Epigallocatechin-3-gallate (EGCG) as a Potential Therapeutic Against Cardiovascular Disease Risk in Mice

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Abstract

Objective: Research is focused on cardiovascular disease (CVD). CVD is the leading cause of death in the world and remains one of the major diseases strongly affected by diet and sedentary lifestyle. The aim of this study was to determine the potential effect of EGCG on blood lipid and glucose profiles in mice exposed to conditions that lead to CVD. Groups of male mice were subjected to different diet, exercise and EGCG supplementation. EGCG was delivered in drinking water for a dose of 30 mg /kg/ day. Blood was collected for lipid and glucose analysis. The data was analyzed using a multivariate approach including one-way analysis of variance test (one-way ANOVA), Tukey –Kramer multiple comparison test and pairwise correlation analysis was used to determine the significance between control and treated groups.

Results: The mice subjected to high-fat diet (Anova p-value = 0.0039) and sedentary lifestyle (Anova p-value = 0.0016) showed elevated levels for total cholesterol, LDL cholesterol triglycerides and glucose. EGCG supplementation in mice undergoing high fat diet and sedentary lifestyle resulted in a significant reduction in the lipid profiles and blood glucose (Anova p-value = 0.0001).

Introduction

Cardiovascular disease (CVD) represents a global problem for human health world_wide, especially in the western countries, where CVD retains a leadership as a top cause of peoples' deaths and invalidation. In 2013, an estimated 1 million deaths were attributable to CVD in sub-Saharan Africa alone, which constituted 5.5% of all global CVD-related deaths and 11.3% of all deaths in Africa [1]. CVD is a class of diseases relate to the heart and or blood vessels that include, stroke, heart failure, hypertension, coronary artery disease, peripheral artery disease, and atherosclerosis caused partly due to dyslipidemia [2].

Multiple risk factors such as high fat diets (HFD), sedentary lifestyle (SL), age, gender, behavior such as smoking, alcohol consumption, diabetes, obesity contribute to CVDs [3].

A HFD can lead to obesity due to deposition of adipose tissues in the body and increased levels of lipids such LDL and total cholesterol (TC) in circulation which forms plaques in the blood vessels causing blockade or stiffnees resulting into high blood pressure and hence CVDs [3]. Lack of physical activity has been associated with obesity, increases endogenous inflammatory molecules, impairment of the physiological balance between inflammatory and oxidative stress reactions thus contributing to lipidemia and hence CVDs [10-13].

Management of CVD may involve avoiding risk factors and the use of antioxidants [13]. Studies have suggested that consumption of green tea (GT) can prevent the incidence of CVD and this has been attributed to epigallocatechin-3-gallate (EGCG) [5]. EGCG is a biologically active polyphenolic flavonoids commonly found in GT [11] and has been known to exert a variety of cardiovascular beneficial effects [2]. The selective use of nutrients, antioxidants and regular exercise in combination which primarily protect against multiple risk factors can be designed to access their protective potential against development of

vascular endothelial dysfunction (VED) due to reactive oxygen species [14, 15], lipidemia that results into atherosclerosis and obesity due to lack of exercise [16]. The purpose of this study was to determine the potential effect of EGCG, diet and exercise on the development of hyperlipidemia which may eventually culminate into CVD in mice. Study showed that a HFD and a SL predispose to hyperlipidemia and high blood glucose levels. Study also proved that EGCG fed to hyperlipidemia mice reduces lipid levels in blood, which confirms that EGCG has therapeutic potential to prevent CVD.

