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Anti-infective properties of Epigallocatechin-3-gallate (EGCG), a component of Green Tea

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Summary:

Consumption of green tea (C*amellia sinensis*) has been shown to cause many physiological and pharmacological health benefits. In the past two decades several studies 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 were described for viruses from diverse families like *Retroviridae*, *Orthomyxoviridae* and *Flaviviridae* and including 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 reports demonstrated antiviral properties within physiological concentrations of EGCG *in vitro*. In contrast, the minimum inhibitory concentrations against bacteria were 10 to 100 fold higher. Nevertheless, antibacterial effects of EGCG alone and in combination with different antibiotics were intensively analyzed against a number of bacteria including multidrug-resistant strains like 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 hints indicating EGCG binds to lipid membranes and has influence on the folic acid metabolism of bacteria and fungi by inhibiting the cytoplasmic enzyme dihydrofolate reductase. This review summarizes the current knowledge and future perspectives about the antibacterial, antifungal and antiviral effects of the green tea substance EGCG.

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 *et al*., 2002). The functional and structural differences of 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 has been considered to have beneficial properties for human health including cardioprotective, anti-carcinogenetic and anti-infective effects. Although a detailed molecular understanding why green tea has these broad protective effects is lacking, the ability of EGCG to bind many biological molecules and influence a variety of enzymes activities and signal transduction pathways at micromolar and nanomolar levels may at least in part be responsible (Lee *et al*., 2002). EGCG is water soluble and high temperature exposure like 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 activity of tea was made over 100 years ago by the British army surgeon Mc Naught, who showed that tea killed the causal organism of typhoid fever

(*Salmonella typhi*) and brucellosis (*Brucella melitensis*) (MC Naught, 1906). However, no further work on this phenomenon was performed in the next decades until the late 1980 when systematic research about antimicrobial and antiviral effects of tea was conducted. Today, a literature search at pubmed.gov shows that over 4000 publications about EGCG and/or green tea were reported. In this review, we will first summarize the antiviral effect of EGCG against different virus families (Table 1) with a focus on hepatitis C virus (HCV) and human immunodeficiency virus (HIV). We will then reflect antibacterial and antifungal activities of EGCG in *in vitro* and *in vivo* model systems. Translations of anti-infective effects into clinically relevant strategies and to achieve physiologically concentration of the molecule at the sites of viral, bacterial and fungal replication are also crucial aspects that need to be considered as EGCG has in general a low bioavailability.

Effect of EGCG against hepatitis C virus

HCV, a positive strand RNA virus of the family *Flaviviridae,* has chronically infected ca. 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 among the leading indications for orthotopic liver transplantation (Brown, 2005). Standard therapy consists of a combination of pegylated interferon alpha with ribavirin (PEGIFN-α/RV). However, PEGIFN-α/RV therapy has differential success rates dependant on infecting viral genotype. The addition of one of two currently licensed viral protease inhibitors, the first generation of direct acting antivirals (DAAs) to current PEGIFN-α/RV combination therapy has substantially increased 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 efficient therapies applicable for all viral genotypes and with fewer side effects are needed. For instance, in the setting of liver transplantation for HCV-associated end stage liver disease, the ability to block viral cell entry would help to minimizing 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 identified EGCG as a potent inhibitor of the HCV entry pathway (Calland *et al*., 2012; Chen *et al*., 2012; Ciesek *et al*., 2011). 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 which identified the green tea molecule as 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 HCV life cycle (Li *et al*., 2006). Three studies by Ciesek et al., Calland et al. and Chen et al. clearly demonstrate that entry of cell culture derived particles (HCVcc) as well as HCV pseudoparticles (HCVpp) are inhibited by EGCG independent of the HCV genotype (Calland *et al*., 2012; Chen *et al*., 2012; Ciesek *et al*., 2011). This was also the case when primary human hepatocytes were used as target cells which resemble more closely the natural reservoir for HCV. 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 was previously suggested that EGCG inhibits the essential NS3/4A serine protease of HCV (Zuo *et al*., 2007), however the assays

