Modulatory effect of curcumin on methionine-induced hyperlipidemia and hyperhomocysteinemia in albino rats

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The present study was designed to investigate the antioxidant effect of curcumin on methionine-induced hyperlipidemia and hyperhomocysteinemia in Wistar rats (200-250 g) of either sex. The vehicle control rats were treated with 1% Tween 80 in normal saline (2 ml/kg, po) for 30 days. Hyperlipidemia and hyperhomocysteinemia was induced by methionine administration (1 g/kg, po) for 30 days. A significant increase in total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C) and homocysteine levels in serum and thiobarbituric acid reactive substances (TBARS) levels in heart homogenates were observed with a concomitant decrease in serum high density lipoprotein (HDL-C) levels in pathogenic control (i.e. group II) rats, as compared to vehicle control (i.e. group I) rats. Further, curcumin (200 mg/kg, p.o.) treatment in methionine treated rats for 30 days significantly decreased the total cholesterol, triglycerides, LDL-C and homocysteine levels in serum and TBARS levels in heart homogenates and increased serum HDL-C levels, as compared to pathogenic control (i.e. group II) rats. The results of biochemical observations were supplemented by histopathological examination of rat’s aortic section. The results of test drug were comparable to that obtained with folic acid (100 mg/kg, p.o.). The results suggest that curcumin has significant antihyperlipidemic and antihyperhomocysteinemic effect against methionine-induced hyperlipidemia and hyperhomocysteinemia in rats.

Keywords: Curcumin, Homocysteine, Hyperlipidemia, Methionine

India has a rich history of using plants for medicinal purposes. Several plant products are known to exhibit creditable medicinal properties for the treatment of various ailments and need to be explored to identify their potential application in prevention and therapy of human ailments. Notable among these, the active principle of turmeric (Curcuma longa Linn., Zingiberaceae) i.e. curcumin has been reported to have anti-hypercholesterolemic, anti-inflammatory as well as anticancer activities. It is also known to scavenge free radicals. In recent times, Traditional Indian System of Medicine uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Ramaswamy et al. have reported that curcumin blocks homocysteine-induced endothelial dysfunction in porcine coronary arteries. Reactive oxygen species (ROS) are continuously produced during normal physiologic events and removed by antioxidant defence mechanisms. Under pathologic conditions, ROS are overproduced and result in lipid peroxidation and oxidative stress. An imbalance between ROS production and antioxidant defense mechanisms leads to oxidative modification in the cellular membrane or intracellular molecules. High homocysteine levels in the blood cause cholesterol to change to oxidized low-density lipoprotein, which damages the arteries by creation of plaque inside artery walls. Some forms of homocysteine have been shown to damage the inner walls of blood vessels directly. Hyperhomocysteinemia, a newly emerged independent risk factor for coronary artery diseases, is one of the main factors that cause various diseases, such as atherosclerosis, diabetes, cancer, and some other aged-related illnesses including Alzheimer’s disease. Homocysteine is formed by demethylation of the essential amino acid methionine. Thus, an imbalance in dietary methionine may contribute to the development of atherosclerosis by increasing homocysteine levels. Hirche et al. have reported that dietary methionine induces hypercholesterolaemia at least in part via an enhanced hepatic cholesterol synthesis. In the present study, we first report the lipid and homocysteine lowering effects of curcumin on methionine induced-hyperlipidemia and hyperhomocysteinemia in rats.
Materials and Methods

Chemicals—Curcumin powder (with purity of 95.42%) was obtained from Kancor Flavours and Extracts Ltd, Angamally, South Kerala, India (sample code TUP 736A).

Methionine and folic acid were obtained from CDH, Bombay. All other chemicals used were of analytical grade and were obtained from Sigma Chemicals (St Louis, MO, USA). Double distilled water was used for all biochemical assays. For oral administration, 200 mg curcumin was suspended in 1% Tween 80 in normal saline.

Experimental animals—The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Hamdard University, New Delhi, which was registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India (Registration no. 173/CPCSEA, dated 28 January, 2000). Male adult Wistar albino rats, weighing 200–250 g, were procured from the Central Animal House Facility, Hamdard University, New Delhi and were acclimatized under standard laboratory conditions at 25° ± 2°C, RH 50 ± 15% and (12 hr light/dark cycle) for 7 days. Commercial pellet diet (Nav Maharstra Chakan Oil Mills Ltd, Delhi, India) and water were provided ad libitum. After acclimatization, 40 rats were randomly divided into five groups with eight animals each and subjected to respective treatment. Group I (vehicle control) received only Tween 80 (1%) in normal saline (2 ml/kg, po) for 30 days. Group II (Pathogenic control) rats were administered with methionine (1 g/kg, po) for 30 days. Group III (curcumin per se) rats received only curcumin (200 mg/kg body wt) orally for 30 days. Group IV (folic acid per se) rats received only folic acid (100 mg/kg body wt) orally for 30 days. Group V (curcumin treatment) rats received curcumin (200 mg/kg body wt) orally for 30 days co-administered with methionine. Group VI (folic acid treatment) rats received folic acid (100 mg/kg body wt) orally for 30 days co-administered with methionine.

