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REVIEW

Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea

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The consumption of green tea (C*amellia sinensis*) has been shown to have many physiological and pharmacological health benefits. In the past two decades several studies have reported that epigallocatechin-3-gallate (EGCG), the main constituent of green tea, has anti-infective properties. Antiviral activities of EGCG with different modes of action have been demonstrated on diverse families of viruses, such as *Retroviridae*, *Orthomyxoviridae* and *Flaviviridae* and include important human pathogens like human immunodeficiency virus, influenza A virus and the hepatitis C virus. Furthermore, the molecule interferes with the replication cycle of DNA viruses like hepatitis B virus, herpes simplex virus and adenovirus. Most of these studies demonstrated antiviral properties within physiological concentrations of EGCG *in vitro*. In contrast, the minimum inhibitory concentrations against bacteria were 10–100-fold higher. Nevertheless, the antibacterial effects of EGCG alone and in combination with different antibiotics have been intensively analysed against a number of bacteria including multidrug-resistant strains such as methicillin-resistant *Staphylococcus aureus* or *Stenotrophomonas maltophilia*. Furthermore, the catechin EGCG has antifungal activity against human-pathogenic yeasts like *Candida albicans*. Although the mechanistic effects of EGCG are not fully understood, there are results indicating that EGCG binds to lipid membranes and affects the folic acid metabolism of bacteria and fungi by inhibiting the cytoplasmic enzyme dihydrofolate reductase. This review summarizes the current knowledge and future perspectives on the antibacterial, antifungal and antiviral effects of the green tea constituent EGCG.

Abbreviations

AUC (0-∞), area under the concentration–time curve from 0 h to infinity; AZT, zidovudine; CLSI, Clinical and Laboratory Standards Institute; Cmax, maximum plasma concentration; DHFR, dihydrofolate reductase; EBV, Epstein–Barr virus; EC, epicatechin; ECG, epicatechin-gallate; EGC, epigallocatechin; EGCG, epigallocatechin-3-gallate; EHEC, enterohaemorrhagic *Escherichia coli*; EMEA, European Medicines Agency; FDA, Food and Drug Administration; FICI, fractional inhibitory concentration index; HBV, hepatitis B virus; HBVeAG, hepatitis B virus e antigen; HCV, hepatitis C virus; HCVcc, hepatitis C virus cell culture; HCVpp, hepatitis C virus pseudoparticles; HFMD, hand, foot and mouth disease; HPV, human papilloma virus; HSV, herpes simplex virus; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; NNRTI, non-nucleoside reverse transcriptase inhibitors; NPC1L1, Niemann-PickC1-like 1; PBMC, peripheral blood mononuclear cells; PBP2a, penicillin-binding protein 2a; PEGIFN-α/RV, pegylated interferon alpha with ribavirin; RT, reverse transcriptase; SINV, sindbis virus; SR-BI, scavenger receptor type B class 1; t½z, apparent terminal elimination half-life; Tmax, time to reach Cmax; YFV, yellow fever virus

Introduction

Tea is the most commonly consumed drink in the world after water. Depending on the manufacturing process, tea can be classified into three major classes: non-fermented green tea, semi-fermented oolong tea, and fermented black and red teas (Cabrera *et al.*, 2006). Non-fermented green tea from the plant *Camellia sinensis* is dried and steamed to prevent oxidation, which is not the case for black and red tea (Cabrera *et al.*, 2006). The natural compound epigallocatechin-3-gallate (EGCG) is an active polyphenolic catechin and accounts for approximately 59% of the total catechins from the leaves of the green tea. Other catechins in green tea include epigallocatechin (EGC) (19%), epicatechin-gallate (ECG) (13.6%) and epicatechin (EC) (6.4%) (McKay and Blumberg, 2002). The functional and structural differences between these catechins are attributed to the number of hydroxyl groups on the B-ring and the presence or absence of a galloyl moiety (Figure 1).

In traditional Chinese medicine, green tea is considered to have beneficial properties for human health including cardioprotective, anti-carcinogenetic and anti-infective effects. Although a detailed molecular understanding as to why green tea has these broad protective effects is lacking, the ability of EGCG to bind many biological molecules and influence the activity of a variety of enzymes and signal transduction pathways at the micromolar and nanomolar level may, at least in part, be responsible for these effects (Lee *et al.*, 2002). EGCG is water soluble and exposure to high temperatures such as boiling water does not greatly influence the stability of the molecule (Wang *et al.*, 2008). Notably, EGCG and various green tea preparations are available as an over the counter remedy in many countries and are inexpensive. The first documented report of an anti-infective effect of tea was made over 100 years ago by the British army surgeon Mc Naught, who showed that tea killed the organism that causes typhoid fever (*Salmonella typhi*) and brucellosis (*Brucella melitensis*) (MC Naught, 1906). However, this effect of tea was not studied further until the late 1980s when systematic research on the antimicrobial and antiviral effects of tea was conducted. Today, a literature search at pubmed.gov shows that over 4000 publications on the effects of EGCG and/or green tea have been reported. In this review, we firstly summarize the antiviral effect of EGCG on different viral families (Table 1) with a focus on hepatitis C virus (HCV) and HIV. We then discuss the antibacterial and antifungal activities of EGCG in *in vitro* and *in vivo* model systems. EGCG has in general a low bioavailability, therefore, the translation of its anti-infective effects into clinically relevant strategies and the procurement of physiologically concentrations of the molecule at the sites of viral, bacterial and fungal replication are also crucial aspects that need to be considered.

Effect of EGCG against hepatitis C virus

HCV, a positive strand RNA virus of the family *Flaviviridae*, chronically infects about 160 million individuals (Lavanchy, 2011). These patients are at risk of potentially life-threatening hepatic complications including cirrhosis, liver failure and hepatocellular carcinoma. In fact, chronic HCV infection is associated with about 30% of liver cancers worldwide and is among the leading indications for orthotopic liver transplantation (Brown, 2005). Standard therapy consists of a combination of pegylated interferon-α with ribavirin (PEGIFN-α/RV). However, PEGIFN-α/RV therapy has different success rates dependant on the viral genotype of the infection. The addition of one of two currently licensed viral protease inhibitors, the first generation of direct acting antivirals to

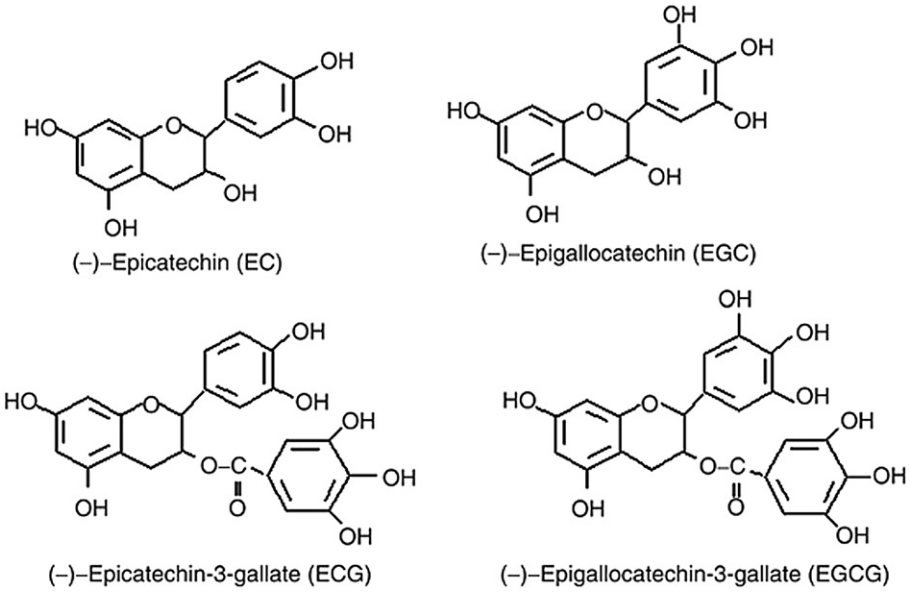

Figure 1
Chemical structure of the four major catechins in green tea.