were performed in a cell-free system and this observation could not be validated in an HCV replication setting (Calland *et al*., 2012; Ciesek *et al*., 2011). Another study reported a slight inhibition (2-3 fold) of HCV RNA-replication with JFH1 and Con1 constructs in tissue culture, but only at a very high concentration of 80 µM EGCG (Chen *et al*., 2012). Other catechins like EGC, EC and ECG had not such a strong inhibitory effect compared to EGCG suggesting that inhibition of HCV entry is unique to EGCG and not shared by other green tea catechins (Ciesek *et al*., 2011).

By testing other viruses it could be demonstrated that Herpes simplex virus (HSV) infection was inhibited as described earlier (Isaacs *et al*., 2008; Isaacs *et al*., 2011), but no effect could be observed for bovine viral diarrhoea virus (BVDV) or yellow fever virus (YFV), which also belong as HCV also to the family of *Flaviviridae*, or 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. It was shown that infection via cell-to-cell spread was refractory to neutralization by E2 monoclonal antibodies and that 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 agarose or incubated with neutralizing antibodies to prevent the extracellular route of infection (Calland *et al*., 2012; Ciesek *et al*., 2011). HCV entry is a complex multistep process involving many host factors 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, the antiviral activity was assessed by administration of the molecule at different time points during the early phase of infection. From these experiments, it was suggested that EGCG acts on the virus particles and inhibits virus entry by impairing virus binding to the cell surface (Calland *et al*., 2012; Ciesek *et al*., 2011)

(Figure 2). In line with these results, no effect of EGCG on target cells in pre-treatment experiments was observed, but inhibition of the primary attachment of ^{35}S-labeled HCV virions to cells (Ciese*k et al*., 2011). Importantly, the green tea molecule was also able to clear HCV from cell culture. At the concentration of 50 µm three cell passages led to undetectable levels of infectious virus in the supernatant of human cells (Calland *et al*., 2012) and Chen et al. observed even clearance of the virus after two passages at the same concentration (Che*n 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 in the setting of liver transplantation of chronically infected patients. 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 via the HCV co-factor cyclophilin (Ciese*k et al*., 2011). Future clinical trials will reveal how effective EGCG will be at reducing viremia in naïve patients with chronic hepatitis C and in preventing graft re-infection in patients undergoing liver transplantation.

Effect of EGCG against Human Immunodeficiency virus

Human immunodeficiency virus 1 (HIV-1) is a lentivirus of the family of *Retroviridae* and the etiologic cause of the acquired immunodeficiency syndrome (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 (Simo*n 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 HIV drug resistance development 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.

Green tea EGCG has been reported in different studies to have antiviral effects against HIV-1 infection. Interestingly, several mechanisms for this inhibitory effect have been proposed (Nanc*e et al*., 2003). Nakane et al. (1989) initially described inhibition of HIV-1 replication by EGCG in human peripheral blood mononuclear cells (PBMCs) *in vitro* (Nakan*e et al*., 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 reverse transcriptase inhibitors (NNRTIs) (L*i 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 is inhibited and NNRTI-resistant viruses were still susceptible to EGCG. Synergistic inhibition was observed with 3'azido-3'-deoxythymidine (AZT) (L*i et al*., 2011). Additionally, and in a similar fashion to HCV, a number of different studies also report an interference of EGCG with the viral envelope of HIV-1. Fassina et al. report HIV-1 infectivity was decreased in the presence of EGCG via lysis of viral particles (Fassin*a et al*., 2002). In a study by Yamaguchi et al. the possible antiviral effects of EGCG for every step of the HIV-1 life cycle were investigated (Yamaguch*i 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 similar manner to polymixin B on bacterial membranes (Ikiga*i et al*., 1993; Yamaguch*i 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 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, can prevent attachment of HIV-1 virions by blocking the interaction of gp120 and CD4 on T helper cells (Kawa*i et al*., 2003). EGCG in concentration ranging from 25-250 µmol/L downregulated the cell surface receptor expression by binding to CD4, presumably at a binding site recognized by gp120 (Kawa*i et al*., 2003; Nanc*e 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 (Geleziuna*s et al*., 1994). HIV-1 infection per se induces CD4 down-regulation by proteasomal degradation (Aike*n et al*., 1994). The molecular details 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 could be ruled out as process (Kawa*i et al*., 2003). However, the crucial mechanism of action of EGCG in HIV-1 entry inhibition seems to be the interferences of EGCG with gp120 as a ligand for CD4 and thereby preventing the initial attachment of viruses to CD4 T cells. The characteristics of EGCG-CD4 binding was further investigated by nuclear magnetic resonance (NMR) spectroscopy and molecular modelling (Williamso*n et al*., 2006). Addition of CD4 to EGCG produced a linear decrease in NMR signal intensity from