After 30 days of treatment schedule, blood samples were collected from retro-orbital plexus using micro-capillary technique from rats of all the groups after overnight fast and serum was separated for biochemical estimation. After blood collection, all animals were sacrificed by cervical dislocation and heart was dissected out for biochemical estimation and aorta for histopathology.

Biochemical analysis—Serum homocysteine levels were estimated using the methods of Primus et al. Total cholesterol and triglycerides levels in serum were estimated by the methods of Demacher et al. and Foster & Dunn respectively, using commercial diagnostic kits from SPAN Diagnostics, Udhna, Surat, India. HDL-C content was estimated by the method of Burstein et al. using a commercial diagnostic kit from Reckon Diagnostics Pvt. Ltd. Baroda, India. Homogenate (10%) of heart tissue in ice cold KCl (0.15 M) was used for the assay of the malondialdehyde (MDA) according to the method of Ohkawa et al.

Histopathological studies—At the end of the experiment, aortic tissues from all the groups were subjected to histopathological studies. The tissues were fixed in formalin (10%), processed following routine method and embedded in paraffin wax. Paraffin section (5 μm) were cut on glass slides and stained with hematoxylin and eosin (H & E) after dewaxing, and examined under a light microscope.

Statistical analysis—Statistical analysis was carried out using Graphpad Prism 3.0 (Graphpad software; San Diego, CA). All data were expressed as mean ± SE. Groups of data were compared with an analysis of variance followed by Dunnett t test. Values were considered statistically significant at P<0.01

Results

Methionine treated (i.e. group II) rats showed a significant increase in total cholesterol, triglycerides and LDL-C levels and a decrease in HDL-C levels, as compared to vehicle control rats (i.e. group I). Curcumin (group IV) and folic acid (group V) treatment showed a significant decrease in total cholesterol, triglycerides and LDL-C levels and a significant increase in the HDL-C levels, as compared to pathogenic control (i.e. methionine only treated) rats (Table 1).

Methionine treated (i.e. group II) rats showed significant increase in serum homocysteine and myocardial TBARS levels, as compared to vehicle control (i.e. group I) rats. Curcumin (group IV) and folic acid (group V) treatment showed a significant decrease in serum homocysteine and myocardial TBARS levels, as compared to pathogenic control (i.e. group II) rats. However, no significant changes in serum homocysteine and myocardial TBARS levels were observed with curcumin and folic acid per se rats (Table 2).
Histopathological studies—Photomicrograph of vehicle control group revealed a normal architecture with regular morphology of aorta (Fig. 1a). Photomicrograph of pathogenic control group showed intimal thickening characterized by proliferation of smooth muscle cells and increased amount of matrix. There were disruption of fibrillar pattern in the medium and diffuse medial calcification (Fig. 1b). Photomicrograph of curcumin per se group showed normal aortic intima (Fig. 1c). Photomicrograph of folic acid per se group showed normal aortic intima (Fig. 1d). Photomicrograph of curcumin treated pathogenic group showed a decrease in intimal thickening of aorta (Fig. 1e). Photomicrograph of folic acid treated pathogenic group showed normal architecture with regular morphology of aortic intima (Fig. 1f).

Discussion

The present study was designed to assess the anti-hyperhomocysteinemic and anti-hyperlipidemic effects of curcumin in animals treated with methionine. Our study demonstrated an increase in homocysteine, total cholesterol, triglycerides and LDL-C levels in serum and TBARS levels in myocardial homogenates in methionine-treated animals. Further, a notable decrease in HDL-C levels was also seen in animals treated with methionine. Homocysteine, a thiol containing amino acid derived from demethylation of dietary methionine, may generate partially reduced ROS that are able to stimulate the lipid peroxidation involved in atherosclerotic process. Thus, an imbalance in dietary methionine may contribute to the development of atherosclerosis by increasing homocysteine levels. The mechanisms associated with homocysteine-induced endothelial dysfunction are mediated by increased oxidative stress, leading to increased levels of oxidized LDL and reduced NO availability. This concurs with the present findings wherein the levels of TBARS (a measure of MDA) were found to be significantly increased in animals subjected to methionine.

