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Curcumin Oligosaccharides (Gluco-oligosaccharides) Penetrate the Blood-Brain Barrier in Mouse Brain: Glycoside (Polysaccharide) Modification Approach for Brain Drug Delivery Across the Blood-Brain Barrier and Tumor Drug Delivery

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Abstract

In order to expand our drug delivery technique by glycosylation of chemical into brain delivery, it was demonstrated that oligosaccharide and monosaccharide modifications of curcumin enhanced its crossing ability of the blood-brain barrier (BBB) in mice. The brain sample prepared by glycosidase-catalyzed hydrolysis of brain tissue homogenates of mice, to which curcumin gluco-oligosaccharides were intraperitoneally injected, contained curcumin at 116 ng/1 g of tissue of brain, indicating that curcumin modified with gluco-oligosaccharides residues can smoothly cross the BBB in mouse brain. The brain samples of mice, which were treated with curcumin monosaccharide or curcumin itself, contained curcumin at 18 ng and 0 ng per 1 g of tissue of brain, respectively. On the other hand, after the administration of curcumin gluco-oligosaccharides to C57BL mouse with a large tumor for 5 days, the tumor disappeared.

Keywords

drug delivery technique, blood-brain barrier, curcumin glucooligosaccharide, glycosylation, brain drug delivery, tumor drug delivery

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Curcumin has been known to possess a wide spectrum of biological actions and be used as a useful drug in many diseases. For instance, curcumin has been reported to have therapeutic benefits such as not only anticancer and anti-inflammatory properties but also neuroprotective properties such as antiamyloid effect in Alzheimer's disease and antioxidative effect in the brain.[1,2] However, due to its extremely poor aqueous solubility and less distribution property, it has just low bioavailability, which does not allow its proper absorption in the body and brain.[3] In order to solve these issues, although the drug delivery systems (DDSs) by using chemical modification such as nanoparticle technology and PEGylation has been ever developed,[4,5] it is still difficult to regard it as fully resolved in terms of compatibility between biosafety and penetration effects.

We have ever developed the DDS by chemical and biological glycosylation. Paclitaxel is one of the most useful anticancer agents. Because of its water insolubility, paclitaxel is not efficient for the delivery system using liposomes as DDS vehicles. The glycosylated paclitaxel was successfully incorporated in liposomes.[6]

Using our drug delivery technique by glycosylation of chemicals, we studied the crossing ability of curcumin

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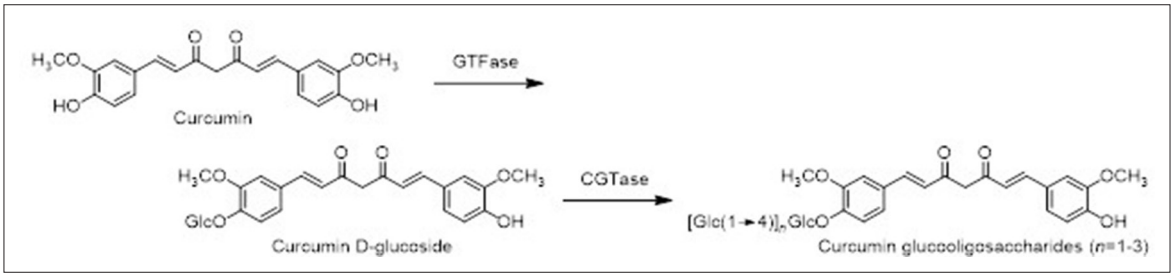

Figure 1. Synthesis of curcumin oligosaccharides by glycosylation of curcumin.

glycosides through the blood-brain barrier (BBB) in mice, resulting in that curcumin oligosaccharides (gluco-oligosaccharides) crossed the BBB in mice.

In this article, we report, for the first time, that gluco-oligosaccharide modification of curcumin enhanced its crossing ability of the BBB in mouse brain.

Curcumin was subjected to the glucosylation by glucosyltransferase (GTFase). After incubation of the enzyme with curcumin for 24 hours, the products were extracted with *n*-butanol (*n*-BuOH). *n*-BuOH extracts were purified by high-performance liquid chromatography (HPLC) to give curcumin β-D-glucoside. After incubation of curcumin β-D-glucoside, soluble starch, and cyclodextrin glucanotransferase (CGTase) from *Bacillus macerans* for 24 hours, the mixture was desalted with Sephadex G-25 column chromatography. Lyophilized eluate was redissolved and purified by HPLC to give a mixture of curcumin gluco-oligosaccharides (Figure 1; Supplemental Material).

