Orijinal Araştırma (Original article)

Deney hayvanlarındaki iskemi-reperfüzyon hasarı modellerinde yeşil çayın etkileri

Effects of Green Tea in Ischemia-Reperfusion Injury Models of Experimental Animals

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Özet

İskemi-reperfüzyon hasarı beyin, çizgili kas, kalp, bağırsak, karaciğer, böbrekler, retina, pankreas ve testis gibi farklı dokuları kapsayan çeşitli birçok klinik durumda ortaya çıkmaktadır. Her ne kadar oksidatif stresin iskemi-reperfüzyon hasarının etiyolojisinde merkezi bir rol oynadığı gösterişmiş olsa da, inflamasyon, lökosit infiltrasyonu ve apoptozis gibi birçok mekanizma da dahil edilmiştir. İskemi-reperfüzyon hasarını azaltmak için bugüne kadar çeşitli maddeler test edilmiştir. Ancak, hiçbiri bu endikasyonda kullanılmak üzere medikal ürün olarak henüz geliştirilmemiştir. Bu bağlamda, yeşil çay içeriklerinin farklı formdaki hayvan iskemi-reperfüzyon hasarı modellerinde faydalı etkiler gösterdikleri bulunmuştur. Bu derlemede, yeşil çay içeriklerinin hayvan iskemi-reperfüzyon hasarı modelleri üzerindeki etkileri tarihsel ve dokuya spesifik bir şekilde tartışılmıştır.

Anahtar Kelimeler: Epigallokateşin gallat; yeşil çay; iskemi-reperfüzyon hasarı

Abstract

Ischemia-reperfusion injury appears in various types of clinical conditions involving different tissues such as brain, skeletal muscle, heart, gut, liver, kidney, retina, pancreas and testis. Although oxidative stress has been shown to have a pivotal role in the aetiology of ischemia-reperfusion injury, several mechanisms including inflammation, leukocyte infiltration and apoptosis have been included as well. In an attempt to reduce ischemia-reperfusion injury, various substances have been tested so far. However, none of them have evolved as a medicinal product to be used in these indications yet. In this context, green tea contents have been shown to possess favourable effects in distinct forms of animal models relating to ischemia-reperfusion injury. In this review, the effects of green tea contents on ischemia-reperfusion injury models of animals have been discussed in a historical and tissue-specific manner.

Key Words: Epigallocatechin gallate; green tea; ischemia-reperfusion injury

1. INTRODUCTION

Ischemia-reperfusion (I/R) injury is responsible for the increased morbidity and mortality due to a variety of clinical conditions seen in different organ systems, including the brain (stroke), skeletal muscle (tourniquet application, myocutaneous tissue transfer, replantation of amputated parts, crush injury), heart (coronary arterial disease, thrombolytic therapy, balloon angioplasty, cardio-pulmonary bypass, occlusive arterial disease), gut (trauma and haemorrhagic shock), liver (transplantation, trauma and haemorrhagic shock), kidney (transplantation), retina (central retinal artery occlusion, ischemic optic neuropathy, diabetic retinopathy, retinopathy of prematurity, glaucoma), pancreas (islet transplantation), and testis (testicular torsion). There seems to be a common set of events responsible for I/R injury seen in these distinct systems that are oxidative stress, inflammation, impaired energy metabolism, Ca^{2+} overload, apoptosis, etc. Although there are several treatment options with different efficacies in clinical use, more effective tools are needed for a better outcome of patients. Hence, a huge amount of agents have been tested against I/R injury in different animal models and research on this field is still contemporary activity. However, none of the agents used so far have evolved as a medicinal product to be used in these indications yet. In this context, green tea contents have been shown to possess favourable effects in distinct forms of animal models relating to ischemia-reperfusion injury. Being the most active part of green tea, epigallocatechin gallate (EGCG) has been shown to possess potent anti-oxidant activity. However, several mechanisms, other than anti-oxidant activity, accounted for the protective effects of EGCG and/or green tea extract (GTE) have emerged during these investigations. In this review, studies carried out with these compounds in I/R injury have been discussed in a historical and tissue-specific manner.

