Modulatory effects of green tea on HEK-293 cell energy metabolism: implications in diabetic nephropathy

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ABSTRACT

The consumption of green tea is epidemiologically associated with a reduction in diabetic nephropathy; however its prophylactic effect remains unclear in an oxidative stress-associated diabetic milieu. The energy metabolism of HEK-293 cell, pretreated with variable concentrations of green tea, was evaluated under different hydrogen peroxide (H₂O₂) concentrations using the MTT assay. Green tea modulated the energy metabolism in renal cell line under different hydrogen peroxide challenge. In the absence of hydrogen peroxide, green tea at concentrations of 0.25 mg/mL and 0.50 mg/mL significantly increased the energy metabolism of HEK-293 cell by 81.5 % (P < 0.01) and 56.1 % (P < 0.05), respectively. Green tea at a concentration of 3 mg/mL significantly reduced (P < 0.05) the energy metabolism in HEK-293 cell by 36.6 % and 20.7 % when incubated in the presence of 200 μM and 500 μM H₂O₂, respectively. At high concentration, H₂O₂ and green tea have shown the ability in reducing the energy metabolism of HEK-293 cell. Conclusively, moderate consumption of green tea could form part of a healthy lifestyle that might ameliorate features of metabolic syndrome and subsequent risks for diabetic nephropathy, an outcome that can be determined by further clinical studies.

KEY WORDS: Diabetes; Green tea and wellness; Antioxidant; Nephropathy; Metabolic syndrome

INTRODUCTION

Diabetes mellitus is a group of disorders characterized by hyperglycemia and glucose intolerance, due to insulin deficiency, impaired effectiveness of insulin action or both. It has been one of the leading causes of death due to its related complications such as nephropathy, cardiovascular diseases, retinopathy and neuropathy. Robertson postulated that persistent hyperglycemia could cause oxidative stress via a number of pathways¹,². Under normal physiological condition, glucose would undergo glycolysis and oxidative phosphorylation, whereas under hyperglycemic condition, glucose would overwhelm glycolytic process and inhibit glyceraldehyde catabolism, thereby causing fructose-1,6-bisphosphate and glyceraldehyde-3-phosphate to be
channelized to other pathways that lead to enolization, α-ketoaldehyde formation, dicarbonyl formation and glycation, sorbitol and hexosamine metabolism. These biochemical pathways trigger oxidative stress, which has been reported to play a major role in the pathogenesis of diabetic nephropathy. Diabetic nephropathy, one of the most severe complications of diabetes, has been associated with increased morbidity and mortality. It remains a major microvascular complication of diabetes and the most common cause of end-stage renal disease, affecting approximately 30–47 % of diabetic patients. The incidence of diabetic nephropathy has increased by more than two-fold in the past decade, due to escalating prevalence of type 2 diabetes. A number of redox-sensitive events such as glomerular and tubular hypertrophy, mesangial cell injury, extracellular matrix accumulation, thickening of glomerular or tubular basement membranes, as well as podocyte dysfunction, could ultimately lead to proteinuria, glomerulosclerosis and tubulointerstitial fibrosis.

Tea has long been considered a palatable drink before the scientific community emphasized its therapeutic potential in the pharmaceutical field. Green tea contains mainly catechins (flavan-3-ols), such as (-)-epigallocatechin-3-gallate; (-)-epigallocatechin; (-)-epicatechin-3-gallate and (-)-epicatechin in addition to gallic, chlorogenic and caffeic acids, and flavonols such as kaempferol, myricetin and quercetin. The mechanisms underlying antioxidant effects of flavonoids largely include the scavenging of reactive oxygen species, chelation of redox active transition metal ions (such as iron and copper), inhibition of ‘pro-oxidant’ enzymes (such as inducible nitric oxide synthase, lipoxygenase, cyclooxygenase and xanthine oxidase) and induction of antioxidant enzymes (such as superoxide dismutase). These antioxidant properties have been related to the prophylactic effects of flavonoids in managing diabetic nephropathy.

Several studies have shown the renoprotective effects of green tea in diabetes. According to Shin et al, green tea can protect against L-arginine-induced toxicity in mesangial cells, suggesting that its polyphenols protect renal cells against oxidative injury. The progression of diabetic nephropathy can be delayed by tea catechins via a reduction in 24-hour urinary albumin excretion rate and a decrease in interstitial fibrosis. Wu et al have reported that a supplementation of green tea for 12 weeks ameliorates insulin resistance and increases glucose transporter IV content in a fructose-fed rat model. Priyadarshi et al found that green tea could attenuate hypertension, cardiac hypertrophy in nephrectomized rat and could prevent free radical generation in cardiac myocytes.

