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Epigallocatechin-3-gallate prevents cardiac apoptosis by modulating the intrinsic apoptotic pathway in isoproterenol-induced myocardial infarction

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ABSTRACT

(−)Epigallocatechin-gallate (EGCG) is an emerging natural therapy. This study examined the cardioprotective effect of EGCG on isoproterenol-induced myocardial damage and apoptosis and EGCG's role in modulating the expression of apoptotic signaling proteins. Experimental myocardial infarction was induced in albino Westar rats by isoproterenol (ISO) administration (100 mg/kg, s.c.) at an interval of 24 h on the 6th and 7th day. EGCG (15 mg/kg, i.p.) was administered seven days before ISO. EGCG pretreatment significantly showed an anti-lipidemic effect and protected the cell membrane integrity, as shown by the blocking of changes in serum levels of CK-MB, LDH, ALP, ALT and troponin T. EGCG also maintained the redox balance by preventing the inhibition of the activity of SOD and CAT while limiting lipid peroxidation. Pretreatment with EGCG inhibited the stimulation of the pro-inflammatory cytokine, TNF-α, in the serum. In animals treated with EGCG, tissue Bcl-2 expression exceeded the values observed after ISO treatment and down-regulated the expression of pro-apoptotic signaling proteins, including Bax, caspase-9 and 3. This is accompanied by the protection of genomic integrity by inhibiting DNA fragmentation coincident with the down-regulation of P53. In conclusion, EGCG protected against cardiac damage by decreasing apoptosis in myocardium tissue by 1) maintaining the balance of anti-apoptotic / pro-apoptotic signaling proteins in the mitochondrial pathway of cell death, 2) limiting oxidative stress while performing antioxidant and anti-inflammatory effects, and 3) protecting DNA integrity, sustaining cardiac health. Therefore, EGCG is potentially beneficial as an early intervention in cardiac attack.

1. Introduction

Green tea polyphenols and their major type Epigallocatechin-3-gallate (EGCG) constantly demonstrated functional potential in cases characterized by oxidative stress, inflammatory stressors and cell death (Liaudet et al., 2014; Deka and Vita, 2011). Therefore, the protection of myocardium from oxidative stress and apoptosis by EGCG is a putative cardioprotective therapeutic strategy.

Green tea (*Camellia sinensis*) is the one of the most universally consumed beverages in the world. The primary green tea flavonoids are (-)- epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epicatechin (EC), and catechin (Cooper et al., 2005). EGCG is the main free form of polyphenol found in plasma in large amounts (Chow et al., 2001). It is reported that catechins function as tocopheroxyl radical scavengers in biological systems and can protect these systems from oxidative damage, as reported for vitamin-C (Mukai et al., 2005). EGCG expresses antioxidant properties due to the presence of phenolic groups in its molecular structure (Yao et al., 2008). Researchers have shown that EGCG can protect the heart, brain, and kidney from oxidative injury (Afzal et al., 2015; Safer et al., 2015; Pan et al., 2015; Saeed et al., 2015). Green tea polyphenols protect the heart from ischemia-reperfusion (IR) injury by preventing cytosolic Ca (2+) overload and control adherens and gap junction protein expression and distribution (Liou et al., 2010). Green tea polyphenol can also bind to cardiac troponin C and modulate myofilament Ca (2+) sensitivity in cardiac muscle to ameliorate myocardial dysfunction in I/R injury (Hsieh et al., 2009).

Myocardial infarction (MI), frequently known as a heart attack, is the primary cause of morbidity and mortality worldwide; hence, it is a major public health concern. It is commonly demonstrated in ischemic heart disease and associated with an increase in the production of reactive oxygen species and the development of oxidative stress (Bagatini et al., 2011). The most documented effect of increased oxidative stress is the oxidation damage of lipids, membranes, proteins, and DNA in tissue and subcellular organelles, potentially leading to cell necrosis and/or apoptosis. The events of apoptotic mitochondrial pathway can be regulated and controlled through Bcl-2 family proteins which govern mitochondrial membrane permeability (Elmore, 2007).

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The p53 has a crucial role in regulation of the Bcl-2 family proteins. These actions lead to activation of caspases that lead to rapid cell death (El-Missiry et al., 2015). The administration of exogenous catecholamines is an essential intervention in the inadequate circulation in acutely ill patients as a short-term therapy. However, sustained adrenergic stress is harmful to the cardiovascular system and can induce substantial cardiomyopathy (Liaudet et al., 2014). Isoproterenol (ISO) is a synthetic catecholamine and β-adrenergic agonist that produces intense stress in the heart, resulting in infarct-like necrosis of the heart myocardium (Patel et al., 2010). The oxidation of catecholamine and formation of quinone compounds generates superoxide anions with the subsequent formation of hydrogen peroxide, which, in the presence of iron, forms highly devastating hydroxyl radicals (Dhalla et al., 2000). The pathophysiological events that occur in the heart of this myocardial infarcted rat model are similar to those observed in human myocardial infarction (Rona, 1985; Lobo Filho et al., 2011). Although quantification of reactive oxygen species is promising biomarkers of heart disease, the short lifetime make them secondary to more stable indicator such as malondialdehyde that may reflect systemic oxidative stress (Ho et al., 2013). Several mechanisms have been suggested to explain ISO-induced cardiotoxicity, including excessive oxidative stress (Patel et al., 2010), hyperlipidemia and disorganization of subcellular organelles (Farvin et al., 2006; Devika and Mainzen Prince, 2008; Devika and Prince, 2008). Myocardial oxidative stress, apoptosis, inflammatory mediation, were altered in ISO-induced MI in rats (Hassan et al., 2015) and are considered to be the major consequences of ischemic heart disease (Sahu et al., 2015). Therefore, treatment with an agent possessing antioxidants or free radical scavenging activity is a potential strategy to prevent the oxidative stress associated with cardiac disease.