2. BRAIN

Although reductions in the lipid peroxidation injury in synaptosomes and iron-induced oxidative stress in the rat brain by EGCG had been reported (1, 2), the first in vivo study showed that EGCG significantly prevented cell damage in the hippocampal CA1 region of gerbils (3). In another study published within the same year, infarction volume in the ipsilateral hemisphere of I/R animals was less in the 0.5% GTE pretreated animals compared to that in left hemisphere of non-treated animals (4). In harmony, GTE also reduced I/R-induced eicosanoid concentrations, hydrogen peroxide level, lipid peroxidation products, 8-oxodG formation (a marker

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of oxidative DNA damage) and apoptotic cell number (4, 5). Similarly, tea catechins were shown to decrease malondialdehyde (MDA) concentration in serum and brain tissue and to improve blood brain barrier injury in a rat I/R injury model (6). In a 2004 publication, EGCG significantly reduced infarction volume, neurological deficit total score, MDA levels and oxidized/total glutathione ratio as compared to those of the control group (7). A study, specifically, demonstrated that EGCG suppresses matrix metalloproteinase-9 activation, which leads, by the breakdown of neuronal cellular matrix, to edema, bleeding and neuronal death in cerebral ischemia, and reduces the development of delayed neuronal death after transient global cerebral ischemia in mouse brain (8, 9). In a recent study designed to find out the optimal dose of tea polyphenols against global cerebral I/R injury, 200 mg/kg was shown to have a protective effect against oxidative stress (MDA, superoxide dismutase, and total anti-oxidant status were measured) and apoptosis in rats (10). In addition, green tea supplementation, with or without physical exercise, prevented the decrease in glutathione concentration and catalase (CAT) activity and the increase in the concentrations of thiobarbituric acid reactive substances in a rat model of cerebral I/R injury (11). According to these studies, neuroprotective effect of GTE and/or EGCG seems to depend mainly on their anti-oxidant, anti-inflammatory and anti-apoptotic effects (Table 1).

Table 1. Mechanisms of green tea effect in I/R injury of brain and skeletal muscle

BRAIN
Anti-oxidant effects
↓ hydrogen peroxide
↑ superoxide dismutase, catalase
↑ glutathione
↓ oxidative DNA damage
Anti-inflammatory effects
↓ leukotriene C4, prostaglandin E2, thromboxane A2
Anti-apoptotic effects
Inhibition of the breakdown of neurovascular matrix and neuronal extracellular matrix
↓ matrix metalloproteinases-9
SKELETAL MUSCLE
Anti-oxidant effects
↓ superoxide anion
Inhibition of Leukocyte infiltration

Abbreviations: Ischemia-reperfusion (I/R)

3. SKELETAL MUSCLE

In an in vivo I/R injury model of rat, EGCG was shown to scavenge superoxide anion and protect against muscle cell injury and neutrophil infiltration (12) (Table 1). In contrast to the effect on superoxide anion, decreased nitric oxide levels were not influenced by EGCG in a consecutive study (13). It is clear that further investigations are necessary to entirely define the effects of EGCG regarding the aetiology of skeletal muscle I/R injury.

4. HEART

In rats subjected to I/R injury, myocardial injury together with tissue neutrophil infiltration, increased plasma levels of interleukin-6 (IL-6), activation of IκB kinase, cytosol degradation of inhibitor κB-α and subsequent activation of nuclear factor-κB (DNA binding), and phosphorylation of c-Jun and activation of activator protein-1 (DNA binding) were all prevented by the administration of EGCG, suggesting that inhibition of the IκB kinase /NF-κB and AP-1 pathway, which are important transcription factors in the setting of inflammation, may be involved in EGCG effect (14). In parallel, EGCG was shown to protect isolated neonatal cardiac myocytes from apoptotic cell death seen in I/R injury by probably inhibiting STAT-1 phosphorylation, STAT-1-mediated death receptor Fas overexpression and caspase-3 activation (these effects were also confirmed in ex vivo experiments) (15). Additionally, GTE/EGCG was able to attenuate the infarct size and proportion of TUNEL-positive myocytes in the isolated Langendorff-perfused rat heart (15). As the role of stress-activated p38 protein kinase in phosphorylating STAT-1 had been known, phosphorylation of p38 kinase in response to I/R injury of isolated intact heart was shown to be reduced by GTE/EGCG and the involvement of this pathway in the reduction of STAT-1 phosphorylation was suggested (15). In harmony, EGCG was found to directly interact with STAT1 protein, suggesting that this interaction would be responsible for the beneficial effect of EGCG in disease states wherein STAT1 pathway is involved (16). In harmony with this anti-apoptotic effect, caspase-3 activity was shown to be suppressed by EGCG in I/R injury established in a Langendorff heart preparation (17). Regarding anti-oxidant activity, generation of hydroxyl and superoxide radicals was also attenuated by both agents (17). In accordance, GTE was demonstrated to significantly restore cytosolic anti-oxidant activity along with the preservation of cell viability in cultured rat cardiomyocytes exposed to hypoxia/reoxygenation (18). Being the rate-limiting enzyme for glutathione synthesis, glutamate cysteine ligase may protect the heart from oxidative stress in a similar fashion with quinone reductase (19). In addition to a significant increase due to the GTE supplementation in these enzyme activities in both heart and liver, increased levels of thiobarbituric acid reactive substances and reduced total glutathione levels were partially ameliorated (19). In an I/R injury model of isolated rat heart, EGCG was found to be beneficial against myocardial damage as shown by the

