Solid dispersion (SD) system of everolimus (EVR) with Gelucire 50/13 (Stearoyl polyoxyl-32 glycerides) was prepared using melt granulation technique with the aim of improving the physicochemical properties and dissolution rate. The solid state characterization using scanning electron microscopy and X-ray powder diffraction, indicated that the drug was homogeneously distributed in the surfactant carrier in a stable amorphous form. The dissolution rate of EVR from the optimized SD composed of the drug, Gelucire 50/13 and microcrystalline cellulose in a weight ratio of 1:5:10, was markedly rapid and higher than that from the drug powder and the market product (Afinitor®, Novartis Pharmaceuticals) in all dissolution mediums tested from pH 3.0 to pH 6.8. The results of this study suggest that formulation of SD with Gelucire 50/13 using melt granulation procedure may be a simple and promising approach for improving the dissolution rate and oral absorption of the anti-cancer agent without the need for using an organic solvent.

Key Words : Everolimus, Solid dispersion, Gelucire 50/13, Dissolution, Melt granulation

Introduction

Everolimus (EVR, Figure 1), the 40-O-(2-hydroxyethyl) derivatives of the natural product sirolimus, is administered as a once-daily, oral therapy for the treatment of patients with advanced renal cell carcinoma (RCC) after they fail to respond to sunitinib or sorafenib treatment. EVR is an inhibitor of the mammalian target of rapamycin (mTOR), a component of an intracellular signaling pathway that regulates cellular metabolism, growth, proliferation, and angiogenesis. Recently, the drug was also approved by the US Food and Drug Administration for the treatment of advanced hormone receptor-positive, HER2-negative breast cancer, advanced neuroendocrine tumors of pancreatic origin, renal angiomyolipoma with tuberous sclerosis complex, and subependymal giant cell astrocytoma. In spite of these attractive pharmacological effects, the oral absorption of EVR is challenging owing to its poor solubility in the gastrointestinal tract, unfavorable breakage of the drug in the gastric fluid, and intestinal efflux by P-glycoprotein transporter. The solubility of EVR in aqueous medium is below 0.1 mg/mL at 25 °C.

To improve the dissolution rate and oral absorption of the mTOR inhibitor, the pharmaceutical company (Novartis Pharmaceuticals, Basel, Switzerland) developed immediate release tablets (Brand name, Afinitor®) using solid dispersion (SD) with a hydrophilic polymer, hydroxypropyl methyl cellulose (HPMC). In the solvent method, both mTOR inhibitor and HPMC are dissolved in the organic solvents and then spray dried and then pulverized to form the granules. The molecular dispersion of the active substance in a polymeric carrier achieves optimal particle size reduction and surface area enhancement, which result in improved dissolution rates. However, a complex process involving the steps of mixing and dissolving a sirolimus derivative and a polymer in an organic solvent, evaporating the solvent, pulverizing dry residues to obtain particulate matter needs to be performed using the conventional solvent method. This process also introduces the residual solvent, which may bring up environmental issues.

Gelucire® (GLC) is a family of vehicles derived from the mixtures of mono-, di- and triglycerides with polyethylene glycol (PEG) esters of fatty acids. GLC has a wide variety of applications in pharmaceutical formulations as the preparation of SDs, fast release and sustained release formulations with a low melting point (33-65 °C), low toxicity, and wide drug compatibility. In particular, GLC-based SD system can be easily prepared by melt granulation technique, a process in which fine agglomeration is obtained through the melting and/or softening of the surfactant carrier at relatively low temperatures, drug dissolution in the molten carrier, followed by drying. The procedure offers some advantages
over the conventional solvent method, since the steps of addition and evaporation of an organic solvent can be omitted. Moreover, from an environmental perspective, it is also a good alternative that does not need the use of organic solvents.

The purpose of the current study is to formulate and optimize an SD system of EVR in the surfactant carrier (GLC 44/14 or 50/13) using melt granulation method. Physicochemical properties of SDs were characterized with an emphasis on surface morphology, crystallinity and chemical stability. Moreover, dissolution profiles of the mTOR inhibitor from the SD systems were investigated under various conditions in comparison with those of an intact drug alone, and market product (Afinitor®).

**Experimental**

**Materials.** EVR was purchased from Biocon Ltd. (India, purity over 99.4%). GLC 44/14 (Lauroyl polyoxyyl-32 glycerides, melting point 44 °C, hydrophilic-lipophilic balance 14) and GLC 50/13 (Stearoyl polyoxyl-32 glycerides, melting point 50 °C, hydrophilic-lipophilic balance 13) was kindly provided by Gattefosse (Cedex, France). Microcrystalline cellulose (Avicel PH 102) was obtained from FMC Corporation (Philadelphia, USA). All organic solvents were high-pressure liquid chromatography (HPLC) grade and all other chemicals were reagent grade.

