Int.J.Curr.Microbiol.App.Sci (2024) *13*(07): 137-147

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 13 Number 7 (2024)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2024.1307.015

Preparation of Paclitaxel Glycoside-Anionic Nanoparticles, Curcumin Gluco-oligosaccharides, α-Tocopherol Glycoside, Daidzein Glycoside, and Genistein Glycoside and their Application for Treatment of Skin Cancer, Dementia, and Allergy

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Keywords

Anionic Technol PG-paclitaxel glycoside nanoparticles, Anionic phospholipid, Epidermis layer, Permeation of stratum corneum

Article Info

Received:
15 May 2024
Accepted:
26 June 2024
Available Online:
10 July 2024

A B S T R A C T

Biotransformation is a useful tool for preparation of biomedically active compounds. This study reports the preparation of glycoside compounds and their medical applications. Composite nanoparticles, "anionic liposomes", composed of anionic Technol PG and paclitaxel glycoside were prepared by mixing them in water with cholic acid-based surfactants of SC and a subsequent heating/cooling/ultrasonicating process. Small-sized anionic Technol PG nanoparticles (particle size: 3 nm) could be prepared by ultrasonic fragmentation at low temperature of 4°C. Upon addition of paclitaxel glycoside-anionic Technol PG nanoparticles to rat skin tissue (*in vitro*), the nanoparticles "anionic liposomes" (particle size: 3 nm) infiltrated into the epidermis layer penetrating stratum corneum (intercellular space: ca. 100 nm). In addition, during the anti-skin cancer test using mouse model of skin cancer, our study revealed that the numbers of papillomas of the mouse applied with paclitaxel glycoside-anionic Technol PG nanoparticles, "anionic liposomes", to mouse skin were decreased, although those of the mouse applied with paclitaxel glycoside itself to mouse skin (control) were increased. Thus, this study established that since paclitaxel glycoside-anionic Technol PG nanoparticles could permeate stratum corneum and be incorporated into the epidermis layer of mouse, they could also treat skin cancer (*in vivo*). On the other hand, biotransformation of curcumin was achieved by using enzymes as biocatalysts. Curcumin gluco-oligosaccharides, which were intraperitoneally or orally injected to a mouse, could penetrate the BBB of mouse brain and be incorporated into the mouse's brain tissue, and could enhance spatial learning (*in vivo*). In addition, glucoside and galactoside of α-tocopherol showed high anti-allergic activity toward allergen, glutenin. Also, glucoside and galactoside of daidzein and genistein had strong anti-allergic activity against allergen, globulin.

Introduction

Paclitaxel, which is naturally produced in the bark and needles of *Taxus brevifolia*, is a tricyclic diterpenoid compound. It is already one of the most successful and widely used natural anticancer drugs, because of its unique anticancer mechanism. Paclitaxel is used for treatment of coronary heart disease, renal and hepatic fibrosis, inflammation, and axon regeneration (Gelmon, 1994; Luo *et al.,* 2017; Narayanan *et al.,* 2010; Perez, 2009; Uchida *et al.,* 2020; Uchida *et al.,* 2022; Zhu and Chen, 2019). It has scientifically proven anticancer activity toward ovarian, lung, and breast cancers. Skin is frequently exposed to oxidative stress from ultraviolet radiation, which presents a risk for the development of cancers such as melanoma, squamous cell carcinoma, and basal cell carcinoma.

Efficient transdermal delivery of paclitaxel would be useful to cure these serious skin cancers. Skin tissue is composed of stratum corneum, epidermis, and dermis. However, the 10- to 40-μm-thickstratum corneum, consisting of densely packed cells, provides a barrier to protect the underlying tissue from infection, dehydration, chemicals, and mechanical stress. It is difficult to applicate paclitaxel for treatment of skin cancer, because it cannot penetrate the stratum corneum.

Phospholipids are biologically friendly molecules to living body because they are synthesized in the body. Therefore, they are highly biocompatible. However, frequently utilized neutral phospholipids tend to form large-sized vesicles, which sometimes result in insufficient skin penetration. Nanotechnology has attracted biomedical attention for the usefulness of nanoparticles containing paclitaxel, its opportunities, and also future perspective. Thus, preparation of phospholipid-based paclitaxel small-sized nanoparticles is still a challenging problem.

The blood–brain barrier (BBB) exists in the brain as a selective semipermeable border that prevents solutes in the circulating blood from non-selectively crossing the extracellular fluid of the central nervous system where neurons exist (Daneman *et al.,* 2015). It comprises endothelial cells of the capillary wall, astrocyte end-feet ensheathing the capillary, and pericytes fixed firmly in the capillary basement membrane (Ballabh *et al.,* 2004). While the BBB system allows the passage of some small molecules by passive diffusion, it also permits the selective transport of various nutrients, ions, organic anions, and macromolecules, such as glucose and amino acids, crucial to neuronal functioning (Ballabh *et al.,* 2004). It is difficult to applicate curcumin for treatment of dementia, because it cannot penetrate the BBB.

