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Original Article

Attenuating Effect of Curcumin on Diet-induced Hypercholesterolemia in Mice

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Background and Aims: Atherosclerosis is currently a chronic disease in which cholesterols accumulate in large arteries. Many genes such as liver X receptor α (LXRα) are involved in the cholesterol homeostasis. Curcumin, the main active polyphenol component derived from *Curcuma longa*, contribute to anti-inflammation and antioxidant in the treatment of atherosclerosis. Thus, this study intended to determine the role of curcumin in the cholesterol biosynthesis and LXRα gene expression in mice.

Materials and Methods: This study examined the effects of curcumin on the gene expression via LXRα in monocytes of hypercholesterolemia mice, which were treated with the curcumin oral gavage at the dose of 15 mg/kg for 4 weeks, measured using real time polymerase chain reaction. To further investigate the effects of curcumin in hypercholesterolemia as well as non-hypercholesterolemia mice, the total cholesterol, triglyceride, low density lipoprotein and high density lipoprotein in plasma have been also measured.

Results: The average has been calculated by SigmaPlot software, showing that LXRα gene expression causes no significant difference between the control group and the experimental groups ($p<0.05$). Although, treatment with the curcumin decreases cholesterol, total cholesterol and low density lipoprotein of plasma level and significantly increases high density lipoprotein cholesterol of plasma level.

Conclusions: Our findings suggest that curcumin has a special effect on lipid metabolism by increasing the reverse cholesterol transport mechanisms and decreasing low density lipoprotein in the blood. In conclusion, curcumin seems to have potentials to be used as a supplement.

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Introduction

Atherosclerosis is a progressive disease that affects large arteries and the primary cause of fifty percent of human deaths worldwide. Oxidized low density lipoprotein (ox-LDL) is an autoantigen that produces a local pro-inflammatory response [1]. Monocytes are the first inflammatory cells, which have the ability to take up modified low density lipoprotein LDL and regulated by cellular cholesterol content [2]. Cholesterol turnover is closely related to expression of many genes. Many studies show that the liver X receptor α (LXRα) represents important regulation of genes engaged in monocytes and cholesterol biosynthesis [3]. The LXRs induce a rise of genes that protect cells from increased cholesterol transcription. The LXRs are nuclear receptors that are activated by concentration of cholesterol. The LXRs directly bind to specific sequences of target genes as an retinoid X receptor heterodimer [3].

Curcumin is the main active polyphenol extracted from *Curcuma Longa*. Curcumin plays as an anti-inflammation and antioxidation in the treatment of atherosclerosis [4, 5]. Several studies show that the antioxidant activity of curcumin is due to the presence of *o*-methoxy phenolic OH increasing the reactivity of curcumin [6]. In addition, curcumin regulates the expression of nuclear receptor control of the cholesterol homeostasis. Furthermore, it causes affective proteins involved in cholesterol metabolism and reverse cholesterol transport (RCT) pathway [7-9].

The present study attempts to determine the effect of curcumin on the cholesterol homeostasis by the monocyte cells and investigate the effect of curcumin on the oxidized LDL-induced cholesterol accumulation in monocytes, to determine the effect of curcumin on the expression of LXRα and elucidate the molecular basis underlying the Curcumin-mediated modulation in cholesterol accumulation.

Materials and Methods

Animals

This study conforms to the guide for the care and use of laboratory animals published by the US National Institutes of Health [10], and the Animal Care and Utilization Committee of University of Parand, Iran, approved all the animal experiments. Mice were maintained on a 12:12-h light: dark cycle and fed a regular low-fat diet (Chow) that contained 4.5% fat by weight (0.02% cholesterol). 40 nine-week-old male mice were randomly selected and divided into 4 groups, which received daily oral administration of curcumin (15 mg/kg body weight) by gastric gavages [8]. After 4 weeks, mice were anesthetized with ketamine/ xylazine combination at a dose of (1:3), after that, their blood was collected for analysis.

Peripheral blood mononuclear cells (PBMCs) isolation

The isolation of PBMC was achieved after transferring the sample to tubes containing Ficoll solution (Baharafshan, F2637. Iran) and centrifuged at 300 g for 20 min. at 4˚C. Afterward, the buffy coat was slowly separated by a pipette and moved to RNAse free tube.

