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Selective vasodilatation effect of sargahydroquinoic acid, an active constituent of *Sargassum micracanthum*, on the basilar arteries of rabbits

Byong-Gon Park,[a] Woon-Seob Shin,[a] Yumi Um,[a] Sungsik Cho,[a] Gab-Man Park,[a] Dong-Soo Yeon,[a] Seong-Chun Kwon,[a] Jungyeob Ham,[b] Byoung Wook Choi[c] and Seokjoon Lee[a,*]

[a]*College of Medicine, Kwandong University, Gangneung, Gangwon 210-701, Republic of Korea*
[b]*Korea Institute of Science and Technology, Gangneung Institute, Gangneung 210-340, Republic of Korea*
[c]*Department of Applied Chemistry, Hanbat National University, Daejeon 305-719, Republic of Korea*

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Abstract—Sargahydroquinoic acid (**2**), a major active constituent of *Sargassum micracanthum* collected from the coast of the East Sea in Korea, showed a selective vasodilatation effect on the basilar arteries of rabbits. Therefore, treatment with sargahydroquinoic acid may selectively accelerate cerebral blood flow through dilatation of the basilar artery without lowering systemic blood pressure.

Natural products obtained from terrestrial plants and microorganisms play an important role in the development of clinical medicines.[1] Because more than 70% of the Earth's surface is covered by oceans, many marine plants are used for food, a source of minerals, dietary fiber, nutrition, and medicine.[2] Numerous marine natural products have been found to be useful for pharmacological studies to treat various diseases.[3]

Due to the public health challenges caused by vascular-related diseases, including hypertension, stroke, subarachnoid hemorrhage and Alzheimer's dementia, there is an urgent need to develop modulators that control vascular tone.[4,5] Contractility of the vascular system is mainly dependent on the intracellular concentration of Ca^{2+}. Cytoplasmic increases in Ca^{2+} are caused by the opening of L-type Ca^{2+} channels through sympathetic nerve stimulation, and IP_3-mediated Ca^{2+} release from the sarcoplasmic reticulum through vasoconstrictor-induced phospholipase C activation.[6,7] Therefore, calcium channels are effective targets to modulate cytoplasmic Ca^{2+} concentrations treat for the treatment of vascular-related diseases.

In particular, vasoconstriction of the basilar artery is important for the brain's blood supply and blood pressure in physiological and pathophysiological conditions. As reported previously, delayed cerebral ischemia remains an important cause of death and disability in patients who have suffered subarachnoid hemorrhages (SAH).[8] Extracellular Ca^{2+} influx through voltage-operated calcium channels into vascular smooth muscle cells plays a fundamental role in the development and chronic effects of vasospasm after SAH.[9–11] Thus, vasodilatation of the basilar artery could be useful in physiological and pathophysiological situations, such as, mitigation of vasospasm following SAH and cerebral ischemia, and protection of occlusion by blood clots or stenosis in the basilar or cerebral artery.

Therefore, the development of vasodilatation molecules with low toxicity profiles for clinical use is important and urgently needed. In particular, if selected candidates have both potency and selectivity for the basilar artery, they may be promising candidates for the treatment of cerebrovascular diseases.

In the course of searching for vasoactive molecules from a marine natural products library collected from the

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* Corresponding author. Tel.: +82 33 649 7454; fax: +82 33 641 1074; e-mail: sjlee@kwandong.ac.kr

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coast of the East Sea in Korea, we discovered that an organic extract of *Sargassum micracanthum* showed a selective vasodilatation effect on the basilar artery but not the carotid artery of rabbits at concentrations of 20 μg/ml and 2 μg/ml (data not shown).[12] Brown algae of the genus *Sargassum* are known to contain structurally unique secondary metabolites, such as plastoquinones,[13,14] chromanols,[15,16] cyclopentenone,[17] and polysaccharides.[18] Previously, the usefulness of platoquinones and chromene from *Sargassum micracanthum* have been reported to be useful as antioxidant,[19] antiviral,[20] antiulcer agents on gastric lesions,[21] and to inhibit bone resorption.[22] Although its constituents have not been reported to show vasodilatation effects, it was reported that marine-derived halogen-containing gramine analogues induced vasorelaxation in isolated rat aorta.[23]

