THEAFLAVINS IMPROVE CIRCULATORY FUNCTION THROUGH SYMPATHETIC NERVE SYSTEM

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ABSTRACT

Ingestion of black tea was reported to decrease blood pressure, however, effective component in it has been unclear. In this murine study, we examined that hemodynamic changes induced by single or repeated treatment of theaflavin fraction (TFs), which are specific polyphenol components in black tea. First, we evaluated that the hemodynamic changes as mean blood pressure (MBP), heart rate (HR) and blood flow (BF) of cremaster arteriole in rats after vehicle (V) or 10mg/kg TFs for 60 min. At the end of the measurement period, aorta of the animal was dissected and phosphorylated endothelial nitric oxide synthase (*p*-eNOS) was measured. MBP and HR were transient increased after ingestion of TFs. BF was increased significantly soon after ingestion of TFs. Aortic *p*-eNOS was elevated significantly by the TFs ingestion. Next, to compare the efficacy of theaflavins, we measured cremastric BF after ingestion of 4mg/kg each chemical. The efficacy of them was following order; theaflavin 3'-O-gallate >> theaflavin-3-O-gallate> theaflavin = theaflavin-3, 3'-di-O-gallate. In order involvement of sympathetic nerve on the hemodynamic changes of TFs, we also measured MBP and HR with or without non-specific adrenaline blocker as carvedilol (CR). Elevation of HR, MBP and aortic *p*-eNOS observed after ingestion of TFs were reduced by the pretreatment of CR. In addition, we determined MBP in rats 2 weeks after repeated treatment of V or 10mg TFs. MBP was reduced and aortic eNOS level was increased significantly in TFgoups. In conclusion, TFs showed hypotensive effect through improvement of endothelial functions, it was suggested that this effect arise through sympathetic nerve stimulation, and the efficacy of hemodynamic action of theaflavin was dependent on their chemical structure.

1. INTRODUCTION

Theaflavins are a type of polyphenols present in black tea. The chemical structure of the four major theaflavins, theaflavin (TF1), theaflavin-3-O-gallate (TF2A), theaflavin 3'-O-gallate (TF2B), and theaflavin-3,3'-di-O-gallate (TF3) are shown in Fig. 1.

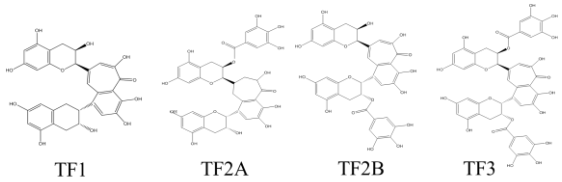

Fig. 1. Chemical structures of theafavins

These compounds are formed from co-oxidation of selected pairs of green tea catechins during fermentation (Haslam E, 2003, Sang S, et al., 2011). Although black tea represents 78% of tea production worldwide, there is only limited research on the biological significance of black tea or theaflavins. Several meta-analyses have also shown that consumption of black tea results in significant primary prevention of cardiovascular diseases as a consequence of decreased levels of plasma LDL cholesterol and blood pressure (Hartley L, et al., 2013, Santesso N, et al., 2014). However, there is limited information on the physiological significance of theaflavins compared with that reported for green tea catechins. In the present study, we evaluated alterations in systemic- and microcirculation caused by a single dose of theaflavin rich fraction or purified theaflavin components. We also examined the involvement of the sympathetic nervous system on hemodynamic changes induced by TF, evaluated using an adrenaline receptor blocker. In addition, we examined the effect of repeated treatment with TFs on the systemic circulation in a murine study.

2. EXPERIMENT

2.1. MATERIALS

The theaflavin fraction was prepared by the method of

previous study (Yamazaki T, et al., 2014). The concentration of each theaflavins in this fraction was TF1 4.6%, TF2A 16.6%, TF2B 11.2%, and TF3 26.2%.

2.2. ANIMALS AND DIETS

The study was approved by the Animal Care and Use Committee of the Shibaura Institute of Technology. All the animals received care under the guidelines of this institution. Male wistar rats weighing 200–250 g (7-10 wks) were obtained from Saitama Experimental Animal Supply (Tokyo, Japan). The diet provided to the animals was MF obtained from the Oriental Yeast Co. Ltd., Tokyo, Japan.

2.3. EXPERIMENTAL METHOD

Experiment 1 A gastric tube was inserted into their stomach under urethane anesthesia (1 g/kg, s.c.). The cremaster muscle was exteriorized and the surface suffused with Krebs-Ringer buffer. Distilled water, 10 mg/kg of the TFs or 4 mg/kg of either TF1, TF2A, TF2B, or TF3 was administered orally to the animals through a gastric tube. Blood flow in the cremasteric artery was determined using a laser Doppler blood flow meter (Periscan PIM-2, Perimed Co. Ltd. Stockholm, Sweden). Another animals were treated with vehicle or 10 mg/kg TF, followed by measurement of MBP and HR every 6 min for 60 min using the tail-cuff method (BP-98A Softron, Tokyo, Japan).

