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Complexation of curcumin with 2-aminoethyl diphenyl borate and implications for spatiotemporal fluorescence monitoring

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ABSTRACT

In this study, we successfully determined spatiotemporal distribution of curcumin in mice via simple and fast fluorescence detection of native curcumin and stabilized curcumin. We used 2-aminoethyl diphenyl borate (DPBA) as a stabilizer of curcumin, which binds to curcumin and enhances its aqueous stability. After intravenous injection, curcumin and DPBA–curcumin complexes showed similar fluorescence intensities in the brain, pancreas, lungs, and kidneys at 15 min. However, stabilized DPBA–curcumin complexes exhibited much stronger fluorescent signals at metabolically active sites such as liver tissues than native curcumin. After incubation for 1–3 h, native curcumin showed significantly rapid reduction of fluorescent signals, compared to DPBA–curcumin complexes, probably due to degradation and reduction. In addition, complicate extraction procedures inhibited precise fluorescent monitoring of unstable curcumin, which result in different biodistribution of curcumin before and after extraction. Direct fluorescent monitoring could allow evaluation of *in vivo* distribution and fate of curcumin, which could be also applied to diverse natural polyphenols with fluorescent signals.

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1. Introduction

Curcumin is an active compound originating from plants and has shown high biological and therapeutic efficacy in a wide range of diseases, *e.g.*, neurodegenerative diseases and inflammatory diseases (Funk et al., 2006; Ireson et al., 2001; Mande et al., 2016; Yang et al., 2005). To better understand biological effects of curcumin *in vivo* and to find new biological applications, precise elucidation of the fate of curcumin in mice after systemic administration is necessary. Previously, curcumin biodistribution was determined by high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and mass spectrometry after extraction of curcumin from tissues (Pan et al., 1999; Tsai et al., 2011). On the other hand, given that curcumin in solution is too vulnerable to oxidation and enzymatic degradation, extraction procedures can affect quantification of such unstable compounds in tissues or organs. For example, curcumin is so unstable that residual amounts of curcumin diminish down to 10% within 1 h at physiological pH (Pan et al., 1999). In addition, not only stability of curcumin but also varying extraction efficiency depending on tissues and extraction methods, *e.g.*, solvents, may affect exact quantification of curcumins in tissues or organs (Perkins et al., 2002). Accordingly, direct evaluation for curcumin distribution in tissues and/or organs with minimized *in vitro* procedures seems to be necessary. Nevertheless, few studies have been published regarding the *in vivo* fate of curcumin without extraction procedures.

Lately, optical imaging of biomolecules *in vivo* is considered a crucial tool for direct monitoring of their functions and localization in tissues and/or organs (Jaffer et al., 2013; Zhang et al., 2015). It is well known that many active phenolic compounds from plants perform important functions such as ultraviolet (UV) light protection *via* absorption of UV–vis light. In addition, some natural phenolic compounds have strong fluorescent signals, which enable fluorescence detection to directly track the locations of polyphenols in plant leaves and roots (Buer et al., 2010; Pietta, 2000). Curcumin has strong absorption at wavelengths of 350–480 nm and intense emission at a wavelength of ~520 nm, which allow for fluorescence imaging of curcumin distribution in cells and sliced tissues (Chen et al., 2015a, 2015b; Patra and Barakat, 2011; Yang et al., 2013). Nevertheless, vulnerability of curcumin to metabolism like reduction and degradation may limit exact monitoring of curcumin biodistribution *in vivo via* fluorescence imaging. To

Abbreviations: BBB, blood-brain barrier; Cre, cremophor EL; CCK-8, cell counting kit-8; DMSO, dimethyl sulfoxide; DPBA, 2-aminoethyl diphenyl borate; FI, fluorescence intensity; MES, 2-(*N*-morpholino)ethane sulfonic acid; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; Umb, umbelliferone.

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enhance curcumin stability, diverse approaches have been reported *e.g.*, the design of curcumin derivatives; complexation with other molecules such as serum albumin, soy proteins, and polysaccharides; and encapsulation of curcumin using nanotechnology (Chen et al., 2015a, 2015b; Tsai et al., 2011; Yang et al., 2013). These approaches greatly improved *in vitro* stability but have not yet been applied to *in vivo* curcumin monitoring *via* fluorescence imaging. The 2-Aminoethyl diphenyl borate (DPBA) is one of the stabilizing agents for phenolic compounds such as dihydroxylated flavonoids, *e.g.*, rutin and quercetin (Matteini et al., 2011). Applications of DPBA are limited only to *in vitro* experiments.

In this study, as a proof of concept, we aimed to develop a method for elucidation of biodistribution of curcumin, one of representative bioactive phenolic compounds originating from natural products, *via* direct fluorescence imaging without any labeling and extraction procedures. As a stabilizer of curcumin, DPBA was assessed in terms of *in vitro* stability and *in vivo* fluorescence monitoring. Biocompatibility of DPBA was examined for two types of cells, macrophages (RAW 264.7 cells) and fibroblast cells (NIH3T3 cells). After intravenous injection, we determined relative abundance of Evan's blue solution (control dye), curcumin, and complexes of curcumin with DPBA (DPBA–curcumin) in each tissue by fluorescence detection using an *in vivo* imaging system (IVIS). Curcumin and DPBA–curcumin in each tissue were also monitored after intravenous injection and incubation for different time intervals (15 min, 1 h, and 3 h). To compare biodistribution of curcumin before and after extraction, each tissue excised from mice injected with curcumin or the DPBA–curcumin complex was quantitatively analyzed using a spectrofluorophotometer.

