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แทนเจอรีตินบรรเทาแอลเนมชักนำการทำงานผิดปกติของหลอดเลือดในหนูแรท

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Tangeretin Alleviates L-NAME Induced Vascular Dysfunction in Rats

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หลักการและวัตถุประสงค์: แทนเจอรีติน (Tangeretin) เป็นสารประกอบฟลาโวนอยด์ที่พบในผลไม้ตระกูลส้ม มีหลายการศึกษาเกี่ยวกับแทนเจอรีตินพบว่าแทนเจอรีตินมีฤทธิ์ต่อต้านการเสื่อมของระบบประสาท ต้านการอักเสบ และต้านอนุมูลอิสระ วัตถุประสงค์ของการศึกษานี้เพื่อตรวจสอบผลของแทนเจอรีตินต่อการทำงานของหลอดเลือดในหนูแรทที่ได้รับสารแอลเนม (L-NAME)

วิธีการศึกษา: หนูแรทถูกแบ่งออกเป็น 4 กลุ่ม กลุ่มที่ 1 คือหนูแรทที่ได้รับสารแอลเนม (40 มก./กก.) ในน้ำกลั่น กลุ่มที่ 2 และ 3 คือหนูแรทที่ได้รับสารแอลเนมร่วมกับสารแทนเจอรีติน (15 และ 30 มก./กก. ตามลำดับ) กลุ่มที่ 4 คือหนูแรทกลุ่มปกติได้รับเพียงน้ำกลั่น (n=7 ต่อกลุ่ม) เมื่อสิ้นสุดการทดลองทำการประเมินการตอบสนองของหลอดเลือดต่อการกระตุ้นโดยกระแสไฟฟ้าและสารที่มีผลต่อหลอดเลือดในหลอดเลือดมีเซนเทอริกและเอออตาร์

ผลการศึกษา: การเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติของการหดตัวตอบสนองต่อการกระตุ้นโดยกระแสไฟฟ้าถูกพบในหลอดเลือดมีเซนเทอริกของหนูแรทที่ได้รับสารแอลเนม ($p<0.05$) แทนเจอรีตินสามารถลดการหดตัวตอบสนองต่อการกระตุ้นโดยกระแสไฟฟ้าได้ในหนูแรทที่ได้รับสารแอลเนมร่วมกับแทนเจอรีตินเมื่อเทียบกับหนูที่ได้รับแค่สารแอลเนม ($p<0.05$) แต่อย่างไรก็ตามการหดตัวตอบสนองต่อนอร์เอพิเนฟริน (Norepinephrine) ไม่มีความแตกต่างกันระหว่างกลุ่ม การตอบสนองคลาย

Background and Objective: Tangeretin is a flavonoid compound found in citrus fruits. Several biological activities of tangeretin have been revealed including; anti-neurodegeneration, anti-inflammation, and anti-oxidation. This study was to investigate the effects of tangeretin on vascular function in L-NAME-treated rats.

Methods: Rats were divided into 4 groups. Groups I; rats treated with L-NAME (40 mg/kg) in drinking water only, Groups II and III; rats treated with L-NAME together with tangeretin (15 and 30 mg/kg, respectively), Groups IV; control rats were received only distilled water (n=7/each group). At the end of experiment, vascular responses to electrical field stimulation (EFS) and vasoactive agents in mesenteric vascular beds and aortic rings were evaluated.

Results: A significant increase in contractile response to electrical EFS was observed in the mesenteric vascular bed isolated from L-NAME-treated rats ($p<0.05$). Tangeretin reduced the augmented response to EFS in L-NAME-treated rats comparing to those of untreated rats ($p<0.05$). However, the contractile response to exogenous norepinephrine was not dif-

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ตัวของหลอดเลือดต่อสารอะซิติลโคลีนในหลอดเลือดมีเซนเทอริกและเอออตาร์ลดลงอย่างมีนัยสำคัญทางสถิติในหนูแรทที่ได้รับสารแอลเนมเมื่อเทียบกับหนูปกติ (p<0.05) แทนเจอรีตินสามารถปรับปรุงการตอบสนองคลายตัวของหลอดเลือดต่อสารอะซิติลโคลีน (Acetylcholine) ให้ดีขึ้นในหนูแรทที่ได้รับสารแอลเนมร่วมกับแทนเจอรีตินเมื่อเทียบกับหนูที่ได้รับแค่สารแอลเนม (p<0.05) ซึ่งการตอบสนองคลายตัวของหลอดเลือดต่อสารโซเดียมไนโตรปรัสไซด์ (Sodium nitroprusside) ไม่มีความแตกต่างอย่างมีนัยสำคัญระหว่างกลุ่ม

