Possible Mechanism of Preventive Effects of Coffee Intake on the Formation of Arterial Occlusive Thrombosis

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Background: Prevalence and incidence of arterial occlusive thrombosis are influenced by life-style. Coffee consumption was shown with a lower incidence of myocardial infarction by Framingham Study. Yet, the mechanism is to be elucidated.
Methods: We examined the effects of coffee intake on the progression of occlusive thrombus formation in mouse cremasteric arteries. After 7 days of free intake of pure water, coffee containing water (5 mg/ml), or caffeine containing water (0.1 mg/ml), endothelial cell function was locally damaged by $FeCl_3$. Circulating platelet and leukocytes were rendered fluorescently by rhodamine 6G. Process of occlusive thrombus growth was continuously visualized by 3-D imaging system equipped with ultra-fast confocal microscopy, and time to vascular occlusion was measured in each mouse.
Results: Platelet accumulation started immediately after $FeCl_3$ exposure in all tested groups. However, arterial occlusion time in taking coffee containing water was significantly longer than those taking pure water. (46.0 ± 17.4 min (n = 5) vs. 12.3 ± 2.6 min (n = 31), $p < 0.05$) Arterial occlusion time in mice taking caffeine (13.8 ± 5.9 min (n = 4)) was not different from those taking pure water.
Conclusion: Coffee, but not caffeine intake, may have preventive effect on arterial occlusive thrombus formation initiated by functional injury of arterial endothelium.

Key words: coffee, atherothrombotic disease, platelet, caffeine

INTRODUCTION

Prevalence and incidence of atherothrombotic diseases such as myocardial infarction is known to influence by life style. Indeed, some of life-style factors such as smoking are proven to influence the risk of myocardial infarction by well designed population cohort studies [1–5, 6].

Although daily food and drink intake may have strong impact on the prevalence of atherothrombotic disease, details are still to be elucidated. Recent subanalysis of Framingham study suggest that regular intake of caffeinated coffee may reduce the risk of myocardial infarction [7]. However, the mechanism of coffee intake to reduce the onset of atherothrombotic diseases such as myocardial infarction is still to be elucidated.

It is well known that platelet play an important role in the onset of atherothrombotic diseases [8, 9]. We have previously established an animal model which reflect occlusive thrombus formation equivalent to the onset of atherothrombosis [10]. Here we tested the effect of continuous intake of coffee and caffeine containing water for arterial occlusive thrombus formation in that model.

METHODS

Mice Model of FeCl3-induced Endothelial Injury and Arterial Occlusive Thrombus Formation.

The details of arterial occlusive thrombus model in mice have been published previously [10]. Briefly, Male ICR mice (CLEA Japan, Inc, Tokyo, Japan), aged from 10 to 11 weeks, were housed 4 or 5 per cage in acclimatized colony rooms on a natural light–dark cycle, with food and water (either containing or not containing coffee and caffeine) continuously available, for at least 1 week before experimental use. Experimental mice were pre-anesthetized by intraperitoneal injection of ketamine, xylazine and atropine sulfate, then anesthetized more profoundly with the use of intra-venous injection of nembutal through the jugular vein. The cremaster muscle of each experimental mouse was prepared on a glass plate rich in saline 5 minutes before starting the experiments. One hundred μl of 0.1% rhodamine 6G containing saline was administered additionally to render platelets and leukocytes fluorescent. To initiate platelet thrombus formation, endothelial injury was induced on the cremasteric artery by putting a 0.2 mm diameter cotton thread containing 0.25M $FeCl_3$ solution for 5 minutes. (left panel of Fig. 1) All experimental procedures were approved by the internal review board of animal care and use committee of the Tokai University School of Medicine.