Because prodrugs and pro-supplements (precursors of drugs and dietary supplements that are metabolized within the body to form the corresponding bioactive materials) have recently attracted a great deal of attention, there is a growing need for techniques that are capable of selective chemical modification of functional compounds. We have focused on glycosylation, particularly glucosylation, as one such type of chemical modification. There have been many studies on the production of useful substances by organic syntheses through routes that incorporate reactions induced by biocatalysts, such as cultured cells or enzymes, and the results of such studies have been applied in the production of a range of fine chemicals, including pharmaceuticals, aroma chemicals, and food additives. Among the biocatalysts that have been used are microorganisms, fungi, yeasts, animal cells, and enzymes extracted from these sources. In addition, biotransformations effected by using cultured plant cells as biocatalysts have recently attracted attention. Plants, which live on land and are generally incapable of movement, produce various secondary metabolites for the purposes of self-defense and signal transduction. As a result, plant cells contain a range of enzymes that have inherent abilities to transform or to synthesize organic substances. With the aim of utilizing the intrinsic ability of plant enzymes to effect biotransformations, we have studied biotransformations of exogenous substances by cultured plant cells and we have succeeded in effecting a range of reactions, including reduction, hydrolysis, isomerization, glycosylation, esterification, and hydroxylation reactions, by using cultured plant cells as biocatalysts.

Glycosylation by plant cells is a particularly important reaction that is involved in the activation of metabolites in cells and might, therefore, be useful in stabilizing various biologically active compounds or in activating various physiological functions. A major advantage of using biocatalysts in organic synthesis is their high selectivity. Furthermore, glycosides, the chemical synthesis of which generally involves a complicated range of procedures, can be obtained by means of a one-step enzymatic reaction with a biocatalyst. For these reasons, the application of cultured plant cells that are capable of highly stereoselective glycosylation reactions

in organic synthesis has been eagerly anticipated. We have attempted to apply glycosylation reactions catalyzed by enzymes and cultured plant cells to the transformation of bioactive compounds into synthetic compounds with improved stabilities and new bioactivities. This review introduces the results of our studies to date on the transformation and activation of bioactive compounds by means of cultured plant cells and enzymes.

Here, we report nanoparticles, "anionic liposomes", of paclitaxel glycoside stabilized with anionic phospholipids of Technol PG. Also, their applications for treatment of skin cancer are reported. Additionally, we report the therapeutic effects of curcumin gluco-oligosaccharides, α-tocopherol glycosides, daidzein glycosides, and genistein glycosides for dementia and allergy.

Materials and Methods

General

Ultrasonication was performed by using a QSonica model ultrasonic homogenizer. The sizes of anionic nanoparticles were measured by using a Horiba model LA-960 laser diffraction particle size analyzer (SALD) or a Malvern model Zetasizer Nano ZSP zeta potential analyzer (DLS).

Preparation of anionic Technol PG-paclitaxel glycosidenanoparticles

For the preparation of Technol PG nanoparticle, "anionic liposomes", Technol PG (5 wt%) and paclitaxel glycoside or cannabidiol were mixed with cholic acid-based surfactants of SC (0.5-5 wt%), CA (5 wt%), or CHAPSO (5 wt%) via a subsequent heating/cooling/ultrasonicating process in water and ultrasonicated at 4°C for 2 min.

In vivo transdermal delivery of paclitaxel glycoside (anionic Technol PG-paclitaxel glycoside nanoparticles) to skin cancer

All animals were housed individually in cages under specific pathogen-free conditions during the experiments. Age- and sex-matched mice were used for the experiments. 8-Week-old male C57BL mice were used. Skin tumors were induced by two-step application of DMBA and 12-*O*-tetradecanoylphorbol-13-acetate. First, 25 μg of DMBA in 100 μL of acetone was applied onto the shaved dorsal skin of the mice on day 7 (1 week). On day 0, topical application of 30 μg of 12-*O*-tetradecanoylphorbol-13-acetate in 100 μL of acetone was initiated and was continued for 20 weeks with a frequency of twice a week. Tumor development was monitored on a weekly basis and lesions greater than 2 mm in length were counted as positive. Technol PG-paclitaxel glycoside (paclitaxel glycoside (7-glycolylpaclitaxel 2"-*O*-α-maltoside) incorporated in anionic nanoparticles (0.2 g/kg)), "anionic liposomes", was applied to the rostral part of the back of mice five times a week. In the control experiment, paclitaxel glycoside itself was administrated in the same method as above.