The efficacy of regular green tea consumption has been supported by conclusive evidence from animal models. This study has been set to assess, on a cellular scale, the effect of green tea in renal cell line under oxidative stress-induced diabetic milieu. Subsequently, the specific objectives of this study have been designed to evaluate the energy metabolism in human embryonic kidney (HEK-293) cells stimulated with various concentrations of hydrogen peroxide.

**METHODOLOGY**

**Chemicals**

Sterile Dulbecco’s Modified Eagle’s Medium (DMEM), trypsin, foetal bovine serum and L-glutamine were obtained from GIBCO (Grand Island, NY). All other chemicals used were of analytical grade.

**Plant Material**

*CAMELLIA SINENSIS* var. *sinensis* (Chinese Jat) was obtained as homogenous green tea bags (finished product) from Bois Chéri Tea.
Estate (Bois Chéri, Republic of Mauritius). The tea bag, containing 2 g of green tea, was manufactured on November 2010. According to Bois Chéri Tea Estate, the leaves of *Camellia sinensis* var. *sinensis* were steamed at 100 °C for 3 minutes to prevent enzymatic oxidation and fermentation. The steamed leaves were then rolled to optimize its organoleptic quality appropriately. Finally, the rolled leaves were dried in a fluid bed dryer to reduce its moisture content to about 3 % before packaging.

**Extraction**

Two grams of green tea (equivalent to 1 tea bag) were infused in 200 mL hot water (100 °C) for 6 minutes. The green tea brew was cooled under running tap water and filtered through a 0.2 μm nylon filter. The brew was diluted to generate different green tea concentrations (0.1 mg/mL to 10 mg/mL).

**Cell culture and the MTT assay**

Human embryonic kidney cell line (ATCC CRL-1573) was cultivated in DMEM supplemented with 10 % foetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100 U/mL). For all experiment, cells were seeded in triplicate at 10⁴ cells/200 μL per well in a sterile 96-well plate. The cells were grown in an incubator (5 % CO₂ and 37 °C) for 24 hours. After the first incubation, culture medium was replaced by a solution containing 150 μL of DMEM (1 % foetal bovine serum) and 15 μL of variable concentrations of green tea extract (0.25 mg/mL - 3 mg/mL). The cells were incubated for the second time for 24 hours. After the second incubation, culture medium was further replaced by a solution containing 150 μL of DMEM (1 % foetal bovine serum) and 15 μL of variable concentrations of hydrogen peroxide (100 μM - 500 μM). Finally, the cells were maintained in the controlled incubator for the third time for 24 hours before conducting MTT assay.

The effect of variable concentrations of green tea extract on the energy metabolism of HEK-293 cell, post-treated under different H₂O₂ concentrations, was evaluated by the MTT assay¹³. The assay is based on the cellular reduction of tetrazolium salt, by NAD(P)H-dependent cytochrome C reductase and succinate dehydrogenase, into insoluble formazan product¹⁴. After the final incubation period, 20 μL of MTT dye (5 mg/mL) was added into each well followed by 3 hours incubation. Culture medium was then carefully removed and 150 μL of dimethyl sulfoxide was added into each well. The 96-well plate was left under gentle agitation in the dark, at room temperature for 30 minutes, to solubilize the formazan crystals. The plate was read using a microplate reader FLUOstar OPTIMA (BMG Labtech, Offenburg, Germany) at 595 nm and 690 nm (for background absorbance). Final absorbance of sample was obtained by subtracting the background absorbance from the optical density at 595 nm. The control samples were cells treated with phosphate saline buffer (1X) (not green tea extracts) and incubated in a DMEM (1 % foetal bovine serum) with variable concentrations of hydrogen peroxide. Results were expressed as mean final absorbance of tested samples with respect to mean final absorbance of control samples.

**Statistical Analysis**

Parametric and non-parametric variables are expressed, after omitting outliers, as mean ± standard deviation and median [interquartile range], respectively. Statistical inference was carried out using MedCalc® for Windows (version 11.6.0.0; Mariakerke, Belgium). Significant differences over time within each group (experimental and control) were determined using paired

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Student’s t-test and where data was not normal the non-parametric alternative Wilcoxon test was used. The level for establishing significant differences was set at 5% and 1% successively. All statistical tests were two-tailed.

RESULTS
The energy metabolism of HEK-293 cells, pretreated with variable concentrations of green tea extract, under different oxidative stress conditions are shown in Figure 1. Under normal condition, the concentration of green tea was inversely proportional to the energy metabolism of HEK-293 cells.