Table 1

Inhibition effect of several representative viruses by EGCG

Virus	Family	Inhibitory effect	Reference
HCV	Flaviviridae	viral entry by interference with binding to target cells	Ciesek *et al.*, 2011, Calland *et al.*, 2012, Chen *et al.*, 2012
HIV-1	Retroviridae	Inhibition of integrase Inhibition of RT Destruction of virions by binding to envelope Binding to CD4 and interference with gp120 binding	Nakane and Ono, 1989, Fassina *et al.*, 2002, Yamaguchi *et al.*, 2002, Kawai *et al.*, 2003, Williamson *et al.*, 2006, Nance *et al.*, 2009, Jiang *et al.*, 2010; Li *et al.*, 2011;
HBV	Hepadnaviridae	Reduction of HBV antigen expression, extracellular HBV DNA and cccDNA	Xu *et al.*, 2008, He *et al.*, 2011
HSV-1/HSV-2	Herpesviridae	Damage and inactivation of virions probably by binding to envelope proteins	Isaacs *et al.*, 2008, Isaacs *et al.*, 2011
EBV	Herpesviridae	Inhibition of transcription of immediate-early genes Rta, Zta and EA-D	Chang *et al.*, 2003
Adenovirus	Adenoviridae	Inactivation of virus particles, inhibition of intracellular virus growth and viral protease	Weber *et al.*, 2003
Influenza virus	Orthomyxoviridae	Alteration of physical integrity of virus particles, inhibition of entry by binding to haemagglutinin	Nakayama *et al.*, 1993, Imanishi *et al.*, 2002, Song *et al.*, 2005
Enterovirus	Picornaviridae	Suppression of viral replication via modulation of cellular redox milieu	Ho *et al.*, 2009

current PEGIFN-α/RV combination therapy has substantially increased the treatment success rates for patients infected with the most prevalent genotype 1. However, this triple therapy cannot be used for all viral genotypes and it is associated with a number of side effects that can compromise patient compliance. Therefore, more effective therapies that are applicable for all viral genotypes and with fewer side effects are needed. For instance, in respect of liver transplantation for HCV-associated end-stage liver disease, the ability to block viral cell entry would help to minimize the currently universal re-infection of the donor liver by virions in the blood.

Recently, in the search for new antiviral molecules, three independent groups have identified EGCG as a potent inhibitor of the HCV entry pathway (Ciesek *et al.*, 2011; Calland *et al.*, 2012; Chen *et al.*, 2012). Ciesek and colleagues were initially working on the influence of semen on HCV infection and became interested in EGCG when it was reported that the green tea molecule counteracts semen-mediated enhancement of HIV infection (Hauber *et al.*, 2009). When they performed the first infection experiments with EGCG, a potent inhibition of HCV infection was noted, and the green tea molecule was identified as a novel HCV entry inhibitor (Ciesek *et al.*, 2011). Calland and co-workers became interested in testing EGCG because it was reported to increase lipid droplet formation and to impair lipoprotein secretion in hepatocytes, two cellular functions known to play a role in the life cycle of HCV (Li *et al.*, 2006). In these three studies it was clearly demonstrated that entry of cell culture-derived particles (HCVcc) as well as HCV pseudoparticles (HCVpp) are inhibited by EGCG independent of the HCV genotype (Ciesek *et al.*, 2011; Calland *et al.*, 2012; Chen *et al.*, 2012). This was also the case when primary human hepatocytes, which resemble more closely the natural reservoir for HCV, were used as target cells. Evaluation of each step in the viral life cycle identified EGCG as an entry inhibitor because RNA replication and release of infectious particles were not affected. It had previously been suggested that EGCG inhibits the essential NS3/4A serine protease of HCV (Zuo *et al.*, 2007); however, these assays were performed in a cell-free system and this observation could not be validated in an HCV replication setting (Ciesek *et al.*, 2011; Calland *et al.*, 2012). Chen *et al.* (2012) reported a slight inhibition (two to three-fold) of HCV RNA replication with JFH1 and Con1 constructs in tissue culture, but only at a very high concentration (80 μM) of EGCG. Other catechins, such as EGC, EC and ECG, did not have such a strong inhibitory effect as EGCG, which suggests that inhibition of HCV entry is unique to EGCG and not shared by other green tea catechins (Ciesek *et al.*, 2011).

On testing its effect on other viruses, it was demonstrated that EGCG inhibited Herpes simplex virus (HSV) infection, as described earlier (Isaacs *et al.*, 2008; 2011), but it had no effect on bovine viral diarrhoea virus or yellow fever virus (YFV), which, like HCV, also belong to the family of *Flaviviridae*, or the on the unrelated Sindbis virus (SINV) (Calland *et al.*, 2012). It has been reported that HCV can be transmitted in cell culture via cell-to-cell spread. This mode of transmission may be particularly relevant *in vivo* in the context of infected liver tissue. Infection via cell-to-cell spread was found to be refractory to neutralization by E2 monoclonal antibodies and it may occur in a CD81-independent manner (Timpe *et al.*, 2008; Witteveldt *et al.*, 2009). EGCG was able to prevent cell-to-cell transmission when infected cells were overlaid by

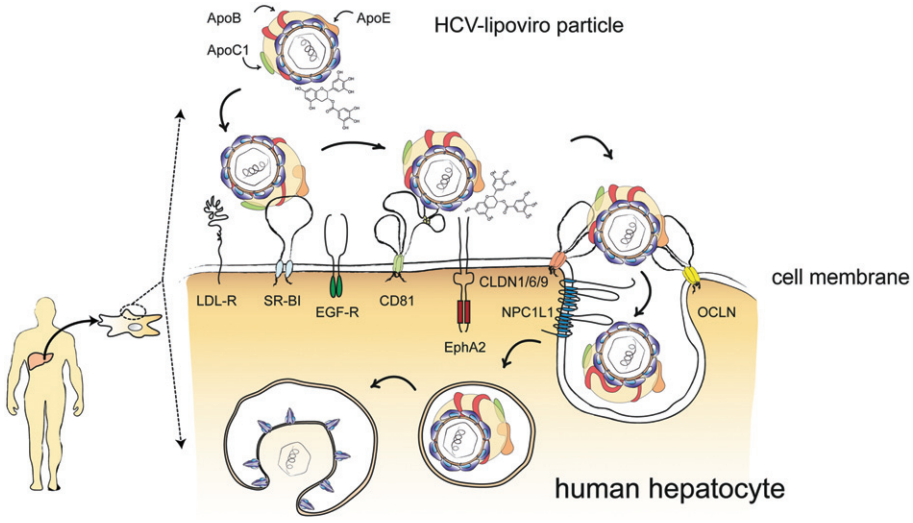

Figure 2

HCV entry into human hepatocytes and interference by EGCG. Cell entry involves an interaction between the extracellular virion that is associated with lipoproteins and several receptors on the host cell membrane. These include scavenger receptor type B class 1 (SR-BI), epidermal growth factor receptor (EGF-R), CD81, claudin 1 (CLDN1), ocludin (OCLN), Niemann-PickC1-like 1 (NPC1L1) and possibly low-density lipoprotein receptor (LDL-R). It has been suggested that the lipoprotein receptors SR-BI and LDL-R act before CD81 and the tight junction components CLDN1 and OCLN. These interactions induce travelling of the virus-receptor complex along the cell surface from the basolateral (blood-side) surface of the hepatic epithelium where LDL-R, SR-BI and CD81 are localized to the tight junction region, where CLDN1 and OCLN are encountered. These events stimulated by virion-mediated activation of receptor tyrosine kinase signalling like EGF-R result in clathrin-dependent endocytosis of the virion. Acidification of the endosome triggers a fusion peptide activity within the glycoproteins E1 or E2, the viral envelope fuses with the endosomal membrane and the nucleocapsid is released into the cytosol. EGCG is suggested to act on the virus particle and inhibits virus entry by impairing virus binding to the cell surface.

agarose or incubated with neutralizing antibodies to prevent the extracellular route of infection (Ciesek *et al.*, 2011; Calland *et al.*, 2012). HCV entry is a complex multistep process involving many host factors and is followed by endocytosis and fusion of the viral membrane with the host membrane (Figure 2). To resolve which step in the entry pathway is blocked by EGCG, its antiviral activity was assessed by administration of the molecule at different time points during the early phase of infection. The results from these experiments suggest that EGCG acts on the virus particles and inhibits virus entry by impairing virus binding to the cell surface (Ciesek *et al.*, 2011; Calland *et al.*, 2012) (Figure 2). In line with these results, pretreatment with EGCG had no effect on target cells, but it inhibited the primary attachment of ^{35}S-labelled HCV virions to cells (Ciesek *et al.*, 2011). Importantly, the green tea molecule was also able to clear HCV from cell cultures. At a concentration of 50 μm EGCG led to undetectable levels of infectious virus in the supernatant of human cells after three cell passages (Calland *et al.*, 2012) and clearance of the virus has even been observed after two passages at the same concentration (Chen *et al.*, 2012).

