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Note

Stability of latanoprost in an ophthalmic lipid emulsion using polyvinyl alcohol

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Abstract

Latanoprost in water is not stable against heat stress due to hydrolysis of the isopropyl ester in the latanoprost molecule. Therefore, the storage condition of latanoprost ophthalmic solution, Xalatan® brand, was in a low temperature (2–8 °C). We formulated a favorable ophthalmic lipid emulsion of latanoprost using polyvinyl alcohol as emulsifier which showed a good heat stability. The assays of the latanoprost ophthalmic lipid emulsions adjusted to pH 5.0, 6.0 and 7.0 were 100.4%, 100.7% and 99.2% after storage for 4 weeks at 60 °C, respectively. The possibility of room temperature storage for the latanoprost ophthalmic lipid emulsion was demonstrated.
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Keywords: Latanoprost; Ophthalmic lipid emulsion; Polyvinyl alcohol; Stability

Formulation studies of ophthalmic preparations have often been confronted with a problem of drug instability in water. Drugs that were unstable in water were formulated by the addition of stabilizing agents such as antioxidant agents and chelating agents (Martin et al., 1993). Epinephrine solution was prevented from oxidative degradation by the addition of ascorbic acid (Thoma and Struve, 1986). Another approach is to keep the drugs in a low storage temperature to suppress decomposition reactions. There are many ophthalmic products that should be stored in a refrigerator at low temperature (PDR-Staff, 2005), but it may impair compliance and the convenience of patients.

Latanoprost ((+)-isopropyl (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate) is an ester prodrug analogue of prostaglangin F2α (Liljebris et al., 1995) that has been investigated for potential treatment of primary open-angle glaucoma and ocular hypertension (Fig. 1) (Camras et al., 1989; Villumsen et al., 1989).

Xalatan® (Pfizer, New York, NY, USA), latanoprost ophthalmic solution, has been exploited commercially in many countries in the world. The storage condition of Xalatan® is at low temperature (2–8 °C). Latanoprost is not stable in water against heat stress (Morgan et al.,

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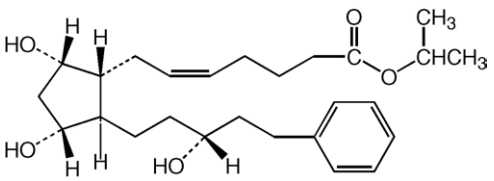

Fig. 1. Structure of latanoprost: (+)-isopropyl (*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate.

2001), which is provably due to hydrolysis of isopropyl ester in latanoprost molecule.

Preparation of an oil-in-water (o/w) type lipid emulsion is one of the approaches to stabilize a drug that is unstable in water if oil/water partition coefficient of the drug is high, because there is little water in the oil phase of the emulsions. Although emulsifier is needed to manufacture lipid emulsions, high concentration of surfactants may cause ocular toxicity (Vandamme, 2002). Water-soluble polymers (Dickinson, 1992) were used as emulsifiers instead of surfactants, since they can form a thick adsorbed layer, which would play a role of stabilizer of oil droplets (Hayakawa et al., 1997). Polyvinyl alcohol (PVA) is widely applied to pharmaceutical preparations due to its history of usefulness and safety (NTP-Study, 1998).

Latanoprost ophthalmic preparation stable at room temperature is a requisite. Therefore, we formulated latanoprost ophthalmic lipid emulsion having good stability against heat stress using PVA as an emulsifier.

Latanoprost (commercially available material) was used. Xalatan® (Pfizer Japan, Shibuya-ku, Tokyo, Japan) was used as control formulation in stability experiments. Medium chain fatty acid triglyceride was obtained from Mituba Boeki (Shinjyuku-ku, Tokyo, Japan). Peanut oil, soybean oil, olive oil, rapeseed oil, cottonseed oil and tung oil were purchased from Nacalai Tesque (Nakagyo-ku, Kyoto, Japan). Polyvinyl alcohol (Gohsenol EG05) was purchased from Nippon Gohsei (Chuo-ku, Tokyo, Japan). Water was purified with an auto still (Model WG220, Yamato, Chuo-ku, Tokyo, Japan). Acetonitrile (HPLC grade) was purchased from Kanto Chemical (Chuo-ku, Tokyo, Japan). Other reagents were HPLC grade or the highest grade commercially available.

