 (gp120) to the CD4 receptor in complex with a chemokine receptor on the T-cell surface.¹ Even though gp120 elicits virus-neutralizing antibodies, HIV-1 eludes the immune system and leads to the onset of AIDS. Ever since the discovery of the virus as the causative agent, there has been an intense effort to develop therapeutic methods to inhibit or prevent infection.²⁻⁴ Among the immune-based strategies designed to prevent the fateful initial binding event between HIV-1 virion and target cell are chemokine analogs, chemokine receptor inhibitors, anti-HIV-1 monoclonal antibodies, fusion proteins, and vaccines.⁵

As reported by Kawai et al⁶ in this issue, a unique candidate in the fight against AIDS has recently been investigated: green tea, one of the world's most frequently consumed beverages. Green tea has been reported to provide a cornucopia of beneficial health activities, such as the prevention of cancer and cardiovascular disease.^{7,8} There are specific components of green tea that have been shown to have antimicrobial activity.⁹ Recently, investigators have found that the antiviral effects can be targeted at HIV-1 infection.^{6,10-12} Although the multiplicity of purported health benefit effects of green tea stretches the length of credibility, the molecular evidence of anti-HIV effects put forth by Kawai et al⁶ warrants further scrutiny.

Green tea is the nonoxidized, unfermented product of leaves from the evergreen plant *Camellia sinensis*. The

Abbreviations used

EGCG: Epigallocatechin gallate
gp120: HIV-1 glycoprotein 120

active components of green tea are the polyphenolic catechins, which include the isomers (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin, and (-)-epigallocatechin gallate (EGCG)¹³ (Fig 1). EGCG accounts for approximately 50% of the total amount of catechins.¹⁰

EGCG binds strongly to many biological molecules and affects a variety of enzyme activities and signal transduction pathways at micromolar or nanomolar levels.¹⁴ It is this specific component of green tea that is thought to be responsible for the vast array of presumed health benefits. Among the properties ascribed to EGCG are anti-tumorigenic, anti-inflammatory, antioxidative, antiproliferative, antibacterial, and antiviral effects.^{8,10,12}

Epidemiologic and preclinical studies have shown that drinking green tea is associated with a lower incidence of human cancer.^{8,15} This protective effect of green tea has been observed in pancreatic, colon, rectal, skin, breast, prostate, liver, and lung cancer.^{8,15} The major component of green tea, EGCG, has been shown to have tumor antimetastatic and antiangiogenic activities, as has been observed in its inhibition of adhesion of carcinoma cells¹⁶ and significant prevention of growth of new blood vessels.¹⁷ EGCG is thought to be the most potent chemopreventive component of the catechins, inasmuch as it possesses both pyrogallol and galloyl moieties¹³ (Fig 1).

In HIV-1 infection, it is EGCG that is responsible for the reported antiviral effects of green tea.^{6,11,12} Several mechanisms for the antiviral effects of EGCG on HIV-1 have been proposed. EGCG inhibits HIV-1 replication in human PBMCs in vitro by inhibiting the biochemical activity of HIV-1 reverse transcriptase, the result being a subsequent decrease in HIV p24 antigen concentration.¹¹ Other studies show that EGCG interferes with HIV-1 viral infection by virion destruction and HIV-1 reverse transcriptase inhibition.¹² Finally, EGCG induces virion destruction in vitro by deformation of phospholipids via binding to the surface of the viral envelope.¹⁰

Kawai et al⁶ have reported yet another anti-HIV-1 effect of EGCG, one that prevents the attachment of the HIV-1 virion, gp120, to CD4 molecules on T-helper cells. The researchers found that in human CD4 T cells, EGCG (25-250 $\mu\text{mol/L}$) downregulated the cell surface expression of CD4 by binding to the CD4 molecule, presumably at a binding site recognized by gp120. Alternate

