Catechins containing a galloyl moiety as potential anti-HIV-1 compounds

Yali Zhao[1,a], Fan Jiang[1,a], Ping Liu[1,a], Wei Chen[1] and Kejia Yi[2]

[1] Chinese PLA General Hospital, 28 Fu Xing Road, Hai Dian District, 100853 Beijing, China
[2] Beijing Science Technology and Management College, Beijing, China

Catechins containing galloyl moieties are important natural antioxidant compounds. In this paper, we review the multiple mechanisms whereby catechins containing a galloyl moiety can target key proteins to inhibit sexual transmission of HIV-1, as well as HIV-1 fusion, HIV-1 reverse transcriptase, HIV-1 integrase and HIV-1 protease. Furthermore, catechins with a galloyl moiety can mediate host cell factors such as nitric oxide synthase, nuclear factor-κB and casein kinase II to inhibit HIV-1 infection. The most significant inhibitory effect is blocking gp120 binding to isolated human CD4+ T cells. The multiple mechanisms underlying the anti-HIV activity of galloyl-containing catechins predict that these catechins could be used as alternative therapies in the treatment of HIV infection.

Introduction

HIV infection begins with viral entry, with the subsequent destruction of T cells that eventually leads to AIDS [1]. According to WHO an estimated 6.6 million people in low- and middle-income countries were receiving antiretroviral therapy (ART) for HIV/AIDS at the end of 2010 (http://www.who.int/mediacentre/news/releases/2011/hivtreatement_20110603/en/index.html). In 2009 alone there were 2.6 million new infections and 33.3 million people worldwide were HIV-positive (http://www.who.int/topics/hiv_aids/en/). Approximately 25% of people are unaware that they are infected with HIV [1].

Antiretroviral drugs have transformed AIDS from a rapid, lethal infection into a chronic condition that can be controlled for many years using combination therapy with different classes of antiviral drugs, which is known as highly active antiretroviral therapy (HAART) [2]. Anti-HIV drugs are classified into different groups according to their activity on the replication cycle of HIV, which can be divided into approximately ten different steps: virus–cell adsorption, virus–cell fusion, uncoating, reverse transcription, integration, virus genome replication, transcription, translation, budding and maturation [2,3]. There are 25 compounds approved and available for the treatment of HIV [2,4]. However, owing to more and more prominent drug resistance and the side-effects of the current drugs [5,6], many investigations now focus on new anti-HIV drugs.

One recent class of compounds that has shown potential anti-HIV activity is the catechin family of polyphenols [7,8]. Catechins are an important group of polyphenols, and they make up approximately 13–30% of the dry weight of green tea leaves. There are five major catechins in green tea: (+)-catechin (C), epicatechin (EC), (−)-epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), all of which are shown in Fig. 1. Out of the five major catechins, EGCG and ECG contain a galloyl moiety (indicated by the D-ring in Fig. 1). EGCG is the most abundant catechin, accounting for approximately half of the total catechins in green tea [9,10]. The physiological functions of catechins are mainly attributed to the galloyl moiety [11,12]. During heat treatment 50% EGC, ECG and EGCG are epimerized to catechin gallate (CG) and gallocatechin gallate (GCG) [12].

Free-radical damage contributes to many chronic health problems, such as cardiovascular disease [13], cancer [14] and AIDS [15]. Viruses require environmental oxidative stress for efficient replication. Viral replication is often enhanced by inflammatory cytokines that also induce oxidative stress [16].

Corresponding authors: Liu, Jiang, F. (jiangfan23@yahoo.com.cn), P. Liu (liuping0707@yahoo.com.cn)
[a] These authors contributed equally to this work.

 1359-6446/06/$ - see front matter. Crown Copyright © 2012 Published by Elsevier Ltd. All rights reserved. doi:10.1016/j.drudis.2012.02.015

