Egyptian Journal of Basic and Applied Sciences xxx (2017) xxx–xxx

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Contents lists available at ScienceDirect

Egyptian Journal of Basic and Applied Sciences

journal homepage: www.elsevier.com/locate/ejbas

Full Length Article

Antioxidant and anti-inflammatory effects of dimethyl fumarate in hypercholesterolemic rabbits

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ARTICLE INFO

Article history:
Received 22 May 2017
Received in revised form 4 July 2017
Accepted 5 July 2017
Available online xxxx

Keywords:
Dimethyl fumarate
Inflammation
CETP
Hypercholesterolemia
Atherosclerosis
Oxidative stress

ABSTRACT

The present study aimed to determine the possible beneficial effects of dimethyl fumarate (DMF) against oxidative stress and inflammation in hypercholesterolemic rabbits. Twenty four New Zealand male rabbits were randomly allocated into 4 groups as the following: **Group I** (control): rabbits received standard rabbit chow; **Group II** high cholesterol diet (HCD): rabbits received 1% cholesterol-enriched chow for 4 weeks; **Group III** (HCD-DMF): rabbits received 1% cholesterol-enriched chow and administered DMF (12.5 mg/kg/day, orally) for 4 weeks; **Group IV** (DMF): rabbits received standard chow plus DMF (12.5 mg/kg/day, orally) for 4 weeks. At the end of experiment (day 30), blood samples were collected for measuring serum total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C) and C-reactive protein (CRP). In addition, the aorta was removed for measurement of malondialdehyde (MDA), superoxide dismutase (SOD), mRNA expression of cholesteryl ester transfer protein (CETP) and histological assessment of intima/media (I/M) ratio. HCD-fed rabbits showed significant increases in TGs, TC, low-density lipoprotein cholesterol (LDL-C), aortic MDA and aortic I/M ratio levels while they significantly exhibited a reduced SOD level relative to control animals. Moreover, HCD rabbits demonstrated upregulated mRNA expression of CETP. DMF administration significantly decreased HCD-induced elevations in serum TC and LDL-C. Additionally, DMF decreased aortic level of MDA while increased SOD level. Moreover, DMF significantly downregulated mRNA expression of CETP and reduced the elevation in I/M ratio.

In conclusion, this study suggests that DMF has the ability to improve HCD-induced vascular irregularities, possibly via its anti-inflammatory and antioxidant effect.

Introduction

Hypercholesterolemia is one of the most important risk factors in the development and progression of atherosclerosis, which is a major cause of mortality in world population [1]. Hypercholesterolemia has been shown to increase reactive oxygen species (ROS) production, enhancing oxidative stress. ROS react with lipoproteins specifically low density lipoprotein (LDL), producing oxidized LDL (ox-LDL) [2]. Oxidative modification of LDL plays an important role in inflammation and atherogenesis [3–5]. Thus, antioxidant supplementation could possess a possible protective action against ox-LDL-mediated inflammation, a cornerstone of atherosclerosis.

Dimethyl fumarate (DMF), an ester of fumaric acid (FA), has been recently approved by the FDA for the treatment of relapsing/remitting multiple sclerosis (MS) under the brand name Tecfidera. It is presently employed for the treatment of psoriasis [6].

DMF is rapidly metabolized in the gastrointestinal tract by esterase enzyme into monomethyl fumarate (MMF) which is the main metabolite exerting the therapeutic effects. Half-life of DMF is about 12 min while that of MMF is 36 h. Therefore, orally administered DMF is not detected in the blood stream [7]. DMF has been reported to exhibit an antioxidant effect due to its ability to enhance expression of multiple antioxidant enzymes, including superoxide dismutase (SOD), heme oxygenase-1 (HO-1), glutathione-S-transferase (GST) and catalase (CAT), resulting in reduced oxidative stress possibly via activation of the nuclear factor (erythroid-derived2)-like2-Nrf2 transcriptional pathway, which is the principal regulator of antioxidant enzymes [8] . In addition to possessing antioxidant effect, DMF has anti-inflammatory effect as

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http://dx.doi.org/10.1016/j.ejbas.2017.07.003
2314-808X/

Please cite this article in press as: Nour OA et al. Antioxidant and anti-inflammatory effects of dimethyl fumarate in hypercholesterolemic rabbits. Egyp. Jour. Bas. App. Sci. (2017), http://dx.doi.org/10.1016/j.ejbas.2017.07.003

it reduces inflammatory gene expression and increases anti-inflammatory gene expression [9–11]. Therefore, the present study aimed to investigate the role of anti-inflammatory and antioxidant properties of DMF in preventing oxidative stress and inflammation induced by atherogenic diet in rabbits.

Materials and methods

Materials

Cholesterol, DMF and thiobarbituric acid (TBA) were purchased from Sigma Aldrich chemical Co. (St. Louis, MO, USA). All other chemicals used in this study were of fine analytical grade.