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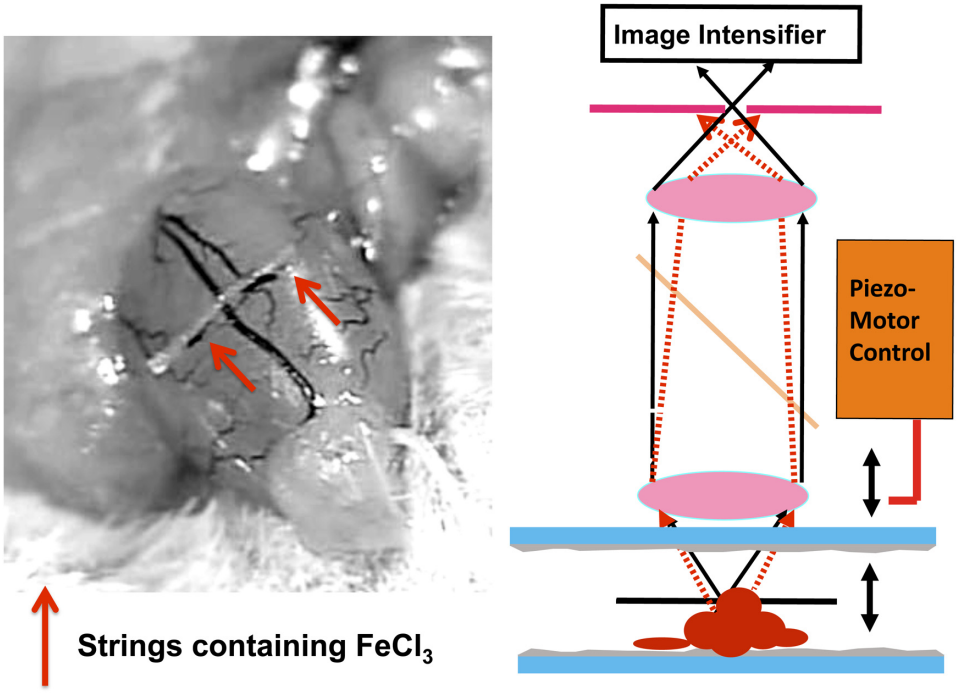

Fig. 1 *Method for cremasteric arterial thrombus formation and detection by 3-D imaging.*
Left panel. Preparation of cremasteric artery was done by procedures described in the results section. A 0.2 mm diameter cotton thread containing 0.25M $FeCl_3$ solution (shown by arrow) was exposed from out-side of the cremasteric arteries to initiate thrombus formation.

Experimental Protocol

For testing the effects of coffee and caffeine intake for the development of arterial occlusive thrombus formation, experimental mice were housed with continuously available pure water, or pure water with instant coffee at a concentration of 5 mg/ml, or pure water containing 0.1 mg/ml of caffeine for 7 days. Water intake was measure in each mouse.

Time to Complete Arterial Occlusive Thrombus Formation.

As shown in the previous publication [10], platelet immediately started to adhere on the endothelial cells stimulated by $FeCl_3$. The three-dimensional growth of thrombi was continuously monitored by our original 3D-imaging systems (right panel of Fig. 1) until arterial occlusive thrombus formation was completed [10-12]. Time to complete occlusion of cremasteric arteries by thrombi was measured in each experiment.

Statistics

All the numerical results were shown as mean ± SD unless otherwise specified. Students' un-paired t-test was performed to test the statistical significance between each experimental group. A p-value less than 0.05 was considered as statistically significant.

RESULTS

Water intake

Total intake of water in mice housed with pure water containing coffee and caffeine of 9.3 ± 0.3 and 9.4 ± 0.5 ml, respectively was larger than those housed with pure water of 8.1 ± 0.5 ml ($p < 0.05$ for both comparison).

Platelet Adhesion and Occlusive Thrombus Formation

Platelet started to adhere at site of $FeCl_3$-induced endothelial injury immediately no matter whether the mice were housed with pure water, pure water containing coffee or pure water containing caffeine (Fig. 2).

As shown in Fig. 2, arterial occlusion time by thrombi in mice housed with pure water containing coffee of 46.0 ± 17.4 min (n = 5) was significantly longer ($p < 0.01$) than those housed with pure water of 12.3 ± 2.6 min (n = 31). Of note, arterial occlusion time by thrombi in mice housed with pure water containing caffeine of 13.8 ± 5.9 min (n = 4) was not significantly different from that of mice housed with pure water.

DISCUSSION

In addition to the epidemiological clarification of the effects of caffeinated coffee intake in prevention of atherothrombosis [7], we demonstrated herein that preventive effect of seven days intake of caffeinated coffee containing water for experimental arterial occlusive thrombus formation in mice. In spite epidemiological study demonstrated the specific preventive effects of caffeinated coffee intake not shared by decaffeinated ones [7], our experimental results did not show inhibiting effects of equivalent dose of caffeine intake in arterial occlusive thrombus formation though caffeine may have theoretical inhibiting effects on platelet activation by increasing intra-cytosolic cyclic AMP (c-AMP).

