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Research Article

Vasorelaxant Effects of Chlorella on Blood Circulation in Healthy Rats

Kagota S[1]*, Norii M[1], Morikawa K[1], Maruyama-Fumoto K[1], Yamada S[2] and Shinozuka K[1]

[1]Department of Pharmacology II, School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Japan

[2]Center for Pharma-Food Research, Graduate School of Pharmaceutical Sciences, University of Shizuoka, Japan

***Corresponding author:** Satomi Kagota, Department of Pharmacology II, School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Japan

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Abstract

Aim: Chlorella is used in Asia and Europe as a functional food. Chlorella is reported to decrease blood pressure in hypertensive individuals and enhance peripheral blood circulation in elder individuals, but the mechanism underlying remains unclear. Therefore, we examined Chlorella-induced relaxation in isolated thoracic aortas and mesenteric arteries to determine whether vasodilation was responsible for its effect on circulation.

Methods: We measured the relaxation using organ bath techniques, and the effects of Chlorella on systemic and peripheral circulation parameters examined using a photo plethysmographic tail-cuff system and a non-contact laser tissue blood flow meter in Wistar rats.

Results: Chlorella induced dose-dependent relaxation in thoracic aortas and mesenteric arteries pre-contracted with phenylephrine. Chlorella-induced relaxation was partially inhibited by treatment with antagonists of Nitric Oxide (NO) synthase and soluble guanylyl cyclase, and endothelium removal. The relaxation in endothelium-denuded preparations of these arteries was not altered by treatment with potassium channel blockers or a beta-adrenergic receptor antagonist. Chlorella did not affect contractile responses induced by potassium chloride in both arteries. Oral administration of Chlorella (900 mg/kg/day for 1 week) did not alter systolic blood pressure; heart rate; and tail blood flow, mass, or velocity in Wistar rats on 1st and 8th days of administration.

Conclusion: Chlorella-induced vasorelaxation occurred via endothelial NO-dependent and NO-independent pathways; however, the vasodilatory activity did not have any positive impact on systemic and peripheral circulation in healthy rats. These results suggest that Chlorella can be used without the occurrence of serious adverse events, e.g., temporary lightheadedness or orthostatic hypotension in healthy conditions.

Keywords: Blood Flow; Blood Pressure; Chlorella; Nitric Oxide; Vasorelaxation

Abbreviations

Ach: Acetylcholine; Flow: Blood Flow; Gli: Glibenclamide; 4-AP: 4-aminopyridine; KCl: Potassium Chloride; L-NAME: NG-nitro-L-arginine methyl ester; NO: Nitric Oxide; ODQ: 1H-[1,2,4] oxadiazolo-[4,3-a]quinoxalin-1-one; Prop: Propranolol; TEA: Tetraethylammonium

Introduction

The prevalence of lifestyle-related health problems, such as high blood pressure and abnormal lipid and glucose metabolism, has been increasing worldwide. Left untreated, they may lead to the onset of organ damage including the heart, kidney, and brain damage; hence, it is important to protect against or minimize damage by these conditions [1,2]. One of the mechanisms involved in the development of organ damage is insufficient blood supply to the organ, resulting from decreased blood flow in the arteries. The vascular endothelium regulates vascular tone by releasing many vasoactive factors [3]. Nitric Oxide (NO) is an important, endothelium-derived, vasorelaxant factor; therefore, a decrease in NO production or availability as well as impaired response of vascular smooth muscle cells to NO can contribute to impaired blood circulation in various diseases such as hypertension, atherosclerosis, and diabetes [4,5]. To ameliorate the symptoms resulting from impaired peripheral circulation, researchers have investigated both drugs and dietary components that increase or restore NO activity [6-8].