Experiment 2 The animals received primary oral administration of 10 mg/kg carvedilol (CR), as an adrenaline receptor (AR) blocker, other rats received distilled water. Ten minutes after administration of carvedilol, 8 of the 16 rats received oral administration of vehicle, while 8 were treated with 10 mg/kg TF, and 16 animals pretreated with distilled water were received similar treatment. The MBP and HR of the animals were then determined every 6 min for 60 min. After the measurements, the aorta was removed and determined aortic *p*-eNOS level by western blotting.

Experiment 3 The animals were fed either vehicle or 10 mg/kg TF daily for 2 weeks. The MBP and HR were determined before and after treatment by the tail cuff method using a MK-2000ST (Muromachi Kikai, Tokyo, Japan). After the measurements, the aorta was removed by dissection under anesthesia, snap frozen in liquid nitrogen and stored at -80^{o}C until analysis. We determined aortic eNOS level by western blotting.

2.4. STATISTICAL ANALYSIS

The data were expressed as mean and standard deviation. Statistical analyses were performed by one or two way ANOVA, or Students' t-test, and post hoc comparisons with the vehicle group were made by the two-tailed followed by Dunnett's test. A probability of $P < 0.05$ was considered to be statistically significant.

3. RESULT

Experiment 1 Ingestion of TFs elevated in HR and MBP transiently, and rise of cremastric BF was shown soon after treatment of TFs (Fig. 2). TFs also significant elevated aortic phosphorylated eNOS (Fig. 2d).

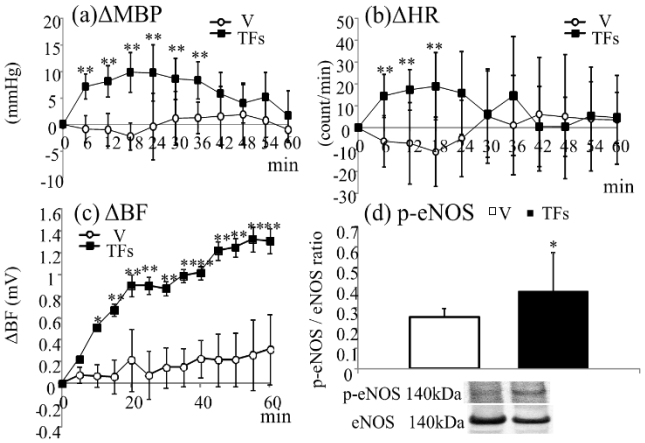

Fig. 2. Changes of MBP (a), HR (b), BF (c) and aortic *p*-eNOS (d) after single treatment of TFs. *p<0.05, **p<0.01 vs V.

TF2B was most effective among the TFs and TF2A showed slight effect on the blood flow elevation, while, TF1 and TF3 did not show any changes (Fig. 3).

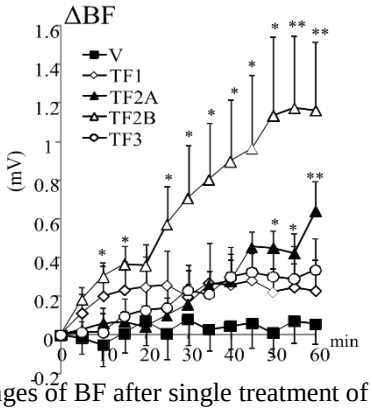

Fig. 3 Changes of BF after single treatment of 4mg/kg purified theaflavin. *p<0.05, **p<0.01 vs V.

Experiment 2 The hemodynamic changes induced by TFs were disappeared by the combination treatment of CR and TFs (Fig. 4).

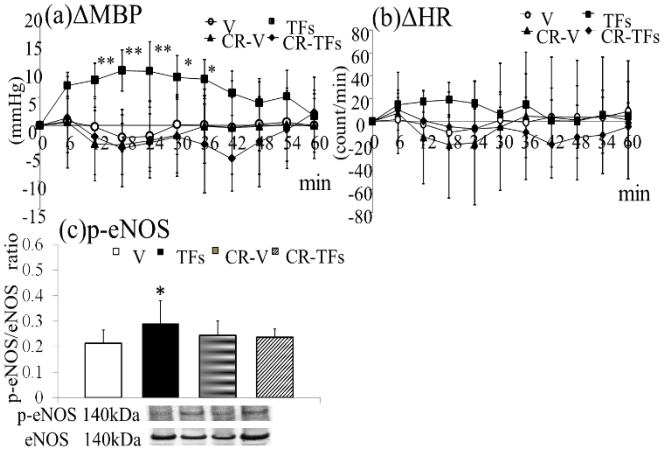

Fig. 4 Changes of MBP (a), HR (b) and *p*-eNOS (c) after single treatment of TFs with or without CR.*p<0.05, **p<0.01 vs V.

