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Full Length Research Paper

Mechanisms of endothelial cell protection by quercetin in hypercholesterolemia

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Mechanism of quercetin 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 with a standard diet; the positive control group was fed with the same diet with 2% cholesterol; the quercetin group was fed with the same diet with 2% cholesterol and quercetin 50, 100 or 150 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 tissue content cyclic GMP with vessels from normal rabbits (negative control). In cholesterol-fed rabbits, quercetin treatment decreased MDA in plasma production, improved endothelium - dependent relaxations to acetylcholine, and increase cyclic GMP production. These results suggest that quercetin not only improves endothelium-dependent relaxations but also reduces lipid peroxidation (malondialdehyde) in the aorta and enhanced the tissue content cyclic GMP in hypercholesterolemic rabbits. These findings suggest that quercetin might play an important role in the protective effect on endothelial dysfunction in hypercholesterolemia.

Key words: Quercetin, malondialdehyde, endothelium-derived relaxing factor (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 EDRF, NO or an NO related substance, followed by an increase in the cyclic GMP content in smooth muscle (Sausbier et al., 2000; Fujitani et al.,1993; Karaki et al.,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 et al., 2005). It has shown that short-term 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). 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 plasmaa (Inoue et al., 1998; Mahfouz et al., 1997; Margurite et al., 2003). Quercetin is considered to be a strong antioxidant due to its ability to

Abbreviations: MDA, malondialdehyde; **EDRF,** endothelium-derived relaxing factor; **NO,** nitric oxide; **TBARS,** thiobarbituric acid reactive substances; and **SOD,** superoxide dismutase; **TCA,** trichloroacetic acid; **BW,** body weight.

scavenge free radicals and bind transition metal ions.

These properties of quercetin allow it to inhibit lipid peroxidation (Hollman and Katan, 1997; Sakanashi, 2008). Lipid peroxidation is the process by which unsaturated fatty acids are converted to free radicals via the abstraction of hydrogen (Young and McEneny, 2001). As a result, quercetin may aid in the prevention of certain diseases, such as cancer, atherosclerosis, and chronic inflammation (Hollman and Katan, 1997; Murota and Terao, 2003). The purpose of our studies was to investigate the molecular mechanisms by which quercetin protected endothelial cell in hypercholesterolemia.

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 quercetin group was fed the same diet with 2% cholesterol and quercetin 50 mg/kg BW/day, 100 mg/kg BW/day or 150 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 of 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, ethylenediaminetetraacetic 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°C 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

MDA levels measured by TBARS assay. The aortic samples were homogenized in cold 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 min. 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 min. Then the strips were incubated with concentration of 1 µM acetylcholine. After 20 s 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) trichloro acetic acid solution and homogenized in a Potter glass homogenizer on ice. The homogenates were centrifuged at 1700 × g for 15 min at 4°C. The supernatants were extracted 3 times with 3 volumes of water-saturated ether, and cyclic GMP contents were measured by ELISA kit from Cayman Chemical Co. (Ann Arbor, MI, U.S.A).

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 of quercetin 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 quercetin at dose 50, 100 and 150 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 quercetin at dose 100 and 150 mg/kg BW but not at dose 50 mg/kg BW markedly reduced aorta TBARS in hypercholesterolemia which was significantly different from the positive control ($p<0.05$) (Figure 1).

Effect of quercetin 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 compared to those in the negative control group ($p<0.05$). The aorta from hypercholesterolemic rabbits treated with quercetin at dose 100 and 150 mg/kg BW but not at dose 50 mg/kg BW showed marked improvement of the impaired endothelium-dependent relaxation which was significantly different from positive control group ($p<0.05$).

Effect of quercetin 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 quercetin at dose 50