In order to isolate active molecules from the MeOH extract (1 g), it was partitioned on a C_{18} reverse phase column with an aqueous MeOH gradient (60%, 70%, and 80%). The third fraction eluted with the 80% MeOH aqueous solution retained cerebrovascular relaxation activity, and was successively separated by silica HPLC (Phenomenex Luna 5 μ silica column, 10 × 250 mm, *n*-hexane/$CHCl_3$/EtOAc = 8:2:1). The separation of the C_{18} reverse phase by HPLC (Phenomenex Aqua 5 μ) with 85% MeOH aqueous solution yielded sargaquinoic acid (**1**, 61 mg)[24] and sargahydroquinoic acid[25] (**2**, 214 mg) as shown in Figure 1. These are identical to structures in reported papers.[13,26]

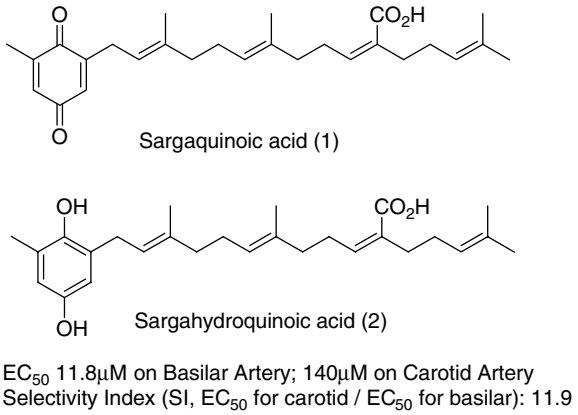

Figure 1. Sargaquinoic acid (**1**) and sargahydroquinoic acid (**2**) purified from ethyl acetate layer of *Sargassum micracanthum*.

Although it is known that sargaquinoic acid (**1**)[14] exhibits Cox 1 and 2 inhibitory effects,[27] anticholinesterase effect,[28] nerve growth factor (NGF)-dependent neurite outgrowth promoting activity,[29] and insect growth inhibition,[30] it did not show a relaxation effect in the basilar artery. However, sargahydroquinoic acid (**2**), with similar biological properties as compound **1**, selectively dilated the basilar artery in our organ bath system.[31–33]

When treated with high K^+ (50 mM), the basilar and carotid arteries were isometrically contracted by an increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$), reaching a steady-state within 20 min. The concentration–response relationship for sargahydroquinoic acid-induced vasodilatation was measured by a single application of sargahydroquinoic acid (**2**) in a log scale concentration in the basilar artery. However, the concentration–response relationship of sargahydroquinoic acid (**2**) was obtained by cumulative application in the carotid artery. Sargahydroquinoic acid (**2**) induced vasodilatation in the basilar and carotid arteries in a concentration-dependent manner. The curve fittings of the concentration–response relationships using the Hill equation revealed that the concentration of half maximal dilatation (EC_{50}) for the basilar and carotid