Animals

Adult male New Zealand white (NZW) rabbits with an average body weight of 1.5–2 kg were obtained from Urology and Nephrology Center, Mansoura University, Egypt. The animals were individually housed in cages with food and water available, maintained under standard conditions of temperature about 25 ± 2 °C with a 12 h on/off light schedule. Standard diet pellets were prepared weekly (El Nasr Chemical Co., Abou-Zaabal, Cairo, Egypt). Animals were handled according to rules of Committee of Ethics of Scientific Research, Faculty of Pharmacy, Mansoura University, Egypt. This is in agreement with the Principles of Laboratory Animal Care (NIH publication No. 85–23, revised 1985).

Experimental protocol

Rabbits were randomly distributed into four groups each containing 6 rabbits as the following: **Group I** (control), rabbits received standard rabbit chow; **Group II** (HCD), rabbits received 1% cholesterol-enriched chow for 4 weeks [12]; **Group III** (HCD-DMF), rabbits received 1% cholesterol-enriched chow and administered DMF (12.5 mg/kg/day, orally) for 4 weeks, the dose was converted from rat to rabbit according to Paget and Barnes [13,14], **Group IV** (DMF), rabbits received standard chow plus DMF (12.5 mg/kg/day, orally) for 4 weeks. DMF was given to rabbits as a suspension in 0.5% carboxymethyl cellulose (CMC). Both control and HCD groups received 0.5% CMC (1 mL/kg/day, orally) during the treatment period.

Blood samples were collected at the end of experiment (day 30) from marginal ear vein for measurement of serum triglycerides (TGs), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and C-reactive protein (CRP). To obtain serum, blood samples were allowed to clot for 90 min before centrifugation at 3000*g*. Rabbits were euthanized by an overdose of sodium pentobarbital and exsanguinated for aortic assessment. Aorta was removed for measurement of aortic malondialdehyde (MDA), superoxide dismutase (SOD), histopathological examination, intima/media (I/M) ratio and mRNA expression of cholesteryl ester transfer protein (CETP).

Assessment of serum lipid profile

Allain and co-workers method was used for determination of TC [15] while Fredrickson and colleagues method was used for determination of TGs [16]. Finley et al. method was designed to determine serum HDL-C enzymatically [17]. According to the method described by Friedewald and co-workers, low-density lipoprotein cholesterol (LDL-C) was calculated [18]. Commercial kits (Stanbio Laboratory, Boerne, Texas, USA) were used.

Assessment of serum CRP

For quantitative assessment of CRP in serum, rapid latex agglutination test was used. Commercial kit (Biomed Diagnostics, Badr city, Egypt) was used.

Assessment of aortic antioxidant status

Preparation of aortic homogenate

The aortas were isolated, weighed and homogenized in phosphate buffered saline (PBS, pH 7.4) as 10% (w/v) using a mini handheld homogenizer (Omni international, USA). The supernatants collected after centrifugation of tissue homogenates (1000*g*, 4 °C, 10 min) were used for assay of MDA and SOD.

Assessment of aortic MDA levels

According to the method of Ohkawa et al., thiobarbituric acid reactive substances (TBARS) were measured as MDA. The concentrations were expressed as nmol/g tissue [19].

Assessment of aortic SOD activity

According to the method of Marklund, the enzymatic activity of SOD was assessed. SOD activity was measured by the degree of inhibition of the auto-oxidation of pyrogallol at an alkaline pH by SOD. The change in absorbance was measured at 420 nm and activity was expressed as U/g tissue [20].

Quantitative real-time polymerase chain reaction (RT-PCR)

The aorta was rapidly isolated, weighed and preserved in RNA Later (Qiagen, Germany) (50–100 mg tissue/1 ml RNA later). Total RNA was extracted from aortic tissues using TRIzol reagent (Invitrogen, USA). One microgram from each sample RNA was reverse transcribed into complementary DNA (cDNA) by using revert aid first strand cDNA synthesis kit (Thermo Scientific Rockford, IL, USA). RT-PCR was performed with Rotor Gene Q thermocycler (Qiagen, Hilden, Germany), using HOT Firepol Evagreen qPCR mix plus kit (Solis BioDyne, Tartu, Estonia).

The mRNA level of CETP was normalized relative to GAPDH ribosomal RNA (Housekeeping gene) in the same sample. The sequences of sense and antisense primers used for amplification are shown as the following: (see Table 1).

The results were expressed as an *n*-fold change of the relative expression levels of target genes from control group using ΔΔCt method [21].

Histopathological analysis

At the end of the experiment, the thoracic aorta was carefully removed, fixed in 10% neutral buffered formalin solution (pH 7.4), inserted in paraffin wax, sectioned transversely (5 μm) and

Table 1
The sequences of sense and antisense primers used for amplification.

	Sense	Antisense	Amplicon size
GAPDH	CATCATCCCTGCCTCCACT	GCCTGCTTCACCACCTTCTT	180
CETP	AGACATCGGGTGGACATTT	TTCTTGTGCGTGAAGTGACC	93

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