Evaluation of Dissolution Profile between Original and Generic Products of Zolpidem Tartrate by Microdialysis-HPLC

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Received August 20, 2018; accepted November 7, 2018

The evaluation of the dissolution profile of hypnotic drugs is important to promote switching from original products to generic products by removing distrust in generic hypnotics. In this study, we investigated differences in the dissolution profiles between original and generic products (GE-D, GE-S, and GE-T) in commercially available zolpidem tartrate (ZOL) products using the HPLC method using a connected microdialysis probe (microdialysis-HPLC method). Although the degree of hardness and the disintegration time were not different among the original, GE-S, and GE-T, GE-D was 1.4 times harder than the other products. The disintegration time of GE-D was approximately twice as long as that of the original product. Generic products dissolved rapidly as compared with the original product, however, the dissolution rate in the ZOL powder (milled ZOL product) was not different between the original and generic products. Macrogol 6000 (polyethylene glycol (PEG)-6000) was used in the generic products, and this additive was the only PEG difference from the original product. We investigated whether the PEG in the product affected the solubility of ZOL and found that the addition of PEG-4000 or PEG-6000 significantly increased the dissolution rate. These results suggest that the solubility of ZOL may be increased by PEG when the product is disintegrated, resulting in the increased dissolution rate in the generic products. In conclusion, we found that the difference of PEG affected the dissolution profile in the disintegration process using the microdialysis-HPLC method. This finding can help ensure the safety of milled products and the selection of additives.

Key words zolpidem tartrate; polyethylene glycol; dissolution profile; microdialysis-HPLC method; generic product

Introduction

The national cost of medical care exceeded 41 trillion yen in Japan for 2015, and the promotion of using generic products is one approach for reducing the cost of medical care.1) As of September 2017, generic products account for 65.8% of medical products used in Japan, and the Ministry of Health, Labour, and Welfare of Japan has set a goal of increasing this to at least 80% by September 2020.2) Generic products are marketed after the patent term of the original products expires, and these products can reduce research and development spending. Drug prices of generic products are usually about 60% lower than those of the original products. Accordingly, popularization of generic products has reduced the financial burden on patients and lowered health insurance costs.3) Generic products have the same active pharmaceutical ingredients, quantities, and administration routes as their original products. In addition, the effects, efficacy, usage, and dosages are essentially identical between original and generic products. However, the maximum concentration (Cmax) and time (Tmax) of drugs can be different between original and generic products, and it has been reported that the difference in the dissolution profile of active ingredients affected the therapeutic effect in clinical studies using prednisolone products4) and danazol products.5,6) As such, some patients may feel suspicious about switching from original products to generic products. In particular, patients with insomnia can feel the difference strongly, since the time until induction of sleep may increase. In fact, authors are asked often by some patients about the difference of sleep-inducing time, in switching from original products to generic products in the pharmacy. The drug effect in the oral formulation express after the absorption in the intestine. Therefore, it is hypothesized that the drug with low dissolution slow the drug absorption, resulting in delaying the time for induction of sleep. Therefore, evaluation of the formulation properties (dissolution profile) of hypnotic drugs is important to clarify to promote switching from original products to generic products and to remove distrust in generic hypnotic products.

Dissolution testing is a core performance test in pharmaceutical development and quality control, and the testing used to evaluate differences in the formulation properties between original and generic products.7) The traditional HPLC dissolution method has many steps, such as filtration, collection, and replenishment of sample solutions. We previously designed a dissolution test using microdialysis method (microdialysis-HPLC method) that can omit many steps. In addition, this method can evaluate the dissolution behavior in more detail than the traditional method. Therefore, the microdialysis-HPLC method is suitable to elucidate the differences between the dissolution behaviors of original and generic products.