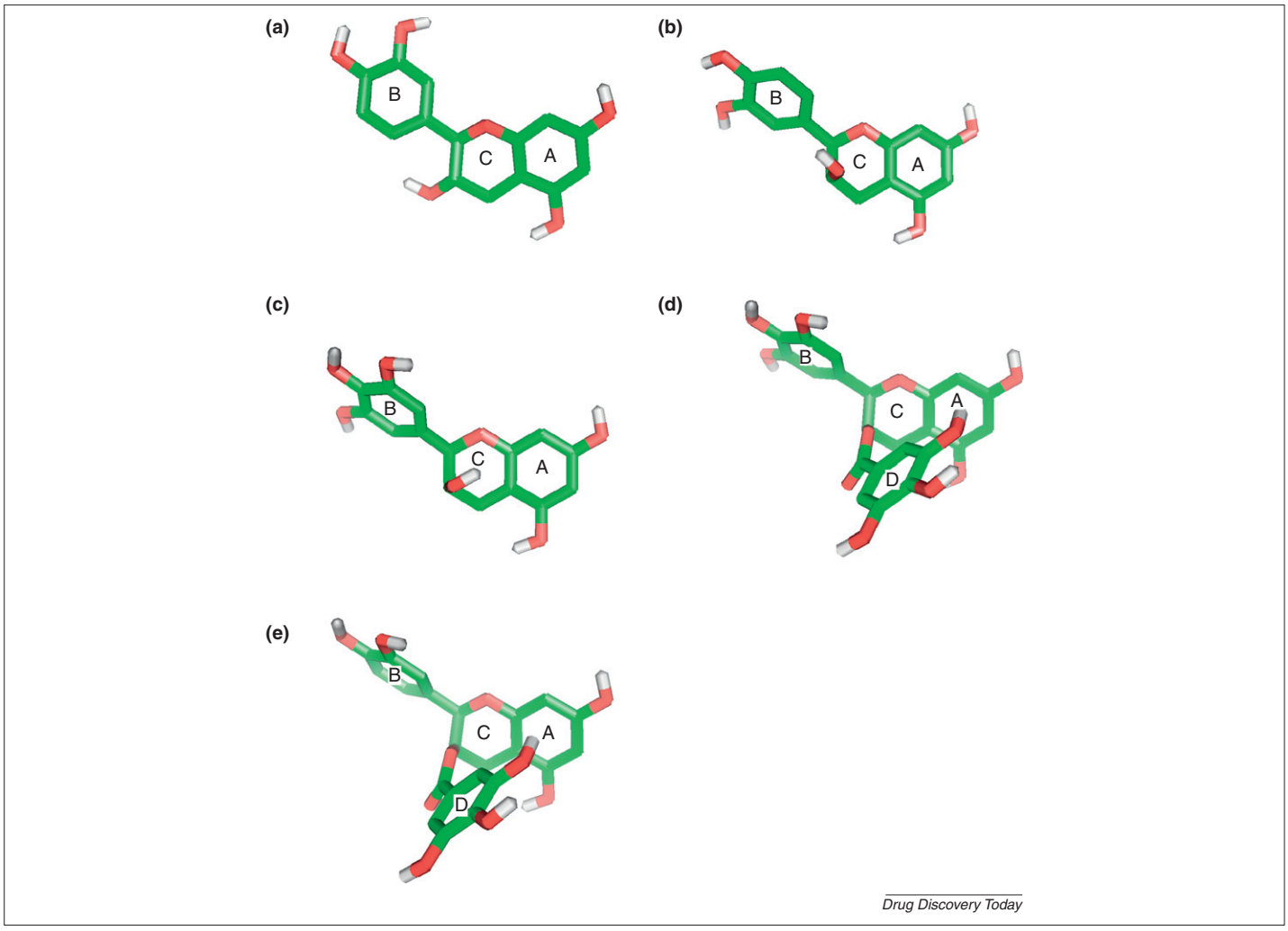

FIGURE 1

Three-dimensional (3D) structures for five major catechins. Green represents carbon, red represents oxygen and white represents hydrogen. **(a)** Shows the 3D structural of C; **(b)** the 3D structure of EC; **(c)** the 3D structure of EGC; **(d)** the 3D structure of ECG and **(e)** the 3D structure of EGCG. The 3D sdf files of EC (CID_72276), EGC (CID_72277), ECG (CID_107905) and EGCG (CID_65064) and the 2D sdf file of C (CID_107957) were obtained from the PubChem compound database (http://pubchem.ncbi.nlm.nih.gov/). The 3D structures of the catechins, with chirality, were built using the Dundee PRODRG2 Server. Energy minimization for each ligand was performed using the steepest descent for a minimum of 50 000 steps. An ffgmx GROMACS force-field was developed using the Dundee PRODRG2 Server [61].

The antioxidant activity of catechins from tea that contain a galloyl moiety could have an important role in the treatment of HIV-1. In a study by Liu *et al.* [7], GCG and EGCG were found to inhibit the HIV-1 laboratory subtype HIV-1IIIB. Furthermore, in 2009 Nance *et al.* [8] reported that EGCG significantly inhibited HIV-1 p24 antigen production across a broad spectrum of HIV-1 clinical isolates and laboratory-adapted subtypes. Importantly, however, catechins without a galloyl moiety did not provide effective inhibition of HIV-1.

The polyphenolic structure of tea catechins with a galloyl moiety also makes them good donors for hydrogen bonding [17]. This hydrogen bonding capacity enables tea catechins to bind strongly to proteins and nucleic acids. Thus, catechins with a galloyl moiety have already been reported to inhibit various stages in the HIV-1 lifecycle by targeting key HIV enzymes [7,18–24], in addition to effecting host cell factors [18,25,26]. In this paper, we review the multiple mechanisms underlying the inhibition of HIV-1 by catechins containing a galloyl moiety and find out which mechanism has the most significant effect. Thus, we show the potential for this class of safe [27–29] and healthy [13,14] natural compounds as candidate alternative therapies for the control of HIV-1 infection.

Multiple mechanisms for inhibition of HIV-1 by catechins containing a galloyl moiety

Inhibition of virus entry

Inhibiting sexual transmission of HIV-1

Genital exposure to semen (SE) contaminated with HIV-1 accounts for most HIV transmissions worldwide [30]. Thus, SE represents a major vector for the dissemination of HIV-1. Fragments of the abundant SE protein prostatic acidic phosphatase (PAP) form amyloid structures that capture HIV-1 virions and promote their attachment to target cells [31].

