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Curcumin as an Environmental Potent Antioxidant Decreases Risk of Arthrosclerosis

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Background & Aims of the Study: Oxidative stress increases platelet-derived growth factor (PDGF) gene expression in endothelial cells that contributes to vascular dysfunction and atherosclerosis. Oxidative stress generates by dys-regulated redox balance between ROS producing systems and antioxidant systems. Also, Curcumin (Cur) as a main part of turmeric has anti- inflammatory, antioxidant, anticancer and antitumor effects. This study was conducted to test the Curcumin as an environmental potent antioxidant decreases risk of arthrosclerosis.

Materials and Methods: This experimental study was conducted during 2015 in Iran. Cultured bovine aortic endothelial cells were incubated with hydrogen peroxide (H_2O_2) (20, 40 and 80 µM) and Curcumin (10 µM) for 24h. Then, the level of PDGF gene expression was analyzed by Real-Time PCR in untreated and treated cells.

Results: The results demonstrated significant increase in the level of PDGF gene expression in H_2O_2 treated groups versus control. Also, treated groups with H_2O_2 -Curcumin showed notable decrease in the level of PDGF gene expression compared with H_2O_2 treated groups.

Conclusion: Our results support valuable data about the application of Curcumin for protection against atherosclerosis.

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Background

Atherosclerosis or plaque build-up in the arteries as one of the serious health problems causes by various conditions such as high blood pressure and high level of cholesterol (1). Recent studies demonstrated remarkable data about the role of oxidative stress in atherosclerosis (1,2). There is an increase on the level of ROS production during cardiovascular diseases (2). The initial step of atherosclerosis is production of free radicals which can induce endothelial cells dysfunction. Oxidation of LDL is the most important cause of oxidative stress. Two most sources of ROS are smooth muscle cells and macrophages (2,3). This fact suggests powerful ability of herbal antioxidant for reducing oxidative stress and risk of oxidative stress-induced atherosclerosis in capable person. Turmeric rhizome has yellow color and uses widely as a spice and for treating different pathogenic conditions such as body pain, intestinal worms, diarrhea, intermittent, fevers, hepatic disorders, biliousness, urinary discharges, dyspepsia, inflammations, constipation, leucoderma and amenorrhea (4-6). Furthermore, the potent effects of turmeric rhizome are related to its

components with strong anti-oxidant activities including Curcumin (7,8).

The levels of enzymatic anti-oxidant improve following Curcumin treatment in animals undergoing irradiation. Curcumin treatment before irradiation shows a significant increase in glutathione concentration and activities of both the glutathione peroxidase and superoxide dismutase in the irradiated mouse skin. Also, lipid peroxidation as a main mark of oxidative stress decreased in the skin of these animals (8). There is an evidence that shows Curcumin acts as potent free radicals scavenger in vivo in the brain. This antioxidant has neuroprotective effects against oxidative stress. Curcumin can inhibit parkinsonian neurotoxin –induced neuronal death in brain by anti-oxidant activity (9). In addition, Curcumin reduces DNA fragmentation, enzymes activities and lipid peroxidation in liver of lambda cyhalothrin (LCT)-intoxicated rats. Prevent DNA fragmentation, protect against oxidative stress and scavenge free radicals are the main results of Curcumin (10). Recently, based on the result of different study, Curcumin has significant effects on high fat diet and high cholesterol (4,5). Dietary Curcumin modulates high fat diet-induced atherosclerosis, steatohepatosis (4) and expression of genes involved in leukocyte adhesion and trans-endothelial migration in mice (5).

Aims of the study:

Our aim was determination of inhibitory effects of anti-oxidant on PDGF-mediated atherosclerosis in the endothelial cells which exposed to oxidative stress.

Materials & Methods

This experimental study was conducted during 2015 in Iran.

Cell culture

Cell lines of bovine aortic endothelial cells (BAECs) were cultured in DMEM (Gibco, Germany) containing FBS (Gibco, Germany) and penicillin/streptomycin (Gibco, Germany).

Incubation and Treatment

Suspension (containing 12×104 cells) was incubated for 24-48 h in CO_2 incubator (Jeltaghiz, Iran). Then, culture media of each well was exchanged with fresh culture media containing FBS and plate was incubated for 24-48h.

Also, all sample preparation was performed in a laminar flow hood (class II, Jeltaghiz, Iran). Cell sample was treated with Curcumin and H_2O_2 (Merck, Germany).

Cell collection

Cells were collected from culture after incubation (Incubator, NAPCO, USA). So, whole culture media was collected from surface of plate wells. Then, trypsin (Idehzist, Iran) was added to wells and plate was incubated. Culture media (Gibco, Germany) was added to wells while the component of wall was centrifuged (ROTOFIX32A, Hettich, Germany). Then, plate was used for RNA extraction and Real-Time PCR.