BBB penetrating ability test of curcumin gluco-oligosaccharides

cDNA of glucosyltransferase from *P. americana* (*Pa*GT) was cloned into pQE30, and the resulting plasmids were transformed into *E.coli* M15 cells. The purified enzyme solution was dialyzed with 50 mM Tris-HCl (pH 7.2) containing 5 mM dithiothreitol, and stored at –80 °C. Glucosylation reactions were performed at 35 °C for 24 hours in 5 mL of 50 mM potassium phosphate buffer (pH 7.2) supplemented with curcumin, UDP-glucose, and enzyme *Pa*GT. The incubation was stopped by adding 1.5% trifluoroacetic acid; the reaction mixture was analyzed by HPLC. The resulting curcumin glucoside was applied for further glycosylation by CGTase to give curcumin gluco-oligosaccharides.

The mice were orally injected once with curcumin glucoside, curcumin gluco-oligosaccharides, or curcumin (control) to test their BBB penetration abilities. One hour later, they were sacrificed by cervical dislocation, after which their brain tissue samples were quickly processed by rinsing with cold sodium phosphate buffer, then frozen and stored at −20°C. Subsequently, curcumin was extracted, after which its concentration in the brain sample was determined using HPLC. Tissue samples were first homogenized in sodium acetate buffer, and tissue homogenates were ultrasonicated in 0.1% Triton X-100.

Then, in a flask containing the homogenate mixture, α-glucosidase, β-glucosidase, and β-glucuronidase were added and incubated at 36°C for one hour. Organic compounds were finally extracted with ethyl acetate. After three extraction steps, ethyl acetate was evaporated. Samples were dissolved in methanol to give brain

extracts sample. Finally, the extracted curcumin was quantified by HPLC. The mice, which were intraperitoneally injected once with these compounds, were tested in the similar method with orally injected mice.

BBB penetration of curcumin gluco-oligosaccharides and Y-maze test

A Y-maze with three arms was constructed with gray plastic, then it was equipped with a partition that isolates an arm. The experiment involved a 5-min trial 1, separated by a 40-min interval, followed by a 5-min trial 2. During the familiarization phase (trial 1), one arm (arm C: novel arm) of the Y-maze was closed with a partition. Then, while we placed one SAMP8 in one arm (arm A) of the two remaining arms (arms A and B) and the mouse allowed to explore the maze for five minutes, the partition was removed after a 40-min interval. Afterward, for five minutes, the mouse had free access to all three arms during the retrieval phase (trial 2). The time of the novel arm (arm C) exploration was only recorded when the mouse put his hind feet in that arm. Then, the percentage of time spent in the novel arm C was calculated. Finally, curcumin was orally injected every day for five days to mouse (one injection per day) (the control), whereas curcumin gluco-oligosaccharides were orally injected every day for five days to mouse (one injection per day (200 mg/kg)) (the curcumin-oligosaccharides-treated mouse). In case of intraperitoneal injection of the gluco-oligosaccharides to mice, injection and Y-maze test were demonstrated in the same method as described above.

Anti-allergic activity of α-tocopherol glycoside and daidzein glycoside against glutenin and globulin

Glucosylation reactions were performed at 35 °C for 24 hours in 5 mL of 50 mM potassium phosphate buffer (pH 7.2) supplemented with α-tocopherol, daidzein, or genistein, UDP-glucose, and enzyme *Pa*GT. When using amylase as a biocatalyst, the mixture of acetonitrile and water was used for a solvent. The incubation was stopped by adding 1.5% trifluoroacetic acid; the reaction mixture was analyzed by HPLC. In case of biotransformation of these compounds using plant cells as biocatalysts, each compound was administered to the flask containing the suspension cultured cells and the cultures were incubated at 25°C for 7 days on a rotary shaker. After incubation, the cells were harvested and extracted by homogenization with MeOH. The yield of the products was calculated on the basis of the peak area from HPLC using the calibration curves prepared by the HPLC analyses of authentic glycosides.

Effects of compounds on O_2^- generation from rat neutrophils were examined as follows. Male Wistar rats, each weighing 250 g, were used. Under ether anesthesia, whole blood was collected from the carotid artery and diluted twice with Hanks' balanced salt solution (HBSS). Neutrophils were purified by Percoll density gradient centrifugation. O_2^- generation from rat neutrophils was measured by the cypridina luciferin analog-dependent chemiluminescence. Neutrophil suspensions were incubated for 3 min in HBSS containing cypridina luciferin analog and sample at 37°C in the dark. Five seconds later, fMLP was added into the assay mixture. Cypridina luciferin analog-dependent chemiluminescence was monitored. The results are expressed in terms of the percentage reduction of the O_2^- generation from rat neutrophils at 5 min after the administration of fMLP by test compounds.