In summary, EGCG potently inhibits HCV entry of all genotypes to hepatoma cell lines and in primary human hepatocytes by preventing viral attachment to target cells. Therefore, EGCG could provide a new approach to prevent HCV infection, especially after liver transplantation of chronically infected patients. The combination of EGCG with other antiviral compounds targeting HCV replication in an interferon-free regimen is possible, as strong and additive inhibition of HCV infection was demonstrated when the molecule was combined with a NS3/4A protease inhibitor or cyclosporine A, which inhibits HCV replication by interfering with the HCV co-factor cyclophilin (Ciesek *et al.*, 2011). Future clinical trials will reveal how effective EGCG is at reducing viraemia in naïve patients with chronic hepatitis C and in preventing graft re-infection in patients undergoing liver transplantation.

Effect of EGCG on human immunodeficiency virus

HIV-1 is a lentivirus of the family of *Retroviridae* and the aetiological cause of AIDS. An estimated 33 million people are infected with HIV worldwide. HIV/AIDS persists as a major cause of morbidity in developed and non-developed countries. In the absence of a protective vaccine or a cure, prevention and access to antiretroviral treatments are the best options against HIV-1 (Simon *et al.*, 2006). Significant advances in antiretroviral therapy have been made since the introduction of zidovudine (AZT) in 1987. However, these drugs frequently cause severe side effects and the development of drug resistant HIV is rapidly emerging. Globally, with the lack of effective treatment regimens HIV/AIDS continues to be a major public health crisis. It is therefore important to develop more potent and conceptually novel drugs and therapies for the treatment of this infection.

In several different studies green tea EGCG has been reported to have antiviral effects against HIV-1 infection. Interestingly, various mechanisms for this inhibitory effect have been proposed (Nance and Shearer, 2003). Nakane and Ono (1989) initially demonstrated inhibition of HIV-1 replication by EGCG in human peripheral blood mononuclear cells (PBMCs) *in vitro* (Nakane and Ono, 1989). EGCG was shown to block the enzymic activity of the HIV-1 reverse transcriptase (RT) resulting in a decrease in p24 antigen concentration. Recently, it was confirmed that EGCG acts as an allosteric RT inhibitor, with time of addition assays revealing a similar inhibitory profile to non-nucleoside RT inhibitors (NNRTIs) (Li *et al.*, 2011). However, the mechanism of inhibition seems to be different from those of currently approved NNRTIs, as HIV-2 with another binding pocket was inhibited and NNRTI-resistant viruses were also still susceptible to EGCG. Synergistic inhibition was also observed with AZT (Li *et al.*, 2011). Additionally, and similar to HCV, in a number of different studies EGCG was also found to interfere with the viral envelope of HIV-1. The reduced HIV-1 infectivity in the presence of EGCG was also shown to be due to increased lysis of viral particles (Fassina *et al.*, 2002). In another study, the possible antiviral effects of EGCG for every step of the HIV-1 life cycle were investigated (Yamaguchi *et al.*, 2002). Again, EGCG destroyed virions in a dose- and time-dependent manner and inhibited RT activity. Mechanistically, viral lysis was facilitated via EGCG binding to the surface of the viral envelope and deforming membrane phospholipids in a manner similar to the effect of polymixin B on bacterial membranes (Ikigai *et al.*, 1993; Yamaguchi *et al.*, 2002).

HIV-1 entry is initiated by the attachment of the gp120 envelope protein to the CD4 receptor and subsequent interaction with the co-receptors CCR5 or CXCR4. Fusion of host and virus membrane occurs with the help of the fusion peptide located in the gp41 of HIV-1. After membrane fusion, the capsid is released into the cytoplasm. Kawai *et al*. investigated the effect of EGCG on the expression of CD4 molecules and noted that EGCG, but not ECG, prevented the attachment of HIV-1 virions by blocking the interaction of gp120 and CD4 on T helper cells (Kawai *et al.*, 2003). EGCG in a concentration ranging from 25–250 μmol L^{-1} down-regulated the cell surface receptor expression by binding to CD4, presumably at a binding site recognized by gp120 (Kawai *et al.*, 2003; Nance *et al.*, 2009). Supporting this observation, EGCG was shown to compete with anti-CD4 monoclonal antibodies. Cell-surface CD4 expression is regulated via multiple mechanisms, including CD4 endocytosis, intracellular retention of the molecular complex and shedding from the cell surface (Geleziunas *et al.*, 1994). HIV-1 infection *per se* induces CD4 down-regulation by proteasomal degradation (Aiken *et al.*, 1994). Details of the molecular mechanism by which EGCG modulates CD4 down-regulation at the cell surface are not fully understood, although CD4 shedding from the cell surface, and CD4 endocytosis are unlikely to be involved (Kawai *et al.*, 2003). However, the crucial mechanism by which EGCG inhibits HIV-1 entry seems to be its interference with gp120, a ligand for CD4, and thereby prevent the initial attachment of viruses to CD4 T cells. The characteristics of the binding of EGCG to CD4 were further investigated by NMR spectroscopy and molecular modelling (Williamson *et al.*, 2006). The addition of CD4 to EGCG produced a linear decrease in the NMR signal intensity from EGCG, but not from the control molecule catechin, providing clear evidence for high-affinity binding of EGCG to the CD4 molecule with a K_d of approximately 10 nmol L^{-1} (Williamson *et al.*, 2006). A physiologically relevant concentration of EGCG (0.2 μmol L^{-1}) inhibited the binding of gp120 to isolated human CD4 T cells and molecular modelling studies suggested a binding site for EGCG in the D1 domain of CD4, the pocket that binds gp120 (Williamson *et al.*, 2006). The HIV-1 integrase protein is responsible for the insertion of HIV proviral DNA into the genome of infected cells. Recently, the ability of EGCG to inhibit the HIV-1 integrase was also evaluated in an ELISA (Jiang *et al.*, 2010). It was shown that catechins with a galloyl moiety were able to reduce HIV-1 integration by binding between the integrase and the viral DNA, so disrupting this interaction. However, further studies with infectious viruses are needed to validate these *in vitro* data.

In conclusion, EGCG appears to interfere with several aspects of the HIV-1 life cycle, including virion destruction via interaction with the viral envelope, prevention of viral replication via inhibition of reverse transcription, inhibition of proviral genome integration and CD4 receptor down-regulation. Most conclusively, competition with gp120 for CD4 binding was validated in several independent studies. Importantly, physiological EGCG concentrations were able to reduce the attachment of gp120 to CD4 by a factor of 20-fold. Further *in vivo* studies are needed to determine whether EGCG has potential as a future antiretroviral therapy.

Effect of EGCG on other viruses

With respect to RNA viruses, EGCG was tested against two other viruses, enterovirus 71 belonging to the family of *Picornaviridae* and influenza viruses, which are members of the family of *Orthomyxoviridae*.