Chlorella, a green alga, contains a variety of nutrients and has been used as a dietary supplement in Asia and Europe for many years. Chlorella has antioxidant effects [9] and helps to maintain healthy lipid levels [10,11] and glucose metabolism [12,13]. A meta-analysis of randomized clinical trials indicates that supplementation with Chlorella improves cardiovascular risk factors by decreasing blood lipid and glucose levels [14]. Furthermore, some clinical trials indicate that supplementation with Chlorella significantly reduces blood pressure in hypertensive individuals [14-16]. These antihypertensive effects are reproduced in animal models of hypertension such as NG-nitro-L-arginine methyl ester (L-NAME)-treated hypertensive rats [17], spontaneously hypertensive rats [18], and stroke-prone spontaneously hypertensive rats [19]. Furthermore, supplementation

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with Chlorella has been reported to increase peripheral circulation without any effect on systemic blood pressure; for instance, a Chlorella supplement decreases the brachial-ankle pulse wave velocity in middle-aged and older individuals [20]. However, few studies have investigated the effects of Chlorella on blood pressure and peripheral circulation or the mechanism underlying its vasorelaxant activity. Therefore, the present study investigated the effect of oral administration of Chlorella on blood pressure and systemic and peripheral circulation in Wistar rats by exploring the mechanisms responsible for Chlorella-induced vasodilation in isolated rat aortas and mesenteric arteries.

Materials and Methods

Chemicals

Dried, powdered Chlorella was provided by Sun Chlorella Co. (Kyoto, JAPAN) (Lot No. C127). The products were diluted to the desired concentrations in distilled water for *in vitro* experiments (200 mg/mL) and *in vivo* experiments (90 mg/mL). Other reagents used in the present study were as follows: Acetylcholine (ACh) chloride (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan); L-phenylephrine hydrochloride, NG-nitro-L-arginine methyl ester (L-NAME), Glibenclamide (Gli) (Sigma-Aldrich Co., LLC., St. Louis, USA); Tetraethylammonium (TEA), KCl (Nacalai Tesque Inc., Kyoto, Japan); 1H-[1,2,4]oxadiazolo-[4, 3-a]quinoxalin-1-one(ODQ, Tocris Bioscience, Inc., Bristol, UK); and 4-aminopyridine (4-AP) and dl-propranolol hydrochloride (Prop) (Wako Pure Chemical Ind., Ltd., Osaka, Japan). All other chemicals of analytical reagent grade were purchased from Nacalai Tesque Inc.

Animals

Male Wistar rats were purchased at 10 weeks of age from Japan SLC, Inc. (Hamamatsu, Japan) and used at 11-13 weeks of age (n=18) for *in vitro* studies and at 11 weeks of age (n=12) for *in vivo* studies. Rats were provided a standard chow diet (CE-2; Clea Japan Inc., Tokyo, Japan) and water ad *libitum* during the experimental period. All protocols involving the care and use of animals were approved by the animal ethics committee and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals at Mukogawa Women's University (Protocol number: P-12-2019-02-A).

Vasodilation Induced by Chlorella in Isolated Rat Arteries *in vitro*

The thoracic aorta and superior mesenteric artery were removed from each rat under sodium pentobarbital anesthesia (120 mg/kg, i.p.) and immediately placed in oxygenated Krebs-Henseleit solution (118.4 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 11.1 mM glucose; pH 7.4, 37 ˚C). Arterial relaxation and contractile responses were measured using the myographytechnique, as previously described [21].

Ring preparations of aortas and mesenteric arteries were contracted by the addition of 2×10^{-7} M and 10-6 M phenylephrine, respectively, generating approximately 80% of the maximal contraction. After a stable contraction was obtained, relaxation was elicited using Chlorella (10^{-5} to 10^{-2} g/mL) or ACh (10^{-10} to 3×10^{-6} M) in the presence or absence of inhibitors, namely, L-NAME (10^{-4} M, 20 min), ODQ (10^{-5} M, 20 min), TEA (10^{-5} M, 20 min), Gli (10^{-5} M, 20 min), 4-AP (10^{-4} M, 20 min), and Prop (10^{-5} M, 20 min), or after endothelial removal [E(-)]. Endothelial denudation of the preparation was confirmed by the absence of relaxation induced by 10^{-6} M ACh. Complete inhibition of the contraction induced by phenylephrine by Chlorella or ACh was considered 100% relaxation. In another endothelium-denuded ring preparation [E(-)], contraction was elicited using KCl (10 to 60 mM) in the presence or absence of Chlorella (3 mg/mL, 20 min) to assess the effects of Chlorella on contractile responses. Individual concentration-response curves were analyzed using nonlinear regression curve-fitting of relaxant drug concentrations to determine the negative log of EC_{50}, the maximum response (E_{max}), and the Area Under the Curve (AUC) using GraphPad Prism ver. 5.0 (GraphPad Software, Inc., San Diego, CA, USA).