EGCG, but not from the control molecule catechin, demonstrating clear evidence of high-affinity binding of EGCG to the CD4 molecule with a K_d of approximately 10 nmol/L (Williamso*n et al*., 2006). Physiologically relevant concentration of EGCG (0.2 µmol/L) inhibited binding of gp120 to isolated human CD4 T cells. Molecular modeling studies suggested a binding site for EGCG in the D1 domain of CD4, the pocket that gp120 binds (Williamso*n et al*., 2006). The HIV-1 integrase protein is responsible for the insertion of HIV proviral DNA into the genome of infected cells. Recently, EGCG was also evaluated for the ability to inhibit the HIV-1 integrase in an ELISA assay (Jian*g 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 disrupting this interaction. However, further studies validating these *in vitro* experiments with infectious viruses should be performed.

In conclusion, EGCG appears to interfere with multiple aspects of the HIV-1 life-cycle, including virion destruction via interaction with the viral envelope, abrogation of viral replication via inhibition of reverse transcription, inhibition of proviral genome integration and CD4 receptor downregulation. 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 and further studies *in vivo* are required to judge if EGCG has promise as a potential 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 every 10–50 years of unpredictable severity (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 hemagglutinin) 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 U.S. alone. Vaccines are the most widely used intervention for influenza infection prophylaxis, but their effectiveness depends on the type of influenza virus and they also have the drawback of limited supply (Colli*n et al*., 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. Antiviral activity of EGCG was reported against influenza virus already 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 (Nakayam*a 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 an inhibition of influenza virus growth in tissue culture (Imanish*i et al*., 2002). These studies were extended by Song et al. who tested the structure-activity relationship of the different green tea polyphenolic compounds EGCG, ECG and EGC against influenza (Son*g et al*., 2005). They found that ECG and EGCG were more effective than EGC and the molecules also exerted

an inhibitory effect on the neuraminidase in a biochemical assay. Influenza viral RNA synthesis analyzed 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 nursing home residents (Yamada *et al*., 2006). In addition, another randomized, double-blind, placebo-controlled trial consuming catechins for 5 months had a statistically significant preventive effect on clinically defined influenza infection and was well tolerated (Matsumoto *et al*., 2012). These trails raise hope for the protective effect of catechins against influenza virus, however, large-scale studies are needed to confirm this effectiveness.

Enterovirus 71 is a single stranded, RNA virus and one of the causative agents for 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 1990s. Recently, enterovirus 71 repeatedly caused life-threatening outbreaks of hand-foot-mouth disease with neurological complications in Asian children. The neurological manifestations progress very quickly and range from aseptic meningitis to acute flaccid paralysis and brainstem encephalitis. It could be demonstrated that EGCG inhibited enterovirus 71 replication and formation of infectious progeny virus (Ho *et al*., 2009). There was a positive correlation between antioxidant capacities of catechins (Yang *et al*., 1994) and their antiviral activity (Ho *et al*., 2009). These findings suggested that EGCG may suppress viral replication via modulation of the cellular redox milieu.