measurements of lactate dehydrogenase and infarct size (20). Similarly, EGCG significantly inhibited the lipid peroxidation ratio and increased the expression of Mn-superoxide dismutase (SOD) and CAT compared to those in I/R injury group (20). As to the effect on apoptosis, the expressions of Bax (pro-apoptotic protein) and Bcl-2 (anti-apoptotic protein) protein levels were prominently decreased and increased by EGCG, respectively (20). Anti-apoptotic effect was supported by the finding that EGCG decreased the ratio of cleaved caspase-3, an important indicator of apoptotic cell death (20). On the basis of these results, it was concluded that EGCG protection against cardiac I/R injury occurred by the prevention of apoptosis via regulation of Bcl-2 and Bax expression and by anti-oxidant effects (20). Two weeks oral pretreatment with EGCG of rats was demonstrated to preserve cardiac function after I/R injury and this effect was related to its anti-oxidative and anti-apoptotic properties, which were, respectively, evaluated by 8-hydroxy-2'-deoxyguanosine index and p38 activation and active caspase-3 expression (21). The reduction seen in infarct size due to the administration of EGCG was inhibited by wortmannin (phosphatidylinositol-3-OH kinase (PI3K) inhibitor), indicating a role for PI3K-Akt pathway in the protective effect of EGCG (22). Regarding the pro-survival kinase signaling cascades (i.e., reperfusion injury salvage kinases, RISK pathway), EGCG was able to increase the phosphorylation of Akt (active state) and glycogen synthase kinase-3β (GSK-3β) (inactive state) (22). Phosphorylation of the latter one, which inhibits the opening of the mitochondrial permeability transition pore and hence apoptosis, was blocked by wortmannin, showing that EGCG phosphorylates GSK-3β through Akt-dependent pathway (22). In contrast, EGCG attenuated the phosphorylation of mitogen activated protein kinases (MAPKs), p38, and c-Jun NH2-terminal kinases (JNK), which are important pro-apoptotic (death) pathways (22). In summary, EGCG exerts its protective effects through activation of pro-survival kinases such as PI3K-Akt/GSK-3β and the attenuation of cell death pathway p38 and JNK (22).

