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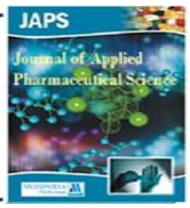

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Mechanisms of endothelial cell protection by curcumin in hypercholesterolemia

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ABSTRACT

Mechanism of curcumin for protection of endothelial cell was studied in cholesterol-fed rabbits. Thirty rabbits were randomly divided into five groups. The negative control group was fed a standard diet, the positive control group was fed the same diet with 2 % cholesterol , the curcumin group was fed the same diet with 2 % cholesterol and curcumin 100 mg/Kg BW/day, 200 mg/Kg BW/day or 400 mg/Kg BW/day. The cholesterol-rich diet significantly increased Malondialdehyde (MDA) in the aortic blood vessels, as reflected by Thiobarbituric Acid-Reactive Substances (TBARS), inhibited endothelium-dependent vascular relaxations to acetylcholine and decrease cyclic GMP were compared with vessels from normal rabbits (negative control). In cholesterol-fed rabbits, curcumin treatment decreased MDA in plasma production, improved endothelium - dependent relaxations to acetylcholine and increase cyclic GMP production. These results suggest that dietary treatment of rabbits with curcumin may prevent superoxide anion (O^{2-}) induced inactivation of endothelium-dependent relaxing factor (EDRF), improve the endothelium-dependent relaxation to acetylcholine in the aortic blood vessels and increase cyclic GMP content in aortic of cholesterol-fed rabbits.

Keywords: Curcumin, Malondialdehyde, EDRF, cyclic GMP.

INTRODUCTION

The vascular endothelium is important in a number of homeostatic functions including the regulation of blood flow, vascular tone and local platelet function (Shimokawa,1999). Endothelium-dependent relaxant effects on vascular smooth muscle is thought to be mediated by releasing endothelium-derived relaxing factor (EDRF), NO or an NO related substance, followed by an increase in the cyclic GMP content in smooth muscle (Sausbier *et al.*,2000; Fujitani and Karaki,1993; Karaki and Sudjarwo,1993). Endothelium dependent vascular relaxations are impaired in numerous disease states, including hypercholesterolemia, atherosclerosis, hypertension, and chronic heart failure (Shimokawa,1999; Verbeuren *et al.*,1990). Bioassay experiments have suggested that impaired synthesis or release of endothelium-derived relaxing factor might contribute to the abnormal endothelium-dependent relaxation in hypercholesterolemic animals (Stephanie and Cor, 2005). It has shown that shortterm cholesterol feeding in rabbit increases endothelial O^{2-} production, seemingly from xanthine oxidase. Thus, there is substantial evidence that hypercholesterolemia can impair endothelium-dependent relaxation via oxidative inactivation of endothelium-derived relaxing factor (Ohara *et al.*,1992; Jiang *et al.*,2001). In cholesterol-fed rabbits, curcumin treatment normalized endothelium-dependent relaxations to

acetylcholine (Sudjarwo, 2002). Administration of polyethylene glycolated superoxide dismutase (SOD) to increase vascular SOD levels improved endothelium-dependent relaxation in atherosclerotic rabbits (Siekmeier *et al.*, 2007; Valko,2007; Rui-Li *et al.*,2008). Also administration of antioxidant such as Vitamin E, Vitamin C and probucol could improve endothelium-dependent relaxation, normalized endothelial O^{2-} production in hypercholesterolemic vessels and reduces lipid peroxidation in the plasma (Inoue and Nishida,1998; Mahfouz et al,,1997; Margurite *et al.*,2003). Curcumin, a major constituent of the yellow spice turmeric derived from the rhizomes of Curcuma spp., is one such compound (Aggarwal *et al.*, 2007). Curcumin has been reported to have several pharmacological effects including anti-tumor, anti-inflammatory, anti-oxidant properties, inhibits oxidation of low density lipoprotein and inhibit lipid peroxidation in vitro (Plummer *et al.*, 2001; Durgaprasad *et al.*,2004).