Experiment 3 Repeated ingestion of TFs significant decreased MBP and increased aortic eNOS level (Fig. 5).

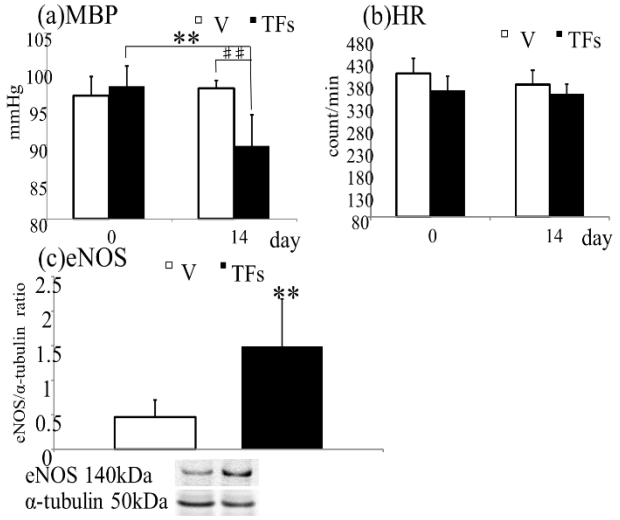

Fig. 5 Changes of MBP (a), HR (b) and aortic eNOS level (c) after repeated treatment of TFs. *p<0.05, **p<0.01 vs V.

4. DISCUSSION

The present study showed that a single dose of TFs resulted potent hemodynamic changes in rats including transient alteration in the systemic circulation such as an increase in MBP and HR (Fig. 2a and b). We also observed changes in the microcirculation as an elevation in BF in the cremaster muscle, with this effect being more persistent than the changes in the systemic circulation (Fig. 2c). The study also showed large differences between theaflavin components in the changes induced in the microcirculation. TF2B increased cremastric BF soon after treatment, a change that was potent and sustainable, whereas TF2A showed lower potency than that of TF2B. In contrast, TF1 and TF3 did not cause any changes in the microcirculation (Fig. 3). These results suggested that the ability of the TFs components to alter the microcirculation was dependent on their chemical structure; the chemicals which had a galloyl group represented persuasive effect, but the chemicals which had no or two galloyl group didn't have any BF changing ability. There are several reports on the bioavailability of theaflavin. Although several methods have been developed for measuring theaflavin, theaflavins were not detected after consumption of black tea or TFs in blood (Neilson AP, et al., 2006, Nishimura M, et al., 2007). These studies indicated that theaflavin was hardly absorbed and distributed in blood unlikely catechins. On the other hand, in the present study, a single dose of TFs showed potent hemodynamic changes despite low bioavailability.

In order to estimate the mechanism of the hemodynamic action of TFs, we examined whether the sympathetic nervous system was involved in this activity using an AR-blocker. The results showed that the transient increase in HR and MBP induced by a single oral dose of TFs totally disappeared after pretreatment with an AR blocker, carvedilol (Fig. 4). Carvedilol is a vasodilating β-blocker, used in therapeutic management of hypertension. According to the significant reduction was shown in the animal with the combination treatment TFs and carvedilol, it was suggest that TFs stimulates sympathetic nerves leading to hemodynamic changes.

Sympathetic nerve system was known to play a key role in multiple physiological changes including systemic circulation (Axelrod J, et al., 1972, Kjaer M, et al., 1987).There is considerable evidence that excitation of sympathetic nerve causes hemodynamic changes rapidly. In addition, we have confirmed that low bioavailable procyanidins, as catechin oligomer as same as theaflavin, revealed blood adrenaline level 2 hr administration (Matsumura Y, et al., 2014). These reports suggested the possibility of the involvement of the sympathetic nerve stimulation after catechin oligomer administration.