In a specific study carried out in isolated mesenteric vascular beds of spontaneously hypertensive rats, EGCG was, with the contribution of nitric oxide, capable of reducing the myocardial infarct size and improving ventricular function (23). EGCG, ultimately, was shown to exert beneficial effects on insulin resistance and adiponectin levels, an adipokine which has anti-inflammatory properties, after 3 weeks therapy (23). In relation with positive myocardial functions, ATP content, the amplitude of transient nitric oxide signals and intramitochondrial Ca^{2+} concentrations were increased whereas the amplitude of transient Ca^{2+} signals was decreased by the pre-treatment of EGCG/GCG (17, 24). In a study, myocardial infarct-reducing effect of EGCG was inhibited by glibenclamide, a nonselective ATP sensitive K^+ channel (K_{ATP}) blocker, and 5-hydroxydecanoate, a selective mitochondrial K_{ATP} (mK_{ATP}) channel blocker (25). In fact, opening of the former channel would stimulate the outward K^+ current suppressing the duration of action potential, which would, in turn, reduce the voltage-dependent Ca^{2+} channel-mediated Ca^{2+} influx and help Na^+/Ca^{2+} exchanger to extrude Ca^{2+} from the cell more effectively (25). Similarly, K^+ efflux from mK_{ATP} would mitigate the driving force for Ca^{2+} influx into the mitochondria, thereby attenuating the harmful Ca^{2+} overload seen due to I/R injury (25). Considering the blockage of infarct limitation effect of EGCG by K_{ATP} and mK_{ATP} channel blockers and the protective effect of the activation of adenosine receptor (ADR) through mK_{ATP} channel on the heart I/R injury, the beneficial effect of EGCG was evaluated in a Langendorff isolated heart perfusion preparation (26). According to the study results, activation of ADR, particularly A_1 and A_{2B} subtypes, seemed to partly be responsible for the infarct reducing effect of EGCG (26). In addition, significantly increased serum cTnI levels after reperfusion period were reduced by the pre-treatment of GTE, indicating that GTE protects against selective proteolysis of elements within the contractile apparatus occurred in response to myocardial I/R injury (27). As alterations in myocardial structures involving selective proteolysis of myofibril proteins in conjunction with decreased myocardial functional recovery has been attributed to the generation of oxygen-derived free radicals and/or by a transient Ca^{2+} overload and the activation of Ca^{2+}-dependent proteases, the effect of GTE on cytosolic Ca^{2+} overload was evaluated and found to be reducing (27). Finally, GTE was found to preserve the expression and distribution of adherens junction proteins (N-Cad and β-catenin) and a gap junction protein (Cx43) organized in the intercalated disc of the myocardium subjected to I/R injury (27).

Depending on the results of these studies, all the potential mechanisms attributed to EGCG are outlined in Table 2.

5. GUT

In a study conducted in I/R injury of the gut, GTE significantly reduced histologically determined tissue injury along with increased tumor necrosis factor (TNF)-α level, myeloperoxidase activity, and expressions of intercellular adhesion molecule (ICAM-1) and P-selectin (28). Beside these pro-inflammatory markers, MDA levels, an indicator of lipid peroxidation, and nitrotyrosine formation, end-product of the interaction of nitric oxide and superoxide anion, were also attenuated by GTE (28) (see Table 3). Obviously, further investigations are needed to ascertain the underlying mechanisms of GTE and/or EGCG in I/R injury of the gut.

6. LIVER

In the first study regarding hepatic I/R injury, dietary supplementation of GTE displayed protective effects, which were evidenced by the reductions seen in the activity of aspartate aminotransferase and the amount of necrosis and leukocyte infiltration in the liver tissue (29). Furthermore, free radical formation, $NF\text{-}_KB$ activation, and mRNA and protein expressions of TNF-α were all attenuated by GTE (29). In addition to its reducing effect on necrotic index and alanine aminotransferase levels, EGCG also decreased total hepatic fat content, palmitic acid levels and linoleic acid levels in another study (30). All these results indicate that EGCG is favorable in I/R injury of the steatotic liver through the mechanisms

Table 2. Mechanisms of green tea effect in I/R injury of heart

HEART
Anti-oxidant effects
↓ superoxide, hydroxyl radical
↑ superoxide dismutase, catalase
↑ glutathione
↑ glutamate cysteine ligase, quinone reductase
Anti-inflammatory effects
↓ cytokines: IL-6
↓: IKK activation → IκB-α (inhibits NF-$_K$B) degradation → NF-$_K$B activation → DNA binding
↓: c-Jun → c-Jun-P → AP-1 activation → DNA binding (IL-6 related mainly)
↑ adiponectin
Anti-apoptotic effects
Inhibition of pro-apoptotic cascades
↓: p38 kinase-P → STAT-1-P → death receptor Fas overexpression → caspase-3 activation
↓: MAPKs (p38)-P + JNK-P
↓ Bax
Stimulation of anti-apoptotic cascades
↑: RISK pathway → PI3K → Akt-P (active state) → GSK-3β-P (inactive state) ⊣ mPTP ⊣ apoptosis
↑ Bcl-2
Other effects
↑ NO
↑: Adenosine → ADR (particularly A1 and A2B subtypes) → K_{ATP}, mK_{ATP}
↓ cytosolic Ca^{2+} overload

