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Research paper

Novel strategy for improving the bioavailability of curcumin based on a new membrane transport mechanism that directly involves solid particles

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ABSTRACT

Amorphization has been widely recognized as a useful solubilization technique for poorly water-soluble drugs, such as curcumin. We have recently reported the novel finding that the membrane transport of curcumin was markedly enhanced when amorphous solid particles of curcumin came into direct contact with the lipid membrane surface, but this was not true for crystalline solid particles. The increase in the permeation of curcumin was found to be independent of the improvements in aqueous solubility brought about by amorphization. Thus, we have identified a novel membrane transport mechanism that directly involves solid particles. In addition, it might represent a novel strategy for improving the bioavailability of curcumin that does not focus on the aqueous solubility of the drug. In this study, the direct effects of the administration of amorphous nanoparticles of curcumin (ANC) on the *in vivo* intestinal absorption of curcumin were investigated. After the intraduodenal administration of a curcumin suspension, the area under the curve of the plasma concentration of curcumin increased in a manner that was dependent on the curcumin concentration of the suspension, while no significant absorption was observed from a saturated solution. This finding is consistent with the results from our *in vitro* transepithelial transport study. In the latter experiment, the bioavailability of curcumin was found to be 1–2%. The intrapulmonary insufflation of ANC powder resulted in a significant increase in the bioavailability of curcumin (it was two orders of magnitude higher than that seen after the application of a crystalline suspension). This was due to the ANC particles coming into contact with epithelial cells in a more efficient manner after the pulmonary application of the ANC powder than after the intestinal application of the ANC suspension. Therefore, the pulmonary insufflation of amorphous powder is a novel approach to improving the bioavailability of curcumin and might be a useful way of increasing the bioavailability of poorly water-soluble drugs, such as curcumin.

1. Introduction

In the last few decades, the number of new chemical entities (including drugs) with poor water solubility has been increasing [1–3]. Needless to say, the good oral absorption typically is the result of appropriate permeability (mostly lipophilicity) and sufficient water solubility. Therefore, it is necessary to improve the water-solubility of drugs in order to increase their oral bioavailability (BA). Along with the increase in the number of such drugs, many solubility-enhancement techniques have also been developed. Among them, amorphous formulations, such as solid dispersions, are a very attractive option [4–6]. However, the number of amorphous drug formulations on the market is limited because their solubility is decreased by recrystallization, which occurs easily and spontaneously. Consequently, many studies have focused on the suppression of recrystallization to maintain supersaturation.

Curcumin, which is a yellow polyphenolic compound produced by turmeric rhizomes, has been drawing much attention as a very promising medicine in recent years. In particular, it has been reported to have antitumor effects by many researchers [7–9]. The BA of curcumin

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is known to be extremely poor [10]. Among the various reasons for its poor BA, its very low solubility in water is the major factor. According to a previous study [11], the aqueous solubility of curcumin from bulk crystalline material is less than 10 ng/mL. Few drugs or chemicals exhibit as low solubility values as curcumin. Therefore, many researchers are focusing on attempts to improve the solubility of curcumin in order to increase its BA. However, the conventional strategy for improving the absorption of poorly water-soluble drugs, which focuses on aqueous solubility, is being reconsidered. It was revealed that the subsequent drug permeation was affected by the condition of the drug molecules dissolved in the solution, including the self-association with two or more molecules and the incorporation into micelles of polymers or surfactants [12,13]. In other words, improved solubility does not necessarily lead to an increase in absorption, which is a limitation of the conventional strategy. In addition, curcumin is categorized into Biopharmaceutics Classification System class 4 (low solubility and low permeability) [14–17], suggesting that it is necessary to improve both its solubility and permeability in order to enhance its BA.

We have recently reported a novel finding regarding the *in vitro* transepithelial transportation of curcumin [18]. Amorphous nanoparticles of curcumin (ANC) were prepared using a microreactor. Although the aqueous solubility of curcumin was 300-fold greater than that of crystalline curcumin, no transepithelial transport of curcumin was observed from a saturated solution. On the other hand, the *in vitro* permeation of curcumin from the suspension was markedly enhanced, and the transport of curcumin was dependent on the concentration of curcumin in the suspension. Enhanced permeation was only observed when the ANC particles came into direct contact with the cell monolayer. For example, the secretory transport (basal to apical) of curcumin across Madin-Darby canine kidney cell monolayers occurs at a significantly lower rate than absorptive transport (apical to basal). This suggests that the BA of curcumin could be enhanced by applying it at a site where large amounts of ANC particles were able to come into contact with the absorptive epithelium. In this study, the lung was selected as the application site in order to increase the absorption of curcumin.

In the present study, based on our previous findings, the BA of curcumin was evaluated in an *in vivo* animal study. First, the elimination of curcumin from the blood after its intravenous infusion was investigated in order to evaluate the absolute BA of curcumin. Second, the intestinal absorption of curcumin was evaluated by changing the concentration of ANC in suspensions. Third, the pulmonary absorption of curcumin was tested after the insufflation of ANC powder into the lungs.