An HPLC system (LC-10A, Shimadzu, Nakagyo-ku, Kyoto, Japan) was composed of an autosampler (SIL-10ADvp), a pump (LC-10ADvp), a column oven (CTO-10ASvp), a UV detector (SPD-10AVvp) and data processing software (CLASS-VP). An octadecylsilica column (Waters Xterra RP18, 150, 4.6 mm i.d., Nihon Waters, Shinagawa-ku, Tokyo) was also used. Analysis of latanoprost was carried out using 35% (v/v) acetonitrile containing 10 mM sodium octane sulfate (pH 3.5 adjusted by hydrochloric acid) as mobile phase at a flow rate of 1.5 ml/min at 25 °C. Detection was performed at 210 nm. The injection volume was 50 μl.

To find the most suitable oil for our formulation, latanoprost (5 mg) was dissolved in medium chain fatty acid triglyceride, peanut oil, soybean oil, olive oil, rapeseed oil, cottonseed oil and tung oil (1 g) by stirring for 30 min at room temperature. After confirming the dissolution by naked eye observation, the oils were filled in 5 ml glass ampoules and were stored for 7 days at 80 °C, latanoprost content was determined by HPLC.

Oil-in-water emulsions adjusted to pH 5.0, 6.0 and 7.0 were prepared containing latanoprost (0.005% (w/v)), medium chain fatty acid triglyceride (1.0% (w/v)) as oil phase, PVA (2.0% (w/v)) as emulsifier, and sodium acetate (0.1% (w/v)) or sodium borate (0.1% (w/v)) as a buffering agent. Preparation of emulsion was performed in three steps. As the initial step, 450 ml of water was placed in 1000 ml glass beaker, and PVA (20.0 g) and glycerin (26.0 g) were then added to the water and dissolved at 70 °C. Separately, latanoprost (0.01 g) was dissolved in medium chain fatty acid triglyceride (10 g) at 70 °C. The medium chain fatty acid triglyceride containing latanoprost was then added to the solution previously heated to 70 °C and emulsified by a homogenizer (Robomics, Tokusyukikaikogyo, Fukusima-ku, Osaka, Japan) at 8000 rpm for 15 min. The coarse-emulsion was obtained after adjusting to a fixed volume by water (500 ml). As the second step, the coarse-emulsion was treated by a high-pressure emulsifier (Microfluidizer M-110EH, Microfluidics corporation, Newton, MA, USA). The inlet pressure was 1.47×10^5 kPa. The individual batches were processed through Microfluidizer M-110EH for 10 discrete volume cycles, and collected into glass beakers. To cool the emulsion, running water, of which the temperature was controlled at 40 °C around the metal coil, dissipated the heat produced during the microfluidization process. After the treatment, the emulsion was cooled to room temperature (25 °C). As the third step, 0.2% (w/v) sodium acetate buffer adjusted at pH 5.0 and 6.0, and 0.2% (w/v) borate

Table 1
Stability of latanoprost in oil at 80 °C

Oil	Stability (assay vs. initial, %)
Medium chain fatty acid triglyceride	104.4
Peanut oil	90.2
Soybean oil	82.4
Olive oil	78.4
Rapeseed oil	70.5
Cottonseed oil	67.3
Tung oil	42.7

buffer adjusted at pH 7.0 were prepared. The emulsion was two-fold diluted by adding the buffer, and then re-adjusted the appropriate pH by hydrochloric acid solution or sodium hydroxide solution.