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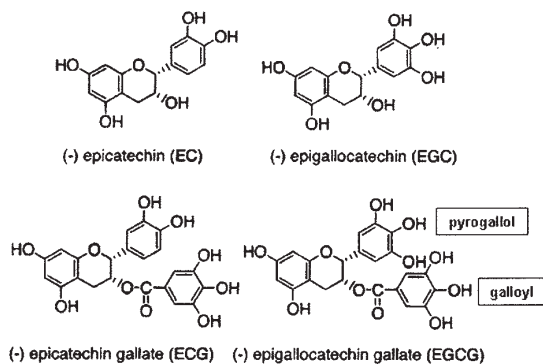


FIG 1. Structure of the polyphenolic catechins found in green tea.

explanations for this theory were ruled out by the demonstrated absence of (1) CD4 shedding from the cell surface, (2) loss of CD4 molecules, and (3) CD4 endocytosis. Kawai et al⁶ also demonstrated that EGCG could antagonize gp120 binding as a ligand for CD4 and demonstrated that EGCG effectively interfered with gp120 binding to lymphocytes. Indeed, the most important of the anti-HIV-1 properties of EGCG might be its blocking effect on gp120 as a ligand for CD4, thus preventing the initial encounter of the HIV-1 virion with CD4 T cells.

HIV-1 entry depends on the sequential interaction of the gp120 exterior envelope glycoprotein with the receptors on the cell, CD4, and members of the chemokine receptor family. Initial binding of the HIV-1 virions to cells involves the interaction of the viral envelope protein gp120 with CD4.¹ Recombinant soluble CD4 proteins bind to the HIV-1 envelope glycoprotein gp120 and inhibit viral infection.¹⁸ High-affinity binding to gp120 occurs at the D1 domain of CD4.¹⁸ The primary sites for HIV-1 interactions are on loops that protrude from the variable-like D1 domain in analogy with immunoglobulin complementarity-determining regions. The D2 domain is intimately associated with D1 but is variable-like.¹⁸

Broadly neutralizing antibodies recognize discontinuous conserved epitopes on gp120, the most abundant of which are directed against the CD4 binding site and block gp120-CD4 interaction.¹⁹ There might be a somewhat similar mechanism for the EGCG-induced disruption of the gp120-CD4 interaction, with the blocking ligand attaching to the CD4 molecule.

The report by Kawai et al⁶ left off at defining the specific molecular binding mechanism involved in the EGCG-induced HIV-1 inhibition. We propose that the binding of the EGCG compound to the CD4 molecule would specifically involve the D1 domain at the sites on the CD4 molecule that are most critical for binding to gp120. Mutational analysis of CD4 has implicated residues from a portion of the extracellular amino-terminal domain (D1) in gp120 binding.²⁰ In HIV-1 infection, interatomic contacts, including van der Waals contacts and hydrogen bonds, are made between 22 CD4 residues and 26 gp120 amino acid residues. These residues are distributed over 6 segments of gp120. On CD4, the

residues span from 25 to 64. The most critical of these residues are Phe 43 and Arg 59, with Phe 43 at the center of the cluster of residues involved in binding. Sixty-three percent of all interatomic contacts come from one span (40-48) in C'C'' of CD4; Phe 43 alone accounts for 23% of the total.^{1,20} Therefore, these might be potential EGCG binding sites, as illustrated in Fig 2.

We examined the structure of CD4 using protein data base:1CDJ²⁰ to model an electron density map at 3 Å, and we highlighted the regions of residues Phe 43 and Arg 59 (Fig 2). Because of the inherent flexibility of the EGCG molecule (due to the 4 key dihedral angles essentially covering the entire 360-degree range of rotational freedom) and the size of the molecule,²¹ there is a wide range of possible conformations for docking into the region flanked by Phe 43 and Arg 59. Although we do not offer this model of EGCG-inhibition of HIV-1 gp120 binding to CD4 as fact, the conformational fit of critical binding sites is plausible. Additional studies to better define the nature and specificity of EGCG binding to the CD4 molecule are needed.

