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Research paper

Comparison of the skin penetration of *Garcinia mangostana* extract in particulate and non-particulate form

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ABSTRACT

The aim of the present study was to solve the water insolubility limitation of the medically and cosmetically interesting substance *Garcinia mangostana* Linn (GML) extract by encapsulation, and to evaluate and investigate the penetration efficacy of free and encapsulated GML in two different vehicles (water and cream) in porcine ear skin. The follicular penetration depth was determined in 50 hair follicles for each of the four formulations by means of fluorescence microscopy. Tape stripping was used to compare the distribution properties of GML with all formulations on the stratum corneum. The results showed that encapsulated and free GML in the cream base penetrated deeper into hair follicles than if applied in an aqueous base. In addition, encapsulated GML could be distributed more homogeneously on the stratum corneum than the free GML. In conclusion, it was found that encapsulated GML in a cream base had the most effective penetration level in porcine ear skin.

1. Introduction

Mangosteen or GML is a traditional fruit which can be found in Southeast Asia, e.g. Indonesia, Malaysia, the Philippines and Thailand and is exploited for medical and cosmetic applications. Ancient medical literature indicates that mangosteen pericarp extract was used for the treatment of abdominal pain, diarrhea, dysentery, wound infection, suppuration and chronic ulcers [1]. Investigations on the mangosteen pericarp extract identified complex phenolic compounds such as tannins, flavonoids and xanthones as chemical constituents, whereby xanthone is the major secondary metabolite. Xanthones have been reported to have antioxidative [2], anti-inflammatory [3] and antiallergenic [4] properties, to protect against skin cancer [5,6], to eradicate Helicobacter pylori [7], and to inhibit Propionibacterium acnes and Staphylococcus epidermidis [2]. While this prompts the medical and cosmetic use of xanthone extracts from the mangosteen pericarp extract, xanthones are water insoluble, thus lowering their bioavailability and limiting their application in water-based systems. The incorporation into drug delivery systems such as micelles, solid lipid nanocarriers or amphiphilic polymeric is assumed to result in increased dispersibility in water, bioavailability as well as stability. Teixeira et al. [8] developed two different drug delivery systems, namely nanospheres and nanocapsules, consisting of poly(DL-lactideco-glycolide) (PLGA). The incorporation of xanthone was realized by a solvent displacement method. The results showed that two different xanthone-loaded systems had been dispersed, which controlled the release in aqueous medium. Khonkarn et al. [9] loaded xanthone into poly(ethylene glycol)-*b*-poly[*N*-(2-hydroxypropyl) methacrylamide-dilactate] mPEG-*b*-p(HPMAm-Lac2) micelles and tested *in vitro* assays with cancer cells. They concluded that the carrier system with xanthone was effective in targeting cancer cells. In the present study, the skin penetration of GML in non-particular and particular form was investigated as skin penetration properties and drug localization in the skin are essential parameters for therapeutic and cosmetic usage. Normally, drugs can penetrate through the skin by means of three pathways: the intercellular, the intracellular, and the follicular pathway [10–14]. However, the intercellular and intracellular pathways are not suitable for particulate drug carriers due to their size limitations. Recently, Lademann et al. [15–17] reported that using nanoparticles can increase the penetration of substances into hair follicles, as the rigid hair shaft may function as a geared pump for moving the particles into the hair follicles, where they could remain intact for up to 10 days [18]. Hair follicles are interesting target structures for drug delivery as they are surrounded by a dense network of blood capillaries and dendritic cells. In addition, they host the stem cells, which are important

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for regenerative medicine [19]. The aim of the present study was to improve the solubility of GML extract in aqueous dispersion by incorporating it into nanoparticles comprised of a polymer-blend of ethylcellulose (EC) and methylcellulose (MC) 1:1 and subsequently evaluating follicular and intercellular penetration. The experiment was designed by testing the differences in penetration between free GML and encapsulated GML in a water base and a cream base on 6 porcine ear skins.

2. Materials and methods

2.1. Materials

GML extract, consisting of 56% α-mangostin, was obtained from the Tipco Group Public Company Limited (Bangkok, Thailand). Ethylcellulose (48% w/w ethoxy content, viscosity 100 cps) and methylcellulose (viscosity 400 cps) were purchased from Sigma–Aldrich (Steinheim, Germany). All other chemicals were locally obtained analytical grade reagents.

2.2. Testing formulations

In total, four different formulations containing GML that was encapsulated into nanoparticles or in free form were prepared in order to test the intercellular and follicular penetration behavior. Formulation A contained encapsulated GML with ethylcellulose blended methylcellulose (ECMC) in cream (GML-EN cream). Formulation B was free GML in cream (GML-Free cream). Formulation C was encapsulated GML with ECMC in water base (GML-EN) and formulation D was free GML in water base (GML-Free).