The etiologic 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 had contact with the virus that 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 that affects over 300 million HBV 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 against HBV, green tea extracts and EGCG were studied in the stable cell line HepG2-N10 expressing HBV antigens (X*u* *et al*., 2008). The authors observed that expression of HBV specific antigens, the levels of extracellular HBV DNA, intracellular replicative intermediates and cccDNA were reduced in a dose-dependent manner (X*u 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 (Zho*u et al*., 2006). Recently, He et al. therefore 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 (H*e et al*., 2011). They observed that HBV replicative intermediates of RNA synthesis were significantly inhibted by EGCG, which resulted in less cccDNA production (H*e 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 allow recapitulation of the complete HBV life cycle.

In case of other DNA viruses, EGCG has been analyzed so far against adenovirus, Epstein-Barr virus (EBV) and Herpes simplex virus (HSV-1), the two latter one belonging to the family of *Herpesviridae*. Adenoviruses are non-enveloped viruses composed of a nucleocapsid and a double-stranded linear DNA genome. There are 57 described serotypes 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. When the antiviral effect of green tea was studied on adenovirus infection, the virus yield could be reduced 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 virus infection (Weber *et al*., 2003). Furthermore, inactivation of purified adenoviruses and inhibition of viral protease activity was noted. The therapeutic value, however, seem to be limited as the effective concentrations were high above the reported serum concentration 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 EGCG concentration exceeding 50 μM decreased expression of EBV lytic proteins, including Rta, Zta and EA-D, but not the expression of EBNA-1 (Chang *et al*., 2003). Moreover, DNA microarray and transient transfection analysis demonstrated that EGCG blocks EBV lytic cycle by inhibiting the transcription of immediate-early genes (Chang *et al*., 2003).

Herpes simplex is a viral disease caused by Herpes simplex virus type 1 (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 titers by more than 1000-fold in 10 to 20 min and reduced HSV-1 titers to the same extent in 30 to 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 could show those purified viruses exposed to EGCG were damaged. As EGCG is stable on 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 causing 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 beta-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* (MSSA)

(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 beta-lactams.

The activity of EGCG as single agent and in combination with beta-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 Ikigai *et al*. investigated the biological activity of green tea components including EGCG against *S. aureus* (Ikigai *et al*., 1993). It was reported that the minimum inhibitory concentration (MIC) values of EGCG were below 100 µg/mL. Initial experiments suggested that negatively charged EGCG exerts its anti-bactericidal activity via 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 detail (Cui *et al*., 2012; Kajiya *et al*., 2008; Kamihira *et al*., 2008; Kumazawa *et al*., 2004; Sirk *et al*., 2008; Uekusa *et al*., 2007). 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 were able to inhibit the production of PBP2 by >90% in a constitutively PBP2 producing *S. aureus* strain. In addition, the production of beta-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).

Combination testing of tea extracts and beta-lactams (methicillin, benzylpenicillin) mostly demonstrated a synergistic antibacterial effect. These results were mainly

confirmed by Zhao and colleagues who showed that 25 µg/mL EGCG was able to reverse the high level resistance of MRSA to all types of beta-lactams, including benzylpenicillin, oxacillin, methicillin, ampicillin and cephalexin (Zhao *et al*., 2001b). Fractional inhibitory concentrations indicies (FICI) of the tested beta-lactams against 25 MRSA isolates were low (0.126 - 0.625), indicating 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 (H*u et al*., 2002; H*u et al*., 2001; Stapleton *et al*., 2004). Furthermore, 12.5 µg/mL EGCG in combination with penicillin revealed a synergistic effect against in 100% of the 21 tested MRSA strains (Zhao *et al*., 2002). As previously reported, also the production of penicillinase from penicillin-resistant *S. aureus* was inhibited by EGCG in a dose-dependent manner.

