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Efficacy of transdermal magnesium ascorbyl phosphate delivery after ultrasound treatment with microbubbles in gel-type surrounding medium in mice

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ABSTRACT

Liquid microemulsions appropriate for topical application were obtained by increasing their viscosity through the addition of thickening agents. The present study first assessed the usefulness of ultrasound (US) plus US contrast agent, microbubbles (MBs), in agarose gel for enhancing transdermal drug delivery. The effect of US plus MBs in agarose gel on the penetration of the skin by magnesium ascorbyl phosphate (MAP) was explored both in vitro and in vivo. In the in vitro experiments, the stability of MBs was investigated by examining the penetration of MAP by the model drug, Evans blue, in two media: an agarose phantom and pig skin. The penetration depth in the agarose phantom and pig skin increased by 40% and 195%, respectively, when treated with US plus MBs in 0.1% agarose solution combined with MAP (UMB1), and by 48% and 206%, respectively, when treated with US plus MBs in 0.15% agarose solution and MAP (UMB2). The skin-whitening effects in C57BL/6J mice in the UMB1 and UMB2 groups over a 4-week experimental period were significantly increased by 63% and 70%, respectively, in the fourth week. The findings of this study suggest that the survival of MBs with US is affected by the viscosity of the surrounding medium, and that in mice, treatment with US plus MBs in a suitable agarose gel can increase skin permeability and enhance transdermal MAP delivery.

1. Introduction

The feasibility of controlled cavitation at high frequency for transdermal drug delivery (TDD) using gas-filled microbubbles (MBs) as ultrasound (US) contrast agents was explored through in vivo experiments in a rat model [1]. Drug or cosmetics solutions were applied on the animal skin after all visible bubbles had disappeared, but it was found that liquid microemulsions were more appropriate for topical application. MB behavior in an US field was recently investigated through numerical simulation in an effort to enhance high-intensity focused US therapies. Both the viscosity and shear elasticity of the medium surrounding the MBs reduced the attenuation of US propagation through the MB mixture [2]. Our previous study demonstrates the penetration depth, concentration, and efficiency of transdermal α-arbutin delivery during 4 weeks after US treatment with MBs solution in mice [3]. The present study explored the feasibility of a gel-type MB compound combined with US for enhancing the penetration of transdermal magnesium ascorbyl phosphate (MAP) delivery in vivo.

US-achieved sonophoresis is known to increase skin permeability, but the fundamental mechanism underlying this effect is still unclear [4]. Shock waves and microjets generated during inertial cavitations are thought to be responsible for transdermal permeability enhancement, with microjets exerting a significantly greater effect than shock waves [5]. Such cavitation occurs at various sites, such as the coupling medium, the skin surface, and the skin tissue [4]. The viscosity, surface tension, density, acoustic impedance, and other bulk and interfacial properties of the coupling medium play an important role in the enhancement of skin permeability [6].

The most common type of drug delivered through the skin using high-frequency sonophoresis is anti-inflammatory medication for joint and muscle pain, with a recently increasing shift in interest from topical steroids to nonsteroidal anti-inflammatory drugs (NSAIDs), including diclofenac, ibuprofen, ketoprofen, ketorolac, and piroxicam [6]. Moreover, oral NSAIDs generally cause gastrointestinal side effects, including nausea, heartburn, gastrointestinal ulcers, and nonspecific colitis [7]. Therefore, the combination of topical NSAID therapy and US is promising; however, the combination of gel-type MBs plus US for TDD is necessary for such therapy.