The purpose of our studies was to investigate the molecular mechanisms by which curcumin protected endothelial cell in hypercholesterolemia.

MATERIALS AND METHODS

Animal preparation

New Zealand White rabbits 6 to 8 weeks old weighing between 1.8 and 2.0 kg, after 1 week of adaptation, were randomly divided into five groups. The negative control group was fed a standard diet, the positive control group was fed the same diet with 2 % cholesterol , the curcumin group was fed the same diet with 2 % cholesterol and curcumin 100 mg/Kg BW/day, 200 mg/Kg BW/day or 400 mg/Kg BW/day. After 8 weeks of dietary treatment, The animals were euthanized by having their necks severed. Median thoracotomy was then performed, and the aorta was removed to obtain the rings for assessing endothelial function, MDA and c GMP content.

Preparations, solutions and measurement of muscle tension

The thoracic aorta was isolated from rabbits, cut into spiral strips (1-2 mm in width and 5-7 mm in length) and placed in normal physiological salt solution which contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8, ethylenediamine-tetraacetic acid 0.01 and glucose 5.5. A high K^+ solution was made by substituting 69.6 mM NaCl with equimolar KCl. These solutions were saturated with a mixture of 95 % O_2 and 5 % CO_2 at 37 ºC and pH 7.4. Muscle tension was recorded isometrically with a force-displacement transducer. Each muscle strip was attached to a holder under a resting tension of 1 g and equilibrated for 60-90 min in a 10-ml muscle bath until the contractile response to the high K^+ solution had become stable.

The functional integrity of the vascular endothelium was assessed by measuring whether 1 μM Acetylcholine induced almost complete relaxation in aortas stimulated with 100 nM norepinephrine (Sudjarwo *et al.*,1992).

Measurement of TBARS levels in aorta

Malondialdehyde (MDA) levels measured by thiobarbituric reactive substances (TBARS) assay. The aortic samples were homogenized in cold trichloroacetic acid (TCA) (1 mg of tissue per mL of 10% TCA). After centrifugation, a portion of the supernatant was added to an equal volume of thiobarbituric acid (0.6% v/v), and the mixture was heated at 100°C for 20 minutes. The MDA concentration was calculated by use of a spectrophotometer, with absorption of 532 nm and the results were expressed in n mol/mg of dry tissue.

Measurement of cyclic GMP

Aortic strips were incubated with krebs solution containing 100 nM norepinephrine for 5 minutes. Then the strips were incubated with concentration of 1 μM acetylcholine. After 20 sec incubation, except where otherwise stated, the preparations were frozen quickly in liquid nitrogen. Aortic strips frozen in liquid nitrogen were transferred to 5 % (W/V) trichloroacetic acid solution and homogenized in a Potter glass homogenizer on ice. The homogenates were centrifuged at 1700 x g for 15 min at 4 ºC. The supernatants were extracted 3 times with 3 volumes of water-saturated ether, and cyclic GMP contents were measured ELISA using a kit from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.).

Chemicals

Curcumin and acetylcholine (Sigma Chemicals, St. Louis, MO, USA) and norepinephrine bitartrate (Wako Pure Chemicals, Tokyo, Japan).

Statistics

Results were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

RESULTS

Effect Curcumin on Lipid Peroxidation in aorta

The lipid peroxidation production was 0.12 ± 0.02; 0.67 ± 0.08; 0.71 ± 0.1; 0.41 ± 0.09, 0.26 ± 0.07 n mol/mg protein in negative control, positive control, treatment curcumin at dose 100 mg/Kg BW, 200 mg/Kg BW and dose 400 mg/Kg BW, respectively. In the positive control (hypercholesterolemic) group, the level of TBARS was significantly increased compared to negative control group ($p<0.05$). Treatment with curcumin at dose 200 mg/Kg BW and 400 mg/Kg BW but not at dose 100 mg/Kg BW markedly reduced aorta TBARS in hypercholesterolemia which was significantly different from the positive control ($p<0.05$) (Figure 1).