Abbreviations: Ischemia-reperfusion (I/R); interleukin-6 (IL-6); IκB kinase (IKK); inhibitor κB-α (IκB-α); nuclear factor-κB (NF-$_K$B); phosphorylation (-P); activator protein-1 (AP-1); signal transducers and activators of transcription-1 (STAT-1); mitogen activated protein kinases (MAPKs); c-Jun NH2-terminal protein kinase (JNK1/2); reperfusion injury salvage kinases (RISK pathway); phosphatidylinositol-3-OH kinase (PI3K); glycogen synthase kinase-3β (GSK-3β); mitochondrial permeability transition pore (mPTP); nitric oxide (NO); adenosine receptor (ADR); ATP sensitive K^+ channel (K_{ATP}); mitochondrial K_{ATP} (mK_{ATP});

associated with lipid metabolism, energy expenditure and antioxidant systems (30). On the assumption that oxidative stress may partly exert its toxic effect through NF-$_K$B activation, EGCG was tested and demonstrated to attenuate NF-$_K$B activation (31). Besides, EGCG was suggested to exert its protective effect by especially suppressing apoptosis via down-regulation of NF-$_K$B and c-Jun expression (31). Finally, GTE was able to significantly attenuate the increased levels of transaminases and lactate dehydrogenase, index of liver damage, index of leukocyte infiltration, and percentage of necrosis (32). Immunohistochemical analysis showed that GTE reduced the number of positively stained hepatocytes for TNF-α and increased the expression of Mn-SOD on hepatocytes (32). In order to evaluate Kupffer cells activation, the phagocytosis of latex beads was monitored and the number of latex bead-positive Kupffer cells per square millimeter was shown to be reduced by GTE (32). Depending on these results, the authors concluded that protective effect of GTE manifests via mechanisms including inactivation of Kupffer cells with subsequently decreased oxidative stress and TNF-α release (32). The underlying mechanisms accounted for the beneficial effects of green tea/EGCG are briefly presented in Table 3.

7. KIDNEY

Since increased production of xanthine oxidase-derived oxidants in the microvasculature had been detected in hypertension, EGCG was investigated in both normotensive and hypertensive rats subjected to I/R injury (33). Accordingly, MDA, blood urea nitrogen (BUN), serum creatinine (sCr), serum creatinine clearance (CrCl) and pathological parameters were deteriorated more severely in hypertensive rats (33). However, animals receiving EGCG were protected from harmful effects in both normotensive and hypertensive rats (35). Similarly, rabbits subjected to I/R showed decreased levels of sCr and urea nitrogen in serum after green tea polyphenols (GTP) treatment (34). Accordingly, EGCG, in addition to its preserving effect on renal function, was shown to reduce MDA levels, pathological score, and the number of cleaved caspase-3 positive cells (a marker of apoptosis) (35). EGCG also attenuated mRNA and protein expressions of MHC class II antigena and toll-like receptor-2/4, pointing out the possibility that EGCG may inhibit the innate immune response in I/R injured kidneys (35). Thus, down-regulation of monocyte chemo-attractant protein-1 mRNA expression, which mediates early monocyte/macrophage infiltration, by EGCG was shown by RT-PCR (35). A macrophage-

Table 3. Mechanisms of green tea effect in I/R injury of gut and liver

GUT
Anti-oxidant effects
↓ peroxynitrite
Inhibition of Leukocyte infiltration
↓ ICAM-1, P-selectin
Anti-inflammatory effects
↓ cytokines: TNF-α
LİVER
Anti-oxidant effects
↑ glutathione
↑ superoxide dismutase
Inhibition of Leukocyte infiltration
Anti-inflammatory effects
↓ cytokines: TNF-α
↓ NF-$_{κ}$B
↓ Kupffer cells activation
Anti-apoptotic effects
Inhibition of pro-apoptotic cascades
↓: activation of c-Jun → cleaved caspase-3