RNA extraction and Real-Time PCR

The aim of this method was the study of PGDF-β mRNA expression as a main marker for atherosclerosis. Total RNA was extracted from samples by RNeasy plus mini (QIAGEN, China), according to manufacturer's catalog.

RNA used for generated cDNA, using the Prime Script 1st strand cDNA Synthesis Kit (Takara, Japan) and amplified by real-time PCR, using a SYBR green Real time PCR (Takara, Japan) and the ABI Step One Plus real-time PCR instrument (Applied Biosystems, USA). Primers list are shown in the Table 1. Primers were designed by Allel ID version 6. Control mixture consisted of PCR mixture without cDNA.

Table 1) Primer sequences used for Real-Time PCR (5' to 3').

Primers	Genes
F; CAGAGCCAGCCGTGATT	**PDGF**
R: GCCACACCAGGGAAGTTAGC	
F: GGACACGGACAGGATTGACAG	**18S**
R: ATCGCTCCACCAACTAAGAACG	

Statistical analysis

The results of Real-Time PCR were analyzed by Delta CT formula and Rest version 2009.

The statistical analyses were performed, using the SPSS 20 (SPSS Inc., Chicago, IL, USA). One-way ANOVA (Tuky Multiple comparison) was used to test differences between various means (post hoc analysis LSD test). All experimental data were presented as the mean±SD. The level of significance for all tests was set at $P<0.05$.

Results

Real-Time PCR was used to evaluate PDGF-β gene expression in treated and untreated Bovine aortic endothelial cells. Comparative gene expression profiling analysis is useful in discovering differentially expressed genes associated with various agents and diseases (11). The levels of PDGF- β mRNA expression were different in experimental groups. The results showed in Table 2.

There were 6 groups in this research as; G1 (Control): untreated group, G2 (Group 2): treated with 20 µM H_2O_2 (for 24h), G3 (Group 3): treated with 40 µM H_2O_2 (for 24h), G4 (Group 4): treated with 20 µM H_2O_2 and 10 µM Curcumin (for 24h), G5 (Group 5): treated with µM H_2O_2 and 10 µM Curcumin (for 24h) and G6 (Group 6): treated with 80 µM H_2O_2 and 10 µM Curcumin (for 24h).

The results demonstrated a significant increase in the level of PDGF- β mRNA expression in group 2 (20 µl H_2O_2) compared with control and group 4 (20 µM H_2O_2-10 µM Curcumin) ($p<0.05$) (Figure 1). But, group 3 (40 µM H_2O_2) didn't show a significant increase versus control.

In addition, 40 µM H_2O_2-10 µM Curcumin treated group (group 5) demonstrated a decrease in PDGF- β mRNA expression compared with 40 µM H_2O_2 treated group (group 3) (Figure 2).

PDGF- β mRNA expression increased in response to 80 µM H_2O_2-10 µM Curcumin (group 6) versus groups 3, 5 and control (Figure 2). But, this change was not significant versus other groups. Unexpectedly, the results of groups 2 and 6 were close.

Table 2) The results of Real-Time PCR in control and treated groups

Groups	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
PDGF-B mRNA expression	1^{a}	3.74 ± 0.37^{b}	$1.82\pm1.15^{a,b}$	0.72 ± 0.59^{a}	$1.64\pm0.32^{a,b}$	$3.28\pm0.64^{a,b}$

Significant differences at $p < 0.05$ are shown by different letters in each column.

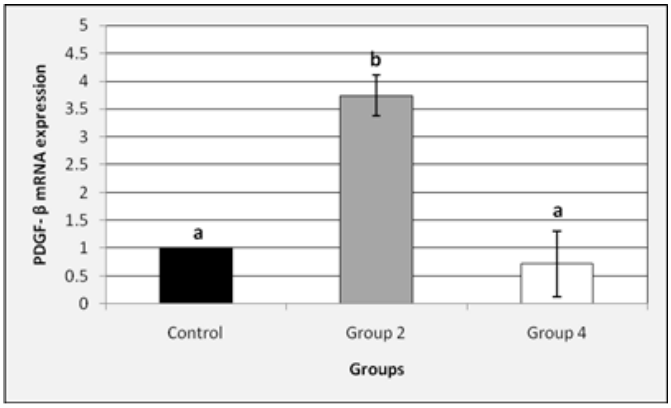

Figure 1) The study of PDGF- β mRNA expression in BAECs by Real-Time PCR.

There was a significant increase in 20 µM H_2O_2 treated cells (group 2) compared with control and 20 µM H_2O_2- 10 µM Curcumin treated cells (group 4) ($p<0.05$). PDGF- β: Platelet-Derived Growth Factor β; BAECs: Bovine aortic endothelial cells; Cur: Curcumin. Significant differences at $p<0.05$ are shown by different letters in each column.

