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Caffeic acid phenethyl ester, a 5-lipoxygenase enzyme inhibitor, alleviates diabetic atherosclerotic manifestations: Effect on vascular reactivity and stiffness

Noura Ahmed Hassan [a], Hany M. El-Bassossy [a,b,*], Mona Fouad Mahmoud [a], Ahmed Fahmy [a]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Zagazig University, Egypt
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, King Abdulaziz University, Saudi Arabia

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ABSTRACT

Atherosclerosis is a major macrovascular complication of diabetes that increases the risks for myocardial infarction, stroke, and other vascular diseases. The effect of a selective 5-lipoxygenase enzyme inhibitor; caffeic acid phenethyl ester (CAPE) on diabetes-induced atherosclerotic manifestations was investigated.

Insulin deficiency or resistance was induced by STZ or fructose respectively. Atherosclerosis developed when rats were left for 8 or 12 weeks subsequent STZ or fructose administration respectively. CAPE (30 mg kg^{-1} day^{-1}) was given in the last 6 weeks. Afterwards, blood pressure (BP) was recorded. Then, isolated aorta reactivity to KCl and phenylephrine (PE) was studied. Blood glucose level, serum levels of insulin, tumor necrosis factor α (TNF-α) as well as advanced glycation end products (AGEs) were determined. Moreover aortic haem oxygenase-1 (HO-1) protein expression and collagen deposition were also assessed.

Insulin deficiency and resistance were accompanied with elevated BP, exaggerated response to KCl and PE, elevated serum TNF-α and AGEs levels. Both models showed marked increase in collagen deposition. However, CAPE alleviated systolic and diastolic BP elevations and the exaggerated vascular contractility to both PE and KCl in both models without affecting AGEs level. CAPE inhibited TNF-α serum level elevation, induced aortic HO-1 expression and reduced collagen deposition. CAPE prevented development of hyperinsulinemia in insulin resistance model without any impact on the developed hyperglycemia in insulin deficiency model.

In conclusion, CAPE offsets the atherosclerotic changes associated with diabetes via amelioration of the significant functional and structural derangements in the vessels in addition to its antihyperinsulinemic effect in insulin resistant model.

1. Introduction

Diabetes mellitus is a chronic disease that affects about 17 million people in the USA [1,2]; there are 170 million cases worldwide, and this figure is expected to be more than double by 2030 [3]. Type II diabetes is the most common form of diabetes, representing about 90% of all diabetes worldwide [4]. Diabetic vascular complications affect many tissues, including microvasculature, macrovasculature, nerve and the heart. These complications are the most common causes of mortality, of end-stage renal disease, and of blindness in diabetic patients worldwide [5]. Atherosclerosis is a major macrovascular complication of diabetes that increases risks for myocardial infarction, stroke, and other vascular diseases. In 2001, the American Heart Association defined several factors involved in diabetic atherosclerosis including metabolic factor, oxidation/glucoxidation, and alteration in vascular reactivity [6,7].

The 5-lipoxygenase enzyme, being activated in diabetes caused by either insulin deficiency or resistance [8,9] is known to catalyze the oxygenation of arachidonic acid [10]. The products of arachidonic acid oxygenation were reported to be a function key mediators

Abbreviations: AGEs, advanced glycation end products; ACh, acetyl choline; CAPE, caffeic acid phenethyl ester; BP, blood pressure; PE, phenylephrine; STZ, streptozotocin; TNF-α, tumor necrosis factor-α; HO-1, haem oxygenase-1; ELISA, enzyme-linked immunosorbent assay; ANOVA, analysis of variance.

* Corresponding author at: Department of Pharmacology, Faculty of Pharmacy, King Abdulaziz University, P.O. Box 80260, Jeddah 21589, Saudi Arabia. Tel.: +966 568751075.

E-mail addresses: helbassossy@zu.edu.eg, helbassossy@kau.edu.eg (H.M. El-Bassossy).

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in the pathogenesis of many inflammatory diseases [11,12] including atherosclerosis [12].

Caffeic acid phenethyl ester (CAPE), an active ingredient of honeybee propolis extract, is a flavonoid-like compound that has been used as a traditional medicine in the Far East treating various ailments [13] due to its anti-inflammatory [14] and antioxidant properties [15], CAPE pharmacological effects are thought to be mediated in 5-lipooxygenase inhibition. Previously, it was shown that CAPE inhibits 5-lipooxygenase enzyme in micromolar concentration [10]. CAPE also has immunomodulatory [16], anti-carcinogenic [17], neuroprotective [14] and anti-atherosclerotic effects [18]. CAPE has the ability to suppress lipid peroxidation [19] and is also a potent inhibitor of NF-κB activation [20].

Precisely the purpose of the present work is to examine the potentially protective effect of CAPE as a selective 5-lipoxygenase enzyme inhibitor against diabetes-induced atherosclerotic manifestations and illustrate the mechanism of this possible protection.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 85–105 g for insulin resistance section and 135–155 g for the insulin deficiency section (Faculty of Pharmacy, Zagazig University, Zagazig, Egypt) were housed in clear polypropylene cages (four rats per cage) and kept on equal duration of dark-light cycle, under constant environmental conditions. Rats received normal rodent pellet diet and water *ad libitum*. Experimental protocol was approved by Zagazig Ethical Committee for Animal Handling.

