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In situ molecular elucidation of drug supersaturation achieved by nano-sizing and amorphization of poorly water-soluble drug

Keisuke Ueda, Kenjirou Higashi, Keiji Yamamoto, Kunikazu Moribe *

Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8675, Japan

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ABSTRACT

Quantitative evaluation of drug supersaturation and nanoparticle formation was conducted using *in situ* evaluation techniques, including nuclear magnetic resonance (NMR) spectroscopy. We prepared a ternary complex of carbamazepine (CBZ) with hydroxypropyl methylcellulose (HPMC) and sodium dodecyl sulfate (SDS) to improve the drug concentration. Different preparation methods, including grinding and spray drying, were performed to prepare the ternary component products, ground mixture (GM) and spray-dried sample (SD), respectively. Although CBZ was completely amorphized in the ternary SD, CBZ was partially amorphized with the remaining CBZ crystals in the ternary GM. Aqueous dispersion of the ternary GM formed nanoparticles of around 150 nm, originating from the CBZ crystals in the ternary GM. In contrast, the ternary SD formed transparent solutions without a precipitate. The molecular-level evaluation using NMR measurements revealed that approximately half a dose of CBZ in the ternary GM dispersion was present as nanoparticles; however, CBZ in the ternary SD was completely dissolved in the aqueous solution. The characteristic difference between the solid states, followed by different preparation methods, induced different solution characteristics in the ternary GM and SD. The permeation study, using a dialysis membrane, showed that the CBZ concentration dissolved in the bulk water phase rapidly reduced in the ternary SD dispersion compared to the ternary GM dispersion; this demonstrated the advantage of ternary GM dispersion in the maintenance of CBZ supersaturation. Long-term maintenance of a supersaturated state of CBZ observed in the ternary GM dispersion rather than in the ternary SD dispersion was achieved by the inhibition of CBZ crystallization owing to the existence of CBZ nanoparticles in the ternary GM dispersion. Nanoparticle formation, combined with drug amorphization, could be a promising approach to improve drug concentrations. The detailed elucidation of solution characteristics using *in situ* evaluation techniques will lead to the formation of useful solid dispersion and nanoparticle formulations, resulting in improved drug absorption.

1. Introduction

Most of the new drug candidates show poor water solubility (Hauss, 2007; Lipinski, 2002), leading to insufficient concentrations in the small intestinal fluid and subsequent poor drug absorption. Various types of additives, such as surfactants, polymers, and lipids, are added to drug formulations to improve drug solubility (Pouton, 2006; Torchilin, 2001). The solubilization of a drug in a drug carrier, such as micelle (Sheu et al., 2003; Torchilin, 2004) and cyclodextrin (Higashi et al., 2010, 2014), results in higher drug concentrations than drug solubility. However, strongly encapsulated drugs cannot permeate through the membrane of the small intestine, resulting in poor permeability (Fischer et al., 2011; Miller et al., 2012; Ueda et al., 2012; Yano et al., 2010). Hence, solubilization, which improves drug solubility, does not always lead to improved absorption of drugs with poor water solubility.

Modification of drug crystal habits, including amorphization (Alonzo et al., 2010; Chiou and Riegelman, 1971) and polymorphic transformation (Pudipeddi and Serajuddin, 2005), also improves the solubility of drugs with poor water solubility owing to its higher energy compared to the intrinsic crystal form. Drug concentrations that are higher than equilibrium solubility, i.e. a drug-supersaturated state, can be obtained by dissolution of these modified drugs. However, a supersaturated drug solution is unstable, and the drug is easily crystallized, resulting in short-term improvements in drug concentrations. Drug crystallization from the supersaturated solution is inhibited by polyvinylpyrrolidone (PVP) (Abu-Diak et al., 2011; Konno et al., 2008), methacrylate copolymers (Eudragit®) (Abu-Diak et al., 2011; Jung et al., 1999), hydroxypropyl methylcellulose (HPMC) (Konno et al., 2008), and

* Corresponding author.
E-mail address: moribe@faculty.chiba-u.jp (K. Moribe).

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hydroxypropyl methylcellulose acetate succinate (HPMCAS) (Friesen et al., 2008; Konno et al., 2008; Tanno et al., 2004). Solid dispersion, in which an amorphous drug is dispersed in a crystallization inhibitor, is an efficient formulation to improve drug concentrations. Drug permeability, from the supersaturated drug solution that is stabilized by the crystallization inhibitor, does not decrease compared to intrinsic drug permeability. This leads to significant improvements in drug permeation in a small intestine model, unlike in a solubilization system (Frank et al., 2014; Miller et al., 2012; Ueda et al., 2012).

Reducing the particle size of drug crystals to increase its surface area is also a promising approach to improve drug solubility and dissolution properties (Kipp, 2004; Liversidge and Cundy, 1995; Merisko-Liversidge et al., 2003). Preparation methods of drug nanoparticles are classified into two categories, top-down and bottom-up processes (Verma et al., 2009). Top-down processes, such as high-pressure homogenization and media milling, produce nanoparticles by size reduction using mechanical stresses (Singh et al., 2011; Van Eerdenbrugh et al., 2008). In contrast, bottom-up processes, such as anti-solvent precipitation, produce nanoparticles from organic solvents dissolving a drug and a stabilizer (Ali et al., 2009; Chan and Kwok, 2011). Appropriate preparation methods and stabilizers are essential to achieve effective size reduction in the drug crystals.