The effects of test compounds on compound 48/80-induced histamine release from rat peritoneal mast cells were examined as follows. Peritoneal mast cells were collected from the abdominal cavities of rats (Male Wistar rats, Nippon SLC) and purified to a level higher than 95%. The purified mast cells were suspended in a physiological buffered solution (PBS) containing NaCl, KCl, $CaCl_2$, glucose, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) to give approximately 10^4 mast cells/mL. Cell viability was always greater than 90% as judged by the trypan blue exclusion test. Mast cells were preincubated with the test compound for 15 min at 37°C, and subsequently exposed to compound 48/80. Histamine release was determined by a fluorometric assay, and was expressed as a percentage of total histamine.

The inhibitory action of test compounds on IgE antibody formation was examined as follows. Glutenin or 7S-globulin was used as the antigen, and $Al(OH)_3$ and pertussis toxin were used as the adjuvants. Sensitization was made by injection of a mixture of the antigen and the adjuvant into the paws of each rat (male).

Paw edema was measured 24 h after injection and the treated rats were divided in groups with an equal average swelling volume. Each sample was dissolved in physiological saline containing 10% Nikkol and the

solution containing test compound was injected daily into each rat for 11 d starting on the day of grouping. Hydrocortisone was used as the positive control. The amount of IgE was measured on the 15th day. The results were expressed as plasma IgE levels.

Results and Discussion

Preparation of anionic nanoparticles

Technol PG consists of a mixture of phosphatidylglycerol having fatty acids which contain C16 and C18 with 0 (C18-0), 1 (C18-1), 2 (C18-2), and 3 (C18-3) of double bonds. The percentage of each component (C16, C18-0, C18-1, C18-2, C18-3) is 14.4%, 4.7%, 13.5%, 61.0%, and 6.0%, respectively.

In this study, we prepared Technol PG-based nanoparticles using easily accessible cholic acid-derived surfactants. Dispersion of Technol PG with sodium cholate (SC) enabled the encapsulation of paclitaxel glycoside(7-glycolylpaclitaxel 2"-*O*-α-maltoside) or cannabidiol. As a typical method, Technol PG powder (5 wt%) was dispersed in water, mixed with SC (0.5-5 wt%), and sonicated for 2 min. When 0.5 wt% of SC was mixed with Technol PG, a cloudy dispersion was observed, similar to Technol PG before the addition of SC. However, when SC was added at 2 wt% and 5 wt%, the Technol PG dispersion became transparent. We performed a dynamic light scattering (DLS) analysis to investigate how the SC surfactant affected the size of Technol PG particles.

The hydrodynamic diameter of Technol PG, around 0.1 to 10 μm, decreased slightly after the addition of 0.5 wt% of SC. The addition of 2 wt% and 5 wt% of SC resulted in Technol PG particles. To clarify the effects of molecular structure of the surfactants. Technol PG with noncharged cholic acid (CA) and 3-[(3-cholamidopropyl) (CHAPSO) having a zwitterionic group. As a result, a cloudy dispersion was observed when CA was added, while a clear dispersion was observed when CHAPSO was mixed, suggesting that the ionic groups in the surfactants were important for the dispersion of Technol PG. In good agreement with the DLS result, microscopic observation of Technol PG (5 wt%) with 2 wt% of SC revealed that Technol PG was well dispersed in the solution, while it showed large aggregates before the addition of SC. DLS analysis of the anionic Technol PG nanoparticles, "anionic liposomes", showed a hydrodynamic diameter of 3 nm (Fig. 1).

In vivo transdermal delivery of paclitaxel glycoside (anionic Technol PG-paclitaxel glycoside nanoparticles) to skin cancer

Mice started to develop papillomas later than 10 weeks after initial 12-*O*-tetradecanoylphorbol-13-acetate treatment. At 14 weeks after initial 12-*O*-tetradecanoylphorbol-13-acetate treatment, mice developed three papillomas and were used for the in vivo transdermal delivery experiment.

The numbers of papillomas in anionic Technol PG-paclitaxel glycoside (7-glycolylpaclitaxel 2"-*O*-α-maltoside) nanoparticles-treated mouse (paclitaxel glycoside incorporated in "anionic liposomes"-treated mouse) were decreased, although those in paclitaxel glycoside-treated mouse (control) were increased (Fig.2).

These observations would explain that anionic Technol PG-paclitaxel glycoside nanoparticles (paclitaxel glycoside incorporated in "anionic liposomes") may contribute as chemo-preventive and anti-skin cancer agents.