In the study by Hauber *et al.* [19], EGCG caused PAP248–286 amyloid fibril degradation at 2.5 mmol/l and EGCG almost completely abrogated the enhancement effect of the SE-derived

enhancer of virus infection (SEVI). In addition, EGCG is stable in acidic solution (pH < 4), which is similar to the conditions in the vaginal environment [32]. Thus, EGCG could be a valuable inhibitor of SEVI, and hence of the sexual transmission of HIV-1 [19], and it has potential as a next-generation microbicide. In addition, in 2006 the FDA approved the marketing of sinecatechins (Veregen®), a topical ointment with a botanical extract containing a mixture of EGCG and other active components for the treatment of external genital and perianal warts (http://www.registrarcorp.com/fda-api/drug-sample/Sinecatechins?lang=ch).

Inhibition of the HIV-1 fusion process

Fusion is a crucial step in the entry process of HIV into a new target cell [33]. It has been demonstrated that catechins with a galloyl moiety inhibit HIV-1-mediated cell–cell and virus–cell fusion [7].

There are two mechanisms whereby catechins with a galloyl moiety inhibit the HIV-1 fusion process: blocking the binding of glycoprotein (gp)120 to CD4 [20,21] and blocking the formation of fusion-active gp41 six-helix bundles [7].

Blocking the binding of gp120 to CD4

CD4 is a cell surface glycoprotein expressed on T cells that has an important role in the recognition of antigens by T cells [34]. HIV-1 envelope protein gp120 can bind to CD4 via its D1 domain [20,35].

Molecules that block the binding of gp120 to CD4 are an appealing method for reducing HIV-1 infectiousness [36]. In an attempt to determine whether EGCG could inhibit gp120 binding to CD4, Kawai *et al.* [21] analyzed the ability of fluorescein isothiocyanate (FITC)-conjugated recombinant gp120 to bind to CD4+ T cells treated with EGCG. EGCG inhibited the binding of gp120 to lymphocytes in a dose-dependent manner, but complete inhibition of gp120 binding was not observed, even at the highest concentration (100 μmol/l) of EGCG tested.

Following the study of Kawai *et al.*, Williamson and co-workers [20] reported that EGCG efficiently inhibited the binding of gp120 to CD4. In their study, EGCG, at a physiologically relevant concentration (0.2 μmol/l), inhibited the binding of gp120 to isolated human CD4+ T cells. High-affinity binding of EGCG to the CD4 molecule was observed with a K_d of approximately 10 nmol/l. Their study showed that the galloyl moiety of EGCG could interact with Trp62, Arg59 and Phe43 of CD4. The residues Arg59 and Phe43 of human CD4 have been reported to interact directly with viral gp120 [37]. The binding of EGCG to the D1 domain of CD4 is shown in Fig. 2.

Blocking the formation of fusion-active gp41 six-helix bundles

The transmembrane protein gp41 has an important role as part of the 'membrane fusion machinery' of HIV. After HIV-1 targets a viral accepter, gp41 undergoes a series of refolding events that ultimately lead to the formation of a six-helix bundle that has the fusion peptide and the transmembrane region present in the merged membrane in its post-fusion conformation [38,39].

Liu *et al.* [7] found that tea catechins with a galloyl moiety significantly inhibited the conformational changes of gp41 by blocking the formation of the fusion-active gp41 six-helix bundles. The half-maximal inhibitory concentration (IC_{50}) values for GCG, EGCG and epigallocatechin 3,5-digallate (EGCDG) for the inhibition of six-helix-bundle formation were 4.32 ± 0.94 μmol/l, 19.91 ± 1.27 μmol/l and 4.58 ± 0.11 μmol/l, respectively. In a docking study, Liu *et al.* [7] showed that catechins with a galloyl moiety interacted with the gp41 *N*-helix-coiled domain to block the formation of the fusion-active gp41 core, resulting in the inhibition of HIV-1 membrane fusion.

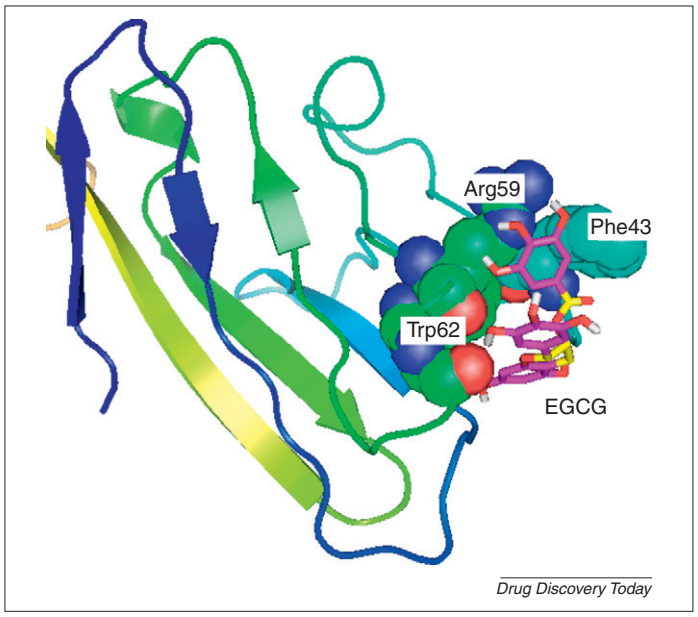

FIGURE 2

The binding of EGCG to the D1 domain of CD4 (PDB ID: 1CDJ) [38] as visualized using the PyMOL program (version 0.97).