Influenza A and B viruses are a major cause of respiratory disease in humans. In addition, influenza A viruses continuously re-emerge from animal reservoirs into humans causing human pandemics of unpredictable severity every 10–50 years (Garcia-Sastre, 2011). Influenza A viruses are negative sense, single-stranded, segmented RNA viruses with an envelope. There are several subtypes known, labelled according to an H number (for the type of haemagglutinin) and an N number (for the type of neuraminidase). The annual flu (also called 'seasonal flu' or 'human flu') results in approximately 36 000 deaths and more than 200 000 hospitalizations each year in the USA alone. Vaccines are the most widely used intervention prophylaxis for influenza infection, but their effectiveness depends on the type of influenza virus and they also have the drawback of limited supply (Collin and de Radigues, 2009). Two main classes of antiviral drugs used against influenza viruses are neuraminidase inhibitors or inhibitors of the viral M2 protein, such as amantadine and rimantadine. These drugs can reduce the severity of symptoms and mortality and can also be taken to decrease the risk of infection. However, viral strains have emerged that show drug resistance to both classes of drug. The antiviral activity of EGCG against influenza virus was reported for the first time in 1993. The green tea molecule affected the infectivity

of influenza virus in cell culture and it was shown to agglutinate the viruses, preventing the virus from absorbing to MDCK cells (Nakayama *et al.*, 1993). Furthermore, green tea extracts including EGCG exerted an inhibitory effect on the acidification of intracellular compartments such as endosomes and lysosomes, resulting in inhibition of influenza virus growth in tissue culture (Imanishi *et al.*, 2002). These studies were extended by Song *et al.*; they determined the structure–activity relationship of the different green tea polyphenolic compounds EGCG, ECG and EGC against influenza and found that ECG and EGCG were more effective than EGC and these molecules also exerted an inhibitory effect on the neuraminidase in a biochemical assay (Song *et al.*, 2005). Influenza viral RNA synthesis analysed by RT-PCR was affected only at very high concentrations (Song *et al.*, 2005). Interestingly, based on these *in vitro* data clinical studies were performed to investigate if green tea catechins can prevent influenza infections in humans. In a small prospective cohort study it was reported that gargling with tea catechins extracts was effective in preventing influenza infection in elderly residents of a nursing home (Yamada *et al.*, 2006). In addition, in another randomized, double-blind, placebo-controlled trial, the consumption of catechins for 5 months had a statistically significant preventive effect on clinically defined influenza infection and was well tolerated (Matsumoto *et al.*, 2012). The results of these trials indicate these catechins have a protective effect against influenza virus; however, large-scale studies are needed to confirm this.

Enterovirus 71 is a single-stranded RNA virus and one of the causative agents of hand, foot and mouth disease (HFMD). This virus causes various clinical manifestations, including cutaneous, visceral and neurological diseases. Large outbreaks have been reported in Taiwan and Malaysia in the 1990s. Recently, enterovirus 71 repeatedly caused life-threatening outbreaks of HFMD with neurological complications in Asian children. The neurological manifestations progress very quickly and range from aseptic meningitis to acute flaccid paralysis and brainstem encephalitis. EGCG was demonstrated to inhibit enterovirus 71 replication and formation of infectious progeny virus (Ho *et al.*, 2009). There was a positive correlation between the antioxidant effects of catechins (Yang *et al.*, 1994) and their antiviral activity (Ho *et al.*, 2009). These findings suggest that EGCG may suppress viral replication via modulation of the cellular redox milieu.

The aetiological agent of acute and chronic hepatitis B is human hepatitis B virus (HBV), a small enveloped virus from the family of *Hepadnaviridae*. Around 40% of the global human population has contact with this virus, which is transmitted parentally, sexually and perinatally (Shepard *et al.*, 2006). Infection results in acute hepatitis and – in some cases – acute liver failure. Chronic hepatitis B, which affects over 300 million, persists even after clinical resolution of acute infection and can be reactivated causing severe disease under conditions of immunosuppression. In contrast to HCV, a preventive vaccine for HBV and specific antiviral drugs are available. However, viral resistance increasingly poses a challenge (Tillmann, 2007). To elucidate the effect of green tea catechins on HBV, the influence of green tea extracts and EGCG was studied in a stable cell line, HepG2-N10, expressing HBV antigens. It was found that the expression of HBV-specific antigens, the levels of extracellular HBV DNA, intracellular replicative intermediates and cccDNA were reduced in a dose-dependent manner (Xu *et al.*, 2008). However, it is difficult to dissect the detailed anti-HBV mechanisms of EGCG using HepG2-N10 cells as the process from cccDNA to antigen expression are strongly affected by transcription of integrated HBV DNA (Zhou *et al.*, 2006). Therefore, more recently, He *et al.* (2011) used an inducible HBV replicating cell line to test EGCG, termed HepG2.117, where HBV precore mRNA can only be transcribed from replicating HBV DNA, but not the integrated HBV DNA. They observed that HBV replicative intermediates of RNA synthesis were significantly inhibited by EGCG, which resulted in a reduction in cccDNA production (He *et al.*, 2011). In contrast, the production of HBV pregenomic RNA, precore mRNA and the translation of hepatitis B e antigen (HBVeAg) were not affected. To elucidate whether the antiviral effect of EGCG is the result of targeting of cellular factors or viral factors, additional studies are required, ideally in cell culture models that replicate the complete HBV life cycle.

In the case of other DNA viruses, so far the effects of EGCG against adenovirus, Epstein–Barr virus (EBV) and HSV-1 have been studied, the two latter viruses belong to the family of *Herpesviridae*. Adenoviruses are non-enveloped viruses composed of a nucleocapsid and a double-stranded linear DNA genome. There are 57 serotypes described in humans, which are responsible for 5–10% of upper respiratory infections in children. Humans infected with adenoviruses display a wide range of responses, from no symptoms at all to the severe infections typical of adenovirus serotype 14. Green tea was found to reduce the virus yield of an adenovirus infection by two orders of magnitude in Hep2 cells (Weber *et al.*, 2003). The molecule was most effective when added to the cells during the transition from early to late phase of viral infection suggesting EGCG inhibits one or more late steps in the viral infection (Weber *et al.*, 2003). Furthermore, inactivation of purified adenoviruses and inhibition of viral protease activity was noted. However, its therapeutic value seems to be limited, as the effective concentrations were much higher than reported serum concentrations of green tea drinkers. This was also the case when EGCG was tested against EBV. EBV is a human herpesvirus causing infectious mononucleosis and is closely associated with Burkitt's lymphoma, nasopharyngeal carcinoma, T-cell lymphoma and Hodgkin's disease (Bravender, 2010). *In vitro*, only an EGCG concentration exceeding 50 μM decreased the expression of EBV lytic proteins, including Rta, Zta and EA-D, but not the expression of EBV nuclear antigen-1 (Chang *et al.*, 2003). Moreover, DNA microarray and transient transfection analysis demonstrated that this concentration of EGCG blocked the EBV lytic cycle by inhibiting the transcription of immediate-early genes (Chang *et al.*, 2003).

Herpes simplex is a viral disease caused by HSV-1 and type 2 (HSV-2). Worldwide rates of HSV infection are between 65 and 90% (Chayavichitsilp *et al.*, 2009). There is no vaccine available or a method to eradicate herpes viruses from the body, but antiviral medications like acyclovir can reduce the frequency, duration and severity of outbreaks. Characterization of the antiviral activity of EGCG against HSV-1 and HSV-2 revealed that EGCG has greater anti-HSV activity than other green tea catechins and inactivated multiple clinical isolates of HSV-1 and HSV-2. Importantly, EGCG reduced

HSV-2 titres by more than 1000-fold in 10–20 min and reduced HSV-1 titres to the same extent in 30–40 min (Isaacs *et al.*, 2008). Similar to HCV, HIV-1 and influenza virus, the anti-HSV activity was due to a direct effect on the virion and incubation of target cells prior to infection had no effect (Isaacs *et al.*, 2008). Using electron microscopy, the authors showed that purified viruses exposed to EGCG were damaged. As EGCG is stable in the pH range found in the vagina, it was proposed that the green tea molecule could be a promising candidate for use in a microbiocide to reduce HSV transmission (Isaacs *et al.*, 2008). Furthermore, EGCG dimers inactivated HSV-1 and HSV-2 more effectively between pHs 4.0 and 6.6 than the EGCG monomer, which has therefore even more potential for reducing spread of HSV *in vivo* (Isaacs *et al.*, 2011).