A crucial aspect of translating the observed effects of EGCG to clinically relevant strategies, as pointed out by Kawai et al,⁶ is the requirement to achieve physiologically relevant concentrations. Inasmuch as tea catechins have poor bioavailability, most of the ingested EGCG does not get into the blood, and a significant fraction is eliminated presystemically.²² At present, phase I clinical trials involving pharmacokinetic studies of EGCG have shown that only a small percentage of the orally administered catechin appeared in the blood. Drinking the equivalent of 2 cups of green tea resulted in the mean peak plasma EGCG level of 0.17 μmol/L after 1.5 hours, and only 4% to 8% of the ingested EGCG was excreted in urine.^{14,23} Therefore, the development of a capsular alternative to green tea or EGCG itself would be beneficial, though even in this form a patient might not be able to achieve the EGCG levels of 25 to 250 μmol/L used in the current in vitro study.⁶

This provocative investigation raises again the question of using natural products in the treatments of serious diseases. By no means should the findings of this study by Kawai et al⁶ be seen as an endorsement of the consumption of green tea (gallons of it, presumably) to counter HIV-1 infection, or, worse, as an alternative therapy to the wonderful life-restoring antiretroviral agents that we now have. Whether green tea could serve as part of a combination therapy, along with other anti-HIV therapies, is an unanswered question thus far. The potential competitive binding properties of EGCG for the CD4 binding sites by gp120, while intriguing, might not translate to an HIV-1 preventative strategy. Nevertheless, it is not improbable that green tea or its extracts will be involved in the future treatment of HIV-1 infection.

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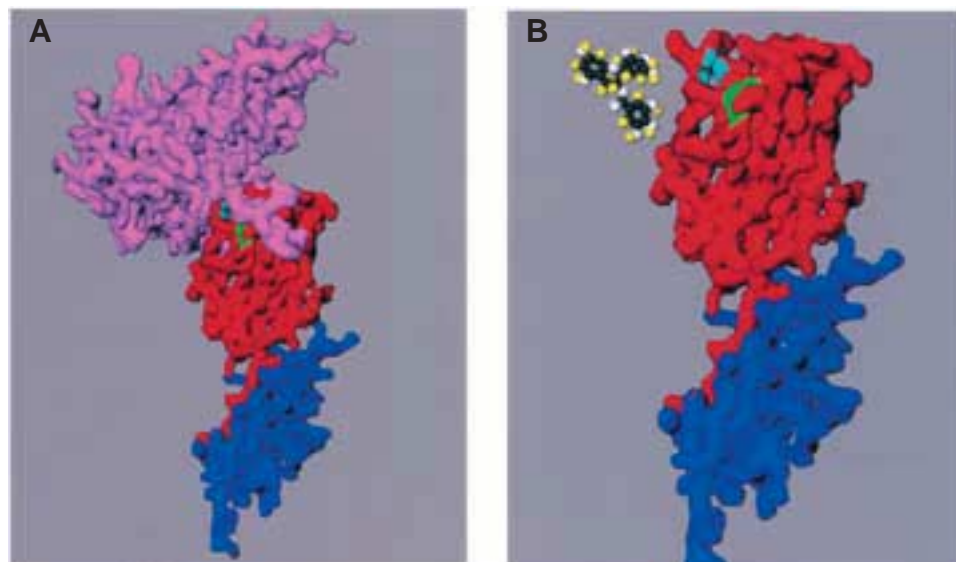


FIG 2. Computer-generated models of CD4 binding. Space-filling model of CD4 domains D1 (red) and D2 (blue). The models demonstrate the extracellular component of the CD4 molecule with residues Phe 43 (cyan) and Arg 59 (green). **A**, Model of HIV-1 gp120 (purple) binding to CD4. **B**, Computer-generated model of EGCG and CD4. The white, black, and yellow spheres represent the oxygen, carbon, and hydrogen atoms, respectively, of the EGCG molecule. Models courtesy of the National Center for Macromolecular Imaging, Baylor College of Medicine, Houston, Tex.

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