Systemic and Peripheral Circulation Parameters Following Oral Administration of Chlorellato Rats

Rats were divided into two groups (n = 6): control and Chlorella groups, and distilled water (10 mL/kg body weight) or Chlorella (900 mg/kg) was orally administered to the two groups once per day, respectively. The dose for the Chlorella group was estimated to be equal to approximately 6 g/day (the conventional dose for humans) of Chlorella in humans. To evaluate the effects of oral administration of Chlorella on systemic and peripheral circulation, each rat was placed in a holder, and systolic blood pressure; heart rate; and tail Blood Flow (Flow), mass, and velocity were measured at room temperature without anesthesia by using a photo plethysmographic tail-cuff system (Model MK-2000; Muromachi Kikai Co., Ltd., Tokyo, Japan) and a non-contact laser tissue blood flow meter (FLO-N1; Omega wave, Inc., Tokyo, Japan), as described previously [22]. Each parameter was measured 1 hour (hr) before and 1, 3, 6, and 24 hr after the administration of Chlorella. To investigate the effects of multiple oral doses of Chlorella, rats received vehicle (distilled water, 10 mL/kg body weight) or Chlorella (900 mg/kg) once per day for 7 days. The above parameters were measured on the 8th day. The parameters for each rat were averaged from at least three consecutive readings under resting conditions. Data were calculated in terms of changes in values, with 100 as the value before the 1st administration.

Data analysis

Data are expressed as means ± Standard Error of the Mean (S.E.M). To analyze differences between groups, statistical analyses were performed using Student's t-test for unpaired comparisons or one-way analysis of variance followed by Bonferroni's post-hoc test *in vitro* studies and with two-way (treatment versus time) analysis of variance followed by Bonferroni's post-hoc test *in vivo* studies (GraphPad Prism software). Results were considered to be significant when $p< 0.05$.

Results

Chlorella induced vasorelaxation in a concentration-dependent manner in the thoracic aortas and mesenteric arteries of Wistar rats (Figure 1). L-NAME, an NO synthase inhibitor, and ODQ, a guanylyl cyclase inhibitor, partly inhibited Chlorella-induced relaxation in aortas (Figure 1A) and mesenteric arteries (Figure 1B). However, ACh-induced relaxation was completely inhibited by these inhibitors in aortas (Figure 1C), but only partly in mesenteric arteries (Figure 1D). There was no significant difference in the AUC of Chlorella-induced relaxation in the presence of L-NAME and ODQ in both arteries (Figure 1E and 1F). Endothelium-denudation [E(-)] partly