EGCG against *Staphylococci*

Staphylococcus aureus is among the most common pathogens to cause community- and hospital-acquired infections. In Europe, *S. aureus* is the second most common causative microorganism for bacteraemia and is one of the leading causes of sepsis worldwide (Biedenbach *et al.*, 2004). Methicillin-resistant *S. aureus* (MRSA) is a type of *Staphylococci* that is resistant to certain antibiotics called β-lactams. Infections with MRSA are more difficult to treat and are therefore associated with a higher mortality rate than those caused by methicillin-susceptible *S. aureus* (Cosgrove *et al.*, 2003). The methicillin resistance in *S. aureus* is primarily mediated by the *mecA* gene, which codes for the modified penicillin-binding protein 2a (PBP2a). PBP2a is located in the bacterial cell wall and has low binding affinity for β-lactams.

The activity of EGCG as single agent and in combination with β-lactams has been assessed in multiple studies. Initially, *in vitro* data from a study performed over two decades ago indicated that tea extracts, at concentrations found in ordinarily brewed tea, inhibited the growth of MRSA (Toda *et al.*, 1989). Subsequently the biological activity of green tea components including EGCG against *S. aureus* was investigated (Ikigai *et al.*, 1993). It was reported that the minimum inhibitory concentration (MIC) values of EGCG were below 100 μg mL^{-1}. Initial experiments suggested that negatively charged EGCG exerts its anti-bactericidal activity by binding to the positively charged lipids of the bacterial cell membrane, causing damage to the lipid layer. Subsequently, the interaction of catechins including EGCG with lipid bilayers has been studied in more detail (Kumazawa *et al.*, 2004; Uekusa *et al.*, 2007; Kajiya *et al.*, 2008; Kamihira *et al.*, 2008; Sirk *et al.*, 2008; Cui *et al.*, 2012).

The mechanism of action of EGCG against *Staphylococci* was further investigated by Yam and co-workers who demonstrated that tea extracts can reverse the phenotypic methicillin resistance in MRSA (Yam *et al.*, 1998). Tea extracts at 25 μg mL^{-1} were able to inhibit the production of PBP2 by >90% in a constitutively PBP2 producing *S. aureus* strain. In addition, the production of β-lactamases was inhibited. In contrast, to the study from Yam *et al.*, suppression of PBP2 could not be detected by Zhao *et al.* either by PBP2 mRNA expression using quantitative PCR or by PBP2 production using latex agglutination (Zhao *et al.*, 2002).

The combination of tea extracts with β-lactams (methicillin, benzylpenicillin) was mostly demonstrated to have a synergistic antibacterial effect. These results were mainly confirmed by Zhao and colleagues who showed that 25 μg mL^{-1} EGCG was able to reverse the high-level resistance of MRSA to all types of β-lactams, including benzylpenicillin, oxacillin, methicillin, ampicillin and cephalexin (Zhao *et al.*, 2001b); fractional inhibitory concentration indices (FICI) of the β-lactams tested alone against 25 MRSA isolates were low (0.126–0.625), indicating that EGCG has a synergistic effect. In additional studies, the combination of EGCG with ampicillin/sulbactam or carbapenems was also shown to exert a synergistic antibacterial effect and MICs were reduced to the susceptibility breakpoint (Hu *et al.*, 2001; 2002; Stapleton *et al.*, 2004). Furthermore, 12.5 μg mL^{-1} EGCG in combination with penicillin revealed a synergistic effect in 100% of the 21 MRSA strains tested (Zhao *et al.*, 2002). As previously reported, the production of penicillinase from penicillin-resistant *S. aureus* was also inhibited by EGCG in a dose-dependent manner.

In addition to EGCG, ECG was also able to reverse β-lactam resistance in clinical MRSA isolates (Stapleton *et al.*, 2004); the gallate moiety of EGC was shown to be essential for oxacillin-modulating activity, as both (-)-epicatechin and (-)-epicatechin-3-cyclohexylcarboxylate were unable to reverse resistance.

Results from Shimamura and co-workers indicated that EGCG binds directly or indirectly to the peptidoglycan of the bacterial cell wall and inhibits the penicillinase activity, protecting penicillin from inactivation (Zhao *et al.*, 2002). Further to its effects when combined with β-lactams, the interactions of EGCG with non-β-lactam antibiotics have been evaluated against MRSA (Hu *et al.*, 2002). The combination of EGCG with antibiotic inhibitors of protein or nucleic acid synthesis was found to be additive or no difference (FICI, 0.5–4.0). In contrast, EGCG tended to have an antagonistic effect on the actions of glycopeptide antibiotics (vancomycin, teicoplanin). These *in vitro* data indicate that the choice of antibiotic in any potential combination therapy consisting of EGCG plus antibiotic against *Staphylococci* is critical to achieve a bactericidal effect.

Two studies from Italy have provided further insights into the effects of EGCG on *Staphylococci* (Sudano Roccaro *et al.*, 2004; Blanco *et al.*, 2005). Blanco *et al.* showed that 50 μg mL^{-1} EGCG was able to reverse tetracycline resistance and appeared to improve the MICs of tetracycline in susceptible staphylococcal isolates. In strains in which tetracycline resistance was due to increased expression of a tetracycline efflux pump protein (Tet(K)), EGCG inhibited the pump activity, which resulted in an increased intracellular retention of tetracycline.

Sudano Roccaro *et al.* (2004) demonstrated that EGCG was able to decrease slime production and inhibit biofilm formation by ocular *S. aureus* and *S. epidermidis* isolates (Sudano Roccaro *et al.*, 2004). These results indicate that in addition to binding to lipid layers and peptidoglycan, EGCG interferes with extracellular polymeric material (glycocalyx).

In experiments using Bagg albino (BALB/c) mice and human PBMCs another interesting antibacterial effect of EGCG was demonstrated, polyphenon, consisting of 50% EGCG, neutralized staphylococcal enterotoxin B in a dose-

and incubation time-dependent manner by binding to enterotoxin B molecules (Hisano *et al*., 2003). Further work is needed to determine the effects of EGCG against different enterotoxins, and whether EGCG has neutralization properties against other staphylococcal superantigens such as toxic shock syndrome toxin.

Taken together, these results indicate that there are multiple mechanisms by which ECGC exerts antibacterial effects against *Staphylococci*, including bactericidal activity, synergism in combination with other antibiotics, anti-biofilm activity and inhibition of β-lactamase production or neutralization of released toxins. However, not all effects of EGCG against *Staphylococci* are beneficial. A recent study demonstrated that short exposure of *Staphylococcus* strains to sublethal doses of EGCG can lead to cross-resistance against antibiotics targeting the bacterial cell wall (vancomycin, oxacillin, ampicillin) (Bikels-Goshen *et al*., 2010). All EGCG-adapted strains were also more heat tolerant. The reason for this phenomenon was studied by transmission electron microscopy analysis, which revealed that bacterial cells in cultures exposed to EGCG showed pseudo-multicellular appearance and had a more than a twofold increase in the cell wall thickness. In summary, the results of this study indicate that EGCG may also contribute to the development and enhancement of bacterial resistance mechanisms. Animal studies are needed to explore whether these observations are reproducible *in vivo*.

EGCG against streptococci and other gram-positive bacteria

Certain *Streptococcus* species are responsible for many cases of meningitis, pneumonia, endocarditis, erysipelas and necrotizing fasciitis. However, many streptococcal species are non-pathogenic, and part of the commensal human microbiome of the mouth, skin, intestine and upper respiratory tract.