Zolpidem tartrate (ZOL) is a short-acting non-benzodiazepine hypnotic that is classified as an imidazopyridine and was commercially marketed in France in 1987 (Stilnox®). Subsequently, it was marketed under the name of Myslee® in Japan in 2000, and it is one of the most frequently used hypnotics in Japan. ZOL enhances the suppressive mechanism of the gamma-aminobutyric acid (GABA) system by acting on the benzodiazepine receptors in the central nervous system, exert-
ing hypnotic and sedative effects. ZOL has weak anti-anxiety, anti-convulsant, and muscle relaxant effects by exerting selective action on omega-1 receptors, which are a subtype of GABA(A) receptor. ZOL is usually used for the treatment of insomnia (excluding insomnia resulting from schizophrenia or manic-depressive psychosis). Generally, the effects of ZOL are equivalent to those of benzodiazepine hypnotics, and patients are less affected by daytime hangover, less likely to have rebound insomnia after interruption, and are less affected in their patterns of sleep. Therefore, ZOL is widely used and has many generic versions in Japan. In this study, we investigated the differences in the dissolution profiles between original ZOL and its generic versions. In addition, we examined the relationship between dissolution profiles and additives in the commercially available ZOL products using the microdialysis-HPLC method.

**Experimental**

**Materials** ZOL original tablets (product) were obtained from Astellas Pharma Inc. (Tokyo, Japan), and three generic products (GE-D, GE-S and GE-T) were purchased from Daiichi Sankyo Esphaco Co., Ltd. (Tokyo, Japan), Sawai Pharmaceutical Co., Ltd. (Osaka, Japan), and Towa Pharmaceutical Co., Ltd. (Osaka, Japan), respectively. Macrogol (polyethylene glycol, PEG) 4000 (PEG-4000) and 6000 (PEG-6000) were provided by Nacalai Tesque Inc. (Kyoto, Japan).

**Measurement of Particle Condition in ZOL Products**

ZOL original and generic products were milled using a mortar (hand-mill method) and a tablet grinder (TS-1OM type, TOSHO Corporation, Japan, auto-mill method) for 1 and 5 min. The milled ZOL was observed using a biological microscope Motic BA210E (SHIMADZU Corporation, Japan), and the particle size distribution was measured according to the Japanese Pharmacopoeia 17th test protocol. The recovery rate was calculated as a ratio of the weight before and after milling.

**HPLC Method**

ZOL concentrations in the samples were determined by HPLC (LC-Net II/ADC system, JASCO Corporation, Tokyo, Japan). The conditions were as follows: mobile phase, consisting of 0.02 M ammonium acetate–acetonitrile (70:30, v/v, pH 8.0); flow rate (PU-2089 Plus), 0.3 mL/min; column, Inertsil® ODS-3 (3 µm, column size: 2.1 × 50 mm) column (GL Science Co., Inc., Tokyo, Japan); column temperature, 35°C; wavelength for detection (UV-2075 Plus), 241 nm.

**Formulation Evaluation by JP Test** The hardness, disintegration, and dissolution tests were performed according to the JP test protocol. The hardness and disintegration in ZOL original or generic products were evaluated using a tablet hardness tester (Monsanto type) and a disintegration tester NT-2H (Toyama Sangyo Co., Ltd., Osaka, Japan), respectively. The disintegration was expressed as disintegration time, and the test solution used purified water. In this study, the dissolution behavior was evaluated using the microdialysis-HPLC method. For the dissolution test, a precise micro-controlled roller pump (EPR-10, Eicom, Kyoto, Japan) connected to a microfiltering probe was used for the measurement of the dissolution rate. The NTR-1000 (Toyama Sangyo Co., Ltd.) and a concentric microdialysis probe (polyethylene membrane, membrane length 0.34 mm, probe diameter 3 mm, pore size 0.3 µm, Eicom) was used. The probe was placed in the test solution (purified water) and collected the sample at 0–570 s. The dissolution of ZOL was expressed as the rate of ZOL powder from original and generic products. ZOL concentrations in the samples were determined using the HPLC method described above.

**Solubility in Combination of ZOL and PEG** The commercially available ZOL product was milled by mortar for 5 min (ZOL powder), and 100 mg of ZOL powder was mixed with 10 mg of lactose, PEG-4000, or PEG-6000. The solubility was evaluated using the microdialysis-HPLC method. The mixture was set in a cell connected to a concentric microdialysis probe (A-1-20-05, 5-mm length; Eicom). After that, 90 µL of purified water was added to the mixture, which was then perfused with purified water through the microdialysis probe at a constant flow rate of 1 µL/min using a micro syringe pump (ESP-64, Eicom). The ZOL concentration in the sample was determined using the HPLC method described above.

