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Pharmaceutical quality evaluation of lipid emulsions containing PGE_1: Alteration in the number of large particles in infusion solutions

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ABSTRACT

There are two generics of a parenteral lipid emulsion of prostaglandin E1 (PGE_1) (Lipo-PGE_1) in addition to two innovators. It was reported the change from innovator to generic in clinical practice caused the slowing of drip rate and formation of aggregates in the infusion line. Thus, we investigated the difference of pharmaceutical quality in these Lipo-PGE_1 formulations. After mixing with some infusion solutions, the mean diameter and number of large particles were determined. Although the mean diameter did not change in any infusion solutions, the number of large particles (diameter >1.0 μm) dramatically increased in generics with Hartmann's solution pH 8 or Lactec® injection with 7% sodium bicarbonate. Next, we investigated the effect of these infusion solutions on the retention rate of PGE_1 in lipid particles. The retention rate of PGE_1 in these two infusion solutions decreased more quickly than that in normal saline. Nevertheless, there were no significant differences among the formulations tested. Our results suggest that there is no difference between innovators and generics except in mixing with these infusion solutions. Furthermore, that monitoring the number of large particles can be an effective means of evaluating pharmaceutical interactions and/or the stability of lipid emulsions.

1. Introduction

Prostaglandin E1 (PGE_1), which has a strong vasodilatory and antiplatelet activity, is clinically used to treat diseases such as periferal arterial occlusive diseases (Makita et al., 1997; Milio et al., 2003) and ductus arteriosus-dependent congenital heart disease (Kramer et al., 1995). However, PGE_1 has a very short half-life in blood and can elicit various side-effects (Golub et al., 1975; Schramek and Waldhauser, 1989). As a result, a lipid emulsion of PGE_1 (Lipo-PGE_1), in which lipid particles incorporating PGE_1 were coated with lecithin, was developed and applied for clinical treatment in Japan (Mizushima et al., 1983; Otomo et al., 1985). Because the lipid particles are efficiently distributed into the vascular lesion site, Lipo-PGE_1 accumulates in the lesion area and is therefore safer and more effective than free PGE_1 (Mizushima et al., 1990; Mizushima et al., 1983). Indeed, Lipo-PGE_1 is widely used to treat a number of conditions other than arterial occlusive diseases, such as cutaneous ulcer with diabetes and improvement of imaging ability for arterial portography. Two innovator formulations and two generic formulations have already been launched. The composition of each formulation is shown in Table 1. Although generic formulations contain olive oil instead of soybean oil, the other additives are the same as those found in the innovator formulations. Hydrochloric acid or sodium hydrate is added appropriately as a pH adjuster, and the pH of each formulation is adjusted 4.5–6.0.

Lipo-PGE_1 can be intravenously administered by bolus injection, or slowly administered as infusions by mixing with infusion solution. Recently, it was reported that the change from innovator to generic formulation in clinical practice caused the slowing of drip rate and formation of aggregates in the infusion line (Sakaya et al., 2005; Goto et al., 2005). This phenomenon was observed under alkaline conditions in the presence of calcium ions. The Lipo-PGE_1 has an approximate pH of 5. There are some cases where Lipo-PGE_1 is mixed into the infusion solutions of relatively high pH (e.g., Hartmann's solution pH 8; 7% sodium bicarbonate) in order to moderate vascular pain or venous inflammation. Furthermore, it has also been reported that generic formulations in saline solution exhibit lower retention rates of PGE_1 in lipid particles and weaker pharmacological activity in animal models than innovator formulations (Takenaga et al., 2007). Therefore, it is important to investigate the difference in pharmaceutical quality between innovator and generic formulations.

In the Japanese Pharmacopoeia, the diameter of lipid particles in a lipid emulsion is defined as being below 7 μm. Included in the tests for the preparation of a parenteral lipid emulsion is "Insoluble Particulate Matter Test for Injections" as well as "Test for Extractable Volume of Parenteral Preparations". The former test defines an examination by "Method 1. Light Obscuration Particle Count Test" or

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Table 1
Formulas of Lipo-PGE_1.

	Alprostadil (PGE1)	Purified soybean oil	Purified olive oil	Highly purified soybean lecithin	Oleic acid	Concentrated glycerin
Formulation #1 (innovator)	5 μg	100 mg		18 mg	2.4 mg	22.1 mg
Formulation #2 (innovator)	5 μg	100 mg		18 mg	2.4 mg	22.1 mg
Formulation #3 (generic)	5 μg		100 mg	18 mg	2.4 mg	22.1 mg
Formulation #4 (generic)	5 μg		100 mg	18 mg	2.4 mg	22.1 mg

"Method 2. Microscopic Particle Count Test". Method 1 is preferably applied to injections and parenteral infusions. However, in cases where the preparation has a reduced clarity or increased viscosity, such as emulsions, colloids and liposomal preparations, the test should be carried our according to Method 2. The Pharmacopoeia of the United State of America (USP), ⟨788⟩ also defines a similar method for parenteral preparations. However, within recent years, ⟨729⟩ "Globule Size Distribution in Lipid Injectable Emulsions" is listed in the second supplement of USP30. This ⟨729⟩ provides two methods, "Method 1. Light-Scattering Method" for the mean diameter of lipid particles, and "Method 2. Measurement of Large Globule Content by Light Obscuration or Extinction method" for the extent of large-diameter particles (>5 μm), and is required to meet both criteria. This is based on the idea that the size of the lipid particles is critical because large-size fat globules can become trapped in the smallest of blood vessels such as capillaries with diameters between 4 and 9 μm (Guyton, 1991). The essential size characteristics of a lipid injectable emulsion include the mean diameter of lipid particles and the range of the various particle diameters distributed around the mean diameter (Driscoll et al., 2001). In this study, we investigated the formation of aggregates and measured the mean diameter and/or number of large-diameter particles. We also monitored PGE_1 retention rate in Lipo-PGE_1 to investigate the difference in the pharmaceutical quality of Lipo-PGE_1 formulations.