RNA extraction and real time polymerase chain reaction (PCR) Analysis

Total RNA was extracted from PBMC using Trizol (Invitrogen, USA). The purification of RNA was determined by the ratio of the absorbance at 260 nm and 280 nm (Nanodrop, Biotek, USA). RNA was stored at -70°C until real time PCR was performed. As we know RNA is highly unstable, it has been converted to complementary DNA (cDNA) by Takara kit (Japan). The mRNA transcripts expression levels were quantified by real time PCR. The real time PCR was performed by the SYBER Green PCR on Rotor Gene 6000 (Corbett, Australia). Direct detection of PCR product was monitored by measuring the fluorescence increased by binding SYBER Green to double-stranded DNA. Specific primers for the LXRα gene, which expressed in PBMC (Forward: AGAGCATCACCTTCCTCAAGG and Reverse: TGCTGATGGCAATGAGCAGAG) were designed by Allel ID (V6) software (Takapozist, Iran). The cycling parameters were 94°C for 15 min. by one cycle, follow by 45 cycles of 9°C for 15 s; 58°C for 30 s and 72°C for 30 s [9]. Efficiency of reaction was checked by LinRegPCR and delta delta CT formulas; the housekeeping GAPDH transcript was used to normalize the amount and quality of the RNAs.

Serum lipid profile analysis

Lipid blood profile levels were measured by clinical spectrophotometric and blood biochemical analyzer kit (Ziest chem Diagnostic, Iran).

Statistical analysis

The entire results are presented as means±S.D. Differences between the groups were determined by one-way ANOVA and Tukey Post-Hoc multiple comparison tests. The level of significance for all statistical analyses was set at $p<0.05$. Analysis was performed using SigmaPlot (V12.2) software.

Results

The effect of curcumin on lipid profile shows a significant decrease of total cholesterol (TC) level, a significant reduction of triglyceride (TG) levels, significant decline of LDL ($p\leq0.04$) and the significant increase in high density lipoprotein (HDL) in the curcumin group (group fed curcumin). Fig. 1A shows a significant decrease (42 mg/dl) on cholesterol in the group, which fed by curcumin against the control group (62.5 mg/dl), and indicates the effect of curcumin on the plasma cholesterol level. Fig.1B shows a significant decrease (70 mg /dl) in TG levels in a group fed by curcumin, compared with the control group (98.5 mg/dl). Fig.1C shows a significant decrease (3 mg/dl) in LDL in a group fed with curcumin, against the control group (7 mg/dl) and indicates the effect of curcumin on the plasma LDL level. A significant increase (142 mg/dl) in HDL is visible in a group fed by curcumin, compared with the control group (133 mg/dl) and showed the effect of curcumin on plasma HDL levels (Fig. 1D).