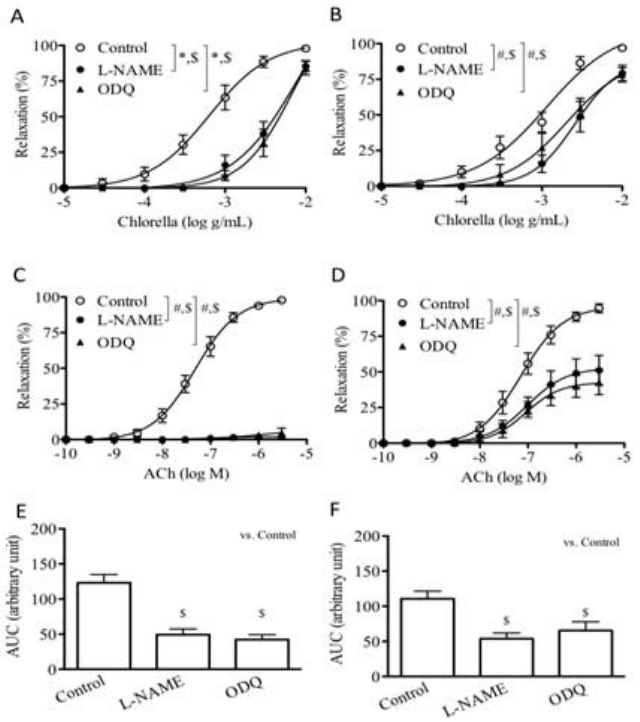

Figure 1: Vasorelaxation in response to *Chlorella* and acetylcholine (ACh) in thoracic aortas (A and C) and mesenteric arteries (B and D), and comparison of the value of the Area Under the Curve (AUC) of vasorelaxation response to *Chlorella* in thoracic aortas (E) and mesenteric arteries (F) isolated from Wistar rats (n = 6). Some ring preparations were pre-treated with N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M, 20 min), a Nitric Oxide (NO) synthase inhibitor, or ODQ (10^{-5} M, 20 min), an NO-selective guanylyl cyclase inhibitor. Results are expressed as means ± standard error of the mean. Statistical analyses were performed by one-way analysis of variance followed by Bonferroni's post-hoc test. *p < 0.05, negative log of EC_{50}; $^{\#}p$ < 0.05, E_{max}; $^{\$}p$ < 0.05, AUC.

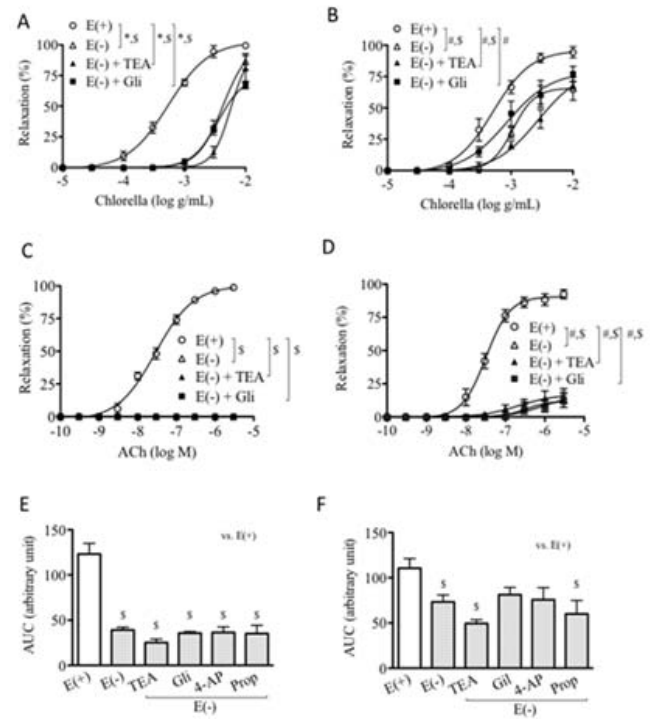

Figure 2: Vasorelaxation in response to *Chlorella* and Acetylcholine (ACh) in thoracic aortas (A and C) and mesenteric arteries (B and D), and comparison of the value of the Area Under the Curve (AUC) of vasorelaxation response to *Chlorella* in thoracic aortas (E) and mesenteric arteries (F) isolated from Wistar rats (n = 6). The endothelial layer of some ring preparations was removed by rubbing the inside of the artery [E (-)]; they were pre-treated with Tetraethylammonium (TEA, 10^{-5} M, 20 min), glibenclamide (Gli, 10^{-5} M, 20 min), and 4-aminopyridine (4-AP, 10^{-4} M, 20 min), all potassium channel blockers, as well as with propranolol (Prop, 10^{-5} M, 20 min), a non-selective beta-adrenergic receptor antagonist. Results are expressed as means ± standard error of the mean. Statistical analyses were performed by one-way analysis of variance followed by Bonferroni's post-hoc test. *p < 0.05, negative log of EC_{50}; $^{\#}p$ < 0.05, E_{max}; $^{\$}p$ < 0.05, AUC.

inhibited Chlorella-induced relaxation in aortas (Figure 2A) and mesenteric arteries (Figure 2B), but abolished ACh-induced relaxation in both arteries (Figure 2C and 2D). In the endothelium-denuded preparation, Chlorella-induced relaxation was not affected by the addition of TEA or Gli, both potassium channel blockers (Figure 2A and 2B). Similarly, neither 4-AP, an ATP-sensitive potassium channel blocker, nor propranolol, a non-selective beta-adrenoceptor antagonist, altered the AUC of Chlorella-induced relaxation in the endothelium-denuded preparation of both arteries (Figure 2E and 2F). There was no significant difference in the contractile response to KCl, regardless of the presence or absence of 3 mg/mL Chlorella, in the aortas and mesenteric arteries (Figure 3A and 3B).