2.2. Study protocol

Rats were randomly divided into four experimental groups (eight animals each) in each model as follows; control, caffeic acid phenethyl ester-treated control (C-CAPE), insulin deficient (ID) or resistant (IR), and caffeic acid phenethyl ester-treated insulin deficient (ID-CAPE) or resistant (IR-CAPE). ID was induced by intraperitoneal injection of streptozotocin (STZ, 50 mg kg^{-1}). ID was confirmed by the development of postprandial hyperglycemia (300–400 mg dl^{-1}) two weeks after STZ injection. IR was induced by adding fructose (10%) to every day drinking water for 12 weeks. We started with rats weighting 85–115 g as they were found to be less resistant to induction of insulin resistance in our previous work [21,22]. Rats with insulin level above 14 μg l^{-1} were considered IR rats. Insulin deficiency was induced by a single intraperitoneal injection of STZ (50 mg kg^{-1}). Rats with moderate hyperglycemia (postprandial glucose level between 250 and 350 mg dl^{-1}) were used in this study. CAPE (30 mg kg^{-1} day^{-1}) was daily administered by oral gavage as suspension in distilled water in the last 6 weeks of study. Control group received distilled water as a vehicle instead.

At the end of the study (twelve hours after the last CAPE administration), body weight and blood pressure (BP) were recorded. Then, the whole blood was collected (under light ether anesthesia) from the retro-orbital plexus, glucose level was determined in blood and the rest of blood was centrifuged (3000*g*, 4 °C, 20 min) to separate serum. Serum was divided into aliquots and stored at −20 °C till analyzed for insulin level, TNF-α and AGEs. The descending thoracic aorta was carefully excised through abdominal opening, placed in a Petri dish containing cold Krebs–Henseleit buffer (KHB) composed of (in mM): NaCl 118.1, KH_2PO_4 1.2, KCl 4.69, $NaHCO_3$ 25.0, $MgSO_4$ 0.5, glucose 11.7 and $CaCl_2$ 2.5. The aorta was cut into 3 rings (of 3 mm length) after cleaning the connective tissue and fat. For each animal, one aortic ring was used for studying vascular reactivity. The two other aortic rings were fixed in 10% formalin for immunohistochemistry techniques to measure HO-1 expression and collagen deposition.

2.3. Biochemical analysis

Glucose was determined in tail blood by a glucose meter (Bionime GmBH) using noble metal electrode strips. Serum AGEs was measured as previously described [23]; the serum was diluted 1:15 with saline and the fluorescence intensity (λex = 370, λem = 440 nm) was recorded by LS45 fluorescence spectrophotometer (PerkinElmer®, Cairo, Egypt). Serum insulin level was measured by enzyme-linked immunosorbent assay (ELISA, Millipore, Cairo, Egypt) that uses plate coated with monoclonal anti-rat insulin antibodies. Serum TNF-α level was determined by ELISA using Quantikine® kit (R&D systems, Cairo, Egypt) using rat TNF-α and antibodies raised against the rat TNF-α.

2.4. Blood pressure measurement

Blood pressure (BP) was indirectly recorded in a slightly restrained conscious rat by tail cuff method as described in our previous work [22]. Rats were trained on the restrainers and the warming chamber for 3 days before measurements for 20 min per day. BP measurements were performed between 7:00 AM and 12:00 AM by the same investigator. After 10 min stabilization in the warming chamber at 35 °C, automated inflation–deflation cycle was repeated 10 times. The mean of 5 readings within a 10 mmHg range was considered as the blood pressure.

2.5. Vascular reactivity

Isolated aortic rings were suspended in individual organ chambers (30 ml) containing KHB at 37 °C and aerated with 95% oxygen, 5% carbon dioxide under 11.2 mN resting tension. Isometric force transducers (Biegestab K30, Hugosachs Elektronik, March, Germany) were used to determine rings tension. The force transducers were connected to a four channel Power Lab Data Interface Module connected to a PC running Chart software (v7, ADI Instruments, Cairo, Egypt). Rings were initially equilibrated for 60 min. During this time, the bath solution was changed twice. Before starting vascular reactivity measurements, vessel viability was assessed by exposing arteries to KCl (80 mM) twice to ensure stable responses. For studying the contractile responsiveness of aorta, cumulative concentrations of KCl (10–100 mM) or phenylephrine (PE, 10–9 M to 10–5 M) were added to the organ bath and the response was recorded.

2.6. Haem oxygenase-1(HO-1) immunohistochemistry

Immunohistochemistry of HO-1 protein in rat aortic sections was performed using the method described by Szocs et al. [24] with slight modification. It depends upon the use of a primary antibody to detect the HO-1 in sections followed by fluorophore conjugated antibody. The sections were deparaffinised in xylene and rehydrated. Endogenous peroxidase activity was saturated by incubating slides in 3% H_2O_2 solution in cold methanol for 30 min. Antigen retrieval solution was performed by incubation with citrate buffer at 90 °C for 30 min followed by rinsing with PBS. After blocking non-specific binding, sections were incubated with 50 μl of the rabbit anti-heme oxygenase-1 primary antibody (dilution 1:200 in blocking solution) overnight in cold room. Then sections were rinsed in 3 changes of 1 × PBS (5 min each) then incubated with 50 μl of the Alexa fluor conjugated gout secondary antibody (dilution 1:10,000 in blocking solution) for 2 h in dark then rinsed with 3 changes of 1 × PBS (5 min each) then the coverslip was mounted with 20 μl of fluorescence mounting media and left in dark before