**Preparation of EVR-loaded SD Formulations.** SDs in various weight ratios of drug to the carrier were prepared by melt granulation method.11 EVR (50 mg) was added to the molten base comprising either GLC 50/13 or 44/14 as listed in Table 1. The blend was heated 10 °C above the melting point of each carrier for 5 min with continuous magnetic stirring. The mass was crushed, ground gently with a mortar and pestle and passed through a 500 µm sieve. For the preparation of F7, microcrystalline cellulose was added to the drug-containing molten solution, followed by continuous blending for 10 min.

**Preparation of EVR and GLC Physical Mixtures.** GLCs are a waxy pellet, and hence these were crushed to fine particles firstly to prepare the physical mixture (PM). PMs of EVR with either GLC 44/14 or 50/13, in a 1:3 weight ratio of drug to the carrier, were prepared by blending them by triturating for 10 min followed by sieving (500 µm).

**Drug Content and Uniformity.** A sample containing 10 mg of the drug was dissolved in 10 mL of acetonitrile (0.25 mg/mL) and sonicated for 10 min. Then the samples were centrifuged at 12,000 rpm for 10 min, and the supernatants were analyzed by the HPLC analysis. The quantitative determination of EVR was accomplished by HPLC using the acetonitrile-phosphate buffer (NaH2PO4·H2O 0.11 w/w%) (40:60) as a mobile phase at a flow rate of 1.2 mL/min. The HPLC system consisted of a UV detector (L-2400), a pump (L-2130), a data station (LaChrom Elite, Hitachi, Japan), and a 15 cm C18 column (Shiseido, Tokyo, Japan). The column eluent was monitored at 275 nm, and the peak of the mTOR inhibitor was separated with a retention time of 7.5 min.

**Scanning Electron Microscopy (SEM).** The samples were coated with a thin gold layer by an automatic magnetron sputter coater system (Joel JSM 201, USA). Then, SEM photographs were taken by a scanning electron microscope (Joel JSM 6510, USA) operated at an acceleration voltage of 15 kV.

**X-ray Powder Diffraction (XRD).** XRD observation of the samples was performed at room temperature with an X-ray diffractometer (Ultima IV, Rigaku Corp., Japan). Monochromatic Cu Kα-radiation (λ = 1.5418 Å) was obtained with a Ni-filtration and a system of diverging and receiving slits of 0.5° and 0.1 mm, respectively. The diffraction pattern was measured with a voltage of 40 kV and a current of 30 mA over a 2θ range of 3–40° using a step size of 0.02°.

**In vitro Drug Release Test.** In vitro dissolution test was performed using USP-24 Type 2 dissolution test apparatus (DST-600A, Fine Scientific Instruments, Korea). Drug powder, SDs and the market product with 10 mg of EVR were placed in the dissolution vessel containing 900 mL of dissolution medium (pH 3.0, pH 4.0, pH 6.8 and water) maintained at 37.0 ± 0.5 °C and stirred at 50 rpm. Aliquots (4 mL) were collected periodically and replaced with fresh and pre-warmed dissolution medium. The samples were centrifuged at 12,000 rpm for 10 min, and the supernatants were diluted with acetonitrile for HPLC analysis.

**Stability Test.** Stability studies were conducted by placing powdered samples in stoppered glass vials and storing in stability chambers maintained at 40 °C and 75%RH for accelerated stability and 25 °C and 60%RH for long-term stability, respectively. Samples were removed after 1 month and tested for changes in the drug content (%).

**Results and Discussion**

**Preparation and Characterization of SDs.** The SDs of EVR in GLCs were prepared by melt granulation technique to enhance the dissolution rate and to minimize the manufacturing and/or environmental issues associated with the solvent method. The melt granulation method did not require the use of organic solvents for the preparation of dispersion system, whereas both the drug and the carrier needed to be dissolved in a sufficient amount of solvent in case of the solvent method. In particular, when HPMC is used as a carrier for SD, a mixture of dichloromethane and ethanol is usually added to dissolve the drug and the carrier as HPMC has a low solubility in ethanol.12,13 However, dichloromethane is classified as a Class II solvent,14 whose usage should be avoided whenever possible.15 The drug content in SD

---

*MCC indicates microcrystalline cellulose (Avicel 102).*

| Table 1. Compositions (mg) of EVR-loaded SD formulations |
|--------------|---|---|---|---|---|---|---|
| F1 | F2 | F3 | F4 | F5 | F6 | F7 |
| EVR | 10 | 10 | 10 | 10 | 10 | 10 |
| GLC 44/14 | 30 | - | - | - | - | - |
| GLC 50/13 | - | 30 | 10 | 50 | 100 | 200 | 50 |
| MCC* | - | - | - | - | - | - | 100 |

*Sun Woo Jang et al.*

---

formulations was almost equal (97.2 to 100.1%) with low values of standard deviation, indicating that the drug was uniformly distributed in the hydrophilic carrier with the melt granulation process without any drug degradation and/or precipitation (Data not shown).