Mice, to which curcumin oligosaccharides, curcumin D-glucoside, or curcumin (control) were intraperitoneally injected for 1 hour, were sacrificed by cervical dislocation and decapitation. The brain was quickly processed and rinsed. After homogenization of tissue samples in sodium acetate buffer, the homogenates were treated with glycosidases. The hydrolysis products were extracted with ethyl acetate (EtOAc) and methanol (MeOH) (95:5) to prepare the brain sample. The product curcumin was quantified on HPLC with a fluorescence detector.

HPLC analysis of brain samples of mice, to which curcumin oligosaccharides (gluco-oligosaccharides) were intraperitoneally injected, detected curcumin at 116 ng/1 g of tissue of brain, indicating that curcumin gluco-oligosaccharides were incorporated into the mouse brain (Figure 2(B)). It was found that a brain sample of mice, which was treated with curcumin itself (control), contained a trace amount of curcumin, showing that curcumin hardly migrated into the mouse brain (Figure 2(C)). The peaks appearing at 3-5 minutes in Figure 2(B) and (C) are considered as substances from the mouse brain because these peaks are found in the HPLC spectrum of the brain sample of mouse, which was not treated with compounds (data not shown). As judged by Figure 2(B) and (C), no curcumin oligosaccharides remained in the brain sample after treatment of brain homogenates with glycosidases (see the section General). It is not clear whether brain glycosidase hydrolyzed curcumin oligosaccharides after penetration of blood-brain barrier (BBB) or not. On the other hand, curcumin was detected at 18 ng/1 g of tissue of brain by HPLC analysis of brain samples of mice, which were treated with curcumin D-glucoside (data not shown). These results suggest that both curcumin gluco-oligosaccharides and curcumin monosaccharide (curcumin D-glucoside), which are intraperitoneally injected into mice, can penetrate the BBB to reach the brain tissue of mice and that curcumin gluco-oligosaccharides can pass the BBB more smoothly than curcumin D-glucoside. All experiments were carried out twice, and almost the same results were obtained. The presentable result is reported here.

Next, antitumor effect of curcumin oligosaccharides on a male C57BL mouse with large spontaneous tumor (tumor volume: ca. 3 cm^3) was investigated. Figure 3 demonstrated that 44.2 g of body weight in a mouse with a large tumor at the initial point was decreased until 33.4 g of body weight by the administration of 13.3 μmol/kg/day of curcumin oligosaccharides for 5 days and that tumor disappeared (tumor volume: 0 cm^3). This effect has been sustained for 14 days of the trial periods. We confirmed that no recurrence of the tumor was observed until 162 days thereafter as far as observed. This result shows that the administration of curcumin oligosaccharides for only 5 days eliminated the large tumor of 30% of body weight ratio in a mouse with a tumor, suggesting that the curcumin oligosaccharides may have the potential to treat the tumor.

Thus, the preparation and evaluation of curcumin oligosaccharides were investigated. Two-step biocatalytic synthesis of curcumin oligosaccharides was achieved using glucosyltransferase and CGTase as biocatalysts. Glycosidase-catalyzed hydrolysis of the brain tissue homogenates of mice, to which curcumin oligosaccharides were intraperitoneally administered, gave curcumin, indicating that curcumin glucooligosaccharides migrated into the brain tissue through the BBB in mouse brain. Recently, the glucosides of ketoprofen and indomethacin have been reported to significantly inhibit the uptake of glucose transporter (GluT1)-mediated uptake of glucose, demonstrating affinity to the transporter.[7] These conjugates could cross