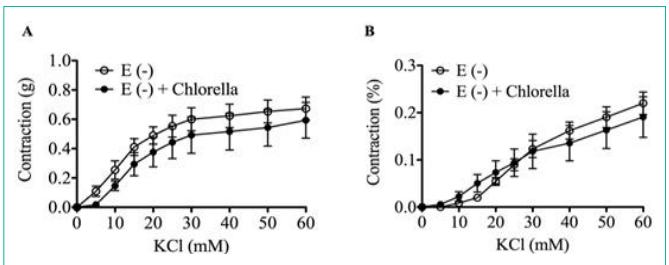

Figure 3: Effects of *Chlorella* (3 mg/mL, 20 min) on vasocontractile responses to potassium chloride (KCl) in thoracic aortas (A) and mesenteric arteries (B) isolated from Wistar rats (n = 6). Results are expressed as means ± standard error of the mean. Statistical analyses were performed by one-way analysis of variance followed by Bonferroni's post-hoc test.

A single oral administration of Chlorella (900 mg/kg) did not significantly affect systolic blood pressure; heart rate; and tail blood flow, mass, or velocity over 24 hr (Figure 4). No significant differences were observed in the systemic and peripheral parameters on the1stor 8thday after the oral administration of Chlorella for 7 days (Figure 5).

Discussion

The present study demonstrates that Chlorella induces vasorelaxation via an endothelial NO-mediated pathway and an NO-independent pathway in aortas and mesenteric arteries isolated from healthy Wistar rats. However, oral administration of Chlorella to healthy rats does not cause any significant change in systemic (blood pressure and heart rate) and peripheral (tail flow, mass, and velocity) circulation parameters on the 1st day and 8th day of treatment. These results do not support the recommendation to use Chlorella for the enhancement of blood circulation in healthy individuals. However, they indicate that Chlorella can be used without the occurrence of

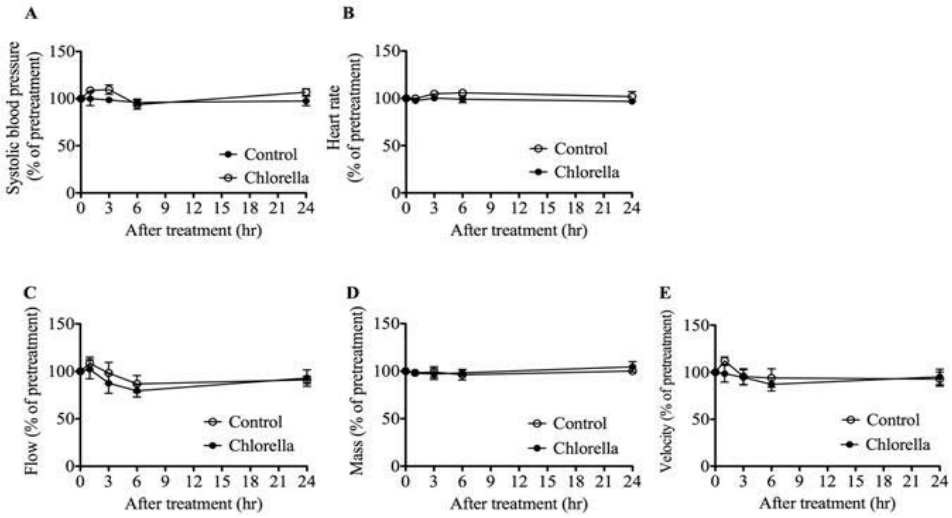

Figure 4: Effects of the oral administration of *Chlorella* (900 mg/kg/day) on systolic blood pressure (A), heart rate (B), tail blood flow (Flow, C), mass (D), and velocity (E) in Wistar rats. Parameters were evaluated 1 hr before (0 min), and 1, 3, 6, and 24 hr after treatment with *Chlorella*. Results are expressed as means ± standard error of the mean (n = 6 per group). Statistical analyses were performed by two-way (treatment versus time) analysis of variance followed by Bonferroni's post-hoc test.