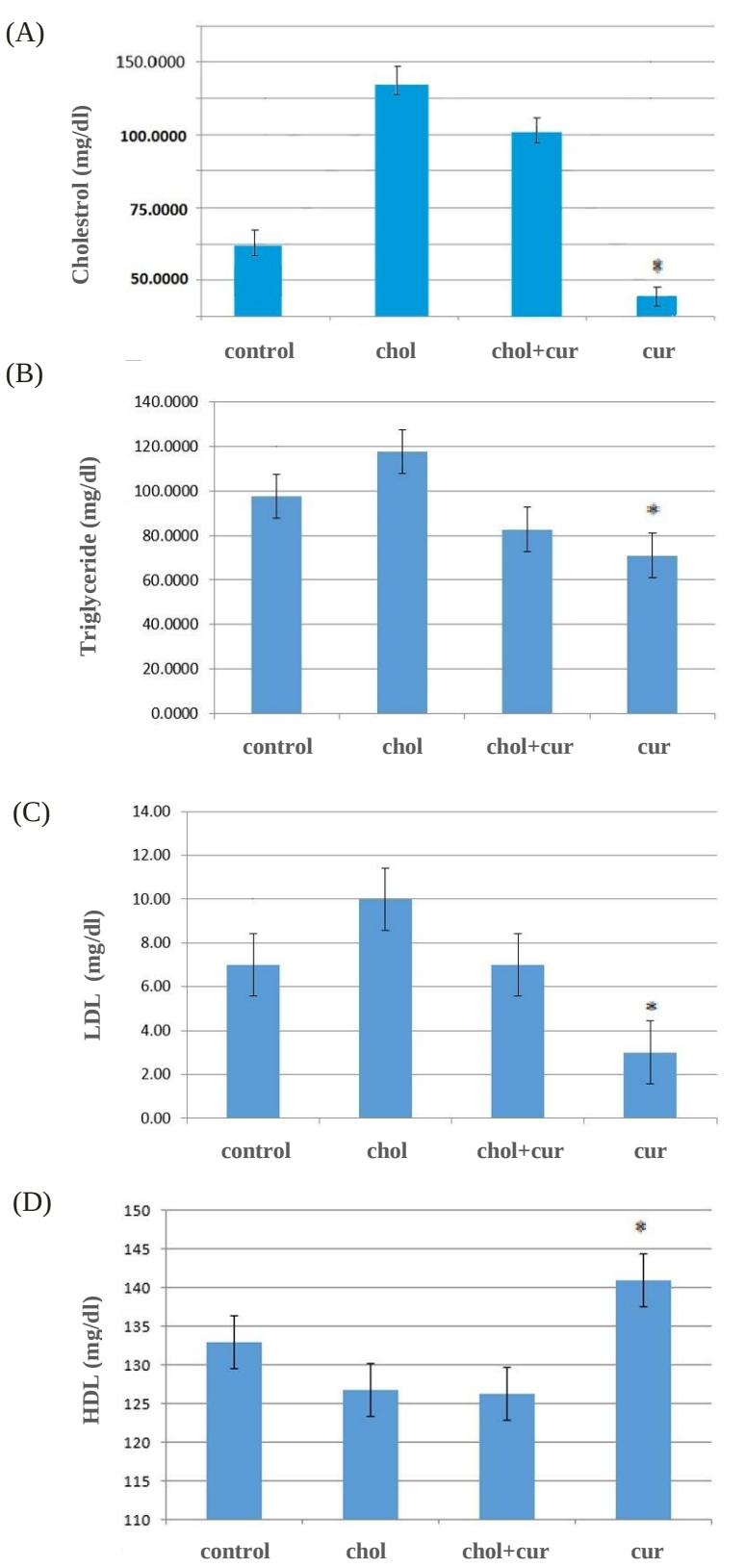

Fig.1. The effect of cholesterol and curcumin on the concentration of lipid profile as total cholesterol (A), triglyceride (B), LDL cholesterol (C) and HDL cholesterol levels (D). The left column shows the control group, The second column shows the cholesterol group which fed a regular low-fat chow diet that contained 4.5% fat in weight (0.02% cholesterol), the third column (the mice were fed cholesterol and curcumin simultaneous) and the last column shows the group which administration of curcumin (15 mg/kg body weight). Data are presented as means±SD; $p<0.05$; chol= cholestrol; cur= curcumin

Effect of curcumin on expression of LXRα

The results of LXRα gene expression showed no significant difference between the control group (the group, which were fed normal food) and the experimental groups. The diagram reported a 3.5-fold increase in the cholesterol group, in 1.36-fold increase in curcumin group and a 0.42-fold decrease in the curcumin-cholesterol group compared with the control group. Thus, the average threshold cycle for groups under investigation showed no significant changes. It should be noted that the delta threshold cycle values were inversely proportional to the amount of target gene expression (Fig. 2). Furthermore, the treatment with the curcumin had no significant effect (p=0.115) on LXRα gene expression.

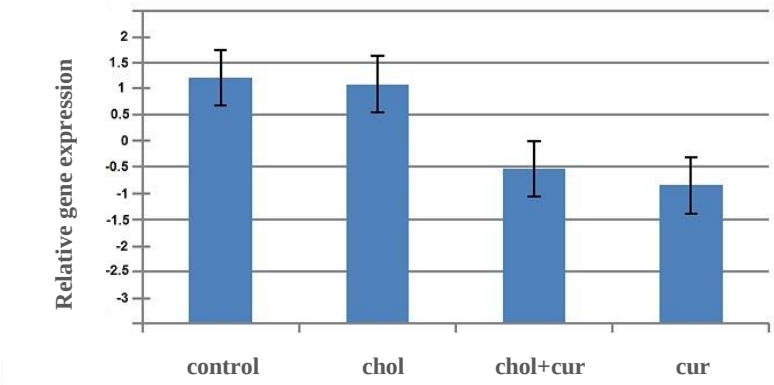

Fig. 2. Effects of curcumin on mRNA levels of the LXRα gene in the PBMCs. Mice were initially pretreated for 24 h with a regular low-fat chow diet that contained 4.5% fat in weight (0.02% cholesterol) and oral administration of curcumin (15 mg/kg body weight) by gastric gavages. The values were normalized with respect to the level of GAPDH mRNA. The results represent means±SD from duplicate determinations, representative of 3 independent experiments compared with control. The statistical difference in comparison with the control group was analyzed by one-way ANOVA followed by Tukey multicomparison test. chol= cholestrol; cur= curcumin