สรุป: ผลการทดลองของการศึกษานี้บ่งชี้ว่าแทนเจอรีตินสามารถยับยั้งการตอบสนองต่อการหดตัวของระบบประสาทซิมพาเธติกและปรับปรุงการคลายตัวของหลอดเลือดจากเอนโดทีเลียมให้ดีขึ้นในหนูแรทที่ได้รับสารแอลเนม

คำสำคัญ: แทนเจอรีติน, การทำงานของหลอดเลือดที่ผิดปกติ, หนูที่ได้รับสารแอลเนม

ferent among groups. Vasorelaxation responses to acetylcholine (ACh) in the mesenteric vascular beds and aortic rings were significantly blunted in L-NAME-treated rats compared to control rats (p<0.05). Treatment with tangeretin improved the vasorelaxation response to ACh in L-NAME-treated rats compared to untreated rats (p<0.05). There was no significant difference in the vasorelaxation responses to sodium nitroprusside among groups.

Conclusions: The results of this study indicated that tangeretin suppresses sympathetic nerve mediated contractile response and improves endothelium-dependent vasorelaxtion in L-NAME-treated rats.

Keywords: Tangeretin, Vascular dysfunction, L-NAME-treated rats

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Introduction

Endothelium derived vasorelaxing factors and sympathetic nerve mediated contractile response play an important role in vascular tone regulation. Long-lasting adrenergic hyperactivity is complicated in the pathogenesis of cardiovascular risk factors since it indirectly induces vascular dysfunction and damage[1]. Previous study reported that nitric oxide (NO) is involved in the sympathetic nervous system (SNS), which may affect arterial pressure[2]. Bergamaschi and co-workers[3] demonstrated that SNS plays a major role in the maintenance of blood pressure in N(G)-Nitro-L-arginine-methyl ester (L-NAME), NO synthase inhibitor, induced hypertension. The mechanism is involved an increase in central sympathetic drive at the rostral ventrolateral medulla neurons. Moreover, Augustyniak and co-workers showed that acute infusion of L-NAME caused renal sympathetic activation in conscious animals[4], indicating NO is involve in modulating sympathetic nerve activity.

It is well established that hypertension is strongly associated with endothelial dysfunction. In the rat model of chronic NO inhibition, the vasorelaxation responses to acetylcholine (ACh) of the aortic rings from rats treated with L-NAME was reduced[5]. This was consistent with a decrease in response to ACh in mesenteric vasculature bed of L-NAME-treated rats[6]. Moreover, Fu and co-workers indicated that rats treated with L-NAME have a decrease in ACh-induced endothelium-dependent vasorelaxation, which mediated by increasing oxidative stress[7]. Recently, it has been reported that oxidative stress is a key factor in the pathogenesis of hypertension since an increase in reactive oxygen species (ROS) is associated in the reduction in the bioavailability of NO[8]. This NO depletion might be involved in the impairment of ACh-mediated vasodilation.

Tangeretin is a flavonoid compound found in citrus fruits. Several biological activities of tangeretin have been revealed including; anti-neurodegeneration, anti-inflammation, and anti-oxidation[9-11]. Tangeretin can alleviate cisplatin-induced acute hepatic injury via the concerted modulation of inflammation and oxidative stress[12]. Furthermore, tangeretin can improve renal function in chronic kidney disease-induced animals with its anti-oxidant effect[13]. However, the effect of tangeretin on SNS mediated contractile response and vascular response to vasoactive agents in L-NAME-treated rats are still unknown.

The aim of the present study was to investigate whether tangeretin could improve vascular dysfunction induced by L-NAME in rats.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 220-240 g were obtained from Nomura Siam International Co., Ltd., Bangkok, Thailand. They were housed at 25 ± 2°C with a 12 h dark–light cycle at Northeast Laboratory Animal Center, Khon Kaen University, Khon Kaen, Thailand. All procedures complied with the standards for the care and use of experimental animals and were

approved by the Animal Ethics Committee of Khon Kaen University, (IACUC-KKU-98/60).