Since atherothrombotic diseases are prevalent in the modern world[13] and the substantial amount of medical resources are being spent for their prevention, lifestyle modification, if proven to be effective, is useful for primary prevention. In this context, Framingham

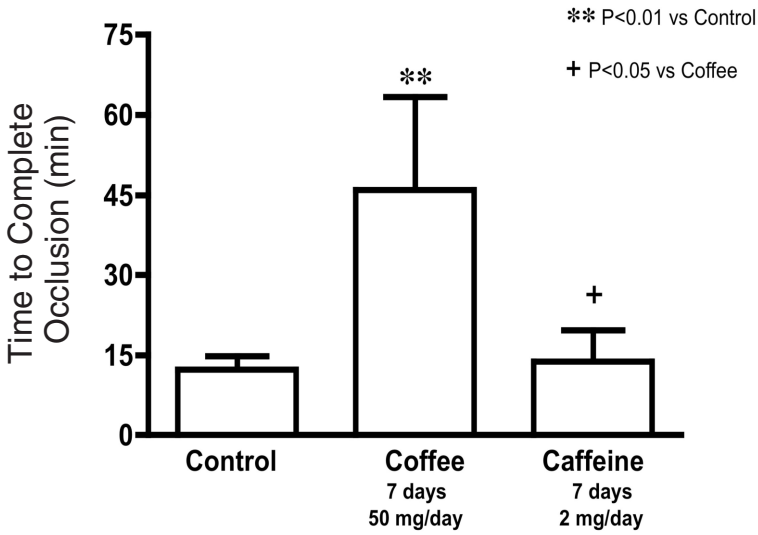

Fig. 2 *Effects of coffee and caffeine on the time to complete occlusion of cremasteric arteries.*

study was successful because it clearly demonstrated the impact of smoking on the onset of atherothreombosis [1–3, 14, 15]. Impact of smoking in the onset of atherothrombosis was confirmed by several other studies [6, 16], thus, this concept is widely accepted in the world. Regarding regular intake of coffee, on the other hand, there are several results reported, but contradicting among them. Relatively old epidemiological studies, for example, suggested that coffee intake might relate to the higher risk of atherothrombosis [17, 18]. However, previous studies did not consider the contributing effects of confounding factors such as smoking with coffee intake. After appropriate adjustment of confounding factors, coffee intake itself does not relate to the higher risk of atherothrombosis [19, 20]. Latest report from Framingham study demonstrating the effects of caffeinated coffee intake for prevention of atherothrombosis is the most well designed study. Though the number of report is a few, results with well designed clinical studies such as recent report from Framingham registry is most trustable to date. [7]Further confirming results are awaited.

From the mechanistic view-points, there are several ingredients contained in coffee, which might contribute to the reduced risk of atherothrombosis. Varani K *et al*. has reported that caffeine, which is methylxanthines known to be present in coffee, causes elevation of cAMP to inhibit platelet activation and inflammatory response [21]. Caffeine and its relatives are initial reasonable candidates for the effects of coffee in prevention of atherothrombosis because similar agents known to increase c-AMP, such as dipillidamol and cilostazol, are known as drugs to prevent atherothrombosis [22–25]. The results of Framingham study demonstrating the reduced risk of heart disease only in population taking caffeinated coffee also support this hypothesis. However, our study, though tested only with limited dosage of caffeine, did not support that caffeine contained in coffee is the important factor to reduce the risk of atherothrombosis, because time for occlusive thrombus formation was not influenced by equivalent amount of caffeine intake as coffee. As shown by various previous studies, there are many possible candidate of active ingredients contained in coffee, including pyridiniums and tetramethylpyrazine [26, 27]. For example, tetramethylpyrazine is speculated to inhibit the vWF-mediated process of platelet thrombus formation [26], however, more studies are needed to specify the candidate.

Our experimental model is unique because arterial occlusive thrombus formation occurs even in the presence of endothelial cell [10]. This might raise limitation of our experimental results in application for clinical medicine because typical atherothrombosis such as myocardial infarction is known to be caused by thrombi formed at site of atheroma rupture (in the absence of endothelial cell). However, as Kawamura *et al*. has reported, thrombi can develop to the size that occlude arterial, even at the site of $FeCl_3$-induced injured endothelial cells [10].

In conclusion, our study support the notion, which was proven by recent Framingham study, that regular intake of caffeinated coffee reduce the risk of atherothrombotic events through inhibition of arterial occlusive thrombus formation. Further studies are awaited to determine the specific chemicals contained in coffee, which is not likely to be caffeine, is the major contribution of the reduced risk of atherothrombosis.

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