BBB penetration by curcumin gluco-oligosaccharides

Curcuma longa Linn. has been used as a spice for centuries worldwide. Also, it has been used in folk medicines for the treatment of a variety of inflammatory conditions. Its intake reduces the risk of certain kinds of cancers and renders other protective pharmacological effects in human.

These medicinal properties have been attributed mainly to the curcuminoids, and the main component present in *C. longa* L. is curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] that has been widely studied for its anticancer, anti-inflammatory, antiaging, antiangiogenic, wound healing, and antioxidant effects. Irrespective of such pharmacological activities, its use as a medicine has been limited, because of its water insolubility and poor absorption after oral administration.

Incubation of glucosyltransferase from *Phytolacca americana* (*Pa*GT) with curcumin gave glucoside as the sole product. Glucosylation of curcumin with *Pa*GT described here is considerably efficient method to give curcumin glucoside rather than chemical glucosylation. Biocatalytic glycosylation of curcumin glucoside with

CGTase was attempted to synthesize curcumin gluco-oligosaccharides (Fig. 3). Curcumin gluco-oligosaccharides were orally injected to the mouse. Mouse brain tissue samples were processed as described in the Materials and Methods. After homogenizing tissue samples in sodium acetate buffer, the homogenates were ultrasonicated and treated by hydrolysis with glycosidases. Afterward, the products were extracted with ethyl acetate to prepare brain extracts. The obtained curcumin was subsequently quantified by HPLC analysis of the brain extracts. Thus, curcumin was detected at 76 ng. The HPLC analysis results of the brain extracts sample indicated that curcumin gluco-oligosaccharides were incorporated into the mouse brain tissue.

Investigations also revealed that the brain extracts sample of the mouse treated with curcumin (control) contained no curcumin, indicating that it hardly migrated to the mouse brain tissue. These results suggest that curcumin gluco-oligosaccharides, which were orally injected into mouse, could penetrate the BBB migrating to the mouse brain. In case of intraperitoneal injection with curcumin gluco-oligosaccharides, curcumin was detected at 116 ng (Fig. 4).

The brain extracts sample of the mouse treated with curcumin (control) contained 0 ng curcumin, suggesting that it hardly migrated to the mouse brain tissue. The brain extracts sample of mice, which were treated with curcumin monosaccharide, contained curcumin at 18 ng. These investigations show that curcumin gluco-oligosaccharides, which were intraperitoneally injected into mice, could smoothly penetrate the BBB in the mouse brain.

BBB penetration of curcumin gluco-oligosaccharides and Y-maze test

In the Y-maze test using SAMP8, the time spent in the novel arm of the Y-maze by the mouse intraperitoneally injected with curcumin gluco-oligosaccharides, was longer than that spent by the control mouse, into which curcumin alone was intraperitoneally injected (Table 1).

In case of Y-maze test using SAMP8 mouse orally injected with curcumin gluco-oligosaccharides, the time spent in the novel arm of the Y-maze was longer than that spent by the control mouse. Investigations also revealed that the percentage of time spent in the novel arm by curcumin gluco-oligosaccharides-treated mouse was higher than that of the time spent by the control mouse (Table 1). These results suggest that curcumin gluco-oligosaccharides penetrated the BBB and were incorporated into the brain tissue of SAMP8, enhancing spatial learning of the mouse.

Anti-allergic activity of α-tocopherol glycoside and daidzein glycoside against glutenin and globulin

The inhibitory activities ofα-tocopherol glucoside, α-tocopherol galactoside, daidzein glucoside, daidzein galactoside, genistein glucoside, genistein galactoside for O_2^- generation from rat neutrophils were 58, 60, 50, 51, 52, and 56%inhibition. Compound 48/80-induced histamine release from rat peritoneal mast cells was inhibited by α-tocopherol glucoside with a %inhibition of 83%. α-Tocopherol galactoside (85% inhibition), daidzein glucoside (62% inhibition), daidzein galactoside (65% inhibition), genistein glucoside (64% inhibition), genistein galactoside (68% inhibition) had strong inhibitory activity toward histamine release. The effects of α-tocopherol glucoside and α-tocopherol galactosideon IgE antibody formation were investigated by abioassay using glutenin as an allergen. It was found that α-tocopherol glucoside and α-tocopherol galactoside showed strong anti-allergic activity (IgE level 96 and 80) against glutenin. Positive control of hydrocortisone exerted IgE level of 320. On the other hand, IgE levels of daidzein glucoside, daidzein galactoside, genistein glucoside, and genistein galactoside against globulin were 128, 96, 96, and 80.