Inhibition of virus replication

Inhibition of reverse transcriptase

Reverse transcriptase (RT) is a key enzyme that catalyzes the reverse transcription of viral RNA into double-stranded DNA [22,40]. For 20 years, catechins with a galloyl moiety have been known to be inhibitors of RT. (−)-Epicatechin-3-gallate was observed to have a non-specific inhibitory effect on HIV-1 RT in the early 1990s [41].

Yamaguchi *et al.* [18] investigated possible anti-HIV-1 activity of EGCG and its mechanisms of action on the virus lifecycle. It revealed that the anti-HIV activity of EGCG results from its interaction with several steps in the HIV-1 lifecycle. Among these several steps, RT inhibition is the most likely site of interaction based on the results of a chemical assay. The IC_{50} was at a physiologically relevant concentration (approximately 25 nmol/l), and the inhibitory kinetics for HIV RT were 5–50-fold higher than for other polymerases (e.g. DNA polymerases α, β, γ and *Escherichia coli* RNA polymerase). These authors identified RT inhibitory effects of EGCG during acute infection *in vitro* during post-adsorption entry and reverse transcription [18].

Tillekeratne *et al.* [22] systematically modified the molecular structures of EC and EGCG to determine the minimum structural characteristics necessary for inhibition of wild-type RT and the A17 mutant RT, which is normally insensitive to most known non-nucleoside inhibitors. In their study, the gallate ester moiety was found to be essential for RT inhibition. One or both of the hydroxyl groups on the aromatic ring of the chromanol moiety is necessary for the inhibitory activity. One or more of the hydroxyl groups on the gallate ring is also required. The 3,4,5-trihydroxy

substitution of this ring was found to be important for the inhibition process. They found that the naturally occurring molecules ECG and EGCG were more active than all of the simplified synthesized analogs (obtained by deleting one ring and changing the number and the nature of the hydroxyl substituents). The IC_{50}s of ECG and EGCG for the inhibition of wild-type RT are 0.76 ± 0.44 μmol/l and 0.73 ± 0.30 μmol/l, respectively. However, both of these natural compounds were inactive against the A17 mutant enzyme (K103N Y181C). Two synthetic compounds that retained the tether structure that joined the catechin and gallate segments were shown to be active against the A17 mutant enzyme (K103N Y181C). Their IC_{50}s against the A17 mutant enzyme were 3.83 ± 0.41 μmol/l and 10.74 ± 1.66 μmol/l, respectively.

Inhibition of integrase

HIV-1 integrase protein is responsible for the insertion of HIV cDNA into the genome of infected cells. There are two main steps in the integration reaction: 3′-processing and strand transfer [42,43].

Tillekeratne *et al.* [23], employing systematic structural simplification of EC and EGCG, demonstrated inhibition of DNA-strand-transfer. These compounds inhibited the polymerization and DNA-strand-transfer processes of wild-type and A17 mutant enzymes. The presence of polar hydroxyl groups on the aromatic ring of the chromanol moiety was found to enhance polymerase inhibition. The complete removal of these hydroxyl groups, or their conversion to less polar methyl ether groups, resulted in a ten- to 80-fold selectivity for DNA-strand-transfer inhibition over polymerase inhibition. Removal of one or more of the hydroxyl groups on the gallic acid moiety led to the loss of polymerase and DNA-strand-transfer inhibitory activities.

In a previous study, we found that four catechins with a galloyl moiety (CG, EGCG, GCG and ECG) inhibited HIV-1 integrase effectively, as determined by ELISA [24]. Their IC_{50}s against HIV-1 integrase were at physiologically relevant concentrations: 0.56 μmol/l, 0.96 μmol/l, 2.4 μmol/l and 3.02 μmol/l, respectively. Compared with catechins with a galloyl moiety, EC, a catechin without a galloyl moiety, demonstrated weak inhibition. The IC_{50} for EC was found to be >344.5 μmol/l. Our docking study [24] showed that when HIV-1 integrase is not combined with virus DNA the four catechins bind to Tyr143 and Gln148, thereby interfering with the flexibility of the Gly140–Gly149 loop, which could inhibit HIV-1 integrase activity. The EGCG docking results for the inhibition of the loop domain in the core domain of HIV-1 integrase are shown in Fig. 3. Moreover, after combining HIV-1 integrase with virus DNA, the four catechins bind between the integrase and virus DNA and disrupt this interaction. Thus, the four catechins can reduce the activity of HIV-1 integrase by disrupting its interaction with viral DNA. In this study, we also analyzed the difference between the inhibition mechanisms of catechins and raltegravir, which is the only integrase-targeted drug approved by the FDA [44]. The influence of the loop domain could be the most variable aspect underlying the inhibition mediated by catechins and raltegravir. In addition, a mixture of the different catechins with a galloyl moiety has shown a stronger inhibitory effect than every individual catechin. This might be because catechins containing a galloyl moiety have a highly cooperative inhibitory effect (IC_{50} = 0.1 μmol/l) [24].

