# Prevention of abdominal aortic aneurysm progression by oral administration of green tea polyphenol in a rat model

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## ABSTRACT

**Objective:** Inflammation-mediated elastin destruction in the aortic medial layer is related to progression of abdominal aortic aneurysm (AAA). Epigallocatechin-3-gallate (EGCG), a major component of green tea polyphenols, reportedly increases elastin synthesis in vitro and may possess anti-inflammatory effects. We used a rat model to investigate whether EGCG could prevent AAA progression.

**Methods:** AAA was induced with administration of intraluminal elastase and extraluminal $CaCl_2$ in male rats. Rats were randomly divided into a control group (n = 30) and an EGCG group (n = 30). In the EGCG group, an EGCG solution (20 mg/d) was administered orally to each rat from 2 weeks before AAA induction and continued 4 weeks beyond induction.

**Results:** The abdominal aortic diameter was significantly smaller in the EGCG group than in the control group on day 28 (2.9 ± 0.2 vs 2.3 ± 0.1 mm; $P < .0001$). The medial layer wall thickness and elastin content were significantly greater in the EGCG group than in the control group on day 28 (68.4 ± 13.6 vs 46.7 ± 13.4 μm [$P < .001$] and 20.3 ± 4.6 vs 9.5 ± 3.6% [$P < .0001$], respectively). Gene expression levels of tropoelastin and lysyl oxidase were significantly higher in the EGCG group immediately before AAA induction, indicating promoted elastoregeneration by EGCG administration (tropoelastin: 0.59 ± 0.36 control vs 1.24 ± 0.36 EGCG [$P < .05$], lysyl oxidase: 0.77 ± 0.45 control vs 1.34 ± 0.4 EGCG [$P < .05$]) (fold increase). Gene expression levels of inflammatory cytokines, including tumor necrosis factor-α and interleukin-1β, were significantly downregulated in the EGCG group (1.82 ± 0.71 vs 0.97 ± 0.59 [$P < .05$] and 3.91 ± 3.24 vs 0.89 ± 0.59 [$P < .05$], respectively). On day 7, gene expression levels and gelatinolytic activity of matrix metalloproteinase 9 were significantly lower in the EGCG group (1.41 ± 0.86 vs 0.51 ± 0.42 [$P < .05$] and 1.00 ± 0.17 vs 0.29 ± 0.12 [$P < .0001$], respectively), whereas gene expression levels of tissue inhibitors of metalloproteinase-1 were significantly higher in the EGCG group (0.96 ± 0.11 vs 1.14 ± 0.09; $P < .05$).

**Conclusions:** EGCG attenuated AAA progression in a rat model by preserving the aortic thickness and elastin content of the medial layer through regeneration of elastin, as mediated by anti-inflammatory effects, and subsequent reduction of matrix metalloproteinase activity. (J Vasc Surg 2016;■:1-10.)

**Clinical Relevance:** Therapeutic options for abdominal aortic aneurysm (AAA) are currently limited to invasive surgical or endovascular repair. Although advances in diagnosis have allowed physicians to detect smaller AAAs, no pharmacologic treatment directly targeting AAA progression has been found thus far. In the present study, we demonstrated that a green tea polyphenol, epigallocatechin-3-gallate, attenuates AAA progression through anti-inflammatory and elastoregenerative mechanisms in a rat AAA model. Regular green tea consumption might be advantageous for patients with a small AAA or as a prophylactic strategy for AAA.

Abdominal aortic aneurysm (AAA) is a life-threatening disease characterized by destruction of aortic structure, which can result in vasodilation and subsequent aneurysm due to continuous exposure to arterial blood pressure. Without treatment, an AAA will eventually rupture, which is a potentially fatal complication for the patient.[1] Although recent advances in disease screening and diagnostics have allowed physicians to detect smaller AAAs,[2] no effective pharmacologic treatment for AAA is currently available, and AAAs with developing size or diameter will eventually require invasive surgical or endovascular therapy.[2,3]

The inflammatory process is considered to be a primary cause of AAA onset and progression. Histologic features of AAA include chronic inflammation of the aorta, inflammatory cell infiltration, reduction of extracellular

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matrix, and vascular tissue remodeling.[4] Specifically, reduction and disruption of the elastin component of the medial layer is considered a key factor in AAA progression.[5] Inflammation triggers the reduction of elastin in the medial layer and the progression of aneurysm by activating the secretion of matrix metalloproteinases (MMPs) from inflammatory cells and vascular smooth muscle cells (VSMCs).[4]

