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Transported amount of diosgenin to the brain is differed by a solvent fat

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Abstract

We previously reported effects of diosgenin on cognitive function in normal mice and Alzheimer's disease model mice and healthy humans. The low oral bioavailability of diosgenin has been well recognized. However, our previous study showed that diosgenin dissolved in olive oil was efficiently distributed in the brain and exerted a memory-enhancing effect in mice after oral administration. This study investigated potencies of olive oil, fish oil and medium-chain triglyceride to transfer diosgenin in the brain. Diosgenin detected in the cerebral cortex after oral administration was very high in case of dissolved in olive oil. Brain penetration of diosgenin dissolved in fish oil was lower than that in olive oil. Medium-chain triglyceride showed very low potential of brain penetration of diosgenin after p.o. administration. We found in this study potencies of transporting diosgenin to the brain were different by fats as solvents.

Keywords : diosgenin, brain transfer, olive oil, fish oil, medium-chain triglyceride

Ⅰ Introduction

We reported effects of diosgenin on cognitive function in normal mice[1, 2)] and Alzheimer's disease model mice[3, 4)] and healthy humans[2)]. The low oral bioavailability of diosgenin has been well recognized. This low absorption has been attributed to the poor solubility of diosgenin in water, and a previous rat study therefore proposed the formation of a complex with cyclodextrin to increase solubility[5)]. However, our previous study showed diosgenin had no effect on memory function in mice, despite successful solubilization when diosgenin was dissolved in a 10% ethanol-5% glucose solution[2)]. In contrast, diosgenin dissolved in olive oil was efficiently distributed in the brain and exerted a memory-enhancing effect in mice[2)]. We previously got evidences showing same potencies of olive oil, sesame oil and soybean oil for diosgenin transfer in the brain. However, it has not been compared that other kinds of fats have an ability of diosgenin transfer in the brain. Therefore, this study investigated potencies of olive oil, fish oil and medium-chain triglyceride (caprylic/capric triglyceride) to transfer diosgenin in the brain.

Ⅱ Materials and Methods

1. Animal studies

All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Toyama. The Committee for Animal Care and Use at the Sugitani Campus of the University of Toyama approved the study protocol (approval number for the animal experiments is A2017INM-1). All efforts were made to minimize the number of animals used.

Male ddY mice (8 weeks old, male) were purchased from Japan SLC (Shizuoka, Japan). All mice were housed in a controlled environment (25 ± 2°C, 12 h light/dark cycle starting at 7:00 am) with free access to food and water. Sixteen hours before administration of the sample, fasting was started.

2. Drug administration

Diosgenin compound was purchased from LKT Laboratories (St. Paul, Minnesota, USA). We dissolved 8 mg diosgenin in 790 µl of each oil using a micromixer. Oils used in this study

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are as follows; Japanese Pharmacopoeia quality standard olive oil (Maruishi Pharmaceutical Co., Ltd, Osaka, Japan). Fish oil from menhaden (Sigma-Aldrich Japan, Tokyo, Japan). Caprylic/capric triglyceride (MCT E6000) from MUSIM MAS (Singapore). Immediately after preparing diosgenin solutions, we orally administered the mice with diosgenin mixed in oil or vehicle solution (olive oil). Our in-house data indicates diosgenin stability in olive oil at this concentration is quite high. Decay rate of diosgenin at room temperature is 9.5% after 45 months.

3. Brain penetration of diosgenin

To assess the blood-brain barrier (BBB) penetration of diosgenin, each sample solution or vehicle solution (olive oil) was orally administered the male ddY mice. The mice were euthanized 6 h after sample administration, and blood was collected from abdominal aorta. Blood was centrifuged at 10,000 × g for 10 min at 4°C to collect plasma. Plasma aliquots (200 µl) were extracted with methanol, dried, and resolubilized in methanol (100 µl). After removing blood, perfusion was performed by infusion of 30 ml saline (0.1 ml/sec speed) from left ventricle with small cutting right auricle. The brain was dissected, and the cerebral cortex was isolated. Methanol (1.5 ml) was added to the cortex, and the tissue was homogenated for 15 sec. Mixing by vortex (1 min) and sonication (5 min) were done. After centrifugation at 12,000 × g for 10 min at 4°C, supernatant was transferred in a new tube, and dry up completely on hot plate at 70°C. The sample was resolubilized in 100 µl of methanol. After filtration with a 0.45-µm pore membrane filter, LC-MS analysis was performed to detect diosgenin. We plotted a standard curve to calculate the diosgenin concentration in the cortex. Briefly, standard solutions of diosgenin were extracted with methanol and subjected to LC-MS analyses. A Thermo Scientific^{TM} Accela HPLC system interfaced with an LTQ Orbitrap XL Hybrid Fourier Transform Mass Spectrometer (Thermo Fisher Co., San Jose, CA, USA) was used to chemically profile diosgenin. LC analyses were performed on a Capcell Pak C18 MGIII (2.0 mm i.d. × 150 mm, Shiseido, Tokyo, Japan) column held at 40°C with a flow rate of 200 µl/min. Ultrapure 0.1% formic acid in water (A) and methanol (B) were used as the mobile phase. We used a linear elution gradient as follows: 0–5 min, 65% B; 5–13 min, 95% B; 14–16 min, 65% B. We used the following electrospray ionization (ESI) parameters: spray voltage, 4.5 kV; capillary voltage, 40.0 kV; tube lens, 150 V; capillary temperature, 330°C; sheath gas flow rate, 50 units; and aux gas flow rate, 10 units. We operated the mass spectrometer in the positive ESI mode, which involves scanning from 50 to 2,000 m/z, and calibrated the instrument using a polytyrosine solution before each experiment.