Effect of Curcumin on Acetylcholine induced endothelium-dependent vasorelaxation.

Table 1 shows the concentration response for the relaxant effect of acetylcholine in norepinephrine stimulated aorta. Endothelium-dependent relaxation evoked by acetylcholine was significantly impaired in aortic ring from the cholesterol-fed (positive control) group as compare to those in the negative control group ($p<0.05$). The aorta from hypercholesterolemic rabbits treated with curcumin at dose 200 mg/Kg BW and 400 mg/Kg BW but not at dose 100 mg/Kg BW showed marked improvement of

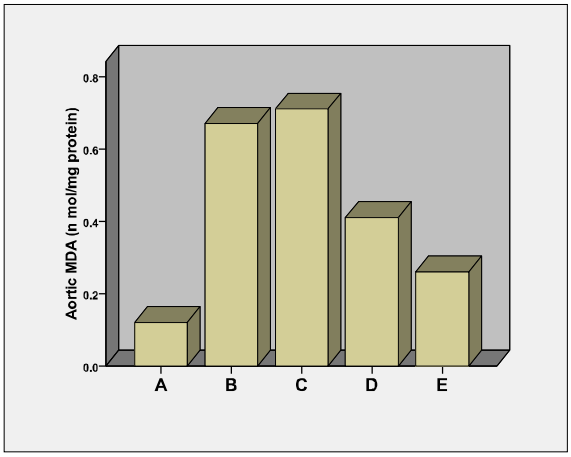

Fig 1. Bar graph showing plasma lipid peroxidation (Malondialdehyde) as determined by thiobarbituric reactive substances (TBARS). Negative control (A), Positive control (B), curcumin treatment at dose 100 mg/Kg BW (C), 200 mg/Kg BW (D) and curcumin treatment at dose 400 mg/Kg BW (E). Each point represents the mean of six experiments.

Table 1. Effect of Curcumin on Acetylcholine induced endothelium-dependent vasorelaxation.

Group	Vasorelaxation of Acetylcholine (%)		
	10 nM	100 nM	1 μM
Negative control	18.9 ± 1.8	67.3 ± 4.4	84.1 ± 4.3
Positive control	4.1 ± 1.9	43.7 ± 3.1	57.6 ± 1.9
Curcumin 100 mg/Kg BW	5.1 ± 1.2	45.1 ± 3.2	59.2 ± 4.1
Curcumin 200 mg/Kg BW	7.3 ± 2.1	51.3 ± 3.7	64.3± 2.9
Curcumin 400 mg/Kg BW	11.8 ± 2.2	59.4 ± 3.5	73.2 ± 4.1

Mean ± SEM, n = 6.

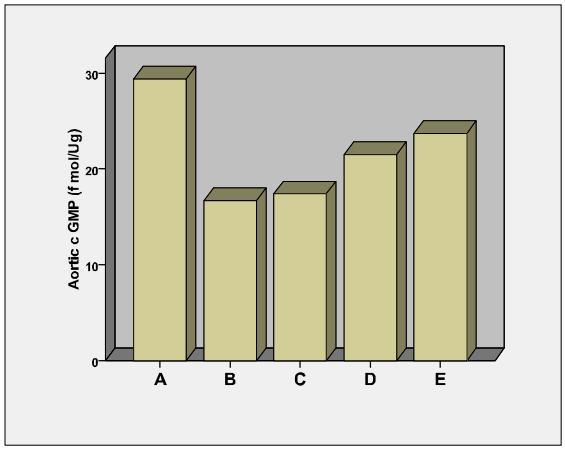

Fig 2. Effect of curcumin at dose 100 mg/Kg BW, dose 200 mg/kgBW and 400 mg/Kg BW on the increase in the cyclic GMP content in rabbits aortic strips after stimulation with 1 μM acetylcholine. Negative control (A), Positive control (B), curcumin treatment at dose 100 mg/Kg BW (C), curcumin treatment at dose 200 mg/Kg BW (D), and curcumin treatment at dose 100 mg/Kg BW (E). Each columns represents the mean of six experiments and SEM, $p<0.05$.

the impaired endothelium-dependent relaxation which was significantly different from positive control group ($p<0.05$).