Experimental protocols

After a week of acclimatization, rats were treated with L-NAME (40 mg/kg/day) in their drinking water for 5 weeks whereas control rats were received distilled water. The rats were randomly divided into 4 groups of 7 rats each. Group I Control + vehicle or propylene glycol (PG) (1.5 ml/kg; p.o.); Group II L-NAME + vehicle or PG (1.5 ml/kg; p.o.); Group III L-NAME + tangeretin (15 mg/kg; p.o.); Group IV L-NAME + tangeretin (30 mg/kg; p.o.). Tangeretin and PG were intragastrically administered daily for the last 2 weeks of the study.

Vascular Function Study

Experimental protocols in isolated mesenteric vascular beds

After exsanguination, mesenteric vascular beds were carefully isolated and then placed on a stainless steel grid in a humid chamber. The preparations were perfused with physiological Krebs' solution at a constant flow rate of 5 ml/min, using a peristaltic pump. Electrical field stimulation (EFS) (5-40 Hz, 90 V, 1 ms, for 30 s at 5-min intervals) was performed. Contractile responses to EFS were detected as changes in mean perfusion pressure (mmHg). After that norepinephrine (NE) (0.15 nmol-15 nmol) was applied to evaluate the contractile responses to exogenous NE. To determine vasoactive performance of resistance small arteries, methoxamine (5-7 μM) was added into Kreb's solution to raise tone (70-90 mmHg above baseline). Subsequently, different doses of vasoactive agents, ACh (ACh, 0.1 nM–0.1 mM) or sodium nitroprusside (SNP, 0.1 nM–0.1 mM) were applied, respectively.

Experimental protocols in isolated aortic rings

The thoracic aorta was rapidly removed and cut into rings 2-3 mm long for tension measurement. They were mounted in 15 ml baths containing Krebs' solution at 37 °C and gassed with a 95% O_2 and 5% CO_2 gas mixture. Isometric contractions were recorded with a resting tension of 1 g using a transducer connected to a 4-channel bridge amplifier and a PowerLab A/D converter and a PC running Chart v5 (PowerLab System, AD Instruments, Australia). ACh (0.001 μM–3 μM) induced endothelial mediated-relaxations and SNP (0.001 μM–3 μM) were assessed by pre-contracting with phenylephrine (10 μM) and relaxation expressed as % of relaxation.

Statistical analysis

Results were reported as means ± S.E.M. Comparisons between groups were performed using one-way ANOVA followed by Fisher's Least Significant Difference tests. A probability value of less than 0.05 was considered statistically significant.

Results

Effects of tangeretin on contractile responses to EFS and exogenous NE in mesenteric vascular beds

EFS at 5-40 Hz produced an increased in perfusion pressure that was frequency-dependent vasoconstriction in all preparations. A significant increase in contractile responses to EFS was observed in the mesenteric vascular bed isolated from L-NAME-treated rats compared to the responses in control rats ($p<0.05$) (Figure 1A). Contractile response to EFS in L-NAME-treated rats-treated with tangeretin was reduced comparing to those of untreated rats ($p<0.05$). However, the contractile response to exogenous NE (0.15 nmol-15 nmol) was not different among groups (Figure 1B).

Effects of tangeretin on vasorelaxation responses to vasodilator agents in mesenteric vascular beds

Vasorelaxation response to ACh (0.1 nM–0.1 mM) in the mesenteric vascular bed was significantly blunted in L-NAME-treated rats compared to control rats ($p<0.05$) (Figure 2A). Treatment with tangeretin improved the response to ACh in L-NAME-treated rats compared to untreated rats ($p<0.05$). There was no significant difference in the vasorelaxation responses to SNP among groups (Figure 2B), indicating normal vascular smooth muscle cell function.

Effects of tangeretin on vasorelaxation responses to vasodilator agents in aortic rings

Endothelium-dependent vasorelaxation responses to ACh (0.001 μM–3 μM) were significantly blunted in aortic rings from L-NAME-treated rats compared to control rats ($p<0.05$) (Figure 3A). Tangerein at dose 30 mg/kg improved vascular response to ACh compared to untreated rats ($p<0.05$). However, vasorelaxation response to SNP, an NO donor, did not differ significantly among groups (Figure 3B).

