Protective effect of cathechin on endothelial cell in hypercholesterolemia

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ABSTRACT

Endothelium dependent vascular relaxations are impaired in numerous disease states, including hypercholesterolemia, atherosclerosis, hypertension, and chronic heart failure. A randomized controlled trial was conducted to evaluate the protective effect of cathechin on endothelial cell in cholesterol fed rabbits. A total 30 white rabbits were randomized into five groups: negative control was fed a standard diet, positive control with 2% cholesterol, cathechin groups were fed with cathechin 100 mg/kg BW/day, 200 mg/kg BW/day and 400 mg/kg BW/day. These results showed that the cholesterol-rich diet markedly increased malondialdehyde (MDA) in the plasma, as reflected by thiobarbituric acid-reactive substances (TBARS), inhibited endothelium-dependent vascular relaxations to acetylcholine compared to vessels from normal rabbits. In cholesterol-fed rabbits, cathechin treatment decreased MDA in plasma production and improved endothelium-dependent relaxations to acetylcholine. These study concluded that dietary treatment of rabbits with cathechin may prevent superoxide anion (O$_2^-$) induced inactivation of endothelium-dependent relaxing factor (EDRF) and improve the endothelium-dependent relaxation to acetylcholine in the aortic blood vessels of cholesterol-fed rabbits.

Keywords : Cathecin, malondialdehyde, hypercholesterolemia, rabbit

INTRODUCTION

The vascular endothelium is important in a number of homeostatic functions including the regulation of blood flow, vascular tone and local platelet function. Endothelium-dependent relaxant effects on vascular smooth muscle is thought to be mediated by releasing endothelium-derived relaxing
factor (EDRF), nitrite oxide (NO) or an NO related substance, followed by an increase in the cyclic GMP content in smooth muscle. 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. It has been 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. Administration of polyethylene glycolated superoxide dismutase (SOD) to increase vascular SOD levels improved endothelium-dependent relaxation in atherosclerotic rabbits. Also administration of antioxidant such as Vitamin E and probucol could improve endothelium-dependent relaxation, normalized endothelial O$_2^-$ production in hypercholesterolemic vessels and reduces lipid peroxidation in the plasma.

It has been reported that cathechin has potent antioxidant effect, inhibits oxidation of low density lipoprotein and inhibit lipid peroxidation in vitro. In the present study, we determine whether cathechin could induce lipid peroxidation (malondialdehyde) decrease and endothelium derived relaxing factor increase in cholesterol fed rabbits.

**MATERIAL AND METHODS**

**Animal preparation**

Thirty male New Zealand white rabbits 2-3 months of age weighing 1.5-2 kg 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 cathechin group was fed the same diet with 2% cholesterol and cathechin 100 mg/kg BW/day, 200 mg/kg BW/day or 400 mg/kg BW/day. After 8 weeks of dietary treatment, the animal was weighed 2.5-3 kg and blood samples were obtained via the marginal ear vein for measurement of lipid peroxidation in plasma.

**Preparations, solutions and measurement of muscle tension**

The rabbits were killed by stunning and bled. The thoracic aorta were isolated, 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. In some experiments, the endothelium was removed by gently rubbing the intimal surface with a finger moistened with the above solution. A high K$^+$ solution was made by substituting 69.6 mM NaCl with equimolar KCl. Ca$^{2+}$ free solution was made by removing CaCl$_2$ and adding 0.5 mM EGTA. These solutions were saturated with a mixture of 95% O$_2$ and 5% CO$_2$ at 37ºC 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 minutes 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.

**Measurement of lipid peroxidation (malondialdehyde) in plasma**

The extent of lipid peroxidation in plasma was measured as thiobarbituric acid-reactive substances (TBARS), expressed as malondialdehyde equivalents, as previously described. Samples were mixed with 1.0 ml of 0.67% thiobarbituric acid acid in 0.05 mol NaOH. After heating at 90ºC for 30 minutes, the samples were centrifuged at 2000 rpm for 10 minutes and the optical density was measured at 532 nm. Fresh tetramethoxypropane, which produces malondialdehyde, was used as a standard.

**Chemicals**

Cathechin and acetylcholine (Sigma Chemicals, St. Louis, MO, USA) and norepinephrine bitartrate (Wako Pure Chemicals, Tokyo, Japan).
Statistics
The results of the experiments are expressed as mean ± S.D. Statistical analysis was examined using analysis of variance (ANOVA). P values of less than 0.05 were considered statistically significant.