Besides EGCG, ECG was also able to reverse beta-lactam resistance in clinical MRSA isolates (Stapleton *et al*., 2004). It was shown that the gallate moiety of EGC was 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). Besides the combination of EGCG with beta-lactams, interactions of EGCG with non-beta-lactam antibiotics have been evaluated against MRSA (H*u et al*., 2002). The combination of EGCG with antibiotic inhibitors of protein or nucleic acid synthesis was additive or indifferent (FICI, 0.5 – 4.0). In contrast to that, the interaction of EGCG with glycopeptide antibiotics (vancomycin, teicoplanin) showed antagonistic tendencies. These *in vitro* data indicate that choice of antibiotic in any potential combination

therapy consisting of EGCG with antibiotics against staphylococci is critical to achieve a bactericidal effect.

Two studies from Italy provided further insights in effects of EGCG against staphylococci (Blanco *et al*., 2005; Sudano Roccaro *et al*., 2004). Blanco et al. showed that 50 µg/mL 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 based on expression of a tetracycline efflux pump protein (Tet(K)), EGCG inhibites the pump activity which results 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 besides the binding to lipid layers and peptidoglycan, EGCG interferes with extracellular polymeric material (glycocalyx).

Another interesting antibacterial effect of EGCG was demonstrated by Hisano et al. In experiments using BALB/c mice and human PBMCs, polyphenon, consisting of 50% EGCG, could neutralize 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 as toxic shock syndrome toxin (TSST) requires investigation.

Taken together, 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 beta-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 sub-lethal 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 2-fold 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 if 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 nonpathogenic, 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 etiologic agents of dental caries (Beighton, 2005). In several studies, it was shown that tea catechins possess antimicrobial effects against oral streptococci. The prevention and reduction of dental caries formation was demonstrated in animal models as well as clinical trials. As the focus of this review is summarising the spectrum of EGCG activity, we would like to refer the reader to Taylor et al. who reviewed the anticariogenic activity of EGCG and its effects on periodontal disease (Taylor *et al*., 2005).

Streptococcus pyogenes has several virulence 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-treated and post-treated cells and could induce *S. pyogenes* cell death (Hull Vance *et al*., 2011). It was concluded that EGCG can 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. Presently, 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 (Yod*a et al*., 2004) because one mode of action of EGCG is binding to peptidoglucan. 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 to the bacterial cell membrane reduce the antibacterial activity of EGCG against gram-negative bacteria (Yod*a et al*., 2004).

The gram-negative non-fermentative bacillus *Stenotrophomonas maltophilia* is intrinsically resistant to beta-lactams and other broad-spectrum antibiotics and has emerged globally as an important nosocomial pathogen (Brooke, 2012). Two studies could show that EGCG exerts antibacterical effects against *S. maltophilia (Gordon et al., 2010; Navarro-Martinez et al., 2005)*. Furthermore, it was demonstrated that EGCG is an efficient inhibitor of *S. maltophilia* dihydrofolate reductase, and acts

synergistically with the folic acid metabolism blocking drug, sulfamethoxazole (Navarro-Martinez *et al*., 2005). The type of inhibition is similar to that of trimethoprim. Therefore, EGCG could represent an effective alternative in combination with sulfamethoxazole in treating *S. maltophilia* infections perhaps also caused by strains being resistant to trimethoprim. The range of the MIC of EGCG for *S. maltophilia* is nearly similar as for *Acinetobacter baumannii*, another multi-drug resistant pathogen causing 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*, enterohemorrhagic *Escherichia coli* (EHEC), *Vibrio cholera*, *Bacillus* spp., *Clostridium* spp. *Shigella* spp. and *Salmonella* spp. (Friedman *et al*., 2006; Lee *et al*., 2009; Mabe *et al*., 1999; Ryu, 1980; Sakanaka *et al*., 2000; Shetty *et al*., 1994; Stoicov *et al*., 2009; Sugita-Konishi *et al*., 1999; Taguri *et al*., 2004; Yanagawa *et al*., 2003). An overview of the existing studies analysing the antimicrobial effects of EGCG against bacteria causing food-borne disease is shown in Table 2.

