The Green Tea Polyphenol, Epigallocatechin-3-Gallate (EGCG)—One Step Forward in Antiviral Therapy Against Hepatitis C Virus

Approximately 170 million patients worldwide are chronically infected with hepatitis C virus (HCV), which represents approximately 3% of the world population. Prevalence rates range from 0.5% in Northern European countries to 28% in some areas of Egypt.¹ Currently there is no vaccine against HCV available for the prevention of this disease. Moreover, HCV persists in 70–80% of infected patients, and require treatment with antiviral agents once chronic infection is established. Current standard of care (SOC) include a combination of pegylated interferon- α (PEG-IFN- α) with ribavirin. In the near future, SOC of HCV infection will include the addition of direct-acting antivirals (DAAs) with a protease inhibitor to the current regimen.² Sustained viral response (SVR) rates have increased from <50% (PEG-IFN- α + ribavirin) to 70% (triple combination of PEG-IFN- α + ribavirin + a protease inhibitor) when the protease inhibitor telaprevir or boceprevir is added to the SOC in HCV genotype-1 patients.² There is ongoing development of new molecules, which may target any of the different steps in the HCV life cycle—prevention of virus entry into the host cell, prevention of translation of viral ribonucleic acid (RNA), inhibition of HCV-RNA polymerase, and prevention of virus assembly and release. Two recent studies suggest that the green tea polyphenol, epigallocatechin-3-gallate (EGCG) potently inhibits HCV entry into the host cell (hepatocyte) and thus could be part of an antiviral strategy aimed at the treatment of HCV infection as well as prevention of HCV reinfection after liver transplantation.^{3,4}

The EGCG is a water soluble molecule produced from the leaves of the plant, *Camellia sinensis*. The polyphenolic compounds, known as catechins, are the major active ingredients of green tea. The catechins include EGCG, epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). Approximately, EGCG accounts for 50% of the total green tea catechins. These catechins have been found to have antiviral and anti-oncogenic properties.^{3,5} The functional and structural differences are attributed to the number of hydroxyl groups on the B-ring and the presence or absence of a galloyl moiety. The inhibition of herpes simplex virus type 1 and 2, enterovirus 71, human immunodeficiency virus, influenza A, and other viruses has been reported with EGCG.³

Using robust methodology, Ciesek and co-workers³ have conclusively demonstrated that EGCG, but not other

green tea catechins, is an inhibitor of cell-culture-derived HCV particles (HCVcc) as well as HCV pseudoparticles (HCVpp). Their findings can be summarized as follows: (i) EGCG inhibits HCVcc entry into hepatoma cell lines as well as primary human hepatocytes thus prevents infection of cells by extracellular virions, (ii) EGCG also prevents cell-to-cell spread; this transmission may be important in vivo and is refractory to neutralization by envelope glycoprotein E2 monoclonal antibodies and occur in a CD81-independent manner,⁶ (iii) EGCG has no effect on HCV-RNA replication or assembly and release, (iv) inhibition of HCV entry seems to be unique to EGCG and not shared by other green tea catechins, (v) EGCG inhibits cell entry mediated by HCV glycoproteins and HCV inhibition is not genotype or isolate specific, and (vi) EGCG inhibits HCV infectivity by inhibiting its primary attachment to cells. They have further demonstrated that pretreatment of cells with EGCG before HCV inoculation did not reduce HCV infection, whereas the application of EGCG during inoculation strongly inhibited HCV infectivity. Expression levels of all known HCV (co-)receptors remained unaltered by EGCG. Ciesek and co-workers finally concluded that EGCG could be part of an antiviral strategy aimed at the prevention of HCV reinfection after orthotopic liver transplantation (OLTx).