Discussion

In this study, it has been shown that curcumin cause significant decrease TC, TG, LDL, and the significant increase in the HDL level in the curcumin group. These results indicated the anti-inflammatory and antioxidative properties of the curcumin. In this study, the curcumin as LXRα gene inducing was used. According to our experiences, it had no significant changes in LXRα gene expression in the experimental groups. These discordant findings suggested a differential effect of curcumin. As we know, oxidative stress causes the endothelial cells to be opened in the artery wall and to enter the macromolecules, such as LDL, into the intima layer. In this layer, reactive oxygen species interaction with the LDL is oxidized and produced auto-genes [11] and activated the monocyte/ macrophage system. They

entered intima layer and converted to the foam cells, and necrotic core was to be shaped [12]. Many genes and proteins, such as LXR α, are involved in the formation of foam cells. The protective effects of curcumin, and the active polyphenol extracted from turmeric in atherosclerosis have been explained [13, 14]. However, the efficacy of curcumin and the possible molecular mechanism involved in atherosclerosis remain elusive. We have detected the novel molecular mechanisms underlying the atheroprotection of curcumin to affect the decrease of cholesterol, TG and LDL levels in monocytes of mice. The LXRα gene expression did not undergo a significant change. Our findings strongly suggest that curcumin has an effect to reduce lipids in monocytes of mice. In other studies, researchers have shown that curcumin can increase ATP-binding cassette transporter A1 (ABCA1) and LXRα gene expression [8] and they have shown the induced curcumin suppresses foam cell formation by LXRα gene expression. Moreover, they have explained the inhibition of the LXRα activation by LXR siRNA diminishes the curcumin-mediated upregulation of ABCA1 [8].

Therefore, our research and various studies show the opposite results and the reasons for the decisions of which are that*:* it seems that after LDL is delivered through scavenger receptors, cholesterol esters molecules of LDL in lysosomes develop into cholesterol as well as free fatty acids. Afterward, it frees cholesterol synthesis of 27-hydroxycholesterol in liver mitochondria by the CYP7α. It is one of the several produced-oxysterol in foam cells and occurs when the LDL level increases into monocytes. The concentration of oxysterol in foam cells greatly increase. That is the most important activator of the LXRα protein that increases the ABCA1 activity. Activation of the LXR alpha regulates its own promoter and binds to the LXRα promoter. Curcumin induces the activity of ABCA1 through LXR α transcription [15] and causes the excess cholesterol to be removed from monocytes of hypercholesterolemia mice though its transfer to HDL, which not only reduces the uptake of LDL and TG levels, but it also increases the HDL cholesterol levels. This would reduce the risk of atherosclerosis [8, 16]. Mice monocytes play an important role in the RCT by the activities of proteins like ABCA1 and ABCG1. The mechanism of monocyte subsets in human is different from mice [17]. Atherosclerosis is reversible by restoring cholesterol from monocytes to HDL, and there are correlations among high HDL, lower blood monocyte counts, and lowered risk for atherosclerosis [18]. Atherosclerosis is a progressive disease characterized by the accumulation of lipids in the large arteries and is a form of chronic inflammation, which is associated with the interaction of monocyte/ macrophage system and formation of m-LDL [19]. Evidence indicates the anti-inflammatory properties of curcumin on the LDL oxidation process. Nakagawa shows that treatment with curcumin causes a significant reduction in the LDL and TG level in serum. It means that the reduction of serum lipid can be associated with curcumin receivers. In addition, curcumin causes the uptake of lipids serum in THP-1

cells to increase [20]. Curcumin activates an effective marker in accumulation of cholesterol in cells. Treatment with the curcumin shows that curcumin is absorbed by monocyte/macrophage THP1, and metabolized into hexahydroxy curcumin. In parallel with the absorption of curcumin observed, the cholesterol is absorbed in THP-1 monocyte/ macrophage [21]. Nakagawa has shown that a dedicated vector within the cells may transport curcumin and cholesterol simultaneously [20]. In this study, it has been shown that curcumin decreases plasma TG and the LDL cholesterol levels, and significantly increases plasma HDL cholesterol levels, while curcumin has a wide therapeutic effect through various mechanisms such as ABCA1, ABCG1 and scavenger receptor B1 protein level.

Conclusion

Our findings suggest that treatment with curcumin has a specific effect on lipids metabolism. On the other hand, coordination between genes involved in lipid metabolism in mice is very complicated and this can explain the different results obtained by various researchers.

Conflict of Interest

The authors declare that they have no competing interests.

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