Despite the complexity of oral flora, oral streptococci, including *S. mutans*, have generally been considered the primary aetiological agents of dental caries (Beighton, 2005). In several studies, it was shown that tea catechins possess antimicrobial effects against oral streptococci. The prevention and a reduction in the formation of dental caries was demonstrated in animal models as well as clinical trials. As the focus of this review is summarizing the spectrum of EGCG activity, we would like to refer the reader to Taylor *et al*. (2005) who reviewed the anticariogenic activity of EGCG and its effects on periodontal disease.

Streptococcus pyogenes has several virulent factors, including cell surface components (lipoteichoic acid, hyaluronic acid capsule, M proteins, laminin and collagen binding proteins), which are responsible for bacterial adhesion to human cells. EGCG was able to inhibit the attachment of bacteria to pre- and post-treated cells and induce *S. pyogenes* cell death (Hull Vance *et al*., 2011). It was concluded that EGCG could be used effectively as an adjunct to conventional antibiotic treatment. However, future studies are needed to elucidate the activity of EGCG against *S. pyogenes* in animal models. At present, no data exist concerning the antibacterial activity of EGCG against *Streptococcus pneumoniae*.

EGCG against gram-negative bacteria

It was supposed that gram-positive bacteria are more susceptible to EGCG than gram-negative bacteria (Yoda *et al*., 2004) because one mode of action of EGCG is to bind to peptidoglucan. The peptidoglucan of gram-negative bacteria is shielded by an outer membrane, that is mainly composed of negatively charged lipopolysaccharides. For that reason it was hypothesized that the physiological function of the outer membrane and low affinity of the also negatively charged EGCG for the bacterial cell membrane would reduce the antibacterial activity of EGCG against gram-negative bacteria (Yoda *et al*., 2004).

The gram-negative non-fermentative bacillus *Stenotrophomonas maltophilia* is intrinsically resistant to β-lactams and other broad-spectrum antibiotics and has emerged globally as an important nosocomial pathogen (Brooke, 2012). Two studies have shown that EGCG exerts antibacterial effects against *S. maltophilia (*Navarro-Martinez *et al*., 2005; Gordon and Wareham, 2010*)*. Furthermore, it was demonstrated that EGCG is an effective inhibitor of *S. maltophilia* dihydrofolate reductase (DHFR), and acts synergistically with sulfamethoxazole, a drug that blocks folic acid metabolism (Navarro-Martinez *et al*., 2005). This type of inhibition is similar to that of trimethoprim. Therefore, EGCG could represent an effective alternative to trimethoprim to combine with sulfamethoxazole for treating *S. maltophilia* infections especially in strains resistant to trimethoprim. The range of the MIC of EGCG for *S. maltophilia* is similar to that for *Acinetobacter baumannii*, another multi-drug resistant pathogen that causes nosocomial infections (Osterburg *et al*., 2009).

It has been reported that the tea catechins have antibacterial activity against various foodborne pathogenic gram-negative bacteria, including *Helicobacter pylori*, enterohaemorrhagic *Escherichia coli* (EHEC), *Vibrio cholera*, *Bacillus* spp., *Clostridium* spp. *Shigella* spp. and *Salmonella* spp. (Ryu, 1980; Shetty *et al*., 1994; Mabe *et al*., 1999; Sugita-Konishi *et al*., 1999; Sakanaka *et al*., 2000; Yanagawa *et al*., 2003; Taguri *et al*., 2004; Friedman *et al*., 2006; Lee *et al*., 2009; Stoicov *et al*., 2009). An overview of the existing studies analysing the antimicrobial effects of EGCG against bacteria causing foodborne disease is shown in Table 2.

H. pylori has been identified as an aetiological agent involved in the development of gastric ulcers, peptic ulcers, gastritis and many other stomach-related diseases. Different *in vitro* and *in vivo* studies explored the activity of tea catechins against *H. pylori*. EGCG was the most potent catechin as the MIC values for 50% of the tested *H. pylori* strains were 8 μg mL^{-1} (Mabe *et al*., 1999). Additive effects were shown when it was administered in combination with amoxicillin, metronidazole and clarithromycin, antibiotics usually used as a first line of treatment for *H. pylori* infections (Mabe *et al*., 1999; Yanagawa *et al*., 2003). However, the bactericidal EGCG activity is limited at pH ≤5.0. Also, in infected Mongolian gerbils, *H. pylori* was only eradicated in 10–36% of the catechin-treated animals (Mabe *et al*., 1999). It is possible that the pH dependency of the antibacterial activity of EGCG or the short gastric transit time of the agent was causative for the low eradication rate observed in this study. Thus, further studies are needed to assess the efficacy of EGCG in combination with a proton pump inhibitor and a drug delivery

Table 2

Overview of existing studies analysing the antimicrobial effects of EGCG against bacteria causing food-borne disease

Bacterium	Type of study	Antibacterial effects of EGCG	Reference
Enterohemorrhagic *Escherichia coli*	*in vitro*	Bacteridical, inhibition of Shiga toxin	Okubo *et al.*, 1998
Helicobacter pylori	*in vitro* and *in vivo*	Bacteridical at pH 7, but not at pH ≤ 5; Eradication in 10–36% in infected Mongolian gerbils	Mabe *et al.*, 1999
Enterohaemorrhagic *Escherichia coli*	*in vitro*	0.05 mg mL^{-1} EGCG inhibits extracellular Shiga toxin release	Sugita-Konishi *et al.*, 1999
Bacillus stearothermophilus *Clostridium thermoaceticum*	*in vitro*	EGCG is antibacteridicial and reduced heat resistance of spores	Sakanaka *et al.*, 2000
Helicobacter pylori	*in vitro*	MIC_{90} of EGCG was 100 μg mL^{-1}; additive effects with amoxicillin, metronidazole and clarithromycin	Yanagawa *et al.*, 2003
Salmonella typhi	*in vitro*	MIC > 100 μg mL^{-1}	Yoda *et al.*, 2004
Bacillus cereus	*in vitro*	EGCG is antibacteridical at nanomolar levels	Friedman *et al.*, 2006
Enterohaemorrhagic *Escherichia coli*	*in vitro* and *in vivo*	25 μg mL^{-1} EGCG decreased biofilm formation, swarm motility and autoinducer 2 concentration; higher survival rate (30%) of nematodes fed with EGCG than without	Lee *et al.*, 2009
Enterohaemorrhagic *Escherichia coli*	Atomic forced microscopy	Sub-MIC EGCG treatment of *E. coli* led to tempory changes of the cell walls (pore-like lesions, collapse), damages were caused by H_2O_2 gernerated from EGCG	Cui *et al.*, 2012

system with prolonged gastric transit time (Mabe *et al.*, 1999). Green tea also has prophylactic properties, as it can prevent gastric mucosal inflammation in animals if ingested prior to exposure to *H. pylori* (Takabayashi *et al.*, 2004; Stoicov *et al.*, 2009).

Shiga toxin-producing *E. coli* is an important pathogen causing haemolytic-uraemic syndrome, including the EHEC O104 : H4 outbreak in Germany in 2011 where 3816 patients were affected (Frank *et al.*, 2011). Even though the MICs of EGCG against *E. coli* 0157 : H7 were quite high (539 ± 22 μg mL^{-1}), it was demonstrated that low concentrations EGCG can inhibit the extracellular release of Shiga toxin and decrease quorum-sensing regulated genes, biofilm formation and swarm motility (Okubo *et al.*, 1998; Sugita-Konishi *et al.*, 1999; Lee *et al.*, 2009). In addition, it was observed that infected gnotobiotic mice fed with green tea extracts had significantly lower Shiga toxin levels than the untreated control group (Isogai *et al.*, 1998). The untreated controls developed neurological and systemic symptoms, usually culminating in death, whereas none of mice receiving dietary green tea extracts exhibited any clinical symptoms or died. Additionally, the combination of green tea extract with levofloxacin increased survival rates and reduced damage to target organs in orally EHEC infected gnotobiotic mice (Isogai *et al.*, 2001). Taken together, these data provide evidence that EGCG has beneficial effects against Shiga toxin-producing *E. coli*. However, more studies are needed to determine the anti-EHEC effects of EGCG in animal models or clinical trials.