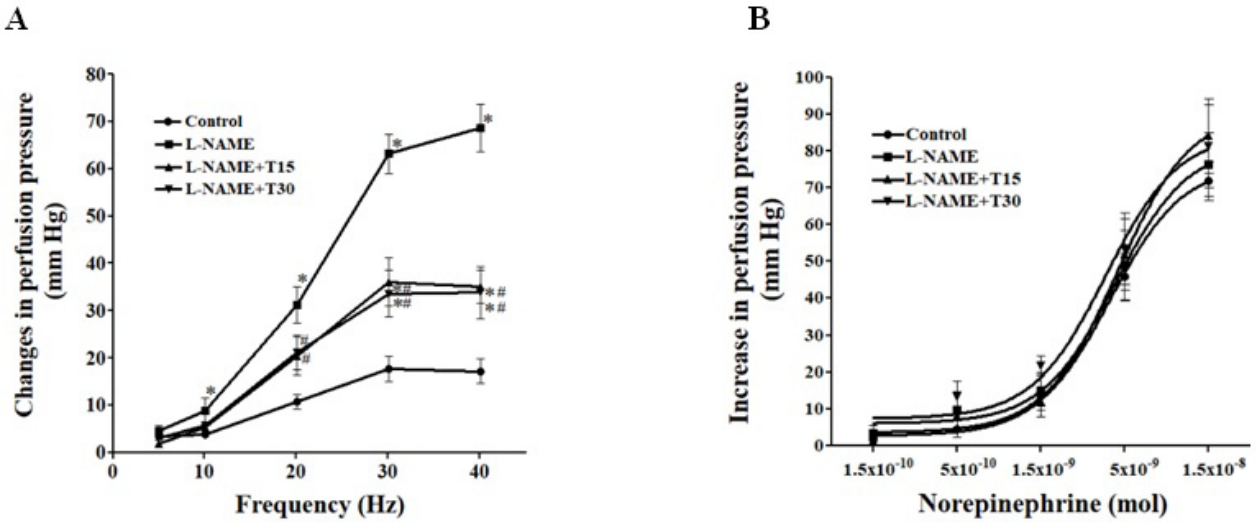

Figure 1 Effect of tangeretin on contractile responses to EFS (A) and exogenous NE (B) in mesenteric vascular beds. Data are presented as mean ± S.E.M. (n = 7/group). *p<0.05 vs. control, # p<0.05 vs. L-NAME.

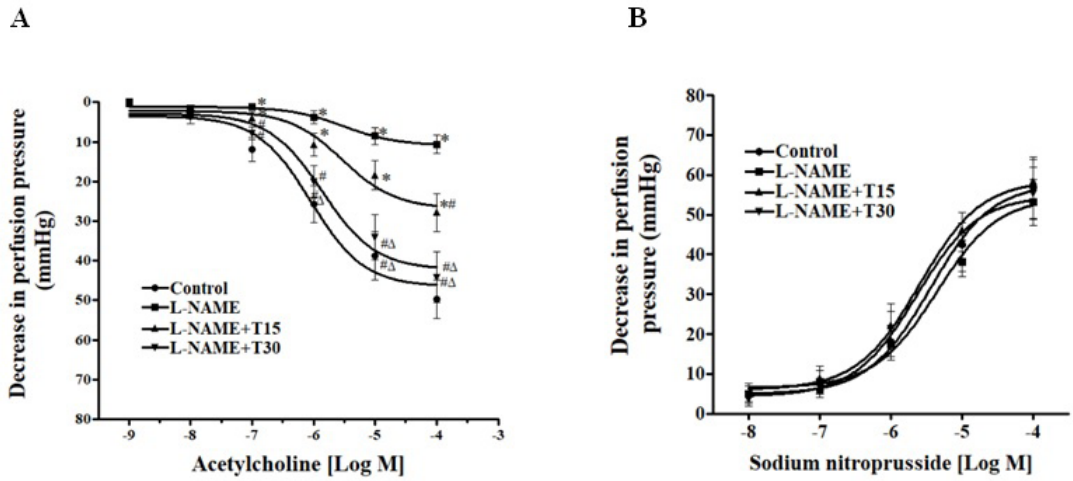

Figure 2 Effect of tangeretin on vascular responses to acetylcholine (A) and sodium nitroprusside (B) in mesenteric vascular beds. Data are presented as mean ± S.E.M. (n = 7/group). *p<0.05 vs. control, # p<0.05 vs. L-NAME, Δ p<0.05 vs. L-NAME+T15.

