Suppression of adipogenesis-relating factors by fermented tea

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Summary

It is known that tea has various beneficial effects on health promotion including anti-obesity. Our previous report demonstrates that green tea and catechins suppress differentiation of adipocytes as an anti-obesity effect. In this study, we investigated whether fermented tea suppressed expression of the adipogenesis-relating factors to improve obesity. Male ICR mice were divided into 4 groups : oolong tea, black tea, pu-erh tea, and water groups. All animals were given tea or water and commercial chow *ad libitum* for one week. At the end of feeding period, fermented tea groups significantly decreased visceral fat without affecting body weight as compare with water group. All fermented teas decreased the expression levels of PPARγ and C/EBPα, while these teas increased phosphorylation of AMPK and UCP1and 2 expression in visceral fat. Tea did not increase the excretion of lipids in fecal. These results indicated that fermented teas prevent obesity through suppression of adipocyte differentiation by down-regulating adipogenesis-relating factors and promotion of energy metabolism by up-regulating energy homeostasis factors.

Introduction

Tea is the most consumed beverage in the world except water. There are four types of tea: green tea (non-fermented), oolong tea (partially fermented), black tea (fully fermented) and pu-reh tea (post-fermented). Recent studies have described the effects of tea on the prevention of obesity [Ueda, Ashida. (2012)]. Especially, the effects of green tea are well documented, but those of fermented teas are not so many. In this study, we investigated the effects of fermented tea on lipid metabolism and elucidated the mechanism.

Materials and methods

Preparation of fermented tea. For preparation of tea extract, 2 g of tea leaves were extracted with 100 ml boiling water for 2 minutes followed by immediate washing with the same volume of boiling water.

Animals and treatments. We carried out animal experiment according to 'Guidelines for the care and use of experimental animals' at Kobe University Rokkodai Campus. Male ICR mice (6 weeks old) were purchased from Japan SLC (Shizuoka, Japan). The mice were housed in air-conditioned room with a controlled environment of 25±1ºC under a 12-hour light-dark cycle with free access to an chemical diet and tap water for 1 week. We divided the mice to following 4 groups of 4 each: water, oolong tea, black tea and pu-erh tea. They were sacrificed under anesthesia after 1 week. Plasma, feces and visceral fat were collected and frozen in liquid nitrogen and stored at -80ºC until use. Cholesterol and triglyceride were measured by the corresponding commercial kit from Wako pure chemical industries (Osaka, Japan). Protein expression was measured by Western blot analysis as described previously [Ueda, *et al*. (2008)].

Statistical analysis of the data. The data are represented as the means ± SE, and significant difference was statistically analyzed by the Dunnet test.

Results and discussion

In this study, an intake of fermented tea did not affect body weight, food intake and beverage intake. Visceral fat weight was significantly decreased in fermented tea groups compared with water group. The

level of plasma total cholesterol in fermented tea groups decreased significantly, whereas the level of plasma triglyceride was unchanged among four groups. These data are consistent with the results of previous reports [Hou, *et al*. (2009), Fujita, *et al*. (2008)]. Fermented tea groups did not affect fecal lipids. To investigate mechanism of the suppressive effect of fermented tea on fat accumulation, we measured the expression level of PPARγ and C/EBPα. All fermented tea groups significantly suppressed PPARγ expression. In the case of C/EBPα expression, pu-reh tea significally suppressed it. These transcription factors regulate the expression of lipogenic enzymes and other adipocyte-specific genes, such as fatty acid synthase (FAS), adiponectin, and GLUT4 [Rosen, *et al*. (2002)]. The expression of these transcription factors is necessary for adipogenesis. Previous study reported that green tea suppressed adipocyte differentiation through adipogenesis-related transcription factors [Ashida, *et al*. (2004)]. Taken our results and previous ones together, tea significantly suppressed adipocytes differentiation.

Next, we investigated phosphorylation of AMPK and expression of UCP1and 2. All fermented teas increased phosphorylation of AMPK, black tea significally increased it. It was reported that (-)-epigallocatechin-3-gallate (EGCg) suppressed lipid accumulation in 3T3-L1 cells [Hyun-suke, *et al*. (2007)]. Amount of EGCg is poor in fermented tea. Our result and previous report indicate that fermented tea contains other substans that is able to stimulate phosphorylation of AMPK. The expression level of UCP-1 was significaly higher in pu-reh tea and black tea groups than that in water group in brown adipose tissue. On the other hand, we demonstrated that all fermented tea groups up-regulated the expression level of UCP-2 in white adipose tissue. The expression of UCPs is strongly induced when thermogenesis is required [Ricquier, *et al*. (1986)]. Thus, up-regulation of UCP-1 and 2 expression may inhibit fat accumulation. Indeed, our study showed that supplementation of fermented tea for 1 week significantly decreased visceral fat weight. In conclusion, improved obesity by suppressing PPARγ and C/EBPα expression and promoting p-AMPK and UCP-1 and 2 expression. It is fermented tea needed to explore the identification of the active compound in fermented tea and to clarify the detailed mechanism of the active compound.

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