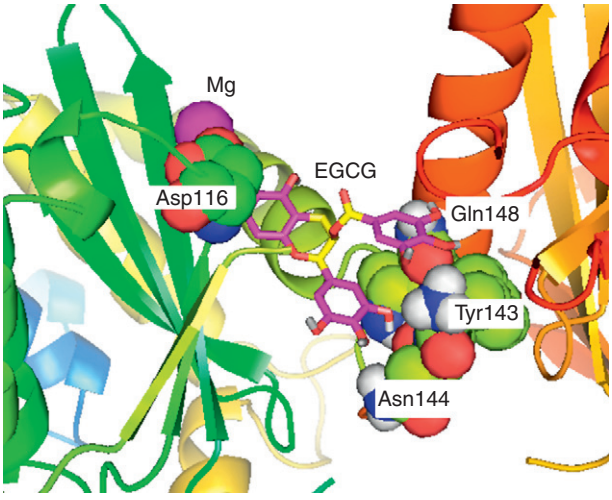

FIGURE 3

The binding of EGCG to the core domain of HIV-1 integrase (PDB ID: 1QS4) [62] as visualized using the PyMOL program (version 0.97).

Inhibition of HIV-1 protease

HIV-1 protease (PR) is encoded by the 5′ portion of the *pol* gene, and it processes the *gag* and *gag-pol* polyproteins to yield mature capsid protein as well as the protease, RT and integrase enzymes [45].

Catechin-mediated direct inhibition of HIV-1 PR has not been substantial. In the study by Yamaguchi *et al.* [18], the inhibition of PR by EGCG was 65% at 100 μmol/l and 30% at 50 μmol/l at 10 min after initial treatment. In the study by Ma *et al.*, the IC_{50} value of the inhibitory activity of epigallocatechin-(4β→8,2β→O-7)-epicatechin against HIV-1 PR was 70 μg/ml [46].

Modification of host cell factors

Effect on nitric oxide synthase and nuclear factor-κB pathways

The antioxidant capacity of catechins can cause them to bind target molecules and activate signaling or metabolic pathways [17]. EGCG can decrease nitric oxide synthase (NOS) activity and decrease the protein levels of inducible NOS (iNOS) by reducing the expression of iNOS mRNA [18]. Blond *et al.* [47] reported that significant induction of the iNOS gene was observed in cultured monocyte-derived macrophages (MDM) concomitantly with the peak of virus replication. The decrease in iNOS levels by catechins is achieved by blocking the nuclear factor (NF)-κB signal transduction pathway. Activation of NF-κB potently upregulates HIV replication [18]. EGCG can inhibit NF-κB activity by blocking the phosphorylation of inhibitor of κB-α (IκB-α). This inhibition decreases the expression of inflammatory gene products including lipoxygenase, cyclooxygenase, NOS and tumor necrosis factor (TNF)-α [25].

Effect on casein kinase 2

Casein kinase 2 (CK2) is a ubiquitous and highly conserved protein kinase with a broad spectrum of target proteins that, upon phosphorylation, function in signal transduction and gene expression to promote cell survival [48].

It has been reported that CK2 can act as a host mediator responsible for promoting viral growth in HIV-1-infected cells.

CK2 modifies the physiologic activity of HIV-1 gene products such as PR, RT, Rev (regulator of expression of virion protein), Vpu (an HIV-1-encoded integral membrane phosphoprotein that enhances viral particle release) and integrase by specifically phosphorylating them *in vitro* [26,49–51]. EGCG was found to inhibit the phosphorylation of PR at the physiologically relevant concentration of 0.1 μmol/l; thus, according to Haneda *et al.* [26], EGCG is a potential inhibitor of CK2-mediated PR phosphorylation.

Discussion

Most environmental stresses effect the production of reactive oxygen species in plants, thus causing oxidative stress. Plants produce secondary metabolites for defense against oxidative damage [52]. Tea catechins are well known plant antioxidants, and humans use these botanical antioxidants to improve health and treat certain diseases. Therefore, a co-evolutionary relationship could exist between humans and tea plants.

In this paper, we review evidence that tea catechins containing a galloyl moiety might have important roles in the treatment of HIV-1. Most of the information presented here is with regard to the antioxidant-independent role of catechins. They can target key proteins, thereby inhibiting the sexual transmission of HIV-1 [19], the HIV-1 fusion process [7,20,21], HIV-1 RT [18,22,41], HIV-1 integrase [23,24] and HIV-1 PR [18,26,46]. Furthermore, catechins containing a galloyl moiety can effect host cell factors, including NOS [18], NF-κB [18,25] and CK2 [26], resulting in inhibition of HIV-1 infection. In these multiple mechanisms the nanomole level of anti-HIV-1 infection includes inhibiting the HIV-1 fusion process [20], HIV-1 RT [22] and HIV-1 integrase [23]. The most significant inhibitory effect might appear to be blocking the binding of gp120 to isolated human CD4+ T cells. High-affinity binding of EGCG to the CD4 molecule was observed with a K_d of ~10 nmol/l [20]. The presence of multiple mechanisms might help catechins with the galloyl moiety continue to inhibit HIV-1 integrase even when viruses have been mutated.