Co-grinding of a drug with a polymer and surfactant enables nanoparticle dispersion in water (Pongpeerapat et al., 2008; Sugimoto et al., 1998), wherein the polymer and surfactant effectively work together to hinder particle agglomeration. On the other hand, the mechanical stress of grinding, especially when a drug is co-ground with a polymer, can induce drug amorphization with size reduction. Solid dispersion has been prepared by grinding a drug with a polymer (Chiou and Riegelman, 1971; Watanabe et al., 2003). These combined effects of amorphization and size reduction by grinding contributed to improved drug concentrations in water. However, the improvement mechanisms for drug concentrations are still unclear.

Precise understanding of the improvement mechanisms for drug concentrations, achieved by drug amorphization and/or nano-sizing, requires *in situ* evaluation of the molecular states of the drug and additive, because the molecular states of a supersaturated drug continuously change owing to drug crystallization. Nuclear magnetic resonance (NMR) is a powerful tool for real-time monitoring of the molecular state over a short period (Guijarro et al., 1998; Schanda and Brutscher, 2005). Detailed information about the molecular state, including molecular environment and mobility, can be obtained from NMR measurements (Fujita et al., 1999; Ma et al., 2007; Singh and Kumar, 2007). NMR monitoring of the molecular state soon after the formation of a supersaturated drug solution could be very informative, since the temporarily high drug concentration improves drug absorption.

Previous studies showed that nanoparticles of approximately 300 nm were prepared using a vibrating rod mill with a ternary mixture of the drug, HPMC, and sodium dodecyl sulfate (SDS) (Moribe et al., 2006). In addition, HPMC was used as a crystallization inhibitor in the solid dispersion to achieve drug supersaturation (Okimoto et al., 1997; Usui et al., 1998). In this study, we attempted to enhance aqueous concentrations of carbamazepine (CBZ), a drug with poor water solubility, using amorphization and/or nano-sizing techniques. A vibrating rod mill and spray drying were used as a comparison study. *In situ* evaluations of CBZ concentrations immediately after supersaturated solution formation were performed using a dissolution/permeation system (D/P system) with a dialysis membrane (Kataoka et al., 2003). Real-time monitoring of the molecular state of CBZ in the supersaturated solution was conducted using the NMR measurements; this revealed the mechanism for achieving high CBZ concentrations. The achievement of drug supersaturation by the drug amorphization and/or nano-sizing was mechanistically examined based on the *in situ* evaluation for the further improvement of drug concentration.

2. Material and methods

2.1. Materials

CBZ and SDS were purchased from Wako Chemicals Co. (Tokyo, Japan). HPMC (type TC-5E) was kindly gifted by the Shin-Etsu Chemical Co. (Tokyo, Japan). All other materials were of reagent grade. The chemical structures of CBZ, HPMC, and SDS are shown in Fig. 1.

2.2. Preparation of ternary complexes

A physical mixture (PM) of CBZ, HPMC, and SDS with a weight ratio of 1:4:1 was prepared by vortex mixing in a vial for 3 min. The PM was ground for 2 h at room temperature using a vibration rod mill (CMT TI-200, CMT Co., Ltd., Fukushima, Japan) to prepare the ground mixture (GM). To prepare the spray-dried sample (SD), the PM was dissolved in dichloromethane/methanol (1/1 = v/v) to a total solid concentration of 5% w/v. The solution was fed into a spray dryer (ADL311S, Yamato Scientific, Tokyo, Japan) at a rate of 4 g/min. Spray drying was performed in the following conditions: inlet temperature, 70 °C; outlet temperature, 45 °C; atomizing pressure, 0.05 MPa; nozzle diameter, 0.7 mm (liquid) and 1.7 mm (gas).

2.3. Powder X-ray diffraction measurement

Powder X-ray diffraction (PXRD) measurements were conducted using MiniFlex II (Rigaku Corp., Tokyo, Japan) in the following conditions: target, Cu; filter, Ni; voltage, 30 kV; current, 15 mA; scanning speed, 4 deg/min; and scanning angle, 10–30°.

2.4. Solid-state NMR measurements

Solid-state NMR measurements were conducted using ECX-400 NMR system (9.4 T, JEOL Resonance Inc., Tokyo, Japan). The ^{13}C

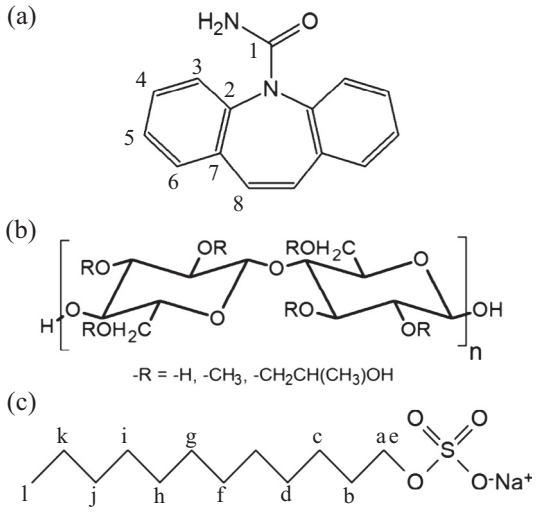

Fig. 1. Chemical structures of (a) carbamazepine (CBZ), (b) hydroxypropyl methylcellulose (HPMC), and (c) sodium dodecyl sulfate (SDS). Carbon numberings of CBZ and SDS represent peak assignment in NMR spectra.