2. Materials and methods

2.1. Materials

Four Lipo-PGE_1 formulations as shown in Table 1 were used in this study. Palux® injection (Formulation #1, lot nos. O17H2 and I07H2, Taisho Pharmaceutical Co., Ltd., Tokyo, Japan), Liple® injection (Formulation #2, lot nos. P625J and P205H, Mitsubishi Tanabe Pharma Corporation, Osaka, Japan), Alyprost® injection (Formulation #3, lot nos. AB07A and AF07A, Nippon Chemiphar Co., Ltd., Tokyo, Japan), Prink® injection (Formulation #4, lot nos. 659109 and 659123, Taiyo Yakuhin Co., Ltd., Nagoya, Japan) were purchased from a drug seller in Japan. Otsuka normal saline, Aminofluid®, Lactec® injection and Meylon® (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), Amicaliq® (Termo Corporation, Tokyo, Japan), Solita®-T No. 3 (Ajinomoto Co., Inc., Tokyo, Japan), Hartmann's Solution pH 8 and Nipro infusion set IS type (Nipro Pharma Corporation, Osaka, Japan), were purchased from a general sales agency for drugs in Japan. Lactec® injection with $NaHCO_3$ was composed of 500 ml Lactec® injection and 20 ml Meylon® (7% $NaHCO_3$ injection). The official PGE_1 reference standard was purchased from the Society of Japan Pharmacopoeia. Slide-A-Lyzer® Dialysis Cassette (molecular weight cutoff: 7 K, capacity: 0.1–0.5 ml) and Buoy used for dialysis method were purchased from Pierce (IL, US). Disposable syringes, 21- and 27-gauge needles were purchased from Terumo Corporation.

2.2. Particle size distribution analysis

A 2 ml aliquot of Lipo-PGE_1 was injected into a 500 ml infusion bag of different solutions. After mixing, the various solutions were incubated at room temperature. At the indicated time point, each mixed solution was collected and analyzed by measurement of dynamic light scattering or single particle optical sizing.

2.2.1. Dynamic light scattering (DLS)

The particle size distribution and mean diameter of each Lipo-PGE_1 after mixing with different solutions were measured using a dynamic light scattering photometer DLS-7000 (Otsuka Electronics Co., Ltd., Osaka, Japan) equipped with a He–Ne laser source (wavelength, 632.8 nm). All DLS measurements were made with a scattering angle of 90°. Mixed solutions were diluted 15-fold with each infusion solution in order to obtain an appropriate scattering intensity. Data were gathered using a counting period of 100 s. Histogram analyses were performed to calculate the average particle size and standard deviation.

2.2.2. Single particle optical sizing (SPOS)

An Accusizer 780A (Particle Sizing Systems, Santa Barbara, CA) was used to determine the number of large-diameter particles in the emulsions. This instrument is based on light extinction (LE) or light scattering (LS) that employs a single-particle optical sizing (SPOS) technique, and was equipped with an automatic dilution system. In this study, the summation mode, which is a combination of LE and LS, was applied to measure the number of particles >0.5 μm in diameter. Before commencing any measurements, the equipment was filled with each infusion solution by using the command "Start Vessel Flush". After ensuring the background count was below 100 counts/s, mixed solutions (about 5 ml) were injected into the sample chamber. Duplicate measurements were made for each sample at the appropriate time point using the following conditions; data collecting time, 60 s; flow rate, 60 ml/min; injection loop volume, 1.04 ml; syringe volume, 2.5 ml; second dilution factor, 40. We ascertained that this dilution factor maintained the per-milliliter counts below the coincidence limit for the sensor, thereby minimizing this source of error. The volume-weighted proportion of fat globules (PFAT) with a diameter of >5 μm ($PFAT_5$) was calculated by the command "Volume Fraction cal".

2.3. Zeta potential

Zeta potential was measured using a Zetasizer NanoZS (Malvern Instruments, Malvern, UK), which is based on laser Doppler velocimetry in an electric field. For each Lipo-PGE_1, 500 μl was diluted using 10 ml distilled water.

2.4. Determination of PGE_1 retention rate

2.4.1. Assay for PGE_1

PGE_1 was measured by high-performance liquid chromatography (HPLC) using a post-column reaction. The HPLC system consisted of two constant pumps (LC-10ADvp, Shimadzu, Kyoto, Japan), a degasser (DGU-14A, Shimadzu), an automated pretreatment system, an autoinjector (SIL-10ADvp, Shimadzu), a UV/VIS detector (SPD-20AD, Shimadzu), a column oven (CTO-10ACvp, Shimadzu), and a system controller (SCL-10Asp, Shimadzu). PGE_1 was detected at 278 nm. The column used in this study was a 15 cm stainless-steel (4.6 mm i.d.) 5 μm Ø Mightysil ODS (Kanto Chem-