Effect of Curcumine on Acetylcholine induced c GMP increase

The cyclic GMP production was 29.4 ± 2.8, 16.7 ± 1.9, 17.4 ± 1.5, 21.5 ± 2.1 and 25.7 ± 1.6 f mol/μg in negative control, positive control, treatment curcumin at dose 100 mg/kg BW, dose 200 mg/Kg BW and dose 400 mg/KgBW, respectively.

In the positive control (hypercholesterolemic) group, the cyclic GMP production was significantly decreased compared to negative control group ($p<0.05$). The treatment with curcumin at dose 200 mg/Kg BW and 400 mg/KgBW but not at dose 100 mg/Kg BW markedly increase cyclic GMP production in hypercholesterolemia which was significantly different from the positive control ($p<0.05$) (Figure 2).

DISCUSSION

In the present study, we demonstrated that in the hipercholesterolemic rabbit induced lipid peroxidation increase. This was associated with production of aortic TBARS. In the hypercholesterolemic rabbit also induced cyclic GMP production decrease and impaired endothelium-dependent relaxation. This is consistent with previous observations that in the hypercholesterolemic rabbit and pig are associated with impairments of endothelium-dependent relaxation and is due, at least in part, to reduced production of EDRF and cyclic GMP by endothelial cells (Fujitani et al,1993; Volker *et al.*,2004; Jiang *et al.*, 2000). In addition, the blunted endothelium-dependent relaxation in hypercholesterolemic animals may also result from the destruction of EDRF by superoxide anion (Ohara *et al.*, 1992; Inoue *et al.*, 1998). The antioxidant such as beta carotene, alpha tocopherol and probucol have been reported to improve endothelium-dependent relaxation in hypercolesterolemic rabbits, suggesting that the free radical scavenging property of these antioxidants might play an important role in the protective effect on endothelial dysfunction (Mahfouz *et al.*,1997; Margurite *et al.*, 2003). Recently, it has been reported that curcumin has potent antioxidant, inhibits oxidation of low density lipoprotein and inhibit lipid peroxidation in vitro effect (Weber *et al.*, 2005; Manjunatha and Srinivasan,2006; Kempaiah and Srinivasan,2004). In our experiments, we also obtain several results indicating that this may be case: 1) in the hypercholesterolemic rabbits significantly inhibited acetylcholine induced endothelium-dependent relaxation, increase lipid peroxidation (malondialdehyde) and decrease cyclic GMP production, 2) the treatment with curcumin in hypercholesterolemic rabbits significantly reduced lipid peroxidation (malondialdehyde) production, augmented acetylcholine induced endothelium-dependent relaxation and increased cyclic GMP production. These results suggest that dietary treatment of rabbits with curcumin may prevent superoxide anion (O^{2-}) induced inactivation of endothelium-dependent relaxing factor (EDRF), improve the endothelium-dependent relaxation to acetylcholine in the aortic blood vessels and increase cyclic GMP content in aortic of cholesterol-fed rabbits.

CONCLUSION

Curcumin not only improves endothelium-dependent relaxations but also reduces lipid peroxidation (malondialdehyde)

in the aorta and enhanced the tissue content cycclic GMP in hypercholesterolemic rabbits. These findings suggest that curcumin might play an important role in the protective effect on endothelial dysfunction in hypercholesterolemia.

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