Materials And Methods

2.1.1. Ethics statement

The procedures and protocols of our study were approved by the Animal Ethics Committee of Kampala International University and Uganda national council of science and technology, Approval number is NS 645. Animals were cared for in accordance with the Guiding Principles in the Care and use of experimental animals of the European Council of the animal and the US National Institutes of Health Guide to the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

2.1.2. Animals and experimental groups

Sixty, three month old Albino swiss male mice were acquired from animal facility of College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB) of Makerere University. The mice were housed in standard cages, maintained at a normal temperature ($27 \pm 2^{\circ}\text{C}$) and humidity ($55 \pm 5\%$), and exposed to a 12-h light/dark cycle. Mice were divided into twelve groups of 5 mice each. The first six groups were exercised with normal diet and the other six received diet treatment as follows: (1) voluntary exercise (VE), (2) chronic exercise (CE), (3) sedentary lifestyle (SL), (4) VE+EGCG, (5) CE+EGCG, (6) SL+EGCG, (7) normal diet (ND) (3% fat), (8) high fat diet (HFD) (16% fat); (9), low fat diet (LFD) (0.2% fat), (10) ND+EGCG, (11) HFD+EGCG and (12) LFD+EGCG (Figure 1 and Table S1) and the experiment was repeated twice.

2.1.3. Animals, preparation, anesthesia, euthanasia, blood collection, glucose and lipid profile measurements

The experiments were carried out in compliance with European animal protection laws. A three-month-old male Swiss Albino mouse for each group was studied after 0, 2, 4 and 6 weeks of the experiment. These mice were anesthetized with isoflurane (Abbott, Cham, Switzerland) 2% (v/v) in a 20% O₂ and 80% air mixture by inhalation in a closed container, then euthanized by manual cervical dislocation and were put on the animal's bed for a blood sample from the cardiac puncture [17-19]. The blood sample was taken for each treatment in order to analyze the lipid and glucose profile. Serum was separated from blood by centrifugation at 3500 rpm for 10 minutes. Plasma lipid profile (TC, LDL cholesterol, HDL cholesterol and TG) and glucose levels were examined using a plasma glucose and lipid profile kit (Sigma-Aldrich) and were measured with an analyzer automatic chemical.

2.1.4. Statistical analysis

All data were shown as mean $\pm$ standard deviation (SD). Results were analyzed using one-way analysis of variance test (one-way ANOVA) followed by Tukey –Kramer multiple comparison test and pairwise correlation. P-value was used to determine the significance level of various diets and exercise in lipid profile and glucose in mice. Most of the statistical tests, the level of significance was fixed at $P < 0.05$.

Results

2.2.1. Evaluation of the effects of EGCG on glucose and lipid profile after diet treatment

In normal and HFD treated mice, we observed elevated levels of total and LDL cholesterol; with twice the concentration in HFD by the 4th and 6th week in comparison to normal diet (Figure 2, Figure S1A&B). However treatment of these mice with the EGCG resulted in a significant reduction in the levels of total cholesterol and LDL by weeks 4 and 6 in mice undergoing high fat diet treatment (Supplementary data: Tables S2 and S3).

Results on the means of TC and LDL cholesterol over the entire treatment period (Figure S2: A & B) showed that there is a statistically significant relationship between diets supplemented with or without EGCG on TC (Anova p-value = 0.0192) and LDL cholesterol (Anova p-value = 0.0134).

The levels of triglyceride and glucose levels were increased as the duration of experiment increased (Figure 2, Figure S1 D&E). EGCG decreased serum triglyceride and glucose levels (Figure 2, Figure 1S and Figure S2: D&E). There was statistically significant association between EGCG treatment and the levels of triglycerides in the serum (Anova p = 0.000317). Similarly we observed high likelihood of development of CVD in untreated mice in comparison to those treated with EGCG (Anova p-value = 0.000143).

2.2.2. Evaluation of the effects of EGCG on glucose and lipid profile after exercise treatment

For exercise, we observed that total and LDL cholesterol levels (Figure 3, Figure S3 A&B) increased gradually in mice subjected to the SL. There was a significant difference in the levels of TC (Anova p-value=0.0062) and LDL cholesterol (Anova p-value=0.0059) between EGCG-treated and untreated mice (Figure S3 A&B). It was also observed that HDL cholesterol, triglycerides and glucose were also increased for the SL (Figures 3, S4).