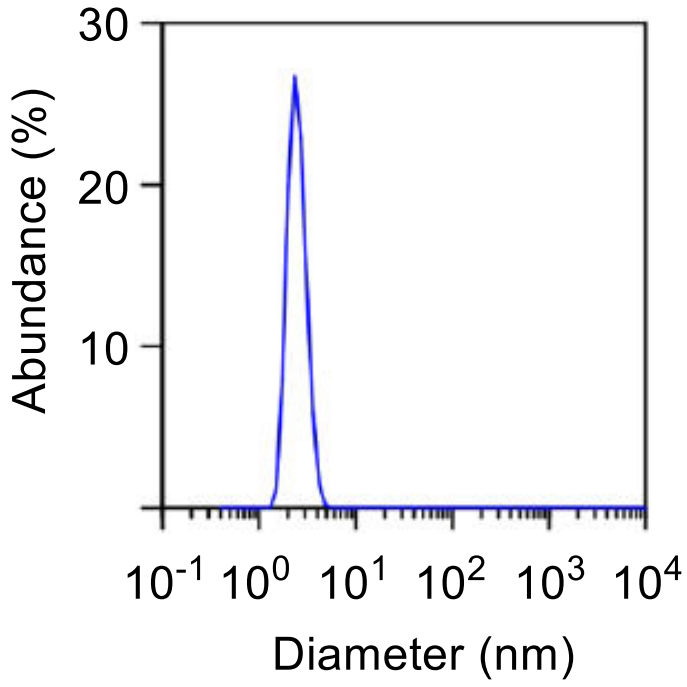
In this study reported paclitaxel glycoside (7-glycolylpaclitaxel 2"-*O*-α-maltoside) nanoparticles stabilized by anionic phospholipids of Technol PG, “anionic liposomes”. The nanoparticles can be fractionated by an ultrasonication treatment to prepare small-sized nanoparticles. When the small-sized Technol PG-paclitaxel glycoside nanoparticles, “anionic liposomes” (particle size: 3 nm), were added to rat skin tissue, they penetrated the skin barrier of stratum corneum (intercellular space: ca. 100 nm). Applications of paclitaxel glycoside, which has no skin permeability, to anti-skin cancer materials have been still challenging because of its difficulty in transdermal delivery. The anionic Technol PG-paclitaxel glycoside nanoparticles (paclitaxel glycoside incorporated in “anionic liposomes”) having skin permeability demonstrated in this study would be a new candidate as effective anti-skin cancer materials, which can infiltrate into epidermis layer decreasing numbers of papillomas.

Table.1 Spatial-learning of SAMP8 treated with curcumin or curcumin gluco-oligosaccharides in the Y-maze test.

SAMP8 group	Time spent in the novel arm (s)	Percentage total time (%)
Oral injection		
Curcumin-treated mouse (control)	95	32
Curcumin gluco-oligosaccharides-treated mouse	131	44
Intraperitoneal injection		
Curcumin-treated mouse (control)	92	31
Curcumin gluco-oligosaccharides-treated mouse	120	40

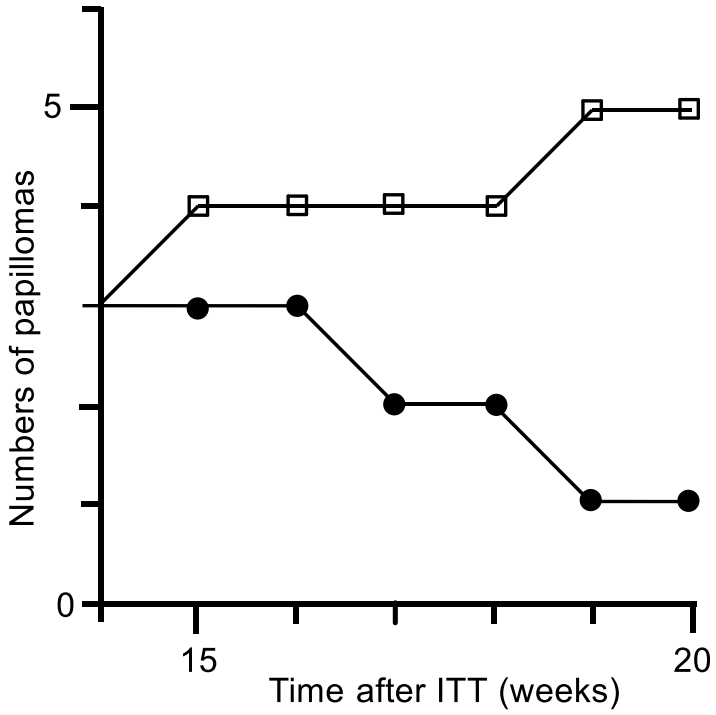
The percentage of time spent in the novel arm by curcumin gluco-oligosaccharides-treated mouse was higher than that of the time spent by the control mouse, suggesting that curcumin gluco-oligosaccharides penetrated the BBB and were incorporated into the brain tissue of SAMP8, enhancing spatial learning of the mouse.