2. Materials and methods

2.1. Materials

Crystalline curcumin (CC) and dithiothreitol (DTT) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polyvinylpyrrolidone (Kollidon 12PF) was obtained from BASF (Ludwigshafen, Germany). Dulbecco's phosphate-buffered saline (D-PBS), urethane, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, U.S.A.). All other reagents used in this study were commercially available and of analytical grade.

2.2. Methods

2.2.1. Preparation of amorphous nanoparticles of curcumin (ANC)

ANC were prepared for the experiments as reported previously [18]. Briefly, CC and Kollidon 12PF were dissolved in ethanol using CLEARMIX® (M TECHNIQUE, Osaka, Japan). The solution was fed into an ULREA® microreactor (M TECHNIQUE, Osaka, Japan) and mixed with distilled water. The precipitated particles were recovered from the solution by centrifugation. After the solid particles had been washed with distilled water and then subjected to vacuum drying, their physicochemical properties were analyzed in the same manner as in our previous study. The prepared ANC were confirmed to be amorphous and nano-sized.

2.2.2. In vivo pharmacokinetic study

Male Wistar/ST rats (Shimizu Laboratory Co., Ltd., Kyoto, Japan), weighing 280–340 g (10 weeks old), were used in all experiments. All animal experiments were approved by the experimental animal committee at Doshisha Women's College of Liberal Arts and performed in accordance with the institutional guidelines for animal experiments. All rats were fasted but allowed free access to water overnight for 18 h before each experiment. They were anesthetized with intraperitoneal injections of urethane (1.0 g/kg), and the right femoral artery was cannulated with polyethylene tubing (SP-31, Natsume, Tokyo, Japan) for blood sampling. The intestinal absorption of curcumin was examined using an *in situ* closed-loop method, as reported previously [19]. The intestine was exposed through a midline abdominal incision. After incising the duodenum, a polyethylene tube was inserted, and the bile duct was ligated and sealed. The end of the ileum was incised, and the ileal lumen was washed with D-PBS (pH 6.5). After the remaining buffer had been expelled with air, the exit was sealed with tubing. Then, 4 mL of ANC or CC that had been suspended for 10 min were introduced into the ileal loop. Blood samples (0.2 mL) were obtained from the cannula at 15, 30, 60, 90, 120, 180, and 240 min after the administration of the suspensions. After being collected, the blood samples were centrifuged at 12,000 rpm for 10 min, and the resultant supernatant was deproteinized by adding acetonitrile. The mixture was then recentrifuged, and the supernatant was evaporated to dryness. The evaporated sample was stored at −30 °C until the analysis. To investigate the effects of the mucus layers found on the surfaces of the intestinal epithelial cells, the small intestine was pretreated with a mucolytic agent, DTT, before the administration of the test compounds. The subsequent treatment was carried out as described above. The experiment involving intrapulmonary administration was performed by modifying a previously reported method [20]. The trachea was exposed by making an incision in the middle of the neck, and 2 cm of PE (SP110) tubing were inserted into the trachea thorough the incision. CC or ANC (1 mg) were sprayed into the lungs through the tracheal PE tubing using compressed air in a syringe. A device prepared from a syringe fitted with 2 cm vinyl tube (SV45) at the tip of the needle was used for the insufflation. A continuous intravenous infusion was also administered to obtain the absolute BA of curcumin. One mL of ANC dissolved in D-PBS containing 5 w/v% BSA was administered into the right jugular vein using a winged needle for 10 min. Blood samples were obtained at 2, 5, 10, 12, 14, 16, 18, 20, 30, 45, 60, and 90 min after the administration of the test compounds. All pharmacokinetic parameters were calculated using non-compartmental analysis (WinNonlin® software Version 6.3, Pharsight Corporation, Mountain View, CA, USA). The BA of curcumin was calculated using the following equation (Eq. (1)):

$$\mathrm{BA}\ (\%) = \frac{\mathrm{AUC}_{\mathrm{intestinal/pulmonary}} \times \mathrm{D}_{\mathrm{i.v}}}{\mathrm{AUC}_{\mathrm{i.v}} \times \mathrm{D}_{\mathrm{intestinal/pulmonary}}} \times 100 \quad (1)$$

where AUC and D are the area under the curve of the blood concentration-time profile of curcumin and the dose of curcumin, respectively.

2.2.3. Analysis of curcumin

The quantification of curcumin was performed by modifying a previously reported method [21]. The concentration of curcumin was fluorometrically determined with high-performance liquid chromatography- fluorescence detector (LC-20A and RF-10A, Shimadzu Co., Kyoto, Japan) equipped with an octadecylsilyl column (COSMOSIL $5C_{18}$-MS-II; internal diameter: 4.6 mm, length: 150 mm; NACALAI TESQUE, Inc., Kyoto, Japan). The mobile phase was composed of