2.2.3. Correlation between diet and exercise on lipid and glucose profile in mice

Study of correlation between diet and exercise revealed that there exists significant association between diets and exercise in mice when treated with or without EGCG for TC, LDL cholesterol, triglycerides and glucose. The level of significance of mean value for all treatments is less than 5% level of significance (Supplementary data: Table S3). We observed high concentration of total and LDL cholesterol in high fat diet and sedentary lifestyle. However, these reduced significantly when the mice subjected to CE were

treated with EGCG (Anova p-value = 0.000809 for TC and Anova p-value = 0.001382 for LDL cholesterol: Figures S5 and S6).

Discussion

We investigated the protective or enhancing effects of EGCG for HFD and for a SL in mice in relationship of development of CVD. We formulated a HFD [20] and we designed a SL model for mice that were fed for 6 weeks and then given EGCG. Mice fed a HFD and mice subjected to a SL showed increased lipid and glucose levels in blood. Study suggests that a high cholesterol diet influences the deposition of cholesterol in the aorta and other tissues as cholesterol esters [21]. Another study has shown that HDL cholesterol levels were found to be decreased in rats fed a pro-atherogenic diet [22]. The increased levels of TC and LDL cholesterol observed in mice fed a HFD and sedentary mice may be due to a decrease in LDL receptor activity which reduces LDL catabolism in animals fed cholesterol. This hypothesis is also pointed out by other studies [23]. Yu et al. [24] reported that serum TC and TG increased significantly in rabbits receiving a HFD. Risk of CVD has been reported to be related to increased consumption of saturated fatty acids and percentage of calories from fat [25], which are positively associated with cholesterol intake. Our relationship demonstrated a risk of developing CVD in mice fed a HFD and in mice subjected to a SL, since we found high levels of TC, LDL cholesterol, TG and glucose. Our results are confirmed by other studies [26].

Study assessed the effects of exercise and diet first and secondly exercise and diet supplemented with EGCG. We determined whether the plasma lipid and glucose levels left by these procedures were maintained in the presence or absence of a HFD and a SL. Cardiovascular risk factors alter endothelial function [27] but physical exercise have the potential to slow down the endothelial damage associated with aging and CVD [28]. The study reported that regular voluntary exercise and chronic physical exercise protects the installation and development of CVD in mice unlike HFD and SL [29].

A study in inactive mice showed that consumption of the HFD between 9 and 12 months led to obesity, hyperglycemia, hyperinsulinemia and hypercholesterolemia [20]. The found effects almost similar to those in the study above. We have noticed an increase in plasma lipid and glucose levels. A 3-month HFD induced obesity [28], tripled the level of circulating cholesterol, induced oxidative plasma stress [29], induced hyperglycemia and hyperinsulinemia in mice [30].

Mice that are fed a HFD and mice that are SL supplemented with EGCG compared to those that were not supplemented with EGCG had low total cholesterol, LDL cholesterol, triglycerides and glucose, which confirmed the preventive role of EGCG on CVD. Xu et al. [31], demonstrated the effectiveness of EGCG (100 mg/kg) in an atherosclerosis disease model induced by feeding atherogenic diet to Wistar rats. Results of the tissue morphometric analysis and lipid profile of the EGCG treated atherogenic diet fed rats showed that there was a reduction in total cholesterol, triglycerides, low-density and very low density lipoprotein cholesterol fractions as compared to those untreated atherogenic diet fed rats [32]. Our findings support a role for regular consumption of dietary EGCG such as green tea in day-to-day life will

reduce the risk of CVD, and that represents a potential therapeutic agent for the prevention of atherosclerosis and related CVD.