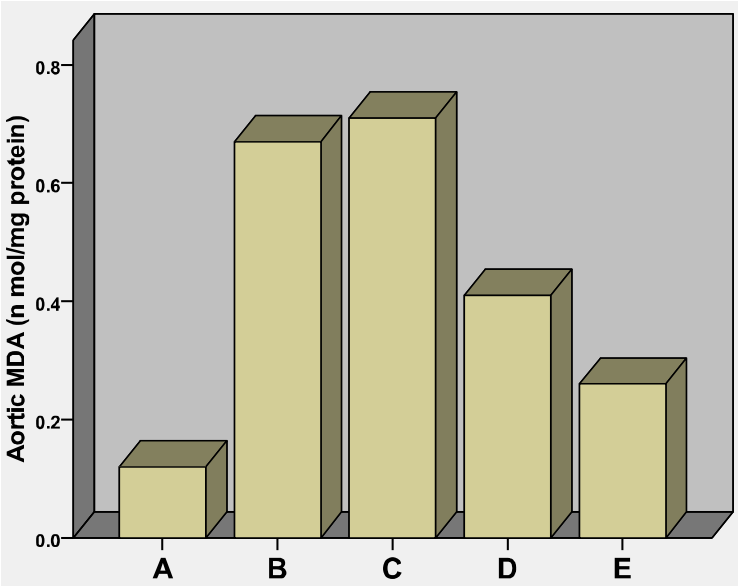

Figure 1. Bar graph showing plasma lipid peroxidation (Malondialdehyde) as determined by thiobarbituric reactive substances (TBARS). Negative control (A), Positive control (B), quercetin treatment at dose 50 mg/Kg BW (C), 100 mg/Kg BW (D) and quercetin treatment at dose 150 mg/Kg BW (E). Each point represents the mean of six experiments.

Table 1. Effect of quercetin on acetylcholine induced endothelium-dependent vasorelax**ation.**

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
Quercetin 50 mg/kg BW	5.1 ± 1.2	45.1 ± 3.2	59.2 ± 4.1
Quercetin 200 mg/kg BW	7.3 ± 2.1	51.3 ± 3.7	64.3± 2.9
Quercetin 400 mg/kg BW	11.8 ± 2.2	59.4 ± 3.5	73.2 ± 4.1

Mean ± SEM, n = 6.

mg/kgBW, dose 100 mg/kg BW and dose 150 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 quercetin at dose 100 mg/kg BW and 150 mg/kgBW but not at dose 50 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 hypercholesterolemic, rabbit induced increase in lipid peroxidation. This was associated with the production of aortic TBARS. In the hypercholesterolemic, rabbit also induced decrease in cyclic GMP production 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 hypercholesterolemic rabbits, suggesting that the free radical scavenging property of these antioxidants might

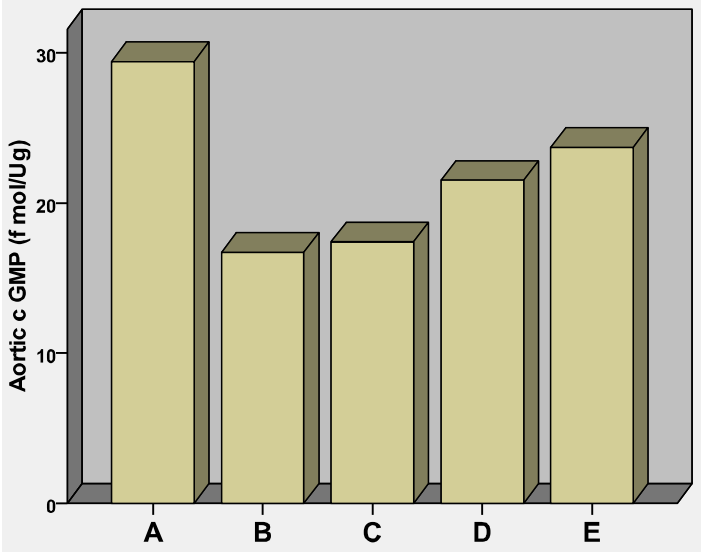

Figure 2. Effect of quercetin at dose 50 mg/kg BW, dose 100 mg/kgBW and 150 mg/kg BW on the increase in the cyclic GMP content in rabbits aortic strips after stimulation with 1 µM acetylcholine. Negative control (A), Positive control (B), quercetin treatment at dose 50 mg/kg BW (C), quercetin treatment at dose 100 mg/kg BW (D), and quercetin treatment at dose 150 mg/kg BW (E). Each columns represents the mean of six experiments and SEM, $p<0.05$.

play an important role in the protective effect on endothelial dysfunction (Mahfouz et al.,1997; Marguerite et al., 2003). Recently, it has been reported that quercetin has potent antioxidant due to its ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation (Hollman and Katan, 1997; Sakanashi et al., 2008). Lipid peroxidation is the process by which unsaturated fatty acids are converted to free radicals via the abstraction of hydrogen (Young and McEneny, 2001). 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 quercetin 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 quercetin may prevent superoxide anion (O^{2-}), induced activation of EDRF, improve the endothelium-dependent relaxation to acetylcholine in the aortic blood vessels and increase cyclic GMP content in aortic of cholesterol-fed rabbits.

In conclusion, quercetin not only improves endothelium-dependent relaxations but also reduces lipid peroxidation (malondialdehyde) in the aorta and enhanced the tissue content cyclic GMP in hypercholesterolemic rabbits. These findings suggest that quercetin might play an important role in the protective effect on endothelial dysfunction in hypercholesterolemia.

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