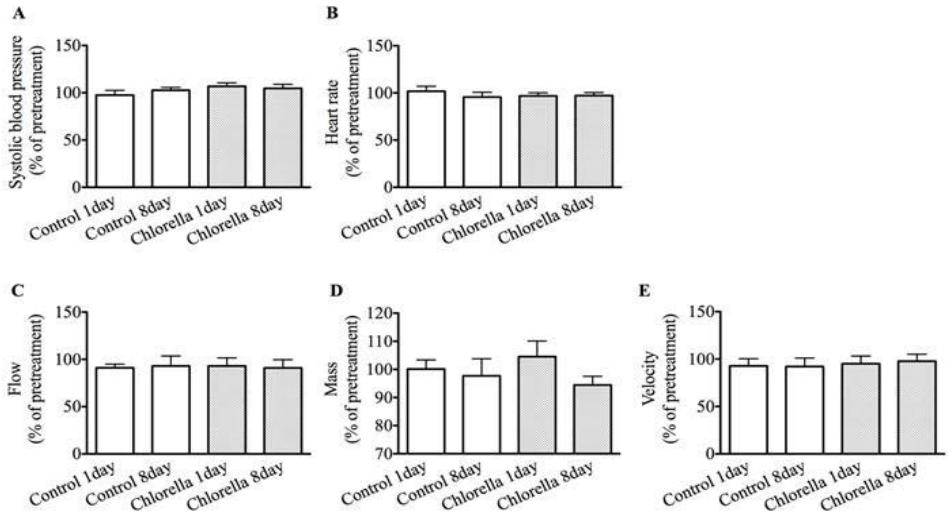

Figure 5: Effects of oral administration of *Chlorella* (900 mg/kg/day) for 7 days on systolic blood pressure (A), heart rate (B), tail blood flow (Flow, C), mass (D), and velocity (E) in Wistar rats. On the 8th day, these parameters were evaluated. Results are expressed as means ± standard error of the mean (n = 6 per group). Statistical analyses were performed by two-way (treatment versus time) ANOVA followed by Bonferroni's post-hoc test.

serious adverse events, e.g., temporary light headedness or orthostatic hypotension, at this dosage, which equals to approximately 6 g/day in humans.

The present study demonstrates that Chlorella induced vasorelaxation via an endothelial NO-soluble guanylyl cyclase pathway in the aortas and mesenteric arteries isolated from Wistar rats, as evidenced by the inhibition of Chlorella-induced vasorelaxation by L-NAME, ODQ, and endothelial removal. The lack of significant differences in inhibitory effects between L-NAME and ODQ treatments indicates that the endothelium-dependent pathway was completely dependent on vascular endothelial NO and guanylyl cyclase in vascular smooth muscle cells. Furthermore, this study demonstrates that Chlorella did not relax vascular smooth muscle by inhibiting calcium channels based on the absence of any effect on the vasoconstriction induced by depolarization with high KCl concentrations. As TEA, Gli, 4-AP, and propranolol did not affect Chlorella-induced relaxation in endothelium-denuded rat arteries, the involvement of potassium channels and beta-adrenergic receptors of vascular smooth muscle cells can also be excluded. The detailed mechanism of Chlorella-induced relaxation via the NO-independent pathway remains unclear, but to the best of our knowledge, this is the first study to demonstrate that Chlorella has direct vasodilatory effects.

Based on the results of the *in vitro* experiments, Chlorella might have a positive impact on systemic or peripheral circulations because of its vasodilatory activity. However, the oral administration of Chlorella

(900 mg/kg, which is estimated to be equal to the conventional dose in humans) to Wistar rats did not alter systolic blood pressure and heart rate on the 1st day and after 1week of administration. The vasorelaxant constituent(s) in Chlorella may be poorly absorbed from the gastrointestinal tract or rapidly metabolized in the healthy body. Similarly, no effects were reported for a Chlorella supplement (30 tablets/day) administered for 4 weeks on blood pressure and heart rate in healthy young men [23]. However, Chlorella has been found to have beneficial effects on blood pressure under hypertensive conditions. A meta-analysis of randomized controlled trials demonstrated that administration of 10 g of a Chlorella supplement for 2 to 3 months significantly reduced blood pressure in subjects with high blood pressure [14,15]. Dietary supplementation with 10 g Chlorella tablets for 2 months reduced or stabilized sitting diastolic blood pressure in subjects with mild to moderate hypertension [16]. The elevation in blood pressure was inhibited by a diet containing 8% Chlorella in L-NAME-treated hypertensive rats [17] and a diet containing 10-20% Chlorella in stroke-prone, spontaneously hypertensive rats [19]. These reports using a diet containing Chlorella raise the possibility that the blood-pressure-lowering effects of Chlorella are mediated by the inhibition of angiotensin I-converting enzyme activity. Peptides with angiotensin I-converting enzyme inhibitory effects have been purified and identified in Chlorella protein hydrolysates [18]. These findings suggest that high-dose long-term administration of Chlorella could exert significant blood-pressure-lowering effects, especially, in the hypertensive state.