Figure.1 Particle size analysis of anionic Technol PG nanoparticles, "anionic liposomes", prepared by mixing Technol PG with cholic acid-based surfactants of SC and a subsequent heating/cooling/ultrasonicating process.



AnionicTechnol PG nanoparticles, "anionic liposomes", were fractionated to 3 nm-sized nanoparticles after an ultrasonication treatment at low temperature of 4°C.

Figure.2 Numbers of papillomas of anionic Technol PG-paclitaxel glycoside nanoparticles (paclitaxel glycoside incorporated in "anionic liposomes")-treated mouse (●) and paclitaxel glycoside-treated mouse (control, □).



ITT: initial 12-*O*-tetradecanoylphorbol-13-acetate treatment. The numbers of papillomas in anionic Technol PG-paclitaxel glycoside nanoparticles-treated mouse (paclitaxel glycoside incorporated in "anionic liposomes"-treated mouse) were decreased, although those in paclitaxel glycoside-treated mouse (control) were increased, suggesting that anionic Technol PG-paclitaxel glycoside nanoparticles (paclitaxel glycoside incorporated in "anionic liposomes") may contribute as chemo-preventive and anti-skin cancer agents, which can infiltrate into epidermis layer decreasing the numbers of papillomas.

Figure.3 Preparation of curcumin gluco-oligosaccharides by *Pa*GT and CGTase.

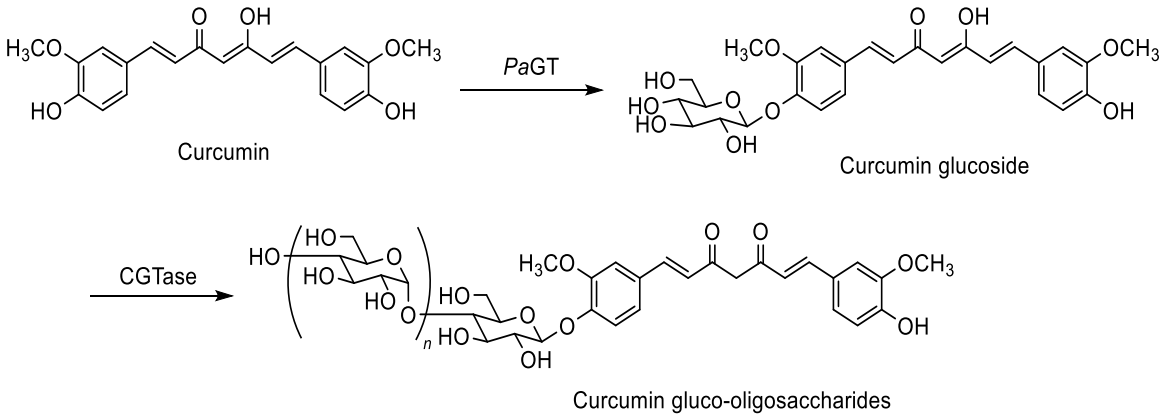
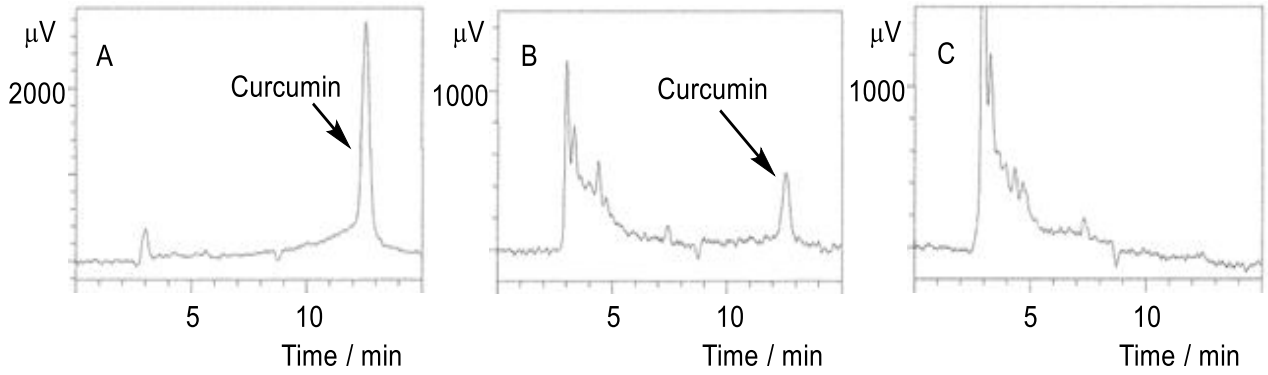


Figure.4 HPLC analyses of (A) authentic curcumin, (B) glycosidase-processed-brain extracts of mouse treated with curcumin gluco-oligosaccharides, and (C) glycosidase-processed-brain extracts of mouse treated with curcumin.