H. pylori has been identified as an etiologic 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 (Mabe *et al*., 1999). Additive effects were shown in combination with amoxicillin, metronidazole and clarithromycin, antibiotics usually used as 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. In infected Mongolian gerbils, *H. pylori* was only eradicated in 10 to 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 efficiency of EGCG in combination with a proton pump inhibitor and a drug delivery system with prolonged gastric transit time (Mab*e et al*., 1999). Green tea had also prophylactic abilities, as it can prevent gastric mucosal inflammation in animals if ingested prior to exposure to *Helicobacter pylori* (Stoico*v et al*., 2009; Takabayash*i et al*., 2004).

Shiga-toxin producing *E. coli* is an important pathogen causing haemolytic-uremic syndrome, including the EHEC O104:H4 outbreak in Germany in 2011 where 3816 patients were affected (Fran*k et al*., 2011). Even though the MICs of EGCG against *E. coli* 0157:H7 were quite high (539 +/- 22 µg/mL), it was demonstrated that at low concentrations EGCG inhibits extracellular release of Shiga-toxin and could decrease quorum-sensing regulated genes, biofilm formation and swarm motility (Le*e et al*., 2009; Okub*o et al*., 1998; Sugita-Konish*i et al*., 1999). In addition, Isogai et al. observed that infected gnotobiotic mice fed with green tea extracts had significantly lower Shiga-toxin levels than the untreated control group (Isoga*i et al*., 1998). Untreated controls developed neurologic and systemic symptoms, usually culminating in death. In contrast, 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 (Isoga*i et al*., 2001). In conclusion, these data provide evidence that EGCG has beneficial effects against Shiga-toxin producing *E. coli*. However, more studies are necessary to determine the anti-EHEC effects of EGCG in animal models or clinical trials.

As previously reported in *S. maltophilia*, EGCG was shown to act as a bisubstrate inhibitor of the bacterial dihydrofolate reductase in *E. coli* (Spina et al., 2008). Furthermore, atomic force microscopy results demonstrated that sub-MIC EGCG

treatment of *E. coli* 0157:H7 led to temporary changes in the cell walls, such as pore-like lesions or their collapse (Cu*i et al*., 2012). By measuring the intracellular oxidation levels in bacteria after EGCG treatment, it was indicated that the morphological changes of gram-negative bacterial cell walls induced by EGCG depended 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 (Arakaw*a et al*., 2004). In conclusion, increasing H_2O_2 levels resulting in higher oxidative stress is also one mechanism of the bactericidal action of EGCG against gram-negative bacteria. EGCG has not only direct antibacterial properties on microorganisms. Sub-inhibitory concentrations of EGCG blocked or significantly diminished the transfer of conjugative R plasmid between *E. coli* isolates in a dose-dependent manner (Zha*o 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 demonstrating the inhibitory effects against the plasmid-mediated gene transfer of resistance factors in *in vitro* and *in vivo* models.

EGCG also had selective immunomodulatory effects on pathogens, as was shown for *Legionella pneumophila* (Matsunag*a 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, without any direct antibacterial effect on the pathogen. The replication was reduced due to selectively altering 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 more modern and interventional medicine and the increase 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 molds such as dermatophytes. Currently, data concerning 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. Candidemia is the fourth most common source of bloodstream infection in the US and is associated with high morbidity and mortality (Pappas *et al*., 2009; Rangel-Frausto, 1999).

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 first study analyzing fungicidal activities of EGCG against *Trichophyton mentagrophytes*, *T. rubrum*, *Cryptococcus neoformans* and *C. albicans* was performed in 1991 (Okubo *et al*., 1991). Low concentrations of EGCG at 2.5 mg/mL

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* (Toyoshim*a et al*., 1994). EGCG was shown to inhibit the germination of conidia and subsequent hyphal growth. After three days EGCG treatment, the conidia changed their morphological characteristics in terms of deformation and swelling and after five 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 an antidermatophytic effect against *T. mentagrophytes*.