**Statistical Analysis** Statistical comparisons were performed using Dunnett’s multiple comparison using JMP (SAS Institute Inc., Cary, NC, U.S.A.). p < 0.05 was considered significant.

**Results**

**Hardness Test, Disintegration Time, and Dissolution Profile in ZOL Products** Figure 1 shows the hardness degree and disintegration time of ZOL original and generic products. The degree of hardness was not different among the original, GE-S, and GE-T. However, GE-D was harder than the other products, and the hardness level was 1.4 times that of the other products. The hardness degree and disintegration time in GE-D were higher in comparison with the original, GE-T, and GE-S.
of the original product. In addition, the disintegration time for GE-D was also longer than the other products. The disintegration time for GE-D was 194 s, approximately twice as long as the original product. Figure 2 shows the dissolution profile of ZOL original and generic products. In the traditional method, disintegrated solid particles (non-dissolution samples) were taken by syringe in initial experiments (for 0–90 s in the ZOL), and these solid particles prevented the accurate measurement (it was observed vary widely). On the other hand, the microdialysis-HPLC method using in this study can estimate the dissolution behavior in more detail in comparison with the traditional method. The dissolution rates increased with dissolution time for each product, and generic products dissolved rapidly as compared with the original product. Moreover, the dissolution rate in GE-D seem to be lower than GE-S and GE-T at several time point such as 30 s or 120–330 s.

Changes in Recovery Rate and Particle Size for Milled ZOL Products

Although the disintegration time of the original product was similar to those of GE-T and GE-S, the dissolution rate of the original product was lower than that of GE-T and GE-S. Therefore, we investigated whether the difference in particle size after disintegration affected the difference in the dissolution rate between the original and generic products. Figure 3 shows microscope images, recovery rates, and mean particle sizes of the ZOL products after mill treatment. No significant difference was observed in the recovery rate between the original and generic products, and the recovery rates of ZOL original, GE-D, GE-S, and GE-T were 80.7 ± 0.9, 85.1 ± 4.0, 81.9 ± 2.1, and 80.5 ± 2.7%, respectively. In the mill treatment for 1 min, the particle size of ZOL original was very small as compared with the generic products, and the particle size of ZOL original, GE-D, GE-S, and GE-T was 3.4 ± 0.2, 31.4 ± 7.4, 20.9 ± 1.6, and 46.9 ± 6.9 µm, respectively. In both cases, the extension of treatment time lead to a decrease in the particle size. Figure 4 shows the dissolution rate of the ZOL powder from the original and generic products. The dissolution rate was not different between the powders of the original and generic products.

Relationship between Additives and Dissolution Profile in ZOL Powder

The additive of the original product is only PEG, as opposed to PEG-6000 in GE-D (Table 1). Therefore, we investigated whether the PEG in the product affected the solubility of ZOL. Figure 5 shows the effect of PEG-4000 and PEG-6000 on the dissolution speed of ZOL. The addition of PEG-4000 or PEG-6000 to ZOL powder significantly enhanced the dissolution rate. In addition, the dissolution speed of ZOL powder when PEG-6000 was added was faster than that when PEG-4000 was added. On the other hand, the ZOL levels in the both of PEG-4000 and PEG-6000 groups reached saturation in over 4 s, and the profiles showed the plateau.

Discussion

In present clinical practice, it is important to remove the anxiety of patients against using generic products in order to

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**Fig. 2.** Comparison of Dissolution Profiles between ZOL Products

The dissolution rate in the original (○), GE-D (●), GE-S (▲), and GE-T (■) products were determined using the microdialysis-HPLC method. The data are presented as means ± S.D. of 3 experiments. *p < 0.05 vs. original for each category. The dissolution rates in the generic products were higher than in the original product.