The fact that liberated curcumin was only detected in HPLC analysis of glycosidase-processed-brain extracts of mouse, which had been treated with curcumin gluco-oligosaccharides, suggested that curcumin gluco-oligosaccharides intraperitoneally injected into mouse could penetrate the BBB migrating to the mouse brain(B), and that curcumin itself hardly migrated to the mouse brain(C).

Previous studies have reported that the glucosides of ketoprofen and indomethacin could significantly inhibit the glucose transporter (GluT1)-mediated uptake of glucose, indicating its affinity to the transporter (Gynther *et al.,* 2009; Berardi *et al.,* 2009).

In addition, these glucoconjugates could temperature-dependently penetrate the BBB, indicating that the glucosylation of drugs enhances their BBB-crossing ability and that the brain uptake of the conjugates is carrier-mediated (Gynther *et al.,* 2009).

Consistent with these studies, we also observed that curcumin oligosaccharides can cross the BBB in the mouse brain and be incorporated into brain tissue. It has been shown anti-dementia drug such as memantine decreases β-amyloid levels via increase in secretion of amyloid precursor protein and activation of α-secretase (Niles *et al.,* 2006; Shan *et al.,* 2014; Hashemi *et al.,* 2022). On the other hand, the hippocampus is a critical brain area for cognitive and memory functions, making it a sensitive area in Alzheimer's (Berardi *et al.,* 2009). Anti dementia drug has been shown to improve learning and memory in several pharmacological models of Alzheimer's disease.

For example, the study of the effects of such drug on locomotor activity, social behavior, and spatial learning assessed in a transgenic mouse model of Alzheimer's disease indicated that it improves hippocampus-based spatial learning in a transgenic mouse model of Alzheimer's disease without producing nonspecific effects on locomotion/exploratory activity (Evers *et al.,* 2004; Minkeviciene *et al.,* 2004; Rimando *et al.,* 2004). These previous findings are consistent with our study, which suggests that curcumin oligosaccharides are chemopreventive agents that can protect neurons against the β-amyloid-induced disruption of spatial learning and memory in the hippocampus of SAMP8 and enhance spatial learning. Therefore, based on these results, our findings suggest that the gluco-oligosaccharide modification of neuroprotective chemicals, such as curcumin, enhances their crossing ability through the BBB in the brain, thus, proposing that the brain–drug-delivery technique of neuroprotective chemicals by glycoside (gluco-oligosaccharide) modification is useful for preparing new anti-dementia drugs.

α-Tocopherol glucoside, α-tocopherol galactoside, daidzein glucoside, daidzein galactoside, genistein glucoside, and genistein galactoside inhibited O_2^- generation from rat neutrophils. These compounds suppressed histamine release from rat peritoneal mast cells. Also, they regulated IgE antibody formation. These findings indicate that suppression of O_2^- generation caused inhibition of signal transduction of histamine release, resulting in reduction of IgE antibody formation.

Further studies on the anti-skin cancer property of anionic Technol PG-paclitaxel glycoside nanoparticles, "anionic liposomes", are now in progress in our laboratory.

Author Contributions

Hiroki Hamada: Investigation, formal analysis, writing—original draft. Daisuke Uesugi: Validation, methodology, writing—reviewing. Kohji Ishihara:—Formal analysis, writing—review and editing. Ryusuke Hosoda: Investigation, writing—reviewing. Kei Shimoda: Resources, investigation writing—reviewing. Atsuhito Kuboki: Validation, formal analysis, writing—reviewing. Yuya Kiriake: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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How to cite this article:

Hiroki Hamada, Daisuke Uesugi, Kohji Ishihara, Ryusuke Hosoda, Kei Shimoda, Atsuhito Kuboki and Yuya Kiriake. 2024. Preparation of Paclitaxel Glycoside-Anionic Nanoparticles, Curcumin Gluco-oligosaccharides, α-Tocopherol Glycoside, Daidzein Glycoside, and Genistein Glycoside and their Application for Treatment of Skin Cancer, Dementia, and Allergy. *Int.J.Curr.Microbiol.App.Sci.* 13(7): 137-147.
doi: https://doi.org/10.20546/ijcmas.2024.1307.015