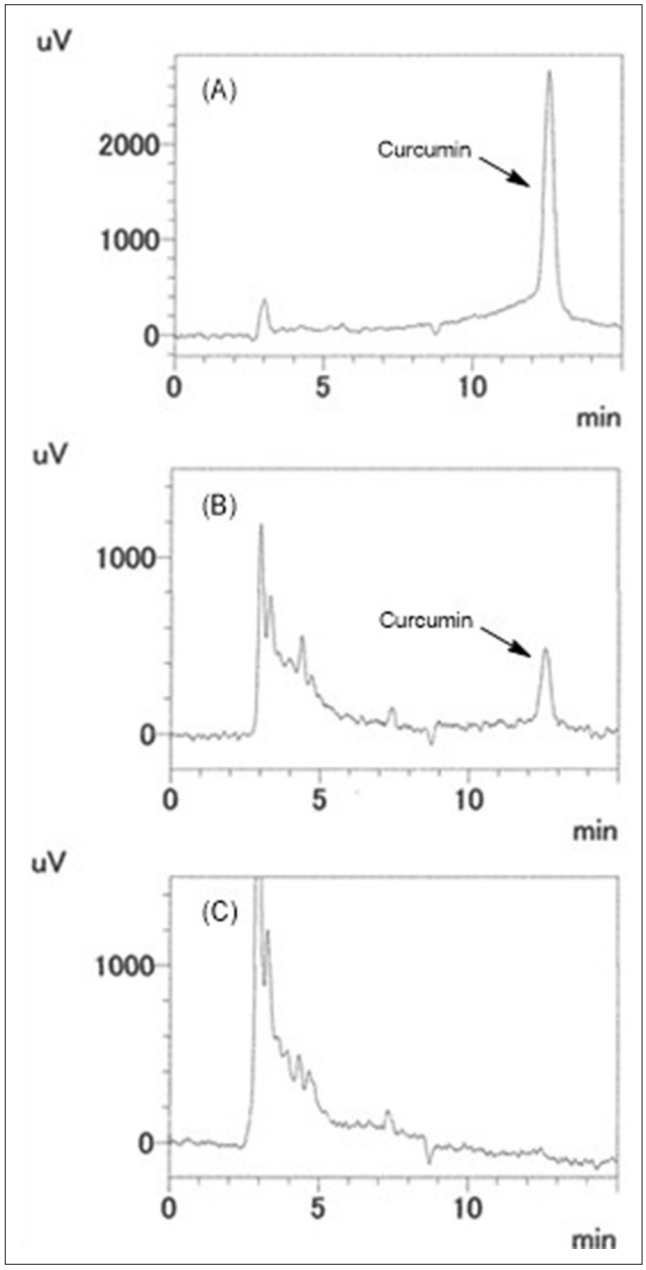

Figure 2. High-performance liquid chromatography analyses of (A) authentic curcumin, (B) brain sample of mice, which were treated with curcumin oligosaccharides, and (C) brain sample of mice, which were treated with curcumin (control).

the BBB in a temperature-dependent manner, suggesting that the glucosylation of drugs can enhance their BBB-crossing ability and that the brain uptake of the conjugates is carrier mediated.[7] In the present study, curcumin gluco-oligosaccharides might penetrate BBB in the mouse brain and be incorporated into the brain tissue. On the other hand, it was found that curcumin gluco-oligosaccharides possess a high potential to decrease tumor volume of mouse with a large tumor. Further studies on the transporter, which recognizes

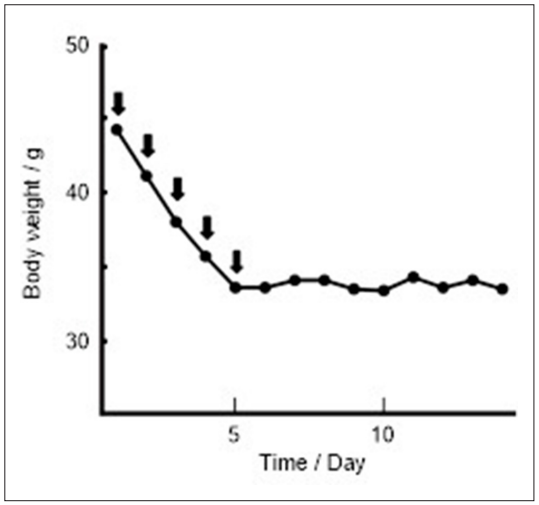

Figure 3. Changes in body weight of a C57BL mouse with a large tumor. C57BL male mouse with a tumor was administered 13.3 μmol/kg/day of curcumin oligosaccharides for 5 days. The bold arrows indicate the administration of curcumin oligosaccharides.

oligosaccharide conjugates as substrates, are now in progress in our laboratory.