It took over 15 years before the next study investigated the *in vitro* activity of EGCG against clinical isolates of dermatophytes (Par*k et al*., 2011). The susceptibility of 35 dermatophytes was tested against wide range of EGCG concentrations 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 µg/mL and 4-8 µg/mL, respectively. Interestingly, *T. rubrum* was more susceptible than *T. mentagrophytes* and *Microsporum canis*. It was suggested to perform *in vivo* or *ex vivo* experiments to verify a potential effect of EGCG.

While infections with dermatophytes only present sometimes 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 (Hirasaw*a et al*., 2004). At a pH of 7.0 the MIC_{90} of EGCG ranged between 15.6 and 250 µg/mL. The combination of

EGCG with antifungal agents (amphotericin B, fluconazole) inhibited the growth of different reference strains indicating additive or synergistic effects. Further *in vivo* experiments are needed to test whether a combination treatment of a catechin with an antimycotic would be beneficial for effective *Candida* treatment.

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 the previous announced study (Par*k et al*., 2006). The MIC_{90} of EGCG against *C. albicans* was >16 µg/mL whereas *C. glabrata*, *C. guilliemondii* and *C. parapsilosis* exhibited the highest susceptibility (MIC_{90}; 1-16 µg/mL). As expected, most antifungals revealed lower MIC values against *Candida* spp. than EGCG. It was suggested to use EGCG as possible agent or adjuvant for antifungal therapy in candidiasis. However, the mechanism of antifungal effect has not been defined and *in vivo* experiments are currently lacking.

Indeed a limitation of these studies is the fact that only *in vitro* testing of EGCG was performed. Besides *in vitro* testing, another important issue is studying the underlying mechanisms of action of EGCG. Furthermore, it would be desirable to confirm the *in vitro* test results in *in vivo* experiments. Up to now, three studies tried to address these issues:

In an *in vitro* study, it was shown that EGCG, EGC and ECG causes metabolic instability to *C. albicans* cultures even at physiological polyphenol concentrations found in green tea (Evense*n et al*., 2009). EGCG was found to be the most potent agent of the three catechins in its ability to retard 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 cellular metabolic and structural disruptions of this yeast.

A study by Navarro-Martínez and colleagues explored the mechanism of inhibition 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. (Hirasawa *et al*., 2004). In addition, it was demonstrated that EGCG is a pH-independent inhibitor of the *C. albicans* dihydrofolate reductase (DHFR) (K_i= 0.7µM), a key enzyme in the biosynthesis of purines, pyrimidines and several amino acids. The combination testing of EGCG with azole antifungals (ketonazole and itraconazole) or inhibitors of the ergosterol biosynthesis pathway was mainly synergistic. By disturbing the folate metabolism in *C. albicans* cells, EGCG could inhibit ergosterol production. As EGCG also had activity against an azole-resistant isolate, it was proposed that EGCG might be an alternative for the treatment of *C. albicans* infections. This investigation brought new knowledge in the mode of action of EGCG. It was shown that EGCG can not only indirectly disrupt the ergosterol synthesis pathway through disruption of the folate cycle but also causes inhibition of ergosterol biosynthesis due to the reduction of cellular pools of the methyl donor S-adenosyl-methionine.