Without any effects on systemic blood pressure and heart rate, a Chlorella supplement (30 tablets/day) for 4 weeks increased forearm blood flow and decreased the brachial-ankle pulse wave velocity in middle-aged and older individuals [20]. After the Chlorella supplementation, a negative correlation was observed between changes in the brachial-ankle pulse wave velocity and plasma concentrations of NO metabolites; therefore, the authors speculated that the Chlorella supplement activated NO production by increasing blood arginine concentration [20]. According to the analysis of the supplier, the powdered Chlorella used in the present study contains approximately 3 g of L-arginine in 100 g. L-arginine, a substrate of NO synthase, could be responsible for inducing the relaxation of rat aortas via endothelial NO formation. In contrast, Chlorella-induced NO-independent relaxation might be supported by a previous report that L-arginine induces relaxation of rat aortas through non-endothelial NO formation [24]. However, this speculation is contradicted by a report that oral L-arginine did not cause endothelium-dependent dilation of the brachial artery in healthy young men [25]. The present study indicates that oral administration of Chlorella (900 mg/kg) to Wistar rats did not alter peripheral circulation parameters, i.e., tail blood flow, mass, and velocity, either on the 1st day or after 1week of administration. There is a possibility that higher doses of Chlorella or accumulation after long-term treatment of Chlorella may be needed to increase peripheral circulation in healthy individuals. Chlorella-induced vasorelaxation may be triggered in the presence of low NO release or production occurring in aged individuals with high blood pressure.

Conclusion

The present study demonstrates that Chlorella induced vasorelaxation via endothelial NO-mediated and NO-independent pathways in healthy rat arteries. However, Chlorella did not alter blood pressure and peripheral circulation parameters on the 1st and 8th days of administration in healthy rats. Therefore, the presence of endothelial dysfunction in elderly and hypertensive subjects may influence the beneficial impact of Chlorella on systemic and peripheral circulation.