2. Material and methods

2.1. Materials

Curcumin, dimethyl sulfoxide (DMSO), ethyl acetate, umbelliferone (umb), 2-aminoethyl diphenyl borage (DPBA), Evan's blue dye, sodium dodecyl sulfate (SDS), and cremophor EL (Cre) were purchased from Sigma-Aldrich (St. Louis, MO, USA). A 2-(N-morpholino)ethanesulfonic acid (MES) Buffer (1 M, pH 6.0) was obtained from bio-solution (Yongin, Korea). Phosphate-buffered saline (PBS) was acquired from Gibco BRL (Grand Island, NY, USA). Cell counting kit-8 (CCK-8) reagent was obtained from Dojindo Laboratories (Kumamoto, Japan). ICR mice (6-week-old, female) were purchased from Orient Bio Inc. (Seongnam, Korea). Isoflurane was purchased from Piramal (USA). All other chemicals were of analytical grade.

2.2. Measurement of UV–vis absorption

Curcumin (100 μg) and umb (100 μg) were dissolved in DMSO at a concentration of 0.1 mg/mL and diluted with PBS (pH 7.4) and 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 6.0), respectively. To prepare DPBA complexes, curcumin (100 μg) and umb (100 μg) in DMSO were mixed with DPBA (8 μg) in DMSO by vortexing and incubated for 5 min at room temperature. After dilution of DPBA–curcumin and DPBA–umb in DMSO with PBS (pH 7.4) and MES buffer (pH 6.0), respectively, at a concentration of 50 μg/mL, the absorption spectra were measured on a UV–vis spectrophotometer (Molecular Devices, CA, USA) at wavelengths of 300–600 nm at room temperature (Zou et al., 2015). Curcumin (50 μg) was mixed with DPBA in DMSO at various DPBA/curcumin weight ratios (0, 0.04, 0.08, 0.2, and 1.0) by vortexing and incubated for 5 min at room temperature. After dilution of DPBA–curcumin with MES buffer (pH 6.0), absorption spectra were measured using the UV–vis spectrophotometer at wavelengths 300 to 600 nm.

2.3. The in vitro stability assay

Curcumin (5 μg) in DMSO was mixed with DPBA at various DPBA/curcumin weight ratios (0, 0.04, 0.08, 0.2, and 1.0) by vortexing and incubated for 5 min at room temperature. Different DPBA–curcumin complexes were diluted with PBS (pH 7.4) and MES buffer (pH 6.0), respectively. During incubation for 0 and 8 h, curcumin samples were protected from light, according to one study (Pietta, 2000). After incubation, fluorescence intensities of DPBA–curcumin complexes at pH 6.0 were measured using a spectrofluorophotometer at excitation and emission wavelengths of 430 and 520 nm, respectively.

2.4. Analysis of biocompatibility of DPBA

RAW 264.7 and NIH3T3 cells were seeded at the density of 3×10^4/well and 1×10^4/well in a 96-well plate and incubated for 24 h at 37 °C. The cells were treated with DPBA in DMSO at various DPBA concentrations (0, 0.05, 0.1, 0.2, 0.5, and 2.5 mg/mL) in 10% serum-containing media for 1 h, and the cells were washed with a fresh medium. For assessment of cell viability on a longer time scale, cells were treated with DPBA for 1 h and further incubated for 24 h. Cell viability was measured by the CCK-8 assay.

2.5. Intravenous injection and optical fluorescence imaging of curcumin

All animal care and experimental procedures were approved by the Animal Care Committee of Konkuk University. ICR mice were anesthetized by means of isoflurane and then injected intravenously with freshly prepared curcumin or DPBA–curcumin complexes (DPBA/curcumin weight ratio = 0.08) in PBS containing 13.5% and 23.5% (v/v) each of Cre and DMSO at a curcumin dose of 20 mg/kg per mouse according to the manufacturer's protocol. Fifteen minutes after the administration, the mice were euthanized and perfused with 0.9% saline to flush out blood. After that, relevant tissues were collected and washed with PBS. Fluorescent images of each tissue were analyzed using the *in vivo* imaging system (IVIS, Caliper Life Sciences Lumina II, Massachusetts, USA) at excitation and emission wavelengths of 430 and 509 nm, respectively. The total flux of the whole tissues in the region of interest was also quantitatively analyzed after subtraction of background signals using the following formula: total flux in each tissue treated with samples minus total flux in tissues from untreated mice. ICR mice were also injected intravenously with freshly prepared 1% Evan's blue solution in PBS or PBS containing 13.5% and 23.5% (v/v) each of Cre and DMSO. After incubation for 15 min, fluorescent images of Evan's blue in each tissue were acquired using the IVIS instrument at excitation and emission wavelengths of 535 and 705 nm, respectively.

2.6. Time-dependent biodistribution

After we dissolved each sample in PBS containing 13.5% and 23.5% (v/v) each of Cre and DMSO, ICR mice were injected intravenously with freshly prepared DPBA (40 μg), curcumin (500 μg), or DPBA–curcumin complexes with the curcumin amount of 500 μg. After wait periods (15 min, 1 h, and 3 h), various organs were collected and analyzed using the IVIS instrument at excitation and emission wavelengths of 430 and 509 nm, respectively. Total flux in each organ was analyzed using the IVIS software. Then, samples were frozen and stored at −70 °C until analysis.

To quantify the curcumin content of different organs including the brain, lungs, spleen, pancreas, and kidneys, curcumin was extracted from the organs using ethyl acetate (Kang et al., 2016).

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