Abbreviations: Ischemia-reperfusion (I/R); intercellular adhesion molecule (ICAM-1); tumor necrosis factor (TNF)-α; nuclear factor-κB (NF-$_{κ}$B)

derived pro-inflammatory cytokine, IL-18, up-regulation was, indeed, significantly decreased by EGCG (35). As transforming growth factor beta 1 (TGF-β1) signalling pathway is connected to tubulointerstitial fibrosis induced by macrophages, EGCG was demonstrated to down-regulate TGF-β1 mRNA expression (35). In addition, mRNA expression of procollagen Ia1 and tissue inhibitor of metalloproteinase 1, which parallel the activity of TGF-β1, was also decreased by EGCG (35). Furthermore, the fact that differentiation of fibroblasts into myofibroblasts, characterized by de novo α-smooth muscle actin synthesis, is stimulated by TGF-β1 led the authors to investigate the effect of EGCG on α-smooth muscle actin expression and it was found to be suppressed (35). Another parameter related to tubulointerstitial fibrosis, i.e., Kim-1, was also determined to be depressed (35). Moreover, the amount of collagen deposition was significantly less in EGCG-treated group than that in control group (35). Finally, EGCG was shown to augment heme oxygenase-1 mRNA levels, and blockade of this gene induction by tin protoporphyrin increased renal tubular damage and macrophage infiltration (35). The authors concluded that EGCG protection could originate from decreased macrophage infiltration via monocyte chemo-attractant protein-1 down-regulation and decreased renal fibrosis via TGFβ1 down-regulation, which were possibly be mediated by heme oxygenase-1 augmentation (35).

In another study conducted in a small piglet model of extracorporeal circulation, EGCG was determined to improve the alterations seen in histomorphology of renal tissue and functional parameters such as total protein amount, sCr and serum urea concentrations (36). Upon immunohistological evaluation, increased nuclear translocation of hypoxia-inducible-factor-1-alpha, an intracellular transcription factor and indicator of hypoxic kidney damage, and nitrotyrosine formation were reduced as well (36). EGCG significantly diminished nuclear translocation of apoptosis-inducing factor, an early sign of apoptosis induction, and the staining of poly-ADP-ribose, a highly energy consuming repair mechanism (36). In addition, the decrease seen in ATP, ADP and AMP was prevented by EGCG, indicating that preservation of mitochondrial function of EGCG may contribute its antioxidant, nitric oxide-scavenging and anti-apoptotic activities (36).

Tea polyphenols showed favorable effects in I/R injury of rats that is increased levels of BUN, sCr, MDA, reactive oxygen species, IL-1β, IL-6, ICAM-1, TNF-α, apoptosis rate, and toll-like receptor-4 and NF-$_{κ}$B protein expressions were decreased whereas those of glutathione, SOD, CAT, glutathione peroxidase, glutathione reductase, and IL-10 were increased (37). In relation with the anti-inflammatory effect, activation of NF-$_{κ}$B upon toll-like receptor-4 signalization may lead the synthesis of certain cytokines, which may increase the expression of ICAM-1, and this pathway may be blocked by the presence of tea polyphenols at different steps (37).

As to the anti-inflammatory effect, serum and tissue levels of IL-1β, IL-6, and TNF-α were significantly reduced by the administration of EGCG before the induction ischemia in a recent study (38). Similarly, pro-apoptotic mediators such as Bax and cleavage caspase-3 were attenuated whereas anti-apoptotic mediators like BCL-2 and caspase-3 were increased by EGCG (38). In an attempt to understand the biological mechanisms responsible for anti-inflammatory and anti-apoptotic effects, NF-κB activation was assessed by measuring IκB-α and p65 (38). Increased phosphorylation level of

IκB-α and p65 and degradation amount of IκB-α in the IRI group were diminished by EGCG, indicating that reducing NF-κB activation is one important mechanism (38). A summary regarding the potential mechanisms of these substances in I/R injury of kidney is shown in Table 4.

optic nerve, where many ganglion cell axons are present, the reductions in NF-L and tubulin proteins, proteins expressed by ganglion cells, caused by I/R were also counteracted by EGCG (39). Same group showed, in another in vitro study, that EGCG is able to prevent light-induced cell death, apoptosis, reactive oxygen species