**Fig. 3.** Image (A), Recovery Rate (B), and Particle Size (C) in ZOL Products at 1 and 5 min after Mill Treatment

Opened columns, 1 min-milled product. Closed columns, 5 min-milled products. These data are presented as means ± S.D. of 3 experiments. No difference was observed in the recovery rates among the original and generic products. The particle sizes for the milled products were as follows: GE-T > GE-D > GE-S > original.
promote the use of such products, as the Ministry of Health, Labour, and Welfare in Japan seeks to inhibit the growth of medical spending.\textsuperscript{10} One problem with using generic products is the possibility of difference of dissolution profiles between original and generic products. In particular, in the case of using hypnotics to treat a sleep disorder, different dissolution profiles can psychologically influence the efficacy of therapy. In fact, we are asked often by some patients about the difference of sleep-inducing time, in switching from original products to generic products in the pharmacy. In this study, we compared the dissolution profiles of the original and three generic products of ZOL, a short-acting non-benzodiazepine hypnotic, and found that the dissolution rate in the generic products (GE-D, GE-S, GE-T) was higher than that in the original product. In addition, we showed that the kind of PEG used as an additive may affect the dissolution profile in the ZOL products.

ZOL is the most popular medicine for treating insomnia in Japan at present, and it is known for having a low tolerance, a quick induction time usually within 15 min, and a short half-life of two to three hours.\textsuperscript{11} However, patients can grow distrustful of the effect when the induction time is slow. Therefore, it is important to demonstrate similar dissolution profiles between the original and generic products of ZOL to show the benefits of using generic products. First, we evaluated the hardness and disintegration time of the ZOL products following the JP (hardness and disintegration tests, Fig. 1). GE-D was 1.4 times harder than the original product. Also, the disintegration time in GE-D was 194 s, approximately twice as long as the original product. The difference in composition between the original and GE-D is the kind of PEG (macrogol). It was known that the PEG increases viscosity and is used as a base of ointments.\textsuperscript{12} Therefore, the kind of PEG used may affect the hardness and disintegration time. By contrast, the hardness and disintegration times of GE-T and GE-S were similar to those of the original product. Light anhydrous silicic acid and talc are added to GE-S and these additives increase the variability.\textsuperscript{13,14} In GE-T, carmellose and carmellose calcium are added as disintegrating agents, along with the binding agent hydroxypropylcellulose.\textsuperscript{15,16} This suggests that the hardness and disintegration time may have been affected by the additives in GE-S and GE-T.

Next, we determined the dissolution rates of the original and generic products (Fig. 2). In the generic products, the dissolution rate also followed the result of the disintegration time, and the dissolution rate in GE-D was lower than that in other generic products (GE-S and GE-T) for the 30 s and 120–330 s. The dissolution rate of the original product was significantly lower than that of the generic products.