As EGCG showed activity against *Candida* spp. in *in vitro* experiments, Han conducted the first *in vivo* study investigating the synergic anticandidal effect of EGCG alone and combined with amphotericin B in a murine model of disseminated candidiasis (Han, 2007). Intraperitoneal administration of 1-4 mg/kg EGCG alone or 2 mg/kg EGCG plus 0.5 mg/kg amphotericin B to BALB/c mice before intravenous inoculation of 5x 10^5 *C. albicans* cells demonstrated the activity of EGCG was dose-dependent as the mean survival time was 29.0 days with 4 mg/kg compared to 11.0 days with 1 mg/kg. The combination treatment of EGCG and amphotericin B enhanced resistance of the mice up to 42.1 days compared to the survival rates of the untreated control (10.9 days). These results demonstrated an anticandidal *in*

vivo activity of EGCG, and showed that EGCG has synergism combined with amphotericin B in a murine model with disseminated candidiasis.

In summary, most data concerning the antifungal *in vitro* and *in vivo* activity of EGCG exists against *Candida* indicating that EGCG can be an additional or alternative therapeutic agent against disseminated candidiasis. However, future work is needed to determine the *in vivo* efficiency in different settings and dosages of EGCG.

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 RNA and DNA viruses of various virus families with different replication strategies are affected by the green tea molecule. The underlying mechanisms how different viruses were inhibited by EGCG are relatively diverse and in some cases not known. However, for most of the enveloped virus like HCV, HIV, HSV and influenza virus an alteration or damage of the virus particles were reported that abrogated viral entry. Therefore, it is hypothesized that the primary target of EGCG seems to be the viral membrane while the host cell membrane seemed to be not affected. Other catechins do not have such a strong ability to bind to viral membranes. In analogy to viruses the main underlying mechanisms of EGCG inhibiting growth and killing of bacteria is the disruption the lipid layers of the bacterial cell wall. In addition, for selected gram-negative bacteria and fungi it could be demonstrated that EGCG is an efficient inhibitor of the dihydrofolate reductase resulting in blocking of the folic acid metabolism.

A crucial aspect of EGCG anti-infective effects 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 reported studies with healthy human volunteers, it could be shown that EGCG is safe and very well tolerated with oral doses of 800 mg EGCG per day over a time period of 4 weeks which equals about 8-16 cups of green tea once a day (Cho*w et al*., 2003). The plasma concentration ranged from 0.13 – 3.4 µg/ml which reaches the IC_{50} of EGCG that were determined for example for HCV (Callan*d et al*., 2012; Ciese*k et al*., 2011), but would probably not be high enough to eliminate the virus completely. In another study by Ulmann et al. the safety, tolerability, and pharmacokinetic properties of single dose administration of EGCG that ranged from 50 mg to 1600 mg were analysed (Table 3) (Ullman*n 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 hours after administration. The mean total EGCG area under the concentration-time curve from 0 h to infinity $AUC_{(0-\infty)}$ ranged from 442 to 10,368 ng h/ml 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 were generally well tolerated (Ullman*n et al*., 2003).

In addition, recent attempts have been made trying to enhance the activity of EGCG. For example, the bioavailability of EGCG can be increased by chronic 800 mg administration or for example by peracetylation (Lamber*t et al*., 2006). Acylation enhanced the anti-influenza virus activity of EGCG up to 44-fold (Mor*i 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 (Matsumot*o et al*., 2012). Recently, 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*., 2006). Inhalation of 2 mL tea catechin extract solution (43% of catechins are composed of EGCG) 3 times daily for 7 days led to disappearance of MRSA in sputum in 31% of patients in comparison to 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 case of influenza viruses, a randomized, placebo-controlled trial was conducted showing that consuming catechins for 5 month 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, a mixture of at least five different catechins, polyphenon E, where EGCG is the most abundant component (Clark *et al*., 2006) is very advanced in the clinics. 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 U.S. 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 EGCGs 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. Even. However, a long way is still 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.

Figure legends

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

Fig. 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), 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 tights 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.

Table 1: Inhibition of different viruses by EGCG

Table 2: Overview of existing studies analysing the antimicrobial effects of EGCG against bacteria causing food-borne disease

Table 3: Pharmacological properties of total EGCG dosages (50 to 1600 mg) studied in healthy volunteers (*Ullmann et al. 2003*)

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Conflict of interest

No conflict of interest

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