Although the beneficial effects of EGCG has been reported in various oxidative stress conditions, there are insufficient data about the anti-apoptotic effects of EGCG against myocardial injury and the precise mechanisms involved in this pathological condition. In this context, the present study was designed to evaluate the cardioprotective effects of EGCG on ISO-induced cardiac injury in rats and attempted to understand the mechanisms underlying the therapeutic effects of this polyphenol in terms of its anti-apoptotic and antioxidant abilities.

2. Materials and methods

2.1. Drugs and chemicals

(-)-epigallocatechin gallate (EGCG) and isoproterenol hydrochloride (1-(3,4-dihydroxyphenyl)−2-isopropylaminoethanol hydrochloride) were purchased from Sigma Chemical (St. Louis, MO). All other chemicals were of analytical grade.

2.2. Animals

The current experiments and protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Mansoura University. All experiments were performed in accordance with the standards accepted by the regional experimental animal ethics panel. Wistar male rats weighing 280–300 g with age of 10 weeks were purchased from the Biological Products & Vaccines (VACSERA) of Cairo, Egypt. They were maintained in cages with free access to food and drinking water under hygienic and standard conditions of 22–24 °C with a 12-h light/dark cycle.

2.3. Induction of experimental myocardial infarction

ISO was freshly prepared in normal saline and injected subcutaneously (s.c) (100 mg/kg body weight) at an interval of 24 h for two days to induce experimental myocardial infarction (Stanely Mainzen Prince, 2012).

2.4. Animal treatment and experimental design

The animals were acclimatized for a week and were then randomly divided into four groups of six rats each. The first group was normal control rats. In the 2nd group, rats received EGCG (15 mg/kg body weight, i.p.) daily for a period of 8 days (Zhong et al., 2015). Rats in the 3rd group were injected sc with ISO alone (100 mg/kg body weight) at an interval of 24 h for 2 days (on 6th and 7th days) (Priscilla and Prince, 2009); the 4th group of rats were pretreated with EGCG (15 mg/kg body weight. ip) daily for 7 days and then injected (sc) with ISO (100 mg/kg body weight) for 2 days on the 6th and 7th days.

At the end of the experimental period, all of the overnight fasted rats were euthanized 24 h post ISO injection. Blood was collected and the sera were separated by centrifugation for biochemical determination. Hearts were dissected out, and ventricles were cleared of blood, weighed and immediately transferred to ice cold containers.

Heart weight and body weight and the ratio of the two was determined and calculated as previously described (Patel et al., 2010).

Samples of ventricle tissue were homogenized in chilled Tris-HCl buffer (0.1 M) pH 7.4 and used for the determination of the biochemical parameters. The homogenate was then centrifuged at 6000 rpm at 0 °C using a HERMLE LABORTECHNIK Z, 326 K, Germany cooling centrifuge. The clear supernatant was used for the heart biochemical assay.

2.5. Biochemical investigations

The lipid profiles in the serum, including triglycerides, total cholesterol, and high-density lipoprotein (HDL), were assayed using kits according to the manufacturer's instructions (Spinreact, St. Esteve d′en Bas Girona, Spain). The serum LDL-cholesterol was calculated by the Friedewald formula (Friedewald et al., 1972). The creatine kinase (CK-MB) lactic dehydrogenase (LDH), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities in the serum were determined using commercially available standard enzymatic kits purchased from Elitech (Puteaux, France). Tumor necrosis factor alpha (TNF-α) and troponin T in the serum were assessed using enzyme-linked immunosorbent assay (ELISA) kits provided by R & D Systems (Minneapolis, MN, USA) and Bioscience (San Diego, CA, USA) respectively according to the manufacturer's instructions.

The lipid peroxidation product (malondialdehyde, MDA) was analyzed by the thiobarbituric acid method, (Ohkawa et al., 1979). Superoxid dismutase (SOD) was determined by the previously described method (Mishra and Mishra, 1996). Catalase (CAT) was estimated in the serum and homogenate by the previously described method (Aebi, 1984). The protein concentrations were determined as described by Lowry et al. (Lowry et al., 1951).

2.6. Flow cytometry study

2.6.1. Determination of apoptosis using Annexin V/ PI staining

Samples from the ventricles were prepared as previously described for flow cytometry analysis (Gong et al., 2007). The cells were suspended in PBS with BSA, divided into aliquots and stored at 4 °C for analysis. The flow cytometry analyses were performed on a FACSCalibur™ cytometer (BD eBiosciences, San Jose, CA) using CellQuest Pro software (Becton Dickinson) for data acquisition and analysis (Juan et al., 2012). Apoptosis was assessed using a fluorescein isothiocyanate-conjugated annexin V/PI, ApoAlert kit from Clontech (Palo Alto, CA) according to the manufacturer's instructions.

2.6.2. Bcl-2, Bax, and P53

Cell suspensions were prepared in a PBS/BSA buffer and were then incubated for 30 min with an anti-Bcl-2 [100/D5] antibody and anti-