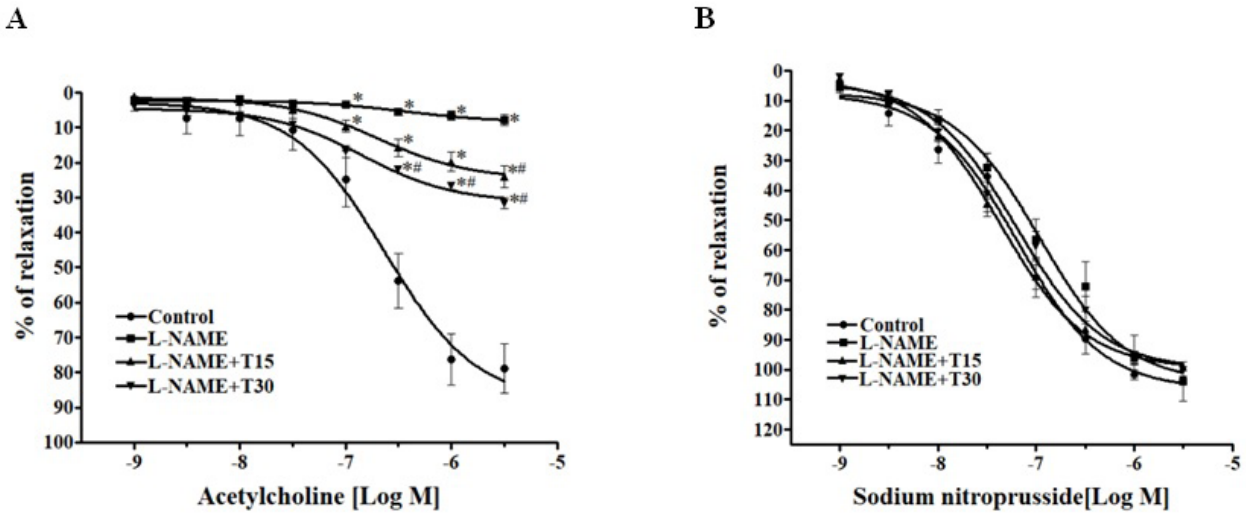

Figure 3 Effect of tangeretin on vascular responses to acethylcholine (A) sodium nitroprusside (B) in aortic rings. Data are expressed as mean ± S.E.M. (n = 7/group). * p<0.05 vs. control, # p<0.05 vs. L-NAME.

Discussion

This study demonstrated that chronic administration of L-NAME in rats increased sympathetic nerve activation as evidenced by increased contractile response to EFS and impaired endothelium dependent vasorelaxation. Tangeretin improved endothelial dysfunction and reduced sympathoexcitation in L-NAME-treated rats.

Sympathetic activation is an important factor to stimulate and maintain high blood pressure via vascular tone regulation. Previous study demonstrated that L-NAME can induce sympathetic activation in rats[4]. In rat mesenteric arteries, vascular tone is mediated by the integrated action of different neurotransmitters, mainly NE from sympathetic nerve terminals[14]. The result of this study showed that there was an enhancement of vasoconsriction responses to sympathetic nerve stimulation without affecting the response to exogenous NE in L-NAME-treated rats, indicating the augmentation of NE release from pre-junctional sites. However, treatment with tangeretin suppressed the nerve mediated contractile response in L-NAME-treated rats. It is possible that tangeretin has antioxidant effect to raise NO bioavailability, which can suppress NE release from sympathetic nerve terminal[11].

The results of this study showed an impairment of endothelium-dependent vasodilation in isolated aortic rings and mesenteric vascular beds of L-NAME-treated rats. Oxidative stress is also involved in L-NAME mediated endothelial dysfunction. Fu and co-workers established that treatment with L-NAME impaired ACh-induced endothelium-dependent vasorelaxation, which mediated by increasing oxidative stress[7]. Previous study demonstrated that L-NAME-treated rats showed impairment of ACh-induced vasorelaxation, and decreased NO bioavailability. NO is also known as a potent vasodilator[15]. High ROS concentrations reduce the quantity of bioactive NO by rapidly reaction with NO to form the potent cytotoxic peroxynitrite which was induced vascular oxidative stress and endothelial dysfunction[16]. Tangeretin also improved endothelial dysfunction in L-NAME-treated rats, probably as an anti-oxidant effect of tangeretin. Previous study showed that tangeretin inhibited ROS production and suppressed the mRNA expression of $p47^{phox}$, $p67^{phox}$, and $gp91^{phox}$ in LPS-stimulated microglia, indicating tangeretin exhibits strong antioxidant activity[9]. Therefore, antioxidative activity of tangeretin might be partially responsible for the alleviation of endothelial dysfunction in NO deficiency rats.

Conclusion

In summary, tangeretin attenuates L-NAME-induced sympathoexitation and endothelial dysfunction in rats. This might be involved with its antioxidant to increase NO bioavailability in L-NAME-treated rats.

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