Green tea extracts at 0.25 mg/mL and 0.50 mg/mL increased significantly the energy metabolism of HEK-293 cells by 81.5 % (P < 0.01) and 56.1 % (P < 0.05), respectively as compared to the control. Green tea did not significantly influence the energy metabolism of HEK-293 cells relative to the control in the presence of 100 μM H₂O₂-induced oxidative stress. Under 200 μM and 500 μM H₂O₂-induced oxidative stress conditions, 3 mg/mL green tea extract significantly reduced (P < 0.05) the energy metabolism of HEK-293 cell by 36.6 % and 20.7 %, respectively, when compared to the control.

Figure 1: The energy metabolism of HEK-293 cell, pretreated with variable concentrations of green tea extract, under different H₂O₂ concentrations. Markers represent mean values of a triplicate experiment. Statistical analyses have been performed, using paired Students t-test, for multiple comparisons. □ Mean value is significantly different from that of control (P < 0.05) (compared within same oxidative stress condition). △ Mean value is significantly different from that of control (P < 0.01) (compared within same oxidative stress condition).
The energy metabolism of HEK-293 cells under different oxidative stress conditions are shown in Figure 2. The hydrogen peroxide concentrations were inversely proportional to the energy metabolism of HEK-293 cell. Hydrogen peroxide at 200 µM and 500 µM decreased significantly the energy metabolism of HEK-293 cells by 56 % (P < 0.05) and 102 % (P < 0.05), respectively compared to the control.

Experiments have shown that MTT reduction involves principally NAD(P)H-dependent enzymes of the endoplasmic reticulum and succinate dehydrogenase of the mitochondrial complex II\textsuperscript{13}. The fact that MTT reduction is also affected by glucose in culture media supports the view that its reduction is related to glycolytic rate per se and thus to NADH production through glycolysis than to respiration\textsuperscript{19,20}. Present findings show that oxidative stress reduced MTT activity and that under normal condition, green tea extract (0.25 mg/mL) significantly increases (P < 0.01) the energy metabolism of HEK-293 cells, possibly, via the induction of glycolytic enzymes gene expression. However, the reduced-transcription factor Sp1 has been shown to be a requisite in activating glycolytic enzymatic genes\textsuperscript{21,22}. Through its antioxidant activity, 0.25 mg/mL green tea extract might regulate cellular redox changes during glycolysis and oxidative phosphorylation. The fate of most intracellular glucose is their conversion to pyruvate and NADH via the glycolytic pathway. In general, NADH is an electron donor for mitochondrial respiratory chain and it is hypothesized that NADH/NAD\textsuperscript{+} ratio increases significantly in hyperglycemic cell\textsuperscript{23}. Indeed, therapies that could decrease chronic glycolysis in hyperglycemic cell would be an advantage while dealing with diabetic complications at cellular level\textsuperscript{24}. Figure 1 also shows that high green tea concentrations could bring down the metabolic activity of HEK cells, a feature that could be attributed to prooxidant effect due to very high levels of phenolic compounds. It has been reported that during aerobic condition, copper and iron catalyze the redox cycling of green tea polyphenols that lead to reactive radical species formation and thereby downregulating the gene expression of glycolytic enzymes\textsuperscript{25}. Green tea extracts supplementation of the HEK cells enabled

Figure 2: The energy metabolism of HEK-293 cell under different hydrogen peroxide concentrations. Markers and error bars represent, respectively, mean values and standard deviations of a triplicate experiment. Statistical analyses were performed, using paired Student’s t-test, for multiple comparisons. *Mean value is significantly different (P < 0.05) from that of control (0 µM H\textsubscript{2}O\textsubscript{2}).

**DISCUSSION**

Intracellular generation of reactive radical species, by mitochondria, has been postulated to be a key player in the pathogenesis of diabetic kidney disease\textsuperscript{15}. An increasing body of evidence has promoted phyto-antioxidants as a supplementary therapy that could manage diabetic kidney disease\textsuperscript{16-18}. The present study reports the energy metabolism of HEK-293 cell, pretreated with variable concentrations of green tea, under different oxidative stress conditions based on the MTT assay. The latter is widely used in cell proliferation and cytotoxicity assays.

maintenance of their viability and protection against oxidative stress-induced mortality, an outcome that may be attributed to the free radical scavenging propensity of the green tea catechins\(^2\). This is largely interesting as it suggests that dietary supplementation with green tea beverage contributes to the viability of renal cells in patients with diabetes, future experimental works can be geared towards extended clinical observations on renal disease biomarkers\(^2\).

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