The solid state of EVR-GLC dispersion was characterized by scanning electron microscopy (SEM). Figure 2 shows SEM pictures of the raw material of the drug, pulverized GLC 50/13, and its corresponding PM and SD (F2). The drug crystals seemed to be irregular fragment in shape and their size ranged from 5-100 µm (Figure 2(a)). Typical appearance of drug powder and GLC 50/13 (Figure 2(b)) were observed in the photomicrographs of the PM (Figure 2(c)). On the other hand, in case of SD, it was difficult to determine the presence of drug crystals (Figure 2(d)), indicating that the drug crystals appeared to be incorporated into molten mass of the carrier at the molecular level. The appearance of SDs prepared using GLC 44/14 (F1) and GLC 50/13 in different ratios (F3, F4, F5, and F6) was almost same to that of F2 (Data not shown). The SEM images of the F7 formula showed that rough surfaces of fibrous microcrystalline cellulose (Figure 2(f)) were appeared to be covered with SD of EVR, and microcrystalline cellulose was white in color (Figure 2(e)).

The XRD patterns of drug powder, the surfactant carrier, and SD prepared by melt granulation method are shown in Figure 3. No diffraction peak of EVR (Figure 3(a)) was observed, while GLC 50/13 exhibited some crystallinity as indicated by the two characteristic peaks of high intensity at 19.26 and 23.50 at 2θ(Figure 3(b)). And the XRD pattern of SDs including F2 (Figure 3(c)) and F7 (Figure 3(d)) was quite analogous as that of the carrier itself, with no other distinctive peaks. This result suggests that EVR itself exists in an amorphous state, and the crystallinity of the drug remained in an amorphous state in the SD formulas, regardless of the presence of microcrystalline cellulose.

**Dissolution Profiles of EVR from SDs.** The dissolution profile of EVR from the drug powder, PMs and SDs with 44/14 and 50/13 in a weight ratio of 1:3 in distilled water are presented in Figure 4. In distilled water, PMs of GLC 44/14 and 50/13 showed dissolution profiles similar to those of the intact drug; after 2 h, approximately 30-35% of the drug was released. On the other hand, the release rate of EVR from SDs (F1 and F2) was significantly higher than that from the drug powder and/or PMs with the same ratio of drug to the carrier. In particular, the greater dissolution enhancement of EVR was achieved with the GLC 50/13-based formula (F2) compared to the GLC 44/14-based formula.

**Figure 2.** SEM micrographs for (a) raw material of EVR, (b) GLC 50/13, (c) physical mixture of drug and GLC 50/13 at the ratio of 1:3, SDs of (d) F2 and (e) F7, and (f) intact microcrystalline cellulose. Scale bars indicate 100 µm.

**Figure 3.** XRD diffractograms for (a) raw drug material, (b) GLC 50/13, and SD of (c) F4 and (d) F7.

**Figure 4.** Dissolution profiles of the drug released from SD systems based on GLC 44/14 (F1, ○) or 50/13 (F2, ●), PMs with GLC 44/14 (△) or 50/13 (▲), and drug powder (×) in distilled water. Data represent mean ± SD (n = 3).
Instead, the SD prepared with 100 mg of microcrystalline cellulose could not produce a free-flowable powder (Data not shown). To improve the dissolution rate and oral absorption of EVR, a novel SD system was prepared by incorporating mTOR inhibitor into GLC 50/13 using the melt granulation technique. The fabrication process was very simple without the need for using an organic solvent. The optimized SD formulation consisted of EVR, GLC 50/13 and microcrystalline cellulose at a weight ratio of 1:5:10. The SD structure was found in accelerated storage conditions. These results indicated that the amorphous drug was further stabilized in the SD system as a consequence of drug-GLC interactions and/or by incorporation into the carrier. However, a further study investigating the stabilization mechanism of the excipients is needed.

### Conclusion

To improve the dissolution rate and oral absorption of EVR, a novel SD system was prepared by incorporating mTOR inhibitor into GLC 50/13 using the melt granulation technique. The fabrication process was very simple without the need for using an organic solvent. The optimized SD formulation consisted of EVR, GLC 50/13 and microcrystalline cellulose at a weight ratio of 1:5:10. The SD structure was significantly improved compared to the raw material and the commercial product.

### Table 2. Drug content (%) of the optimized SD (F7) and drug powder in ambient conditions of 25 °C, 60% RH and in accelerated conditions of 40 °C, 75% RH at 1 month

<table>
<thead>
<tr>
<th>Condition</th>
<th>Drug powder</th>
<th>F7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>99.5 ± 1.1</td>
<td>98.9 ± 1.4</td>
</tr>
<tr>
<td>25 °C, 60% RH at 1 month</td>
<td>93.5 ± 1.1</td>
<td>97.5 ± 0.3</td>
</tr>
<tr>
<td>40 °C, 75% RH at 1 month</td>
<td>72.4 ± 3.6</td>
<td>91.5 ± 0.5</td>
</tr>
</tbody>
</table>

*Values represent mean ± SD (n = 3).*
showed a remarkably higher dissolution rate in all dissolution mediums compared to that of the intact drug and the market product (Afinitor®), and it demonstrated that more than 80% of initially loaded active ingredient was released within 10 min. On the basis of these results, it can be suggested that the SD formulation using GLC 50/13 can be promising for the development of an immediate release tablet of the mTOR inhibitor.

Acknowledgments. The present research was conducted by the research fund of Dankook University in 2013.

References