To evaluate latanoprost concentration in water phase of the lipid emulsion just after manufacturing, the water phase was separated by ultrafilter membrane (Ultrafree-CL (10,000 NMWL), Millipore, Minato-ku, Tokyo), and then the latanoprost concentration in water phase was assayed by HPLC.

Latanoprost ophthalmic lipid emulsion was filled in 5 ml glass ampoules. Latanoprost ophthalmic solution, Xalatan®, was also filled in 5 ml glass ampoules. The emulsion and the solution were stored for an appropriate period at 25 and 60 °C, and the latanoprost content was monitored in a single assay by HPLC during the stability program.

The results of the stability of latanoprost in various oils are summarized in Table 1.

The amount of latanoprost was 104.4% of the initial in medium chain fatty acid triglyceride after storage for 7 days at 80 °C. However latanoprost was not stable in soybean oil and the amount was 82.4% after storage for 7 days at 80 °C. The amount of latanoprost in peanut oil was 90.2%, and amounts in the other oils were under 78.4%, which were not sufficient for preparation of an ophthalmic lipid emulsion. Therefore, we chose medium chain fatty acid triglyceride as the lipid phase in the present study.

We selected PVA, which is a water-soluble polymer, as emulsifier of the lipid emulsion due to its history of usefulness and safety in the ophthalmic area. The lipid emulsion containing 1.0% (w/v) medium chain fatty acid triglyceride and 2.0% (w/v) PVA was prepared using a high-pressure emulsifier. Stability of latanoprost ophthalmic lipid emulsion was examined in glass ampoules. Results of stability were summarized in Table 2.

Latanoprost content in Xalatan® decreased to 76.4% after storage for 4 weeks at 60 °C. In contrast, latanoprost in ophthalmic lipid emulsions with water phase adjusted to pH 5.0, 6.0 and 7.0 was very stable, and the assays were 100.4%, 100.7% and 99.2% after storage for 4 weeks at 60 °C, respectively. Latanoprost concentrations in water phase of the emulsions adjusted at each pH were under detection limit by HPLC (detection limit 51.7 ng/ml, SN = 3). The results suggested that the oil/water partition coefficient of latanoprost was sufficient to confine it in oil phase and pH of the emulsions did not affect the oil/water partition coefficient of latanoprost. Decrease of latanoprost content both in Xalatan® and in ophthalmic lipid emulsions was not observed under experimental condition for 4 weeks at 25 °C, and the contents were 98.5%, 101.2%, 100.5% and 99.8% in Xalatan® and ophthalmic lipid emulsion adjusted at pH 5.0, 6.0 and 7.0, respectively. The results

Table 2
Stability of latanoprost ophthalmic lipid emulsions and Xalatan®

Formulation	Storage condition (°C)	Assay of latanoprost (%)			
		Initial	1 week	3 weeks	4 weeks
Ophthalmic lipid emulsion (pH 5.0)	25	100.0	102.1	99.7	101.2
	60	100.0	103.5	99.7	100.4
Ophthalmic lipid emulsion (pH 6.0)	25	100.0	100.4	99.3	100.5
	60	100.0	99.3	99.1	100.7
Ophthalmic lipid emulsion (pH 7.0)	25	100.0	100.0	98.2	99.8
	60	100.0	99.0	100.3	99.2
Xalatan® (pH 6.5–6.9)	25	100.0	100.7	101.4	98.5
	60	100.0	94.4	86.5	76.4

were supported by the report of Morgan et al. (2001), which mentioned that the stability of latanoprost was strongly temperature-dependent.

In the present study, stability of latanoprost in an ophthalmic lipid emulsion was examined. The stability of latanoprost was improved in the ophthalmic lipid emulsion, although there is limited stability data due to an ongoing investigation. The possibility of the storage at room temperature for the latanoprost ophthalmic lipid emulsion was demonstrated. The physicochemical stability of the ophthalmic lipid emulsion containing latanoprost should be further investigated.

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