Evidence suggests that catechins undergo significant metabolism in the liver, small intestine and colon [53,54]. The biotransformation of catechins leads to the formation of glucuronide and sulfate conjugates and methylated metabolites. Significant biotransformation of green tea catechins (GTCs) occurs in the colon, where the resident microflora degrades them to small phenolic acids, some of which can be absorbed [53]. One clinical study showed that repeated GTC administration, at a daily dose of 800 mg (EGCG) for four weeks, had no effects on cytochrome P450 (CYP)1A2, CYP2D6 or CYP2C9 phenotypic indices; however, it resulted in a 20% increase in the buspirone AUC, suggesting a small reduction in CYP3A4 activity. These clinical data suggest that catechin administration is unlikely to induce relevant metabolic drug interactions for drugs metabolized by these CYP isozymes [55].

Tea catechins are known to be naturally safe compounds. In a randomized, double-blind, placebo-controlled study, repeated dosing of 200 mg, 400 mg or 800 mg EGCG once daily for ten days was well tolerated in humans. No serious adverse event, or any other clinically relevant adverse event, was reported [27]. Another randomized, double-blind, placebo-controlled study showed that single oral doses of EGCG up to 1600 mg in humans were safe and well tolerated. The AUC from 0 h to infinity $AUC_{(0-\infty)}$ for total EGCG varied from 0.97 to 22.64 μmol/h/l. Accordingly, mean maximum plasma concentration (C_{max}) values for EGCG ranged from 0.28 to 7.41 μmol/l and were observed after 1.3–2.2 h. The mean terminal elimination half-life ($t_{1/2z}$) of EGCG was observed from 1.9 to 4.6 h [28]. In the study by Chow *et al.*, EGCG and Polyphenon® E at a daily dose of 800 mg for four weeks were found to be safe and well tolerated in healthy human subjects. Repeated green tea polyphenol administration as a high daily bolus dose (800 mg once daily) resulted in a >60% increase in the systemic availability of free EGCG [29].

HAART is the current treatment for HIV-1-infected patients. For initial use, HAART should include two nucleoside RT inhibitors (NRTIs) and one non-nucleoside RT inhibitor (NNRTI) or a protease inhibitor (PI) [56,57]. In rural areas of China, the mean expenditure per person during the first year of HAART was US$2633 (US$1 = 7 Chinese Yuan). This cost comprises 85.1% for HAART medications, 2.5% for treating adverse drug events and 1.8% for treating opportunistic infections [58]. Catechins with a galloyl moiety could be safe and well tolerated anti-HIV agents. Furthermore, they are inexpensive, naturally occurring compounds. EGCG (purity ≥ 98%) is less than US$5 per gram on the Chinese market. After EGCG is mixed into Polyphenon® E (EGCG purity ≥ 60%) the price is just US$0.06 per gram [24]. Catechins containing a galloyl moiety could decrease the prescription costs for HIV-1 patients; thus, they could become popular alternative drugs in developing countries.

Low oral absorption could be the biggest obstacle blocking the clinical use of catechins containing a galloyl moiety. Nearly 80% of EGCG degrades following 1 h of incubation in simulated intestinal fluid (pH 7.4) [59]. In addition, catechins are poorly absorbed across intestinal membranes. In one study, chitosan nanoparticles (CS NP) were found to enhance *in vitro* EGCG intestinal absorption significantly. Furthermore, this enhancement by CS NP occurs as a result of the stabilization of catechins in the 'door chamber' that subsequently drives their flux across the tissue [59]. Another study has shown that nanolipidic particles can safely increase the oral bioavailability of EGCG for human clinical trials [60]. Therefore, these methods could improve the bioavailability of catechins with a galloyl moiety, enabling their inhibition of HIV-1 to be tested in clinical trials.

Concluding remarks

In this paper, we present the multiple mechanisms and the most significant mechanism of catechins with a galloyl moiety in the inhibition of HIV-1. We have shown that these important, natural, safe and inexpensive antioxidant compounds could become alternative anti-HIV-1 drugs.

References

1 Taege, A. (2011) Seek and treat: HIV update 2011. *Cleve. Clin. J. Med.* 78, 95–100

2 Cos, P. *et al.* (2008) Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection—an update (1998–2007). *Planta Med.* 74, 1323–1337