Ⅲ Results and Discussion

Diosgenin detected in the plasma 6 h after oral administration was very high in case of dissolved in olive oil (Fig. 1). Concentration in plasma of diosgenin dissolved in fish oil (9.781 ± 4.790 µg/ml) was lower than that in olive oil (32.196 ± 3.773 µg/ml). Medium-chain triglyceride showed very low (0.909 ± 0.488 µg/ml) potential of brain penetration of diosgenin.

Diosgenin detected in the cerebral cortex 6 h after oral administration was very high in case of dissolved in olive oil (Fig. 2). Brain penetration of diosgenin dissolved in fish oil (2.024 ± 0.278 µg/g) was lower than that in olive oil (17.576 ± 6.642 µg/g). Medium-chain triglyceride showed very low (0.374 ± 0.408 µg/g) potential of brain penetration of diosgenin.

A critical difference between medium-chain fatty acid and other oils used in this study is fatty acid composition (Table 1). Medium-chain triglyceride just consists of caprylate and capric acid. In contrast, olive oil and fish oil consist of saturated fatty acids, monounsaturated fatty acid and polyunsaturated fatty acid except for caprylate and capric acid. The difference between olive oil and fish oil is high rate of oleic acid and linoleic acid in olive oil. Although we have not known yet the detail mechanism of oil-supported diosgenin transport to the brain, we would like to discuss considered possibility. Diosgenin is highly lipophilic compound (cLogP: 5.7), so it's affinity with lipid

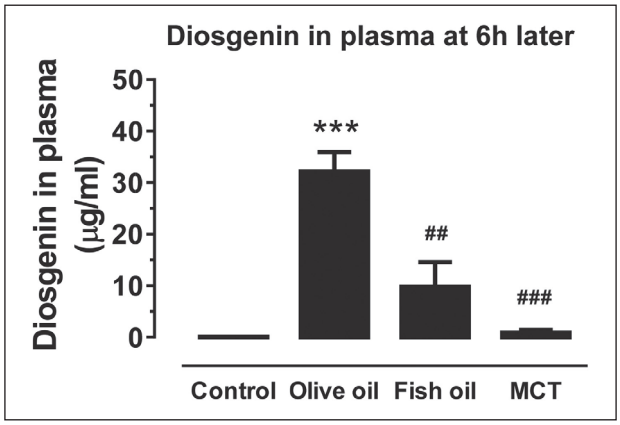

Fig. 1. Concentration of diosgenin in the plasma

Diosgenin was suspended in olive oil, fish oil or medium-chain triglyceride (MCT) and administered to normal ddY mice (male, eight weeks old). Plasma were collected 6 h after oral administration, and the samples were prepared. The diosgenin content of each sample was quantified using liquid chromatography-mass spectrometry. ***P < 0.001 vs Control, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001 vs Olive oil, n = 3. (One-way ANOVA, *post hoc* Tukey test)

solvent is high. Generally, the lymph pathway is known to play an important role in the absorption of highly lipophilic compounds. Very interestingly, Porsgaard and Høy compared lymphatic transport of 9 kinds of fatty acids in rats[6]. Olive oil was the most absorbed in lymph and fish oil from menhaden was not so much absorbed after oral administration. The accumulated doses of fatty acids in lymph at 6 h after the administration were approximately 160 mg and 60 mg in cases of olive oil and fish oil, respectively[6]. Comparing differences of fatty acid property absorbed in lymph between olive oil and fish oil, only difference is high oleic acid quantity in lymph after olive oil administration[6]. It may suggest that oleic acid is possible key fatty acid for delivery of diosgenin. In Fig. 1, diosgenin transferred in the plasma was 3.2 times higher by olive oil than by fish oil. In contrast, diosgenin transferred in the brain was 8.7 times higher by olive oil than by fish oil (Fig. 2). These results suggest that absorption of diosgenin in the systemic circulation and brain is affected by a type of oils. Especially, olive oil contributes to good brain penetration of diosgenin. Since other report identified lymphatic vessels in the brain[7], good direct absorption in lymph might relate to efficient brain accumulation of diosgenin in addition to BBB penetration. Our previous data clearly suggested using LC-MS analysis similarly performed like this paper that diosgenin in water solvent showed low delivery in the cerebral cortex compared with the case of olive oil solvent[2]. Oral administration of diosgenin dissolved in aqueous solution did not enhance memory function in mice, but diosgenin in olive oil significantly enhanced it[2]. Therefore, the detection of diosgenin in the cerebral cortex possibly indicate actual and functional existence of diosgenin in the brain. However, to get more fine date suggesting brain penetration of diosgenin through BBB, *in vitro* BBB model is useful and critical.