2.2.1. Encapsulation of GML (GML-EN)

For formulation A and C, GML was encapsulated into nanoparticles by the solvent displacement method inducing the self-assembly of the polymer-blend of ECMC as previously reported [20]. The polymer-blend and GML were dissolved in 80% (v/v) aqueous ethanol and were brought to the final concentration of polymer-blend and GML at 6 mg/ml. The mixture (50 ml) was put into the dialysis bag (cellulose membrane, MWCO 12,400 Da, size 76 mm × 49 mm, Sigma–Aldrich, Steinheim, Germany) and dialyzed against water (5 × 1000 ml). The GML-loaded particles were subjected to scanning electron microscopy (SEM), transmission electron microscopy (TEM), particle size distribution and zeta potential analysis. Next, the nanoparticles were dried by spray-drying. The amounts of GML in particles were determined by extracting the dry spheres with ethanol. Subsequently, the extraction was filtered with the filtering centrifuge MW cutoff 10,000 Da (Amicon Ultra-15, Millipore, Billerica, MA, USA) and quantified with GML in the obtained liquid using UV visible absorption spectroscopy (UV 2500 UV/VIS spectrophotometer, Shimadzu Corporation, Kyoto, Japan) at 317 nm. The encapsulation efficiency (%EE) and loading capacity (%Loading) were determined as follows:

$$\%EE = ([A]/[B]) \times 100$$

$$\%Loading = ([A]/[C]) \times 100$$

[A] = weight of GML founded in spheres, [B] = weight of GML used, [C] = weight of GML-loaded spheres

Three batches of GML-loaded particles were used for the determination of average encapsulation efficacy and loading capacity.

2.2.2. Preparation of four test formulations containing GML

The water dispersing formulation of GML-EN was prepared by mixing 0.5 g of GML-EN in EC blended MC powder with 100 ml water to bring them to the sonication process (40 kHz, room temperature). The final GML concentration was 2.5 mg/ml. A total of 0.25 g of free GML dispersed in 100 ml water (GML-Free) was prepared by the same method. The final concentration of xanthone was 2.5 mg/ml.

The cream formulations were prepared using encapsulated GML (GML-EN cream) or free GML (GML-Free cream) at 0.5% (W/W) by mixing the GML encapsulation powder or free GML with 100 g cream (Abitima® Clinic, Actavis Deutschland, Langenfeld, Germany; Composition: Aqua, isopropyl myristate, glycerin, sorbitan stearate, lanolin, dimethicone, cetyl alcohol, polysorbate 60, sorbic acid; pH 4.5, oil in water emulsion). Distributions of GML-free and GML-EN in cream were determined with confocal laser scanning microscope (CLSM) (Eclipse T*i* Series microscope, Nikon, Japan).

2.3. Skin model

Porcine ear skin is a highly suitable model for human skin [21,22]. The investigations were carried out on the skin of porcine ears freshly obtained from a butcher. Preparation of the porcine samples consisted of cleaning the porcine ears with cold water and drying them with paper towels. Approval of the experiments had been obtained from the Government Office of Veterinary Medicine in Berlin-Treptow, Germany.

2.4. Study design 1: Determination of the follicular penetration depth

Six porcine ears were used for each formulation test. For each formulation, a skin area of 3 × 2 cm was demarcated with a silicone barrier (C. Kreul window color C2 number 40134, Hallerndorf, Germany) to prevent lateral spreading of the formulations. The cream formulations A and B (GML-EN cream and GML-Free cream) were applied at a concentration of 2 mg/cm^2. The water formulations C and D (GML-EN and GML-Free) were applied onto the designated skin areas at a concentration of 20 μl/cm^2. Subsequent to the application of the formulations, the marked skin areas were massaged for 2 min with a hand-held device (Mini Vibrator; Rehaforum Medical PZN 2427872, Elmshorn, Austria) and stored for a 30 min penetration time at room temperature. After the penetration time, the porcine samples were cut into smaller sizes (0.5 × 0.5 cm), after which they were shock frozen in liquid nitrogen. For histological investigations, the frozen samples were sectioned vertically into 10 μm thin slices using the microtome cryostat (Microm HM 560 Cryostar; MICROM international GmbH, Walldorf, Germany). The thin sections obtained from the hair follicles were marked and examined with the fluorescence microscope (Olympus BX60, Olympus optical Co., EUROPA GmbH, Hamburg, Germany). The penetration depths of GML in μm of all four formulations were measured in 50 hair follicles for each formulation.

2.5. Study design 2: Determination of the intercellular penetration by tape stripping

Tape stripping was performed using adhesive film (Tesafilm® 5529, Beiersdorf, Hamburg, Germany) of a width of 18 mm. The test formulations were applied to six porcine ears, massaged and subjected to a penetration time of 30 min according to study design 1. A further skin area remained untreated and served as a control. After the penetration time, adhesive films were applied to the treated skin areas and to the untreated skin area and pressed onto the skin using a roller (back and forth 10×) in order to stretch the skin surface [23]. Adhesive films were removed and a new film was applied onto the same area. In total, 5 tape strips were removed from each skin area. The tape strips were investigated by laser scanning microscopy (LSM) in the fluorescence mode. The results obtained from the different formulations were compared to the results obtained from the non-treated skin areas.