3 Pomerantz, R.J. and Horn, D.L. (2003) Twenty years of therapy for HIV-1 infection. *Nat. Med.* 9, 867–873
4 Clercq, E.D. (2010) Antiretroviral drugs. *Curr. Opin. Pharmacol.* 10, 507–515
5 Mehta, N. and Reilly, M. (2005) Atherosclerotic cardiovascular disease risk in the HAART-treated HIV-1 population. *HIV Clin. Trials* 6, 5–24
6 Poon, A.F. *et al.* (2011) Transmitted drug resistance in the CFAR network of integrated clinical systems cohort: prevalence and effects on pre-therapy CD4 and viral load. *PLoS ONE* 6, e21189
7 Liu, S. *et al.* (2005) Theaflavin derivatives in black tea and catechin derivatives in green tea inhibit HIV-1 entry by targeting gp41. *Biochim. Biophys. Acta* 1723, 270–281
8 Nance, C.L. *et al.* (2009) Preclinical development of the green tea catechin, epigallocatechin gallate, as an HIV-1 therapy. *J. Allergy Clin. Immunol.* 123, 459–465
9 Song, J.M. and Seong, B.L. (2007) Tea catechins as a potential alternative anti-infectious agent. *Expert Rev. Anti Infect. Ther.* 5, 497–506
10 Cabrera, C. *et al.* (2006) Beneficial effects of green tea—a review. *J. Am. Coll. Nutr.* 25, 79–99
11 Wang, X. *et al.* (2003) The galloyl moiety of green tea catechins is the critical structural feature to inhibit fatty-acid synthase. *Biochem. Pharmacol.* 66, 2039–2047
12 Ikeda, I. (2008) Multifunctional effects of green tea catechins on prevention of the metabolic syndrome. *Asia Pac. J. Clin. Nutr.* 17 (Suppl. 1), 273–274
13 Karasu, C. (2010) Glycoxidative stress and cardiovascular complications in experimentally-induced diabetes: effects of antioxidant treatment. *Open Cardiovasc. Med. J.* 4, 240–256
14 Kryston, T.B. *et al.* (2011) Role of oxidative stress and DNA damage in human carcinogenesis. *Mutat. Res.* 711, 193–201
15 Coaccioli, S. *et al.* (2010) Oxidant/antioxidant status in patients with chronic HIV infection. *Clin. Ter.* 161, 55–58
16 Feng, W.Y. *et al.* (2008) Pycnogenol, a procyanidin-rich extract from French maritime pine, inhibits intracellular replication of HIV-1 as well as its binding to host cells. *Jpn. J. Infect. Dis.* 61, 279–285
17 Yang, C.S. *et al.* (2009) Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. *Nat. Rev. Cancer* 9, 429–439
18 Yamaguchi, K. *et al.* (2002) Inhibitory effects of (−)-epigallocatechin gallate on the life cycle of human immunodeficiency virus type 1 (HIV-1). *Antiviral Res.* 53, 19–34
19 Hauber, I. *et al.* (2009) The main green tea polyphenol epigallocatechin-3-gallate counteracts semen-mediated enhancement of HIV infection. *Proc. Natl Acad. Sci. U. S. A.* 106, 9033–9038
20 Williamson, M.P. *et al.* (2006) Epigallocatechin gallate, the main polyphenol in green tea, binds to the T-cell receptor, CD4: potential for HIV-1 therapy. *J. Allergy Clin. Immunol.* 118, 1369–1374
21 Kawai, K. *et al.* (2003) Epigallocatechin gallate, the main component of tea polyphenol, binds to CD4 and interferes with gp120 binding. *J. Allergy Clin. Immunol.* 112, 951–957
22 Tillekeratne, L.M. *et al.* (2001) Simplified catechin-gallate inhibitors of HIV-1 reverse transcriptase. *Bioorg. Med. Chem. Lett.* 11, 2763–2767
23 Tillekeratne, L.M. *et al.* (2002) Differential inhibition of polymerase and strand-transfer activities of HIV-1 reverse transcriptase. *Bioorg. Med. Chem. Lett.* 12, 525–528
24 Jiang, F. *et al.* (2010) The evaluation of catechins that contain a galloyl moiety as potential HIV-1 integrase inhibitors. *Clin. Immunol.* 137, 347–356
25 Melgarejo, E. *et al.* (2010) Targeting of histamine producing cells by EGCG: a green dart against inflammation? *J. Physiol. Biochem.* 66, 265–270
26 Haneda, E. *et al.* (2000) Biochemical characterization of casein kinase II as a protein kinase responsible for stimulation of HIV-1 protease in vitro. *Biochem. Biophys. Res. Commun.* 275, 434–439
27 Ullmann, U. *et al.* (2004) Plasma-kinetic characteristics of purified and isolated green tea catechin epigallocatechin gallate (EGCG) after 10 days repeated dosing in healthy volunteers. *Int. J. Vitam. Nutr. Res.* 74, 269–278
28 Ullmann, U. *et al.* (2003) A single ascending dose study of epigallocatechin gallate in healthy volunteers. *J. Int. Med. Res.* 31, 88–101
29 Chow, H.H. *et al.* (2003) Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clin. Cancer Res.* 9, 3312–3319
30 Olsen, J.S. *et al.* (2010) Amyloid-binding small molecules efficiently block SEVI (semen-derived enhancer of virus infection)- and semen-mediated enhancement of HIV-1 infection. *J. Biol. Chem.* 285, 35488–35496
31 Kim, K.A. *et al.* (2010) Semen-mediated enhancement of HIV infection is donor-dependent and correlates with the levels of SEVI. *Retrovirology* 7, 55