Experimental

General

Curcumin was purchased from Tokyo Kasei Kogyo Co. Ltd. Curcumin concentrations in brain samples were determined using HPLC with fluorescence detection. Tissue samples (399 mg) were homogenized in sodium acetate buffer (0.1 M, pH 6.0) by using BioMasher I (Nippi Inc., Tokyo, Japan). Tissue samples were incubated with 2 mg/mL α-glucosidase (Amano enzyme Co., Ltd., Aichi, Japan), 20 U β-glucosidase (Oriental Yeast Co., Ltd., Tokyo, Japan), and 1000 U β-glucuronidase (Roche Diagnostics GmbH, Mannheim, Deutschland) at 37 °C for 1 hour. After incubation, 1 mL of extraction solvent (95% EtOAc, 5% MeOH) was added. After 3 extraction steps, the solvent was evaporated to dryness. Samples were solved in 50 μL MeOH to give brain sample. The extracted compound, curcumin, was quantified on the HPLC system (Shimadzu Corp., Kyoto, Japan) with a fluorescence detector (excitation wavelength 426 nm, emission wavelength 536 nm). The mobile phase consisted of 35% deionized water and 65% MeOH with 0.1% trifluoroacetic acid and was delivered at a flow rate of 1.0 mL/min using reversed phased packed column (Mightysil RP-18 GP 250-4.6, Kanto Chemical Co., Inc., Tokyo, Japan) at 40 °C. Curcumin was quantified against external standard curves. C57BL mice (20 g, 6-10 week) were purchased from Sankyo Labo Service Corporation (Tokyo, Japan). The mice had free access to feed and water throughout the experiment and were housed in groups of 6 animals per cage in a conditioned room (12 hours light/dark cycle, temperature, 25 °C; relative humidity 55%). All experiments were carried out by

individuals with appropriate training and experience according to the requirements of the Kyorin University Animal Care Committee.

Preparation of Curcumin Oligosaccharides

Curcumin oligosaccharides were prepared as follows. Curcumin D-glucoside (**2**) was prepared by biocatalytic glucosylation of curcumin with GTFase from *Phytolacca americana*.[8] The substrate curcumin (1 mmol/flask) was administered to 100 mL conical flasks containing glucosyltransferase (1 μmol/flask), and the mixture was incubated at 38 °C for additional 24 hours on a rotary shaker (120 rpm). After incubation, the mixture was centrifuged at 1000 × g. The supernatant was extracted with *n*-BuOH (×3). The *n*-BuOH extract was concentrated by evaporation. The residue was partitioned between water (H_2O) and EtOAc. The H_2O layer was applied to a Diaion HP-20 column, and the column was washed with H_2O followed by elution with MeOH. The MeOH eluate was subjected to HPLC (column: YMC-Pack R&D ODS column [150 × 30 mm]; solvent: MeOH-H_2O [9:11, v/v]; detection: UV [280 nm]; flow rate: 1.0 mL/min). The structure of the product was determined to be curcumin D-glucoside (95% yield) by analyses of its ^{1}H and ^{13}C nuclear magnetic resonance (NMR), and electrospray ionization mass spectrometry. No curcumin diglucoside was found by careful HPLC analysis. The synthesis of curcumin oligosaccharides was performed by incubating the reaction mixture (10 mL) containing 0.2 mmol of curcumin D-glucoside, 5 g of soluble starch, and 200 units of CGTase from *Bacillus macerans* purchased from Amano Pharmaceutical Co. Ltd. in 25 mM sodium phosphate buffer (pH 7.0) at 40 °C for 24 hours. The mixture was centrifuged at 3000 × g for 10 minutes. The supernatant was subjected to a Sephadex G-25 column equilibrated with H_2O. The fractions containing glycosides were lyophilized, resolubilized, and purified by HPLC to give a mixture of curcumin oligosaccharides, that is, curcumin maltoside, curcumin maltotrioside, and curcumin maltotetraoside. These compounds were separable, as their HPLC retention times are 20 minutes (curcumin maltotetraoside), 26 minutes (curcumin maltotrioside), and 33 minutes (curcumin maltoside).

BBB Crossing of Curcumin Oligosaccharides

Body weights were measured at the start of the trial. Mice were intraperitoneally injected once with 665 μmol/kg of curcumin oligosaccharides, curcumin D-glucoside, or curcumin (control) for 1 hour and were sacrificed by cervical dislocation and decapitation. The brain was quickly processed, rinsed with ice-cold sodium phosphate buffer, snap-frozen in liquid nitrogen, and stored at −80 °C.

Antitumor Examination of Curcumin Oligosaccharides

The mouse with a large tumor of 30% of body weight ratio was intraperitoneally injected with 13.3 μmol/kg/day curcumin gluco-oligosaccharides for 5 days, and the body weight was recorded at every day during 14 days of the trial periods.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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