Green tea is a popular beverage, historically consumed in the Eastern world, but has recently become popularized globally.[6] Various health-promoting effects of green tea have been proposed, including prevention of cancer and cardiovascular disease,[7,8] anti-inflammatory[9] or antioxidative effects,[10] improvement in glycemic control,[11] and reduction of low-density lipoprotein cholesterol,[12] among others. A recent cohort study indicated that the consumption of green tea reduces the risk of death from any cause.[13]

The beneficial effects of green tea have been primarily attributed to polyphenol, a notable bioactive ingredient.[14] Epigallocatechin-3-gallate (EGCG), also known as polyphenolic catechin, exhibits the greatest biological activity among the family of polyphenols[13] and is incorporated in green tea far more abundantly than in any other type of tea.[12] A recent in vitro study that used smooth muscle cells obtained from healthy or aneurysmal rat aorta showed that EGCG exhibited potent regenerative properties for elastin.[15] Considering the previously noted literature on the etiology of AAA, this suggests that green tea may have a promising potential for reducing the risk of AAA. In the present study, we aimed to verify the hypothesis that EGCG attenuates AAA progression, as modulated via anti-inflammatory and elastoregenerative properties, in an experimental rat AAA model.

## METHODS

Detailed methods are provided as the Appendix (online only).

**Animals.** The study used male Sprague-Dawley rats (400-490 g body weight; CLEA Japan Inc, Tokyo, Japan). All animal procedures were performed in accordance with the guidelines for animal experiments at Kyoto University Graduate School of Medicine and the *Guide for the Care and Use of Laboratory Animals*.[16] We only used male rats because estrogen has been reported to affect the incidence of AAA progression through attenuation of MMP synthesis.[17]

**AAA model.** The experimental AAA model has been described previously.[18] Briefly, animals were anesthetized with isoflurane, and a 10-mm segment of the infrarenal abdominal aorta was exposed through a midline laparotomy. Subsequently, 30 U of porcine pancreatic elastase (135 U/mg; Elastin Products Company, Owensville, Mo) was administered intraluminally through an SP10 polyethylene catheter (Natsume Seisakusho, Tokyo, Japan) that had been inserted via the right common femoral artery and guided to the aorta. The aorta was wrapped in gauze soaked with 0.5 mol/L $CaCl_2$ (Sigma-Aldrich, Tokyo, Japan) for 20 minutes, with simultaneous administration of elastase (Fig 1, *A* and *B*).

**Green tea polyphenol solution.** Pure form of EGCG was provided by BioVerde Inc (Kyoto, Japan). EGCG was prepared in a solution at a concentration of 1 mmol/L and given as drinking water, according to the methodology established in our previous report.[19]

**Study groups and EGCG administration.** The rats were randomly divided into a control group (n = 30) and an EGCG group (n = 30). For the EGCG group, a 1.0 mmol/L EGCG solution was administered orally from 2 weeks before the induction of AAAs and continued for another 4 weeks or until euthanasia. Six rats were euthanized for histologic or biochemical analyses on days 0, 2, and 7 after induction and 12 rats on day 28. The control group received tap water over the interval (Fig 1, *C*). Fluid consumption was measured daily for each rat.

**Measurement of plasma EGCG levels.** Immediately before AAA induction (pre-day 0), a blood sample was obtained from the jugular vein of each rat that had been given EGCG for 2 weeks. Samples were centrifuged at 3000 rpm for 20 minutes. Plasma was collected (50 μL), mixed with methanol (150 μL), and centrifuged at 13,000 rpm for 4 minutes. After centrifugation, 10 μL of the supernatant was applied directly to the liquid chromatography-tandem mass spectrometry system to measure the plasma EGCG concentration.

**Statistical analysis.** Values are presented as mean ± standard deviation (SD). Statistical analysis was performed using analysis of variance and Student *t*-tests (GraphPad Prism 6.0; GraphPad Software Inc, La Jolla, Calif). *P* values of $<.05$ were considered significant.

## RESULTS

Each rat in the EGCG group received an average of 0.09 mL/g body weight/day of EGCG solution, which is equivalent to 0.04 mg/g body weight/day. After drinking the EGCG solution for 2 weeks (pre-day 0), plasma EGCG levels were 11.2 ± 7.5 ng/mL (n = 12). The increase in body weight did not differ significantly between groups, and the general condition of the rats in both groups was stable.

Representative macroscopic images immediately before AAA induction (control group, pre-day 0) and 28 days after AAA induction (day 28) in both groups are shown in Fig 2, *A*. A difference in the abdominal aortic diameter was observed between groups on pre-day 0 and immediately after (post-day 0) AAA induction. The abdominal aortic diameter in both groups gradually increased after AAA induction, with diameters in the