RESULTS

Effect cathechin on lipid peroxidation in plasma

Table 1 shows the lipid peroxidation production was 3.8 ± 0.76, 7.7 ± 0.61, 6.9 ± 0.77, 5.1 ± 0.86, 4.1 ± 0.68 µM in negative control, positive control, cathechin groups with 100 mg/kg BW, 200 mg/kg BW and dose 400 mg/kg BW, respectively.

Table 1. Effect cathechin on lipid peroxidation in plasma

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA level (µM/ml)</th>
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<tr>
<td>Negative control</td>
<td>3.8 ± 0.76</td>
</tr>
<tr>
<td>Positive control</td>
<td>7.7 ± 0.61</td>
</tr>
<tr>
<td>Cathechin 100 mg/Kg BW</td>
<td>6.9 ± 0.77</td>
</tr>
<tr>
<td>Cathechin 200 mg/Kg BW</td>
<td>5.1 ± 0.86</td>
</tr>
<tr>
<td>Cathechin 400 mg/Kg BW</td>
<td>4.1 ± 0.68</td>
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* Mean ± SD

In the positive control (hypercholesterolemic) group, the level of TBARS was significantly increased compared to negative control group. Treatment with cathechin at dose 200 mg/kg BW and 400 mg/kg BW, significantly reduced plasma TBARS in hypercholesterolemia but not at dose 100 mg/kg BW which was not significantly different from the negative control (Figure 1).

Effect of cathechin on acetylcholine induced endothelium-dependent vasorelaxation

Figure 2 shows the concentration response curves for the relaxant effect of acetylcholine in norepinephrine stimulated aorta. The hypercholesterolemic group inhibited the response to acetylcholine that shifted the curve to the right and downwards. Treatment with cathechin at dose 200 mg/kg BW and 400 mg/kg BW but not at dose 100 mg/kg BW improved the response to acetylcholine which almost was similar to the response of the negative control group.

Figure 1. Bar graph showing plasma lipid peroxidation (Malondialdehyde) as determined by thiobarbituric reactive substances (TBARS). Negative control (1), Positive control (2), cathechin treatment at dose 100 mg/kg BW (3), 200 mg/kg BW (4) and cathechin treatment at dose 400 mg/kg BW (5). Each point represents the mean of six experiments.

Figure 2. The effect of cathechin treatment on concentration-response curves of acetylcholine to induce endothelium-dependent relaxation of norepinephrine stimulated aorta.
Endothelium-dependent relaxation evoked by acetylcholine was significantly impaired in aortic ring of the cholesterol-fed (positive control) group compared to those in the negative control group. The aortic from hypercholesterolemic rabbits treated with cathechin showed marked improvement of the impaired endothelium-dependent relaxation which was not significantly different from negative control group (Table 2).

**DISCUSSION**

In the present study, we demonstrated lipid peroxidation increase in the hypercholesterolemic rabbit. This was associated with production of plasma TBARS. In the hypercholesterolemic rabbit also induced impaired endothelium-dependent relaxation. This is consistent with previous study 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. In addition, the blunted endothelium-dependent relaxation in hypercholesterolemic animals may also result from the destruction of EDRF by superoxide anion.

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 play an important role in the protective effect on endothelial dysfunction. Recently, it has been reported that cathechin has potent antioxidant, inhibits oxidation of low density lipoprotein and inhibit lipid peroxidation in vitro. In our experiments, we also obtained several results indicating that this may be the case: 1) in the hypercholesterolemic rabbits significantly inhibited acetylcholine induced endothelium-dependent relaxation and increased lipid peroxidation (malondialdehyde), 2) the treatment with cathechin in hypercholesterolemic rabbits significantly reduced lipid peroxidation (malondialdehyde) production and augmented acetylcholine induced endothelium-dependent relaxation. The results suggest that cathechin have protective effect on endothelial cell function in hypercholesterolemic rabbits via decreasing endothelial O₂− production.

**CONCLUSION**

Cathechin not only improves endothelium-dependent relaxations but also reduces lipid peroxidation (malondialdehyde) in the plasma. These findings suggest that catechin might play an important protective role on endothelial dysfunction in hypercholesterolemia.

**References**

4. Inoue N, Nishida K. Probucol improves endothelial-dependent relaxation and decreases