As previously reported for *S. maltophilia*, EGCG acts as a bisubstrate inhibitor of the bacterial DHFR in *E. coli* (Spina *et al.*, 2008). Furthermore, results obtained using atomic force microscopy demonstrate that sub-MIC EGCG treatment of *E. coli* 0157 : H7 leads to temporary changes in the cell walls, such as pore-like lesions or their collapse (Cui *et al.*, 2012). By measuring the intracellular oxidation levels in bacteria after EGCG treatment, it was demonstrated that the morphological changes of gram-negative bacterial cell walls induced by EGCG depend on H_2O_2 release. As previously shown, one EGCG molecule can produce up to two molecules of H_2O_2 in phosphate buffer at neutral pH (Arakawa *et al.*, 2004). In conclusion, increasing H_2O_2 levels resulting in higher oxidative stress is also one mechanism by which EGCG induces a bactericidal effect against gram-negative bacteria. EGCG also produces indirect antibacterial effects on microorganisms; sub-inhibitory concentrations of EGCG can block or significantly reduce the transfer of conjugative R plasmid between *E. coli* isolates in a dose-dependent manner (Zhao *et al.*, 2001a). This could be of interest because the horizontal transfer of resistance genes by conjugation via plasmids is one of the major mechanisms for dissemination of resistance genes between bacteria. However, future studies are warranted to demonstrate these inhibitory effects against the plasmid-mediated gene transfer of resistance factors in *in vitro* and *in vivo* models.

EGCG also has selective immunomodulatory effects on pathogens, as was shown for *Legionella pneumophila* (Matsunaga *et al.*, 2001). *L. pneumophila* is an obligate human-pathogenic bacterium that invades and replicates in macrophages. EGCG was demonstrated to inhibit growth of *L. pneumophila* in macrophages at a concentration as low as 0.5 μg mL^{-1}, without any direct antibacterial effect on the pathogen. The replication was reduced due to selective changes in the immune response of macrophages and enhanced production of pro-inflammatory cytokines.

In conclusion, multiple *in vitro* and *in vivo* datasets indicate EGCG has significant direct and indirect anti-pathogenic

effects against foodborne bacteria and other gram-negative rods, including multi–drug-resistant strains.

EGCG against fungi

Over 600 different fungi have been reported to infect humans, ranging from common to fatal infections (Brown *et al.*, 2012). They infect billions of people every year and due to the use of more modern and interventional medicine and an increased number of immunosuppressed patients, the incidence of invasive fungal infections is rising. The antifungal effects of EGCG were mainly studied against yeasts such as *Candida* spp. and moulds such as dermatophytes. Currently, data relating to aspergilli or other human-pathogenic fungi as zygomycetes are lacking.

Yeasts such as *Candida* spp. are generally considered as commensals of the skin, mucosa and gut flora. Superficial infections by *Candida* spp. are commonly present in cases of deferment of bacterial flora or dysfunction of the local defence system. Candidaemia is the fourth most common source of bloodstream infection in the US and is associated with high morbidity and mortality (Rangel-Frausto, 1999; Pappas *et al.*, 2009).

The dermatophytes are a distinct group of fungi, which have the ability to utilize keratin as a nutrition source. These fungi cause superficial infections of the skin, hair and nails of humans and animals.

The problem with the most currently available antifungals is not the existing antimycotic activity; it is more the potential side effects of the different classes of drugs as most of them are nephro- or hepatotoxic. Thus, developing and testing compounds from nature with less toxic effects is desirable.

The fungicidal activities of EGCG against *Trichophyton mentagrophytes*, *T. rubrum*, *Cryptococcus neoformans* and *C. albicans* were first analysed in 1991 (Okubo *et al.*, 1991). Low concentrations of EGCG (2.5 mg mL^{-1}) showed no antifungal effects against *C. albicans* and *C. neoformans in vitro*. However, the tea extract with EGCG inhibited the growth of *Trichphyton* in a dose- and contact time-dependent manner. Using scanning and transmission electron microscopy to study the mode of action, the same research group examined the effects of EGCG against *T. mentagrophytes* (Toyoshima *et al.*, 1994). EGCG was shown to inhibit the germination of conidia and subsequent hyphal growth. After 3 days of EGCG treatment, the morphological characteristics of the conidia were changed in terms of deformation and swelling and after 5 days, most of the ungerminated conidia were broken down. In addition, the hyphal cell walls were exfoliated. It was concluded that EGCG can cause lysis of the conidia and hyphae suggesting that it has an antidermatophytic effect against *T. mentagrophytes*.

It was over 15 years later before the *in vitro* activity of EGCG against clinical isolates of dermatophytes was investigated (Park *et al.*, 2011). The susceptibility of 35 dermatophytes to a wide range of EGCG concentrations was tested using the standard protocol (M38-A2) from the Clinical and Laboratory Standards Institute (CLSI). The MIC_{50} and MIC_{90} of EGCG were 2–4 and 4–8 μg mL^{-1}, respectively. Interestingly, *T. rubrum* was more susceptible than *T. mentagrophytes* and *Microsporum canis*. However, more *in vivo* and *ex vivo* experiments need to be performed to verify a potential effect of EGCG.

While infections with dermatophytes only sometimes present therapeutic challenges, yeasts like *Candid*a spp. possess a substantially higher medical relevance in terms of associated morbidity and mortality.

A study testing the susceptibility of *C. albicans* to catechins as single agents and in combination with antifungal agents by a broth microdilution method showed that EGCG had pH-dependent anti-*C. albicans* effects (Hirasawa and Takada, 2004). At a pH of 7.0, the MIC_{90} of EGCG ranged between 15.6 and 250 μg mL^{-1}. The combination of EGCG with antifungal agents (amphotericin B, fluconazole) inhibited the growth of different reference strains indicating additive or synergistic effects. The results from another investigation evaluating the antifungal activity of EGCG (CLSI M27-A) on 21 clinical isolates of seven *Candida* species *in vitro* was mainly in agreement with those obtained previously (Park *et al.*, 2006). The MIC_{90} of EGCG against *C. albicans* was >16 μg mL^{-1} whereas *C. glabrata*, *C. guilliemondii* and *C. parapsilosis* exhibited the highest susceptibility (MIC_{90}; 1–16 μg mL^{-1}). As expected, most antifungals revealed lower MIC values against *Candida* spp. than EGCG. Hence, it has been suggested that EGCG could be used as an agent or adjuvant for antifungal therapy in candidiasis. However, the mechanism of the antifungal effect of EGCG has not been defined and *in vivo* experiments are currently lacking. So far three studies have been performed to try and address these issues.

In an *in vitro* study, it was shown that EGCG, EGC and ECG cause metabolic instability of *C. albicans* cultures even at physiological polyphenol concentrations found in green tea (Evensen and Braun, 2009). Of the three catechins, EGCG was found to be the most potent at retarding the formation and maintenance of *Candida* biofilm and to disrupt a preformed biofilm. It was demonstrated that higher EGCG concentrations inhibited *C. albicans* proteasomol chymotrypsin-like activity *in vivo* suggesting that the impairment of proteasol activity contributes to the cellular metabolic and structural disruptions of this yeast.