Antioxidant activity of curcumin has been reported as early as 1975. It acts as a scavenger of oxygen free radicals. In vitro, curcumin can significantly inhibit the generation of reactive oxygen species (ROS) such as superoxide anions, H₂O₂, and nitrite radical generation by activated macrophages, which play an important role in inflammation. Curcumin reduces serum and liver cholesterol levels in mice and also reported to have anti-inflammatory activity in standard animal models. It has been reported by Ruby et al. in 1995 while studying the antitumor and antioxidant activity of natural curcuminoids that curcumin inhibits the generation of ROS.
Superoxide radicals. Curcumin also decreases lipid peroxidation in rat liver microsomes, erythrocyte membranes, and brain homogenates. Because ROS have been implicated in the development of various pathological conditions, curcumin has the potential to control these diseases through its antioxidant activity. Several studies have reported the antioxidant property of curcumin, augmenting endogenous antioxidant levels.

The effect of curcumin and/or its parent plant *Curcuma longa* on cardiovascular systems has recently received much attention. It has been reported that curcumin improves the left ventricular function in pressure overloaded rabbits, and that curcumin inhibited the development of atherosclerosis in the apoE/LDLR double knockout mice. It has also been reported that the aqueous extract of *Curcuma longa* shows significant protective effect on ischemia–reperfusion induced myocardial injuries in rats and that water and methanol extracts of five *Curcuma* drugs exhibit significant effect on vasomotion in isolated rat aorta. Recently, Yang has reported that curcumin (1 to 25 μM) produces a concentration-dependent inhibition of platelet-derived growth factor (PDGF)-elicited vascular smooth muscle cell (VSMC) migration, proliferation, and collagen synthesis assessed by chemotaxis, [3H]thymidine incorporation, and [3H]-l-proline incorporation, respectively. It is reported recently that curcumin significantly blocks homocysteine-induced superoxide anion production and nitric oxide synthase down-regulation in porcine coronary arteries.

Present study revealed that curcumin modifies various biochemical markers in methionine-induced hyperhomocysteinemia and hyperlipidemia in rats. Curcumin treatment for 30 days in hyperhomocysteinemic rats produced significant decrease in homocysteine, total cholesterol, LDL-C and triglycerides levels in serum and TBARS levels in myocardial homogenates and a significant increase in serum HDL-C levels, as compared to pathogenic hyperhomocysteinemic rats, thus, effectively protected cell functions and structure suggesting cardioprotective potential of curcumin.

The results of biochemical observations were supplemented by histopathological examination of rat's aortic section. Pathogenic control group i.e. Fig. 1 — Histological examination of aorta in experimental animals. (a) – Vehicle control group (Group I) rat showing normal architecture with regular morphology of aorta (400X); (b) – Pathogenic control group (Group II) rat showing intimal thickening characterized by proliferation of smooth muscle cells and increased amount of matrix. There were disruption of the fibrillar pattern in the media and diffuse medial calcification (400X); (c) – Curcumin (200 mg/kg) per se group (Group III) rat showing normal aortic intima (400X); (d) – Folic acid (100 mg/kg) per se group (Group IV) rat showing normal aortic intima (400X); (e) – Curcumin (200 mg/kg) treated group (Group V) rat showing decrease in intimal thickening of aorta (400X); (f) – Folic acid treated group (Group VI) rat showing normal architecture with regular morphology of aortic intima (400X).
superoxide radicals. Curcumin also decreases lipid peroxidation in rat liver microsomes, erythrocyte membranes, and brain homogenates. Because ROS have been implicated in the development of various pathological conditions, curcumin has the potential to control these diseases though its antioxidant activity. Several studies have reported the antioxidant property of curcumin, augmenting endogenous antioxidant levels. Curcumin also inhibits the induction of nitric oxide synthase in activated macrophages and down regulates nitric oxide formation.

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The results of biochemical observations were supplemented by histopathological examination of rat’s aortic section. Pathogenic control group i.e. group II showed high subintimal deposits of foam cells and extracellular lipids showing the extension and thickness of the aortic arch. However, curcumin and folic acid treatment reduced the intimal thickening of aortic arch. Furthermore, aortic sections of curcumin and folic acid *per se* groups were found to be normal.

These observations indicated that curcumin may have therapeutic potential in oxidative stress and cellular damage mediated by methionine-induced hyperlipidemia and hyperhomocysteinemia.

In conclusion, the present study showed that chronic administration of curcumin (200 mg/kg) along with methionine prevented the rise of serum lipids and homocysteine levels in rats. Individuals with elevated blood levels of either cholesterol or homocysteine are at high risk of CVS disease. Thus, use of curcumin may be useful in the treatment of cardiovascular diseases in which atherosclerosis plays a major role.

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