### Table 1. Composition of Original and Generic Versions of ZOL

| Original product | GE-D | GE-S | GE-T |
|------------------|------|------|------|
| Lactose hydrate  | Lactose hydrate | Lactose | Lactose hydrate |
| Microcrystalline cellulose | Microcrystalline cellulose | Microcrystalline cellulose | Microcrystalline cellulose |
| Hypermellose      | Hypermellose      | Hypermellose      | Hypermellose      |
| Sodium carboxymethyl starch | Sodium carboxymethyl starch | Sodium carboxymethyl starch | Sodium carboxymethyl starch |
| Magnesium stearate | Magnesium stearate | Magnesium stearate | Magnesium stearate |
| Carnauba wax     | Carnauba wax     | Carnauba wax     | Carnauba wax     |
| Red ferric oxide | Red ferric oxide | Red ferric oxide | Red ferric oxide |
| Yellow ferric oxide | Yellow ferric oxide | Yellow ferric oxide | Yellow ferric oxide |
| Titanium oxide   | Titanium oxide   | Titanium oxide   | Titanium oxide   |
| Macrogol (PEG)   | Macrogol 6000 (PEG-6000) | Macrogol 6000 (PEG-6000) | Macrogol 6000 (PEG-6000) |
| Lactose          | Lactose          | Lactose          | Lactose          |
| Microcrystalline cellulose | Microcrystalline cellulose | Microcrystalline cellulose | Microcrystalline cellulose |
| Hypermellose      | Hypermellose      | Hypermellose      | Hypermellose      |
| Sodium carboxymethyl starch | Sodium carboxymethyl starch | Sodium carboxymethyl starch | Sodium carboxymethyl starch |
| Magnesium stearate | Magnesium stearate | Magnesium stearate | Magnesium stearate |
| Carnauba wax     | Carnauba wax     | Carnauba wax     | Carnauba wax     |
| Red ferric oxide | Red ferric oxide | Red ferric oxide | Red ferric oxide |
| Yellow ferric oxide | Yellow ferric oxide | Yellow ferric oxide | Yellow ferric oxide |
| Titanium oxide   | Titanium oxide   | Titanium oxide   | Titanium oxide   |
| Macrogol 6000 (PEG-6000) | Macrogol 6000 (PEG-6000) | Macrogol 6000 (PEG-6000) | Macrogol 6000 (PEG-6000) |
| Light anhydrous silicic acid | Light anhydrous silicic acid | Light anhydrous silicic acid | Light anhydrous silicic acid |
| Talc             | Talc             | Talc             | Talc             |
| Carmellose       | Carmellose       | Carmellose       | Carmellose       |
| Carmellose calcium | Carmellose calcium | Carmellose calcium | Carmellose calcium |
| Hydroxypropylcellulose | Hydroxypropylcellulose | Hydroxypropylcellulose | Hydroxypropylcellulose |
lower than those of the generic products (GE-D, GE-S, and/or GE-T) until 390 s (Fig. 2). The hardness and disintegration time in the original product were similar to those in GE-S and GE-T (Fig. 1). These results suggest that after disintegration the original product may be dispersed in a particle state. From these findings, we prepared ZOL powder by milling treatment and investigated whether the particle size after disintegration affected the dissolution rate. The milled original product was smaller than the milled generic products (Fig. 3), and the dissolution rate was similar in the original and generic products (Fig. 4). These results showed that the particle size after disintegration was not related to the low dissolution rate in the original product.

It is important to clarify the difference between the dissolution profile of the original ZOL and those of generic products. The disintegration time of the original product was similar to that of GE-T and GE-S, approximately 100 s (Fig. 1B). However, the dissolution rate of the original product was lower than that of GE-T and GE-S products at 100 s (Fig. 2). In addition, the difference in particle size did not affect the dissolution rate between the original and generic products (Figs. 3, 4). The difference between the original and generic products was the composition, and the additive of the original product was different only PEG (Table 1). It has been published that GE-D, GE-S, and GE-T contain macrogol 6000 (PEG-6000). On the other hand, the original products contain same macrogol type (PEG-4000 and PEG-6000), and the type and ratio in macrogol are non-public information in original products. Therefore, we investigated whether the PEG in the product affected the solubility of ZOL by using the microdialysis probe. The addition of PEG-4000 or PEG-6000 enhanced the dissolution rate of ZOL (Fig. 5). The results support the previous studies of Vijaya and Mishra, and Zerrouk et al. which mentioned that the drug solubility was enhanced by PEG. Taken together, it is suggested that this method using microdialysis probe is possible to investigate the effect of additives on dissolution profiles. Furthermore, the enhancement of solubility with PEG-6000 was higher than that with PEG-4000 (Fig. 5). Those effects may be caused by the properties of PEG as a water-soluble base. These results show that the solubility of ZOL may be enhanced by the additives in conjunction with the product disintegrating, resulting in the increased dissolution rate in the generic products. Further studies are needed to investigate the relationships of feel suspicious about switching from original products to generic products and the difference of dissolution profile between original products to generic products. In addition, it is important to elucidate this mechanism. Therefore, we plan to measure the effect of PEG addition on drug solubility in other oral medicines by the method using microdialysis probe.

In conclusion, we evaluated the difference of dissolution profile in ZOL tablet by using the microdialysis-HPLC method. In addition, we designed the method using microdialysis probe to investigate the effect of additives on dissolution profiles, and found a difference in how PEG affects the dissolution profile of ZOL in terms of the disintegration process. This finding can help ensure the safety of milled products and the selection of additives.

Conflict of Interest The authors declare no conflict of interest.

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