We found a significant increase in eNOS phosphorylation in the aorta dissected 60 min after a single oral dose of TFs (Fig. 2d). This change also did not occur following pretreatment with carvedilol (Fig.4c). This finding suggested that the phosphorylation of aortic eNOS requires shear and circumferential stress associated with the hemodynamic changes induced by a single oral dose of TFs. BF is known to be correlate significantly with shear stress for endothelial cells (Lu D, et al., 2011, Hoefer IE, et al., 2013). It is also well established that these mechanical forces cause biochemical changes in the endothelium and vessel walls including eNOS phosphorylation (Bevan JA, 1997, Topper JN, et al., 1999). In addition, shear stress, as shown in increase of blood flow, activated integrin on the endothelium surface leading to upregulation of eNOS. There is also considerable evidence that these anti-atherogenic modifications, such as eNOS induction and vascular endothelial growth factor production, occur as a consequence of repetitive intransient mechanical changes, such as exercise (Kojda G, et al., 2005). We confirmed that the repeated ingestion of TFs elevated eNOS expression in vessel as shown in Fig.5c, this change was induced by the repeated mechanical stress after ingestion of TFs. Therefore, we consider that these results indicate that a single dose of TFs may cause transient mechanical stress, and that repetition of this effect induces a significant decrease in MBP.

5. CONCLUSION

The results of this study indicated that TFs causes potent alterations in hemodynamics in rats. The magnitude of these changes was different between theaflavin components. Our study also suggested that the sympathetic nervous system was involved in the mechanism of the hemodynamic changes induced by TFs. Further studies are required to determine how TFs stimulates sympathetic nerves.

6. REFERENCES

Haslam E. Thoughts on thearubigins. Phytochemistry. 2003;64:61-73.

Sang S, Lambert JD, Ho CT, Yang CS. The chemistry and biotransformation of tea constituents. Pharmacol Res. 2011;64:87-99

Hartley L, Flowers N, Holmes J, Clarke A, Stranges S, Hooper L, et al. Green and black tea for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev. 2013;6:CD009934.

Santesso N, Manheimer E. A summary of a cochrane review: green and black tea for the primary prevention of cardiovascular disease. Glob Adv Health Med. 2014;3:66-7.

Yamazaki T, Sagisaka M, Ikeda R, Nakamura T, Matsuda N, Ishii T, et al. The human bitter taste receptor hTAS2R39 is the primary receptor for the bitterness of theaflavins. Bioscience, biotechnology, and biochemistry. 2014; 78:1753-6.

Neilson AP, Green RJ, Wood KV, Ferruzzi MG. High-throughput analysis of catechins and theaflavins by high performance liquid chromatography with diode array detection. J Chromatogr A. 2006; 1132:132-40.

Nishimura M, Ishiyama K, Watanabe A, Kawano S, Miyase T, Sano M. Determination of theaflavins including methylated theaflavins in black tea leaves by solid-phase extraction and HPLC analysis. Journal of agricultural and food chemistry. 2007; 55:7252-7.

Leonetti G, Egan CG. Use of carvedilol in hypertension: an update. Vasc Health Risk Manag. 2012; 8:307-22.

Fongemie J, Felix-Getzik E. A Review of Nebivolol Pharmacology and Clinical Evidence. Drugs. 2015.

Axelrod J, Weinshilboum R. Catecholamines. The New England journal of medicine. 1972; 287:237-42.

Kjaer M, Secher NH, Galbo H. Physical stress and catecholamine release. Bailliere's clinical endocrinology and metabolism. 1987;1: 279-98.

Matsumura Y, Nakagawa Y, Mikome K, Yamamoto H, Osakabe N. Enhancement of energy expenditure following a single oral dose of flavan-3-ols associated with an increase in catecholamine secretion. PloS one. 2014; 9:e112180.

Lu D, Kassab GS. Role of shear stress and stretch in vascular mechanobiology. Journal of the Royal Society, Interface / the Royal Society. 2011; 8:1379-85.

Hoefer IE, den Adel B, Daemen MJ. Biomechanical factors as triggers of vascular growth. Cardiovascular research. 2013; 99(2):276-83. Epub 2013/04/13. doi: 10.1093/cvr/cvt089. PubMed PMID: 23580605.

Bevan JA. Shear stress, the endothelium and the balance between flow-induced contraction and dilation in animals and man. Int J Microcirc Clin Exp. 1997;17:248-56.

Topper JN, Gimbrone MA, Jr. Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype. Mol Med Today. 1999;5:40-6.

Kojda G, Hambrecht R. Molecular mechanisms of vascular adaptations to exercise. Physical activity as an effective antioxidant therapy? Cardiovascular research. 2005; 67:187-97

7. NOMENCLATURE

Subscripts

TFs	:	theaflavins
MBP	:	mean blood pressure
HR	:	heart rate
BF	:	blood flow
p-eNOS	:	phosphorylated endothelial nitric oxide synthase
eNOS	:	endothelial nitric oxide synthase
CR	:	carvedilol, non-specific adrenaline blockers
V	:	vehicle
TF1	:	theaflavin
TF2A	:	theaflavin-3-O-gallate
TF2B	:	theaflavin 3’-O-gallate
TF3	:	theaflavin-3,3’-di-O-gallate
AR	:	adrenaline receptor

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