A study by Navarro-Martínez and colleagues explored the mechanism of the inhibitory effect of tea cathechins on *C. albicans* (Navarro-Martinez *et al.*, 2006). They found nearly the same MICs of EGCG against *C. albicans* as previously shown by Hirasawa *et al.* (2004). In addition, they demonstrated that the inhibitory effect of EGCG on the *C. albicans* DHFR (K_i = 0.7 μM), a key enzyme in the biosynthesis of purines, pyrimidines and several amino acids, was pH-independent. When EGCG was combined with azole antifungals (ketonazole and itraconazole) or inhibitors of the ergosterol biosynthesis pathway it mainly had a synergistic effect. EGCG was shown to inhibit ergosterol production by disturbing the folate metabolism in *C. albicans* cells. In addition, EGCG was also shown to have activity against an azole-resistant isolate and it was proposed that EGCG might be an alternative treatment for *C. albicans* infections. Hence, the results from this investigation provided new information as to the mode of action of EGCG: EGCG not only indirectly disrupts the ergosterol synthesis pathway through disruption of the folate cycle, but also inhibits ergosterol biosynthesis

by reducing the cellular pools of the methyl donor S-adenosyl-methionine.

As the results from *in vitro* experiments suggested that EGCG could be an effective treatment for *Candida* spp., Han conducted the first *in vivo* investigation of the anticandidal effects of EGCG alone and combined with amphotericin B in a murine model of disseminated candidiasis (Han, 2007). It was found that when EGCG, 1–4 mg kg^{-1} (i.p.), was administered alone to BALB/c mice before an i.v. inoculation of 5×10^5 *C. albicans* cells it had a dose-dependent inhibitory effect on the survival of the cells: the mean survival time was 29.0 days with 4 mg kg^{-1} compared with 11.0 days with 1 mg kg^{-1}. In addition, the combination treatment of EGCG, 2 mg kg^{-1}, and amphotericin B, 0.5 mg kg^{-1}, enhanced the resistance of these inoculated mice for up to 42.1 days compared with the survival rates of the untreated control (10.9 days). These results demonstrate that EGCG has anticandidal activity *in vivo*, and further show that it has a synergist effect when combined with amphotericin B in a murine model of disseminated candidiasis.

In summary, most of the *in vitro* and *in vivo* data on the antifungal activity of EGCG were obtained against *Candida* and indicate that EGCG could be used as an additional or alternative therapeutic agent against disseminated candidiasis. However, future work is needed to determine its *in vivo* efficacy in different settings.

Conclusions

In this review, the anti-infective effects of EGCG against viruses, bacteria and different fungi were summarized and discussed. A comparison of the antiviral activity of EGCG (Table 1) shows that the RNA and DNA viruses of various families with different replication strategies are affected by the green tea molecule. The underlying mechanisms by which different viruses were inhibited by EGCG are relatively diverse and in some cases not known. However, for most of the enveloped viruses, like HCV, HIV, HSV and influenza, ECGC has been shown to alter or damage the virus particles and so prevent viral entry. Therefore, it is hypothesized that the primary target of EGCG is the viral membrane while the host cell membrane appears to be unaffected. Other catechins do not have such a strong ability to bind to viral membranes. In analogy to viruses, the main underlying mechanism for the EGCG-induced inhibition of growth and killing of bacteria is disruption the lipid layers of the bacterial cell wall. In addition, for gram-negative bacteria and fungi it was demonstrated that EGCG is an efficient inhibitor of dihydrofolate reductase resulting in blocking of the folic acid metabolism.

A crucial aspect in the anti-infective effects of EGCG is the translation into clinically relevant strategies. In this regard, poor membrane permeability, low chemical stability and rapid metabolism of EGCG pose substantial obstacles that need to be addressed by future studies and possible derivatives of the EGCG backbone. Moreover, testing the safety and tolerability of a drug are very important issues before approval for clinical use. In studies with healthy human volunteers, it was shown that EGCG is safe and well-tolerated at oral doses of 800 mg EGCG day^{-1} over a time period of 4 weeks, which is equivalent to about 8–16 cups of green tea a day (Chow *et al.*, 2003). The plasma concentration ranged from 0.13 to 3.4 μg mL^{-1}, which reaches the IC_{50} of EGCG that was determined, for example, for HCV (Ciesek *et al.*, 2011; Calland *et al.*, 2012), but would probably not be high enough to eliminate the virus completely. In another study the safety, tolerability and pharmacokinetic properties of single-dose administration of EGCG that ranged from 50 to 1600 mg were analysed (Table 3) (Ullmann *et al.*, 2003). EGCG peak concentrations were reached between 1.3–2.2 h. The plasma kinetics of EGCG were assessed at intervals for a time frame of 26 h after administration. The mean total EGCG area under the concentration–time curve from 0 h to infinity $AUC_{(0\text{-}\infty)}$ ranged from 442 to 10 368 ng h mL^{-1} and the mean terminal elimination half-life $t_{1/2z}$ were seen from 1.9 to 4.6 h. Importantly, doses of purified EGCG up to 1600 mg are generally well tolerated (Ullmann *et al.*, 2003).

In addition, recent attempts have been made to try and enhance the activity of EGCG. For example, the bioavailability of chronic administration of EGCG 800 mg can be increased by peracetylation (Lambert *et al.*, 2006). Acylation enhances the anti-influenza virus activity of EGCG by up to 44-fold (Mori *et al.*, 2008). Furthermore, addition of long alkyl chains to EGCG significantly enhanced its *in vitro* activity against several bacteria and fungi, particularly against *S. aureus* including MRSA (Matsumoto *et al.*, 2012). Recently, the first controlled human studies with EGCG have been reported. A prospective randomized controlled study evaluated the effects of tea catechin inhalation on eradication of MRSA in sputum of disabled elderly patients (Yamada *et al.*,

Table 3

Pharmacological properties of total EGCG dosages (50–1600 mg) studied in healthy volunteers (Ullmann *et al.*, 2003)

Parameter	Result
Relative bioavaliability	1.6% at low dose (75 mg kg^{-1} body weight); 13.9% at higher doses (250 mg kg^{-1} and 400 mg kg^{-1} body weight)
Maximum plasma concentration (C_{max})	130–3392 ng mL^{-1}
Time to reach C_{max} (T_{max})	60–115 min
AUC (0-∞)	442–10 368 ng h mL^{-1}
Apparent terminal elimination half-life ($t_{½z}$)	2.2 h after i.v. and 5–6 h after oral administration
Safety and tolerability	was present within a dosage of up to 1600 mg

2006). Inhalation of 2 mL tea catechin extract solution (43% of catechins are composed of EGCG) three times daily for 7 days led to the disappearance of MRSA in the sputum of 31% of the patients in comparison with 12% in the control group (saline). But this difference was not statistically significant (P = 0.091). However, no adverse events of nebulized EGCG were observed during the study. In the case of influenza viruses, a randomized, placebo-controlled trial was conducted showing that consuming catechins for 5 months has a statistically significant preventive effect on clinically defined influenza infections (Matsumoto *et al.*, 2012), but further large-scale trials are needed to confirm these findings.

Interestingly, the therapeutic effects of a mixture of at least five different catechins, polyphenon E, where EGCG is the most abundant component (Clark and You, 2006) is commonly used clinically. This well-defined pharmaceutical mixture is a botanical drug approved by Food and Drug Administration (FDA) and European Medicines Agency (EMEA) as a topical treatment of external genital and anal warts in adults. It is the first prescription botanical (herbal) drug approved by FDA under the 'new' drug amendments of 1962 that required drugs to be proven both safe and effective prior to being marketed in the USA. External genital warts, caused by human papilloma viruses (HPV type 6 or 11), are one of the most common and fastest-spreading venereal diseases worldwide.

In conclusion, the magnitude of EGCG's anti-infective activity differs substantially between different reports probably due to different experimental setups and *in vitro* systems. Most of the data come from *in vitro* studies and future research efforts should focus on the design of animal models for investigating the anti-pathogenic effects of teas and tea ingredients. In addition, extraction procedure and methods of *in vitro* testing should be standardized to allow better comparison and interpretation of results. There is still a long way to go and future work is needed before EGCG can be routinely administered as an anti-infective drug in patients. However, the exciting findings of the past years should stimulate further research on EGCG that ultimately may translate into future therapeutic applications of EGCG and/or related catechins.

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Conflict of interest

No conflict of interest.

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