Calland and co-workers⁴ have also independently demonstrated that EGCG inhibits HCVcc as well as HCVpp entry regardless of the genotype. In contrast to the findings by Ciesek et al, they reported that both ECG and EGC also exhibited an inhibition of HCV infection of approximately 40% and 80%, respectively. They have demonstrated that a concentration of 50 μ M EGCG inhibited HCV infectivity by >90% at an early step of the viral life cycle, most likely the entry step. This inhibition was not observed with other members of the Flaviviridae family tested. The antiviral activity of EGCG on HCV entry was confirmed with HCVpp envelope glycoproteins E1 and E2 from 6 different genotypes. They have further demonstrated that EGCG acts at a very early step of entry probably by inhibiting the docking of the virus to the cell surface hence disrupting the initial step of HCV cell entry. Like the previous study they have shown that EGCG has no effect on viral replication and virion secretion and inhibits HCV cell-to-cell spread. Finally, by successive inoculation of naïve cells with supernatant of HCV-infected cells in the presence of EGCG, they observed that EGCG leads to undetectable levels of infection after four passages. Calland and

co-workers concluded that EGCG could be used in combination with other DAAs.

Thus, both studies have independently demonstrated that EGCG effectively inhibits HCV infectivity at the entry step of the viral life cycle, i.e., it prevents attachment of the virus to the cell surface, has no effect on viral replication and virion secretion, inhibits HCV cell-to-cell spread, and most importantly all effects are genotype independent. These findings might have some applications in HCV therapy. For example, graft reinfection with HCV is universal and long-term outcomes are unsatisfactory in patients undergoing OLTx for end-stage liver disease due to hepatitis C. Infection of HCV in these recipients is characterized by an accelerated fibrogenesis, with approximately one-third of patients developing cirrhosis within 5 years of follow-up. Further, HCV is associated with decreased patient and graft survival when compared with other indications of OLTx.⁷ Prevention of graft reinfection, as routinely practised in the case of hepatitis B,⁸ is a major clinical goal for hepatitis C as well; however, no such efficient preventive measure is available. This would require efficient pharmacological means of preventing viral entry into hepatocytes; EGCG may very well fit for this goal but will require further randomized controlled trials.^{3,4} Also, EGCG could be used in combination with present SOC and with other DAAs; it might improve SVR and lower the relapse rates further by both mechanisms of actions (preventing HCV entry into the hepatocytes and preventing cell [hepatocyte]-to-cell [hepatocyte] spread).

The HCV is a highly variable enveloped RNA virus of the Flaviviridae family; its genome encodes two envelope glycoproteins, E1 and E2, which play a key role in virus entry into the hepatocyte. Its entry is a complex multistep process involving sequential interactions with several cell surface proteins.^{4,9} The virus relies on glycosaminoglycans and on low-density lipoprotein receptor to attach to cells. Furthermore, four specific entry factors—scavenger receptor class B type 1, tetraspanin CD81, claudin-1 and occludin—are sequentially involved after initial virus binding. Finally, HCV enters the cells via clathrin-mediated endocytosis.^{4,10} As the very first step, EGCG targets the HCV cell entry process (i.e., attachment).^{3,4} Silymarin, a polyphenol molecule, has been recently shown to inhibit HCV entry, probably at the fusion step.¹¹

Administration of EGCG is safe and very well tolerated in healthy individuals with oral doses of 800 mg of EGCG per day over 4 weeks, which equals approximately 8–16 cups of green tea once a day.¹² It is also easily available and is inexpensive. Calland et al.⁴ have also determined the toxicity of EGCG on Huh-7 cells. Although some toxicity began to be observed at 100 μ M, a concentration of 50 μ M was shown to have no toxic effect even after 72 hours. The half

maximal inhibitory concentration (IC₅₀) was estimated to approximately 5 μ M and the IC₉₀ was close to 50 μ M. Thus, the therapeutic index of EGCG is about 30.

These new data indicate that EGCG, the new anti-HCV molecule, could efficiently be used in combination therapy with other DAAs. Unlike NS3/4A protease inhibitors, such as, telaprevir and boceprevir, EGCG is not genotype specific and may be useful in nongenotype-1 HCV infection. This could be an ideal molecule to prevent cell-to-cell spread in especially in the setting of liver transplantation of chronically infected HCV patients. Future clinical trial should investigate its role in the management of naïve patients with chronic hepatitis C, management of previously treated patients with chronic hepatitis C, and in the prevention of reinfection in hepatitis C patients undergoing OLTx.

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