Vitamin C (L-ascorbic acid) is a water-soluble vitamin synthesized from D-glucose in the mammalian liver, or in the kidneys in some vertebrates [8]. It can inhibit melanogenesis, promote collagen biosynthesis,

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and prevent the formation of free radicals in the skin [9–13]. It has been considered an interesting ingredient of cosmetic skin-care products, but formulating products containing vitamin C is impractical because it is readily soluble in water and is extremely unstable [9]. Therefore, vitamin C is chemically modified by esterification of the hydroxyl group with long-chain organic or inorganic acids. The derivative, magnesium ascorbyl phosphate (MAP), an inorganic water-soluble acid ester, was formulated to overcome the instability of vitamin C [14]. MAP is a very stable derivative of vitamin C that may be easily used in various types of cosmetic product. MAP decreases melanogenesis by interacting with copper at the active site of tyrosinase and by reducing dopaquinone by blocking dihydrochinindol-2-carboxyl acid oxidation [15]. Shaikh and Mashood determined the effectiveness of treating melasma with a combination of topical 5% MAP and pulsed fluorescent light, and found that combining 5% MAP with pulsed fluorescent light is an effective, well-tolerated, and safe method of treating refractory melasma in Asian patients [16].

The use of US to enhance the delivery of related drugs via the skin surface has been widely studied. However, the penetration of the stratum corneum lipid bilayer by water-soluble drugs is a major challenge. The effect of MBs in combination with US on drug penetration through the skin was evaluated in the present study. In order to facilitate the smearing of MBs on the skin surface, they were formulated in a gel-type composition. Since the viscosity of the agarose increases with concentration. In this study, the efficiency of US plus MBs in different concentrations of agarose gel for enhancement of skin permeability has been demonstrated. The properties of liquid and gel-type MBs used for US enhanced transdermal MAP delivery were demonstrated both in vitro and in vivo. US combined with gel-type MBs was found to induce cavitation and thus enhance TDD.

2. Materials and methods

2.1. Production of albumin-shelled MBs

Albumin-shelled MBs were prepared according to the procedure used in our previous studies [17,18]. Briefly, albumin-shelled MBs were generated by sonication in 10 ml of a solution containing 140 mg of albumin (Octapharma, Vienna, Austria) and perfluorocarbon gas in physiological saline (pH 7.4, 0.9% sodium chloride) using a sonicator (Branson Ultrasonics, Danbury, CT, USA) for 2 min. The number of perfluorocarbon-filled albumin MBs in the solution was measured using the MultiSizer III device (Beckman Coulter, Fullerton, CA, USA) with a 30-μm-aperture probe and measurement boundaries of 0.6–20 μm. The size distribution in the suspension was measured based on dynamic light scattering (Zetasizer Nano, ZS90, Malvern, UK). The albumin-shelled MBs had a mean diameter of 1.2 μm and a mean concentration of 2.3×10^9 bubbles/ml. The albumin-shelled MBs were centrifuged (1200 rpm for 1 min, 128.6 $\times g$), and half of the physiological saline was removed. The final concentration of MBs used in this study was $4.64 \pm 0.05 \times 10^9$ bubbles/ml (mean ± SD) for a size range of 0.8–2 μm, and their mean diameter was 1195 nm.

2.2. Preparation of agarose-gel-type MBs

Two amounts of agarose powder (0.1 and 0.15 mg; FB-HA0604, FocusBio, Burgos, Spain) were dissolved in 99.9 and 99.85 ml of phosphate-buffered saline, respectively, and heated to boiling. The solution was fixed on a rotary shaker (50 rpm; Shaker RS-01, TKS, New Taipei City, Taiwan) for 20 min. The agarose solution was then placed into the sonication tank (Delta ultrasonic cleaner d150h, Delta, Hsin-Chu City, Taiwan) to remove the excess gas, and finally stored at 4 °C in a refrigerator. Before the experiments were performed, the gel-type MBs were prepared in either 0.1% or 0.15% agarose solution at the following four concentrations: 0.23×10^7, 4.6×10^7, 11.5×10^7, and 23×10^7 bubbles/ml. The albumin MBs and gel-type MBs were filtered with a 5 μm syringe filter (Critical Process Filtration, Inc., Nashua, NH, USA) and then hardened by 0.002% glutaraldehyde (Nippon Shiyaku Co., Tokyo, Japan). The morphology of the hardened albumin MBs was studied using scanning electron microscopy (SEM) (Quanta™ 3D FEG, FEI, ORR, USA). The samples were prepared for SEM by coating with platinum. SEM images were recorded on a system at an accelerating voltage of 30 kV.