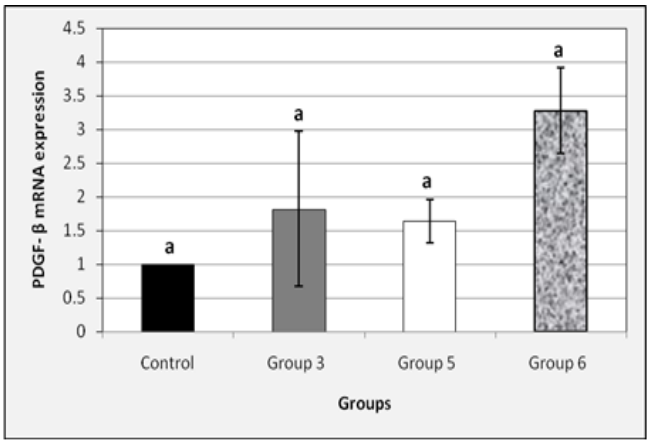

Figure 2) The levels of PDGF- β mRNA expression in BAECs were evaluated using Real-Time PCR

Group 3 (40 µM H_2O_2-10 µM Cur/24h) showed lower levels of PDGF- β mRNA expression compared to 40 µM H_2O_2 incubated cells for 24h (group 2). Also, group 6 which received 80 µM H_2O_2- 10 µM Cur treatment for 24h, demonstrated the highest level of PDGF- β mRNA between all experimental groups. PDGF- β: Platelet- Derived Growth Factor β; BAECs: Bovine aortic endothelial cells; Cur: Curcumin. Significant differences at $p<0.05$ are shown by different letters in each column.

Discussion

Atherosclerosis as a major cause of cardiovascular diseases has several risk factors such as high cholesterol and VSMCs migration (6,12). Also, the side effects of high fat diet and high cholesterol can alleviate by Curcumin (4-6). Curcumin significantly decreases fatty liver development, weight gain, dyslipidemia in Ldlr (-/-) mouse model of human atherosclerosis (4-6). This potent antioxidant modulates atherogenesis by inhibiting expression of aP_2 and CD36 in macrophages (4). Furthermore, Curcumin significantly decreases hepatic complement factor D (Cfd) and systemic CRP levels as main markers of immune complement pathway activation (5). Linton (2003) and Hassan (2011) showed that ApoE-deficient mice null for macrophage aP_2 expression, develop significantly less atherosclerosis than controls wild type for macrophage aP_2 expression (6,13). Recent researches demonstrated strong anti-atherogenic effect of long-term Curcumin treatment in high-cholesterol treated mice. There are multiple mechanisms by which Curcumin induced anti-atherogenic effect such as alteration on lipid concentration, cholesterol concentration and immune gene expression (5). Long-term Curcumin administration has more effective and protective effect on high cholesterol-induced atherosclerosis compares to lovastatin. Most of the anti-atherogenic effects of Curcumin are similar to lovastatin. Curcumin improves lipid infiltration, ICAM-1 and VCAM-1 localization (5) and early atherosclerotic lesions (5,6). In addition, long-term Curcumin decreases plasma cholesterol, triglycerides, Apo B levels, LDL cholesterol and increases plasma HDL cholesterol and liver Apo A-I expression (5). H_2O_2 is one of the major ROS that has important roles on pathologic conditions (14). The shift in balance between oxidant (such as ROS) and antioxidant in favor of oxidants is termed "oxidative stress" (14). Many diseases including cancer (15-18), cardiovascular diseases (18-20), diabetes (20,21) and hypertension (14,22) are induced by oxidative stress (15). So, atherosclerosis can induce by ROS-related oxidative stress (23). Here, we showed that H_2O_2 as a potent oxidant can promote atherosclerosis by changes on atherosclerosis-induced genes including PDGF. H_2O_2 increased the level of PDGF-β expression in endothelial cells. Also, PDGF-β can cause VSMC proliferation and migration (24). The proliferation and migration of VSMC are critical factors for promoting atherosclerosis. Curcumin co-treatment in H_2O_2 incubated cells showed significant decrease on expression of PDGF-β mRNA versus H_2O_2 treated cells. Curcumin reduces the risk of VSMC proliferation and atherosclerosis on oxidative stress-exposed endothelial cells. ROS have been implicated in the pathogenesis of

cardiovascular diseases, in part by promoting VSMC proliferation (25-27).

Conclusion

In this study, we tested the hypothesis that Curcumin as a potent antioxidant decreases the risk of atherosclerosis by reducing H_2O_2-related oxidative stress and PDGF gene expression in endothelial cells. The understanding of the pathophysiology and effective treatment of atherosclerosis can provide new perspectives for preventive and therapeutic strategies. According to these finding, herbal diet supplement such as Curcumin can help patients or person who has risk factor of cardiovascular diseases.

Footnotes

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Conflict of Interest:

The authors declared no conflict of interest.

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