Table 4. Mechanisms of green tea effect in I/R injury of kidney

KIDNEY
Anti-oxidant effects
↓ peroxynitrite
Anti-inflammatory effects
↓ MCP-1
↓ cytokines: IL-1β, IL-6, TNF-α, IL-18
↓: p65-P + IκB-α-P + IκB-α (inhibits NF-$_{K}$B) degradation → NF-$_{K}$B activation
Inhibition of Leukocyte infiltration
↓ TLR4 → NF-$_{K}$B protein expressions → ICAM-1
Anti-apoptotic effects
Inhibition of pro-apoptotic cascades
↓ apoptosis-inducing factor (AIF)
↓ pro-apoptotic mediators (Bax and cleavage caspase-3)
Stimulation of anti-apoptotic cascades
↑ Bcl-2, caspase-3
Anti-fibrotic effects
↑ HO-1
↓ TGF-β1, procollagen Ia1, TIMP-1, α-SMA, Kim-1, collagen deposition
Other effects
↓ HIF-1-alpha (an intracellular transcription factor and indicator of hypoxic kidney damage)
↑ energy-rich phosphates (ATP, ADP, AMP)

Abbreviations: Ischemia-reperfusion (I/R); monocyte chemo-attractant protein-1 (MCP-1); interleukin-1β (IL-1β); tumor necrosis factor (TNF)-α; phosphorylation (-P); inhibitor κB-α (IκB-α); nuclear factor-κB (NF-$_{K}$B); toll-like receptor-4 (TLR4); intercellular adhesion molecule (ICAM-1); apoptosis-inducing factor (AIF); heme oxygenase-1 (HO-1); transforming growth factor beta 1 (TGF-β1); tissue inhibitor of metalloproteinase 1 (TIMP-1); α-smooth muscle actin (α-SMA); hypoxia-inducible-factor-1-alpha (HIF-1)

8. RETINA

It was found, in in vitro part of a study, that hydrogen peroxide-induced death, apoptosis and reactive oxygen species production in a ganglion cell line were prevented by EGCG (39) (Table 5). Regarding in vivo experiments, EGCG was able to inhibit the alterations seen in a and b-waves (informative for the photoreceptors and the ON-bipolar/Müller cells, respectively) measured by the electroretinogram (39). Negative effects of I/R on Thy-1 and ChAT immunoreactivities, associated with ganglion cells and a sub-set of amacrine cells in inner retina, were prevented by EGCG as well (39). Decreased mRNA levels of ganglion cell specific markers Thy-1 and NF-L, and opsin (photoreceptor marker) in retina were reversed by EGCG on the one hand and increased mRNA levels of caspase-3, caspase-8, and glial fibrillary acidic protein (directly related to retinal degeneration) were reduced on the other (39) (Table 5). Similarly, decreased protein levels of NF-L and rhodopsin kinase (photoreceptor marker) in retina were increased whereas increased protein levels of caspase-3 and glial fibrillary acidic protein were attenuated by EGCG (39). In the production, and loss of mitochondrial dehydrogenase activity in retina ganglion cells (40) (Table 5).

In a similar study, EGCG was demonstrated to attenuate retinal I/R-induced retinal ganglion cell loss and to inhibit retinal glial cell activity, which was evaluated by the measurement of glial fibrillary acidic protein expression (41). Indeed, increased TUNEL-positive cells in the I/R injury group was decreased by EGCG, indicating that retinal ganglion cell death is secondary to apoptosis (41) (Table 5). Similarly, EGCG attenuated the increases seen in the expression of neuronal nitric oxide synthase, NADPH activity, and MDA formation (41) (Table 5).

9. PANCREAS

Since the failure in islet cell transplantation had been attributed to the detrimental consequences of hypoxia/reoxygenation and subsequent apoptosis, EGCG was found to inhibit apoptosis occurred under normal

Table 5. Mechanisms of green tea effect in I/R injury of retina, pancreas , umbilical vein and testis

RETINA
Anti-oxidant effects
Anti-apoptotic effects
Inhibition of pro-apoptotic cascades
↓ caspase-3, caspase-8
Other effects
↓ nNOS, NADPH activity
PANCREAS
Anti-oxidant effects
↓ 8-OHdG (a marker of oxidative DNA damage)
Anti-apoptotic effects
UMBILICAL VEIN
Pro-apoptotic effects
Stimulation of pro-apoptotic cascades
↑ cleaved caspase-3
↑ Bax-to-Bcl-2 ratio
↑: MAPKs (JNK1/2)-P → c-Jun-P → AP-1 activation
Inhibition of anti-apoptotic cascades
↓: Akt-P (active state) → Foxo1-P + Foxo3a-P □ apoptosis
↓: MAPKs (ERK1/2)-P
TESTIS
Anti-oxidant effects
Anti-inflammatory effects
↓ TNF-α, IL-6, IL-1β, iNOS, MCP-1
Anti-apoptotic effects
Inhibition of pro-apoptotic cascades
↓ p53, Bax