2.3. In vitro high-frequency US imaging of MBs and gel type MBs

High-frequency US imaging was performed to evaluate the survival of MBs in agarose gel during sonication using a US animal-imaging system (Prospect, S-Sharp Corporation, New Taipei City, Taiwan) at a frequency of 40 MHz using a transducer with a diameter of 7 mm and a fixed focus at 12 mm. A 2% agarose square column $10 \times 20 \times 20$-mm^3 phantom was constructed with a $2 \times 2 \times 20$-mm^3 chamber at its center to load the 4×10^7 bubbles/ml MBs or gel-type MBs. According to our previous study, the enhancement of the penetration depth was greatest for 2-W/cm^2 US and the condition was used either for the in vitro skin penetration or for the animal treatments in this study [3]. During the US imaging, the loaded phantom was sonicated using the 1-MHz US transducer of the sonoporation gene transfection system (ST 2000 V, NepaGene, Ichikawa, Japan) at an acoustic intensity of 2 W/cm^2 for 1 min. The duty cycle was set at 50%, the PRP was set at 250 msec and a 6-mm-diameter transducer was used. The region of interest was drawn over the entire MB-loaded chamber in a two-dimensional imaging plane by an operator, the dynamic range was set at 50 dB and the average pre- and postsonication image intensities were measured in B-mode images.

2.4. Measurement of penetration depth in agarose phantoms

The model drug, Evans blue (0.1 mg; molecular weight = 960.81 Da; E2129, Sigma-Aldrich, St. Louis, MO, USA), was dissolved in 10 ml of physiological saline (0.9% sodium chloride) and then stirred for 1 h at 4 °C. Disk-shaped 0.3% agarose phantoms were constructed with a diameter of 1.2 cm and a height of 5 mm (encircled with US gel to prevent leakage); the round area of each phantom was loaded with Evans blue or MBs. The probe of the sonoporation gene transfection system (ST 2000 V, NepaGene) was placed 5 mm from the top of the phantom. After adding 500 μl of the 0.1% agarose gel, 0.15% agarose gel, or the MBs at four different concentrations (0.23×10^7, 4.6×10^7, 11.5×10^7, and 23×10^7 bubbles/ml) in 0.1% or 0.15% agarose gel, the area was sonicated using the 1-MHz US transducer of the sonoporation system at an acoustic intensity of 2 W/cm^2 for 1 min. The duty cycle was set at 50% and a 1.2-cm-diameter transducer was used. The change in temperature during the US sonication was only ≤0.3 °C, as measured by a thermometer (Optris LS, Optris, Berlin, Germany). The MBs were subsequently removed from the surface and the area was washed three times for 1 min with physiological saline. The Evans-blue solution was then injected into the same area on the phantom and the sample left for 5 min to allow penetration. The Evans blue was then removed and the area was washed three times for 1 min with physiological saline. Sections of the phantom were cut at a thickness of 2 mm and prepared for light-microscopy evaluation.

The penetration depths of the Evans blue were measured using MATLAB (The MathWorks, Natick, MA, USA). At first, the light-microscopy images were converted into grayscale images and image histogram-based binarization was performed [19]. For histogram-based binarization, the peak-and-valley thresholding of target was performed based on the histogram for many experiments [19]. Then, the boundary was detected by Sobel-operator-based edge detection [20]. At this step, the same threshold (100–230) was used when processing all of the images. Finally, the area of the penetration region was measured and the area was divided by the length of the x-axis of the image to get the mean penetration depth (y-axis) of the Evans blue.