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• Research Article

Chungtaejeon, a Korean fermented tea, prevents the risk of atherosclerosis in rats fed a high–fat atherogenic diet

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ABSTRACT

OBJECTIVE: Hypercholesterolemia is one of the well-established risk factors for cardiovascular mortality and morbidity in coronary heart disease. The aim of this study was to investigate the anti-atherogenic effect of Chungtaejeon (CTJ, a Korean fermented tea) aqueous extract on proliferation and migration of human aortic smooth muscle cells (HASMCs) *in vivo* and *in vitro*.

METHODS: The authors used high-fat atherogenic diet (HFAD) to induce hyperlipidemia in Wistar rats in *in vivo* animal experiments and used HASMCs for *in vitro* cell experiments. For the *in vitro* cell experiment, the proliferation of HASMCs was evaluated using the MTT assay. Similarly, the expression of matrix metalloproteinases (MMPs) in HASMCs was measured using gelatin zymography. Antimigratory activity of CTJ was revealed using the wound-healing model and Boyden's chamber assay. In the *in vivo* experiment, CTJ was administered in three different doses for 20 d from the initiation of the HFAD. After 20 d, the serum lipid profile and total lipid contents in liver were measured.

RESULTS: Treatment with CTJ for 24 h dose-dependently inhibited the proliferation and migration of HASMCs and expression of MMP-2 in HASMCs. The oral administration of CTJ at concentrations of 200 and 400 mg/kg decreased the levels of low-density lipoprotein cholesterol, total serum cholesterol and hepatic cholesterol of HFAD-fed rats.

CONCLUSION: CTJ possessed strong antiproliferative, antimigratory, as well as lipid-lowering activities. Thus, CTJ can be considered as a therapeutic option in the treatment of high-fat diet-induced atherosclerosis.

Keywords: hypercholesterolemia; atherosclerosis; Chungtaejeon; proliferation; migration

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1 Introduction

Atherosclerosis, an inflammatory vascular disorder, is described as the accumulation of smooth muscle cells (SMCs), white blood cells and modified lipids in the intima layer of medium- and large-sized arterial blood vessels[1]. These SMCs that accumulate at the tunica intima of the arteries can migrate from the tunica media of the blood vessel; their proliferation in the initma forms an atheroma plaque. The ability of matrix metalloproteinases

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(MMPs) to induce proliferation and migration of SMCs has been confirmed by both *in vivo* and *in vitro* experimental studies[2]. The key role of MMPs in this process is to degrade the elastic lamina barrier of the extracellular matrix (ECM) through its proteolytic activity, leading to pathological conditions such as rheumatoid arthritis, vascular disease and cancer[3,4]. Various studies have reported that the proliferation of SMCs, as well as endothelial cells, is influenced by the surplus expression of MMPs[2,5]. Other endogenous molecules that facilitate the proliferation and migration of SMCs are platelet-derived growth factor (PDGF) and tumor necrosis factor-α (TNF-α)[6,7].

Low-density lipoprotein cholesterol (LDL-C), also known as "bad cholesterol", is actively involved in the progression of numerous diseases, including atherosclerosis, cancer, diabetes mellitus and aging[8]. Oxidative modification of LDL, *via* reactive oxygen or nitrogen species-mediated lipid peroxidation, alters its structure such that oxidized-LDL (ox-LDL) is taken up by LDL scavenger receptors on macrophages, leading to the formation of fatty streak foam cells[9]. Several factors such as sedentary lifestyle, consumption of diets rich in cholesterol, and especially LDL, can lead to hypercholesterolemia[10]. A recent study has shown that hypercholesterolemia is linked with increased oxidative stress from enhanced lipid peroxidation[11]. Increased formation of ox-LDL is a major factor responsible for vascular damage associated with high cholesterol levels[12]. The functions of herbal medicines in lowering high cholesterol level are complex. Cholesterol-lowering activity of berberine acts by increasing hepatic LDL receptor mRNA and protein. Similarly, alcoholic extract of *Panax ginseng* augments antioxidant potency by depleting malondialdehyde levels, as well as elevating erythrocyte superoxide dismutase, a scavenger that reduces high cholesterol[13]. Chungtaejeon (CTJ), a well know Korean fermented tea from *Camellia sinensis* leaves, is rich in polyphenols, volatile oils, vitamins and minerals; the tea is prepared through a step by step processing technique[14].

Regular consumption of *C. sinensis* tea is believed to promote cardiovascular health by improving the cholesterol metabolism[15], attenuating angiotensin converting enzyme, reducing the degree of platelet aggregation[16] and overcoming oxidative stress by anti-oxidant effects. Although *C. sinensis* tea has been served worldwide since ancient times, with the belief that it can help in the management of obesity and hypertension, a major risk factor of atherogenesis, however, scientific research has yet to demonstrate the efficacy of the tea for these purposes. In this study, the authors investigated the ability of CTJ to regulate the serum lipid profile induced by high-fat atherogenic diet (HFAD) in rats. Moreover, the effects of CTJ on proliferation and migration of human aortic smooth muscle cells (HASMCs) were also evaluated. The data generated by this investigation might be helpful to evaluate the above uses of this herbal preparation.

2 Materials and methods

2.1 Processing of CTJ

Leaves of wild *C. sinensis* were dried overnight in a well ventilated room. Steaming was carried out 3 to 4 min after removing impurities associated with the leaves. After pulverization, it was kneaded and given a shape with the help of plastic frames having a diameter of 2.5 cm and a thickness of 0.5 cm. These wafers were dried for 2 to 3 d using bamboo baskets. After punching with the help of bamboo needle of diameter 0.2 cm, fermentation was carried out for 7 d using a fermenter. Finally, they were dried at room temperature and relative humidity of 50% for 20 to 30 d. Thus produced CTJ was wrapped in a hand-made traditional Korean paper from mulberry trees and packaged in a case[17].

2.2 Extraction of CTJ

CTJ extract was prepared according to Park *et al*[17]. A total of 112 g of CTJ were boiled in 3 300 mL of water for 3 h; this was repeated twice. The residues were removed by filtration using Whatman's paper and then the extract was evaporated followed by freeze-drying. The percentage yield of extracts was approximately 12%. In a typical experiment, the extract was dissolved in distilled water to the desired concentrations and used for analysis.

2.3 *In vivo* toxicity study and dose selection

Before the start of real experiment, an *in vivo* toxicity study was carried out in male Wistar rats for 3 weeks to identify the safe effective dose. CTJ extract was administered daily at a high dose of 1 g/(kg·d). At the end of study all rats survive with no sign of toxicity, and no alteration in body weight and food consumption pattern. Finally three safe doses of CTJ (100, 200 and 400 mg/(kg·d)) were selected for the study.

2.4 Administration of diet and treatment

Male Wistar/ST rats (13 weeks old, n=40) were purchased from Central Laboratory Animal Inc., Seoul. Animals were maintained in the Animal Research Center, Mokpo National University, under a strictly controlled environment with alternate light-dark cycle of 12-12 h, 100% fresh HEPA-filtered air, a room temperature of (23±1) °C and humidity of 45%±5%. Animal experiments were conducted after obtaining approval from Mokpo National University-Lab Animal Research Committee (MNU-LARC). Normal basal diet (D12450B rodent diet) was supplied with free access during the 2-week period of acclimation. Rats were then placed in 5 separate enclosures (n=8 rats per enclosure). A normal