Abbreviations: Ischemia-reperfusion (I/R); neuronal nitric oxide synthase (nNOS); 8-Hydroxy-2'-deoxyguanosine (8-OHdG); extracellular signal regulated protein kinase (ERK1/2); tumor necrosis factor (TNF)-α; interleukin-6 (IL-6); inducible NO synthase (iNOS); monocyte chemo-attractant protein-1 (MCP-1)

culture conditions and lactate dehydrogenase secretion in isolated islet cells of rats (42) (Table 5). In harmony, H/R-associated apoptosis, lactate dehydrogenase secretion and 8-Hydroxy-2'-deoxyguanosine, a marker of oxidative DNA damage, were also prevented by EGCG (42) (Table 5). As to β-cell function, depressed insulin release in the rat islets upon stimulation with glucose under H/R conditions was prohibited by EGCG (42).

10. UMBILICAL VEIN

The mechanisms responsible for the beneficial effects of EGCG on human umbilical vein endothelial cells (HUVECs) were evaluated in an in vitro study (43). At first, EGCG was shown to markedly inhibit endothelial cell growth and to regulate cell cycle-related regulators like cyclin D1, p27 and p21 proteins (43). Second, in order to discriminate whether reduced cell numbers could be the result of diminished cell proliferation or increased cell death, I/R-induced apoptosis was demonstrated to be enhanced by EGCG application (43). Along with this pro-apoptotic effect, enhanced expression of cleaved caspase-3 and PARP in response to I/R injury was augmented by EGCG (43). In support of these results, EGCG also enhanced the increased Bax-to-Bcl-2 ratio in I/R, indicating that EGCG can stimulate the activation of mitochondrial apoptotic pathway (43). As reduced AKT activity is frequently associated with apoptotic cell death, decreased AKT phosphorylation (active state) in HUVECs exposed to simulated I/R was further attenuated by EGCG (43). Similarly, decreased phosphorylation of Foxo1 and Foxo3a, major downstream targets of AKT, due to I/R was intensified by EGCG (43). In a further analysis focusing on apoptotic pathways, EGCG was found to augment the inhibitory effect of I/R on extracellular signal regulated protein kinase (ERK1/2) phosphorylation (anti-apoptotic cascade of mitogen-activated protein kinases (MAPKs)) and the stimulatory effect on c-Jun NH2-terminal protein kinase (JNK1/2) phosphorylation (pro-apoptotic cascade of MAPKs) (43). Consistently, c-Jun phosphorylation, a downstream target of JNK signaling pathway that is an obligation for AP-1 dimerization and activation in terms of apoptosis, was further increased by EGCG (43). It seems obvious to the authors that EGCG exerts its apoptotic effects through different apoptotic pathways, the role of which should be confirmed by in vivo experimental models (43) (see Table 5).

11. TESTIS

In a rat I/R injury model, EGCG was shown to protect against testicular damage and to prevent a further decrease in SOD activity, suggesting a protective antioxidant potential (44). In a further study, it was found that germ cell apoptosis and increased expressions of pro-apoptotic genes p53 and Bax were significantly inhibited by EGCG (45). Furthermore, EGCG was able to reduce the elevated levels of TNF-α, IL-6 and IL-1β and to counteract to the increased mRNA expressions of inflammatory markers inducible nitric oxide synthase and monocyte chemo-attractant protein-1 (45). These results clearly demonstrate that inflammatory mediators in conjunction with apoptotic pathways are involved in the favourable actions of EGCG (see Table 5).

12. CONCLUSION

Several studies conducted in different tissues have shown that green tea contents exert favourable effects in I/R injury. The mechanisms attributed to these substances may mainly be classified as follows: i) anti-oxidant effects, ii) anti-inflammatory effects, iii) inhibition of leukocyte infiltration, iv) anti-apoptotic effects and v) anti-fibrotic effects. Obviously, further investigations are needed to entirely figure out the underlying mechanisms responsible for these main effects. In addition, after completely conducting animal studies in terms of efficacy and safety, clinical trials in humans will be carried out in order to make these substances available for certain indications in clinical practice.

13. REFERENCES

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