32 Zhu, Q.Y. *et al.* (1997) Stability of green tea catechins. *J. Agric. Food Chem.* 45, 4624–4628
33 Greenberg, M.L. and Cammack, N. (2004) Resistance to enfuvirtide, the first HIV fusion inhibitor. *J. Antimicrob. Chemother.* 54, 333–340
34 Janeway, C.A., Jr *et al.* (1988) CD4+ T cells: specificity and function. *Immunol. Rev.* 101, 39–80
35 Gray, L. *et al.* (2011) Conformational alterations in the CD4 binding cavity of HIV-1 gp120 influencing gp120-CD4 interactions and fusogenicity of HIV-1 envelopes derived from brain and other tissues. *Retrovirology* 8, 42
36 Schweizer, A. *et al.* (2008) CD4-specific designed ankyrin repeat proteins are novel potent HIV entry inhibitors with unique characteristics. *PLoS Pathog.* 4, e1000109
37 Wu, H. *et al.* (1996) Kinetic and structural analysis of mutant CD4 receptors that are defective in HIV gp120 binding. *Proc. Natl Acad. Sci. U. S. A.* 93, 15030–15035
38 Pejchal, R. and Wilson, I.A. (2010) Structure-based vaccine design in HIV: blind men and the elephant? *Curr. Pharm. Des.* 16, 3744–3753
39 Chan, D.C. *et al.* (1997) Core structure of gp41 from the HIV envelope glycoprotein. *Cell* 89, 263–273
40 Gotte, M. *et al.* (2010) Reverse transcriptase in motion: conformational dynamics of enzyme-substrate interactions. *Biochim. Biophys. Acta* 1804, 1202–1212
41 Moore, P.S. and Pizza, C. (1992) Observations on the inhibition of HIV-1 reverse transcriptase by catechins. *Biochem. J.* 288, 717–719
42 Pommier, Y. *et al.* (2005) Integrase inhibitors to treat HIV/AIDS. *Nat. Rev. Drug Discov.* 4, 236–248
43 Lataillade, M. and Kozal, M.J. (2006) The hunt for HIV-1 integrase inhibitors. *AIDS Patient Care STDS* 20, 489–501
44 Croxtall, J.D. *et al.* (2008) Raltegravir. *Drugs* 68, 131–138
45 Volonte, F. *et al.* (2011) Optimizing HIV-1 protease production in *Escherichia coli* as fusion protein. *Microb. Cell Fact.* 10, 53
46 Ma, C. *et al.* (2000) Inhibitory effects on HIV-1 protease of constituents from the wood of *Xanthoceras sorbifolia*. *J. Nat. Prod.* 63, 238–242
47 Blond, D. *et al.* (2000) Nitric oxide synthesis enhances human immunodeficiency virus replication in primary human macrophages. *J. Virol.* 74, 8904–8912
48 Zwicker, F. *et al.* (2011) A specific inhibitor of protein kinase CK2 delays gamma-H2Ax foci removal and reduces clonogenic survival of irradiated mammalian cells. *Radiat. Oncol.* 6, 15
49 Friborg, J. *et al.* (1995) Functional analysis of the phosphorylation sites on the human immunodeficiency virus type 1 Vpu protein. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 8, 10–22
50 Sargeant, D. *et al.* (2011) HIVToolbox, an integrated web application for investigating HIV. *PLoS ONE* 6, e20122
51 Meggio, F. *et al.* (2001) HIV-1 Rev transactivator: a beta-subunit directed substrate and effector of protein kinase CK2. *Mol. Cell. Biochem.* 227, 145–151
52 Ali, A. and Alqurainy, F., eds) (2006) *Activities of Antioxidants in Plants under Environmental Stress*, Transworld Research Network
53 Feng, W.Y. (2006) Metabolism of green tea catechins: an overview. *Curr. Drug Metab.* 7, 755–809
54 Schantz, M. *et al.* (2010) Metabolism of green tea catechins by the human small intestine. *Biotechnol. J.* 5, 1050–1059
55 Chow, H.H. *et al.* (2006) Effects of repeated green tea catechin administration on human cytochrome P450 activity. *Cancer Epidemiol. Biomarkers Prev.* 15, 2473–2476
56 Maggiolo, F. (2009) Efavirenz: a decade of clinical experience in the treatment of HIV. *J. Antimicrob. Chemother.* 64, 910–928
57 Dagnra, A.Y. *et al.* (2011) High prevalence of HIV-1 drug resistance among patients on first-line antiretroviral treatment in Lomé, Togo. *J. Int. AIDS Soc.* 14, 1–6
58 Zhou, F. *et al.* (2011) Expenditures for the care of HIV-infected patients in rural areas in China's antiretroviral therapy programs. *BMC Med.* 9, 6
59 Dube, A. *et al.* (2010) Chitosan nanoparticles enhance the intestinal absorption of the green tea catechins (+)-catechin and (−)-epigallocatechin gallate. *Eur. J. Pharm. Sci.* 41, 219–225
60 Smith, A. *et al.* (2010) Nanolipidic particles improve the bioavailability and alpha-secretase inducing ability of epigallocatechin-3-gallate (EGCG) for the treatment of Alzheimer's disease. *Int. J. Pharm.* 389, 207–212
61 Schuttelkopf, A.W. and van Aalten, D.M. (2004) PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallogr. D Biol. Crystallogr.* 60, 1355–1363
62 Goldgur, Y. *et al.* (1999) Structure of the HIV-1 integrase catalytic domain complexed with an inhibitor: a platform for antiviral drug design. *Proc. Natl Acad. Sci. U. S. A.* 96, 13040–13043