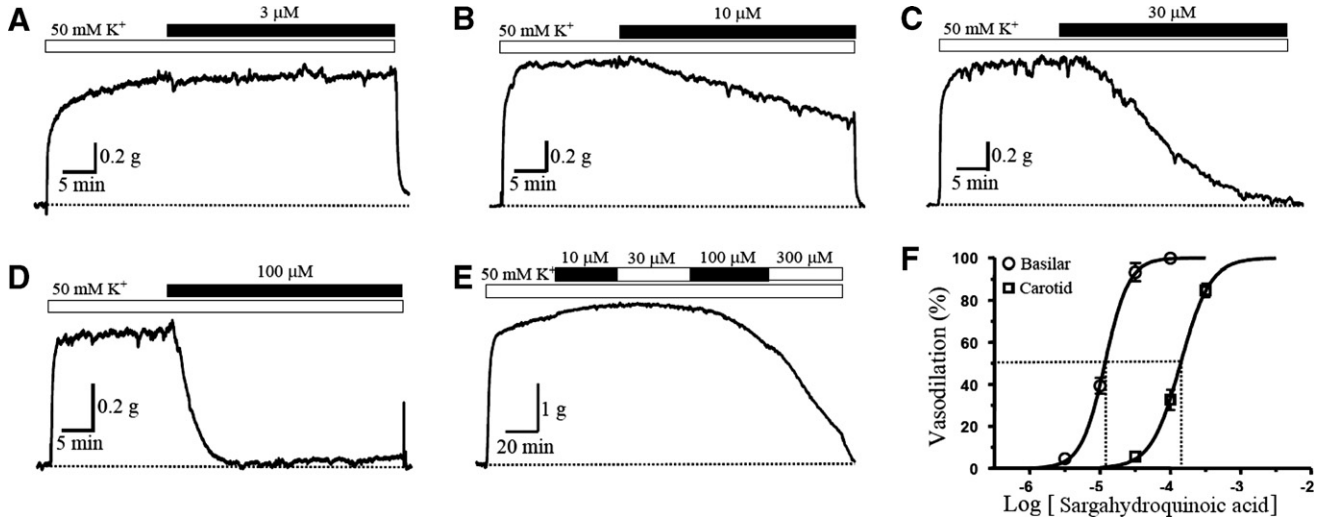

Figure 2. Vasodilatative effects of sargahydroquinoic acid from *Sargassum micracanthum* on high K^+-induced vasoconstriction of the rabbit basilar and carotid arteries. Representative traces were obtained by single application of sargahydroquinoic acid in a log scale concentration ($10^{-5.5}, 10^{-5}, 10^{-4.5}, 10^{-4}$ M) for 40 min after contraction induced by high K^+ (50 mM) reached a steady-state (20 min) on basilar artery (A–D). Representative trace was obtained by cumulative application of sargahydroquinoic acid in serial a log scale concentration for 40 min after high K^+-induced contraction for 20 min on the carotid artery (E). The vasodilatation was plotted as a function of sargahydroquinoic acid concentration and the curves were fitted using the Hill equation, $E = (1 + EC_{50}/[\text{sargahydrquinoic acid}]^n)^{-1}$ (F). Data are represented as means ± SD ($n = 3$).

arteries were 11.8 ± 0.28 and 140 ± 0.6 μM ($n = 3$), respectively. Based on the selectivity index (SI, EC_{50} for carotid/EC_{50} for basilar, 11.9), sargahydroquinoic acid (**2**) selectively dilated the basilar artery more than 10-fold over the carotid artery. Thus, treatment with sargahydroquinoic acid (**2**) may accelerate cerebral blood flow through dilatation of the basilar artery, without influencing systemic blood pressure. Generally speaking, because vasodilatation by sargahydroquinoic acid (**2**) does not decrease the blood pressure in the heart, blood flow through the basilar artery will effectively increase. Our results suggest that compounds that share a similar core structure with sargahydroquinoic acid (**2**), such as plastquinone[34,35] and hydroquinone,[36] may be novel lead compounds for selective pharmacological agents for the human vascular system (Fig. 2).

In conclusion, the activity-guided purification for an organic extract of *Sargassum micracanthum* gave a vasodilatative constituent, sargahydroquinoic acid (**2**), which selectively dilated the basilar artery of rabbits with an EC_{50} of 11.8 ± 0.28 μM. This molecule will be a novel lead compound as well as a drug candidate for the treatment of cerebral vascular diseases.

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25. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.51 (1H, d, $J = 3.0$ Hz), 6.47 (1H, d, $J = 3.0$ Hz), 6.00 (1H, t, $J = 7.2$ Hz), 5.28 (1H, t, $J = 7.1$ Hz), 5.11 (2H, m), 3.30 (1H, d, $J = 7.2$ Hz), 2.60 (2H, dt, $J = 7.2$, 7.2 Hz), 2.27 (2H, t, $J = 7.2$, 7.2 Hz), 2.18 (3H, s), 2.14 (4H, m), 2.09 (4H, m), 1.76 (3H, s), 1.69 (3H, s), 1.61 (6H, d, $J = 7.2$ Hz) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.1, 148.9, 146.6, 145.8, 138.5, 134.9, 132.5, 130.8, 127.9, 125.8, 124.5, 123.7, 121.9, 115.7, 114.2, 39.7, 39.2, 34.7, 30.1, 28.5, 28.1, 26.3, 25.9, 17.9, 16.4, 16.3, 16.2 ppm.
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connective tissue, fat, and adventitia were carefully removed. Basilar and carotid arteries were cut into rings (3 mm) in a dissecting chamber filled with PSS saturated with 95% O_2 and 5% CO_2 mixture. Basilar and carotid rings were mounted using a pair of stainless steel hooks under a resting tension of 0.6 and 1.5 g, respectively, in organ baths containing 15 ml of PSS, which was maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2 mixture. One of the hooks was connected to a force displacement transducer (MLT050; ADInstruments, Colorado Springs, CO, USA) and the tension was recorded with Powerlab/400 on a chart program (ADInstruments). After equilibration for 30 min, each ring specimen was repeatedly exposed to the high K^+ solution (50 mM K^+), prepared by replacing NaCl with an equimolar concentration of KCl, until the responses became stable. Functional endothelial cells were confirmed by the ability of acetylcholine (1 μM) to induce relaxation. Concentration–response relationships were obtained by a single application of sargahydrquinoic acid in a log scale concentration after precontraction induced by high K^+ reached a steady-state on the basilar artery and with cumulative application on the carotid artery.

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