Conclusion

The main topics on which my research is based on CVDs and mechanisms that characterize them: diet, physical exercise and the use of antioxidants such as EGCG, with particular attention for its benefits on cardiovascular health and its therapeutic potential. Study showed that a HFD and a SL predispose to hyperlipidemia and high blood sugar and therefore could be associated with the development of CVD. Study also proved that EGCG administered to hyperlipidemia mice reduced blood glucose and lipid levels, confirming that EGCG has therapeutic potential to prevent CVD.

Limitations

In this study there are two limitations (experimental conditions and ethical reasons). For chronic physical exercise, we used very large cages. The mice were run three times a day for thirty minutes each. It would have been interesting to subject mice to treadmills and compare the effects of physical training between cage and treadmill model on cardiovascular health. It would also have been interesting to see whether the use of an antioxidant in a pathological context in humans with CVD succeeds or not in reversing the effects associated with the disease.

Abbreviations

CE: Chronic exercise

CoVAB: College of Veterinary Medicine, Animal Resources and Biosecurity (Makerere University)

CVD: Cardiovascular disease

EGCG: Epigallocatechin-3-gallate

GT: Green tea

HDL: High density lipoprotein cholesterol

HFD: High-fat diet

LFD: Low fat diet

LDL: Low-density lipoprotein cholesterol

ND: Normal diet

SL: Sedentary lifestyle

TC: Total cholesterol

TG: Triglycerides

VE: Voluntary exercise

VED: Vascular endothelial dysfunction

VLDL: Very low density lipoprotein cholesterol.

Declarations

Author contributions

Banzubaze Emmanuel is the research designer. In addition to the design of the research, this Banzubaze Emmanuel did the experimentation, the analysis of the results, the discussion and the drafting of the manuscript. The other authors were the directors of the research. To do this, they read and made corrections to errors of substance and form in the manuscript.

Declaration

In research, there is no competing interest as the research has been experimental on laboratory animal models (mice).

Ethics Approval

The research has been approved by the Animal Ethics Committee of Kampala International University and the Uganda national council of science and technology, Approval number is NS 645.

Consent to publish

Before submitting the manuscript, there was consent of the authors for its submission

Founding source

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Availability of data and materials

I declare on my honor that the data and materials used in the writing of this manuscript are available in the file entitled "manuscript data submit". I also declare that this data may be made public by the scientific community for research purposes or during review of the manuscript by editors or reviewers.

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Figures

Figure 1

Experimental design for the study.

CE is chronic exercise, EGCG is epigallocatechin-3-gallate, HDL-C is high density lipoprotein cholesterol, HFD is high fat diet, LDL-C is low density lipoprotein cholesterol, LFD is low fat diet, M is mice, ND is normal diet, SL is sedentary lifestyle, TC is Total cholesterol, TG is triglycerides and VE is voluntary exercise.

Figure 2

Effect of EGCG on lipids and glucose of serum in mice after diet treatment.

CHO is total cholesterol, CHO_EGCG is total cholesterol supplemented by EGCG, LDL is low density lipoprotein cholesterol, LDL_EGCG is low density lipoprotein cholesterol supplemented by EGCG, GLU is glucose, GLU_EGCG is glucose supplemented by EGCG, HDL is high density lipoprotein cholesterol, HDL_EGCG is high density lipoprotein cholesterol supplemented by EGCG, TAGS is triglycerides and TAGS_EGCG is triglycerides supplemented by EGCG.

Figure 3

Effect of EGCG on lipids and glucose of serum in mice after exercise treatment.

CHO is total cholesterol, CHO_EGCG is total cholesterol supplemented by EGCG, LDL is low density lipoprotein cholesterol, LDL_EGCG is low density lipoprotein cholesterol supplemented by EGCG, GLU is glucose, GLU_EGCG is glucose supplemented by EGCG, HDL is high density lipoprotein cholesterol, HDL_EGCG is high density lipoprotein cholesterol supplemented by EGCG, TAGS is triglycerides and TAGS_EGCG is triglycerides supplemented by EGCG.

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