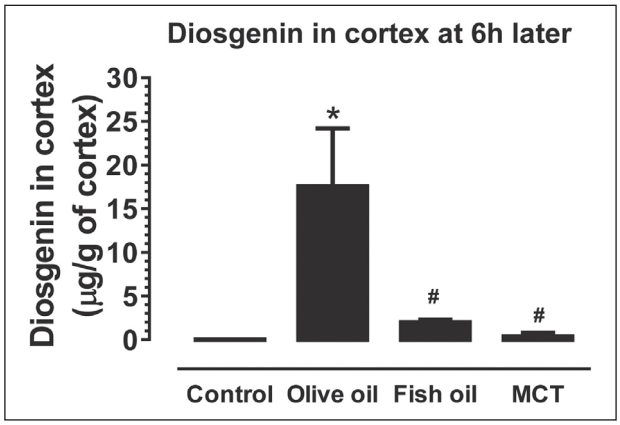

Fig. 2. Concentration of diosgenin in the cerebral cortex
Diosgenin was suspended in olive oil, fish oil or medium-chain triglyceride (MCT) and administered to normal ddY mice (male, eight weeks old). Plasma were collected 6 h after oral administration, and the samples were prepared. The diosgenin content of each sample was quantified using liquid chromatography-mass spectrometry. *P < 0.05 vs Control, #P < 0.05 vs Olive oil, n = 3. (One-way ANOVA, *post hoc* Tukey test)

Generally, fish oil and its main composition, eicosapentaenoic acid and docosahexaenoic acid are recognized as beneficial components for brain health. Therefore some idea to mix diosgenin with those fatty acids may come up simply. However, date shown in this paper indicate appropriate selection of oil as a solvent is required for efficient delivery of diosgenin.

Ⅳ Conclusion

Herein, we report differences of potencies of fatty acids transporting diosgenin to the plasma and brain as vehicle solution. Olive oil shows remarkable transport efficiency to the plasma and brain compared with fish oil and medium-chain fatty acid.

Table 1. Fatty acid composition (%) of used oils

Type	Saturated											Monounsaturated					Polyunsaturated				
Trivial name	Caprylic acid	Capric acid	Lauric acid	Myristic acid	Pentadecanoic acid	Parmitic acid	Heptadecanoic acid	Stearic acid	Arachidic acid	Behinic acid	Lignoceric acid	Myristoleic acid	Palmitoleic acid	Heptadecenoic acid	Oleic acid	Icosenoic acid	Linoleic acid	α-Linolenic acid	Arachidonic acid	Eicosapentaenoic acid	Docosahexaenoic acid
Chain length: unsat. degree	8:0	10:0	12:0	14:0	15:0	16:0	17:0	18:0	20:0	22:0	24:0	14:1	16:1	17:1	18:1	20:1	18:2 (n=6)	18:3 (n=3)	20:4 (n=6)	20:5 (n=3)	22:6 (n=3)
Olive oil	0	0	0	0	0	10.4	0	3.1	0.4	0.1	0	0	0.7	0	77.3	0.3	7	0.8	0	0	0
Fish oil from menhaden	0	0	0	6-9	0	15-20	0	3-4	0	0	0	0	9-14	0	5-12	0	0	0	0	10-15	8-15
Medium-chain triglyceride	60	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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ジオスゲニンの脳移行量は溶媒とする油脂によって異なる

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キーワード : ジオスゲニン、脳移行、オリーブ油、魚油、中鎖脂肪酸トリグリセリド

概　要

我々はこれまでに、ジオスゲニンによる認知機能促進作用を、正常マウス、アルツハイマー病モデルマウス、健常人で示してきた。ジオスゲニンの生物学的利用率は低いことがよく知られているが、我々の以前の研究では、ジオスゲニンをオリーブ油に溶解させて経口投与すると、脳に良く移行し記憶促進作用も認められた。本研究では、オリーブ油、魚油、中鎖脂肪酸トリグリセリドのそれぞれにジオスゲニンを溶解させたときの、経口投与後の血漿移行量と脳移行量を調べた。ジオスゲニンの血漿移行量と脳移行量は、オリーブ油に溶解した場合が極めて高く、魚油はそれに比べると少なかった。中鎖脂肪酸トリグリセリドではさらに、ジオスゲニンの移行量が低かった。本研究において我々は、溶媒として用いる油脂の違いにより、経口投与後に血中および脳へ移行するジオスゲニンの量が異なることを見出した。

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