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References

1. Mourad JJ, Laville M. Is hypertension a tissue perfusion disorder? Implications for renal and myocardial perfusion. J Hypertens Suppl. 2006; 24: S10-16.
2. Dinh T, Scovell S, Veves A. Peripheral arterial disease and diabetes: a clinical update. Int J Low Extrem Wounds. 2009; 8: 75-81.
3. Godo S, Shimokawa H. Endothelial Functions. Arterioscler Thromb Vasc Biol. 2017; 37: e108-e114.
4. Konukoglu D, Uzun H. Endothelial Dysfunction and Hypertension. Adv Exp Med Biol. 2017; 956: 511-540.
5. Bleakley C, Hamilton PK, Pumb R, Harbinson M, McVeigh GE. Endothelial Function in Hypertension: Victim or Culprit? J Clin Hypertens (Greenwich). 2015; 17: 651-654.
6. Pinheiro LC, Tanus-Santos JE, Castro MM. The potential of stimulating nitric oxide formation in the treatment of hypertension. Expert Opin Ther Targets. 2017; 21: 543-556.
7. Monsalve B, Concha-Meyer A, Palomo I, Fuentes E. Mechanisms of Endothelial Protection by Natural Bioactive Compounds from Fruit and Vegetables. An Acad Bras Cienc. 2017; 89: 615-633.
8. Wojtala M, Pirola L, Balcerczyk A. Modulation of the vascular endothelium functioning by dietary components, the role of epigenetics. Biofactors. 2017; 43: 5-16.
9. Shibata S, Natori Y, Nishihara T, Tomisaka K, Matsumoto K, Sansawa H, et al. Antioxidant and anti-cataract effects of Chlorella on rats with streptozotocin-induced diabetes. J Nutr Sci Vitaminol (Tokyo). 2003; 49: 334-339.
10. Shibata S, Oda K, Onodera-Masuoka N, Matsubara S, Kikuchi-Hayakawa H, Ishikawa F, et al. Hypocholesterolemic effect of indigestible fraction of Chlorella regularis in cholesterol-fed rats. J Nutr Sci Vitaminol (Tokyo). 2001; 47: 373-377.
11. Kim S, Kim J, Lim Y, Kim YJ, Kim JY, Kwon O. A dietary cholesterol challenge study to assess Chlorella supplementation in maintaining healthy lipid levels in adults: a double-blinded, randomized, placebo-controlled study. Nutr J. 2016; 15: 54.
12. Mizoguchi T, Takehara I, Masuzawa T, Saito T, Naoki Y. Nutrigenomic studies of effects of Chlorella on subjects with high-risk factors for lifestyle-related disease. J Med Food. 2008; 11: 395-404.
13. Noguchi N, Yanagita T, Rahman SM, Ando Y. Chlorella Protein Hydrolysate Attenuates Glucose Metabolic Disorder and Fatty Liver in High-fat Diet-induced Obese Mice. J Oleo Sci. 2016; 65: 613-620.
14. Fallah AA, Sarmast E, Habibian Dehkordi S, Engardeh J, Mahmoodnia L, Khaledifar A, et al. Effect of Chlorella supplementation on cardiovascular risk factors: A meta-analysis of randomized controlled trials. Clin Nutr. 2018; 37: 1892-1891.
15. Merchant RE, Andre CA. A review of recent clinical trials of the nutritional supplement Chlorella pyrenoidosa in the treatment of fibromyalgia, hypertension, and ulcerative colitis. Altern Ther Health Med. 2001; 7: 79-91.
16. Merchant RE, Andre CA, Sica DA. Nutritional supplementation with Chlorella pyrenoidosa for mild to moderate hypertension. J Med Food. 2002; 5: 141-152.
17. Yang SC, Yang HY, Yang YC, Peng HC, Ho PY. Chlorella pyrenoidosa

ameliorated L-NAME-induced hypertension and cardiorenal remodeling in rats. Eur J Nutr. 2013; 52: 601-608.

18. Lin YH, Chen GW, Yeh CH, Song H, Tsai JS. Purification and Identification of Angiotensin I-Converting Enzyme Inhibitory Peptides and the Antihypertensive Effect of Chlorella sorokiniana Protein Hydrolysates. Nutrients. 2018; 10: 1367.

19. Sansawa H, Takahashi M, Tsuchikura S, Endo H. Effect of chlorella and its fractions on blood pressure, cerebral stroke lesions, and life-span in stroke-prone spontaneously hypertensive rats. J Nutr Sci Vitaminol (Tokyo). 2006; 52: 457-466.

20. Otsuki T, Shimizu K, Maeda S. Changes in arterial stiffness and nitric oxide production with Chlorella-derived multicomponent supplementation in middle-aged and older individuals. J Clin Biochem Nutr. 2015; 57: 228-232.

21. Kagota S, Tada Y, Nejime N, Nakamura K, Kunitomo M, Shinozuka K. Telmisartan provides protection against development of impaired vasodilation independently of metabolic effects in SHRSP.Z-Lepr(fa)/Izm Dmcr rats with metabolic syndrome. Can J Physiol Pharmacol. 2011; 89: 355-364.

22. Liang Y, Kagota S, Maruyama K, Oonishi Y, Miyauchi-Wakuda S, Ito Y, et al. Royal jelly increases peripheral circulation by inducing vasorelaxation through nitric oxide production under healthy conditions. Biomed Pharmacother. 2018; 106: 1210-1219.

23. Otsuki T, Shimizu K, Iemitsu M, Kono I. Multicomponent supplement containing Chlorella decreases arterial stiffness in healthy young men. J Clin Biochem Nutr. 2013; 53: 166-169.

24. Moritoki H, Ueda H, Yamamoto T, Hisayama T, Takeuchi S. L-arginine induces relaxation of rat aorta possibly through non-endothelial nitric oxide formation. Br J Pharmacol. 1991; 102: 841-846.

25. Adams MR, Forsyth CJ, Jessup W, Robinson J, Celermajer DS. Oral L-arginine inhibits platelet aggregation but does not enhance endothelium-dependent dilation in healthy young men. J Am Coll Cardiol. 1995; 26: 1054-1061.