Pakistan J. Zool., vol. 54(4), pp 1927-1930, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20200722050704

Short Communication

Studies on Stability of Alprostadil (Lipo-PGE1 and DN-PGE1) Targeting Preparation

Fan Xu[1,2]*, Cheng Wu[3] and Su Zeng[1]

[1]*Institute of Drug Metabolism and Pharmaceutical Analysis, Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, 310058, China*

[2]*920 Hospital of Joint Logistics Support Force, Kunming, 650032, China*

[3]*Department of Health Statistics, Naval Medical University, Shanghai, 200433, China*

ABSTRACT

Objective of this study was to investigate the compatibility and stability of alprostadil (lipo-PGE1 and DN-PGE1) after compatibility with different volume of 0.9% sodium chloride or 5% glucose, and to provide a basis for drug safe use in the clinic. PGE1 lipid microspheres (lipo-PGE1) and alprostadil dried emulsion (DN-PGE1) were compatibility with different volumes of 0.9% sodium chloride and 5% glucose, respectively. After the compatibility of different concentrations, the changes of pH, particle size distributions, the particles greater than 5μm and encapsulation rate of the compatibility solutions were observed within 8 h by using electronic acidimeter, Malvern zeta sizer nanoparticle size analyzer, accusizer APS 780 particle size analyzer and Agilent 6460 LC-MS/MS mass spectrometer. From the experimental results, 5 μg:50ml group kept good targeting within 6 h. The encapsulation rate of 5 μg:100ml group decreased significantly after 6 h, and the percentage of fat globules > 5 μ m ($PFAT_5$) in 10 μg:10ml group was relatively high. The risk of embolism was easily caused by injection or small pot dripping, which may be related to the insufficient amount of solvent and the incomplete dispersion of lipid microspheres. As for the two dosage forms of lipo-PGE1 and DN-PGE1, the average particle size and $PFAT_5$ after the preparation of DN-PGE1met the USP standard, and the stability indexes were more advantageous. The clinical treatment of alprostadil targeted injection can be prepared by pharmacy intravenous admixture service. The drug concentration should be more than 0.05 μg/ml, and the drug concentration 0.1 μg/ml is the most appropriate. Lipo-PGE1 and DN-PGE1 injection can be administered by intravenous drip. The finished infusion should be used up within 6 h after the dispensing.

Article Information
Received 22 July 2020
Revised 28 August 2020
Accepted 16 September 2020
Available online 12 August 2021 (early access)
Published 24 April 2022

Authors' Contribution
FX and SZ conceived the idea of the research and designed the experiments. FX conducted the experiments. FX and CW analyzed the results. All authors discussed the results and wrote the manuscript.

Key words
Alprostadil injections, Lipo-PGE1, DN-PGE1, Stability

Prostaglandin E1 (PGE1), another name as alprostadil, is an autacoid drug. It is effective in treating patients with various peripheral vascular occlusive disorders (Martin and Tooke, 1982) and with spontaneous pain and sensory disturbance due to diabetic neuropathy and diabetic ulcers (Low *et al*., 1986). However, the clinical application of PGE1 is limited due to its adverse reactions to frequently causes local pain on administration and low bioavailability. To overcome these problems, several new alternative dosage forms of PGE1, such as lipid microspheres (Mizushima *et al*., 1983), cyclodextrin clathrate (Yabek and Mann, 1979), nanoparticles (Ishihara *et al*., 2008) and nano emulsion (Preece, 2017) can prevent PGE1 from inactivation in blood and improve the efficacy of PGE1. Among these new dosage forms, cyclodextrin clathrate prostaglandin E1 (PGE1-CD) injected intravenously is rapidly inactivated in the lungs and a high dose is necessary for the treatment of these disorders furthermore renal toxicity of beta-cyclodextrin limited its clinical use (Lewis *et al*., 1981; Peled *et al*., 1992). PGE1 lipid microspheres (lipo-PGE1) which incorporated PGE1 into lipid microspheres were established by Mizushima *et al*. (1983) and alprostadil dried emulsion (DN-PGE1) which incorporated PGE1 into nanoemulsion and freeze-dried at low temperature by Chongqing Yaoyou Pharmaceutical Co., Ltd. Since they can deliver the encapsulated PGE1 efficiently to disease sites, lipo-PGE1 and DN-PGE1 were used widely in the clinic.

This type of alprostadil targeted formulation clearly states in its instructions: Alprostadil injection is administered once a day for adults, 1-2ml (5-10μg of alprostadil) + 10ml of normal saline (or 5% Glucose) intravenously (Okuno *et al*., 2020; Fan *et al*., 2019). In clinical application, how to formulate this type of infusion to ensure the safety and effectiveness of alprostadil is

* Corresponding author: Xu_fan@126.com
0030-9923/2022/0004-1927 $ 9.00/0

a matter of great concern. In this study, the stability of alprostadil targeted formulations (alprostadil lipid microsphere injection and alprostadil lipid microsphere dry emulsion) in different volumes and different kinds of solvent were investigated by using comparative experimental methods. We aimed to provide a basis for the safe use of this formulation in clinical practice.

Materials and methods

Alprostadil (99%) was purchased from Taiwan Yongguang Chemical Co., China; E0018506 and Alprostadil injection from Beijing Teide Pharmaceutical Co., China; 1B058H, 1B068H, 1B078H and Alprostadil emulsion from Chongqing Youyao Pharmaceutical Co., China; 18220960, 18220190, 18220020 were used for the experiment.

For measurement of precision and accuracy Alprostadil was dissolved in methanol and diluted to 20, 5, and 1 ng/ml and used for injection. Each concentration was measured six times and was injected on different days. Three batches were prepared and measured to calculate intra-batch and inter-batch precision and accuracy. The results showed that the intra-precisions among three concentrations (RSD) were 8.52%, 5.92%, and 7.12%. The inter-precisions among three concentrations (RSD) were 11.14%, 6.83%, and 9.52%. The accuracy of three concentrations (RE) was 12.14%, 6.83%, and -5.52%. The results show that the precision and accuracy of alprostadil is good under the conditions of this study.

For measurement of stability alprostadil was dissolved in methanol and diluted to 20 and 1 ng/ml. Each concentration was prepared for 6 samples. Three samples were placed in the sampler, and measured after being placed for 0 h, 2h, 4h, and 8h; Other three samples were placed at room temperature and measured after being placed for 0 h, 2 h, 4 h, and 8 h. The results showed that the stability of alprostadil was good.

For measurement of appearance and pH three new prostol preparations Kaishi, Mencito, and Youdier were thoroughly mixed with saline injection or 5% glucose injection, to prepare three concentrations of 1.0, 0.1, and 0.05 μg/ml. This operation was reported for 3 batches of each alprostadil targeted preparation product. The appearance of compatibility solution was observed and then pH was recorded using Mettler Seven Easy pH meter, Mettler Toledo Instruments, Switzerland.

Two ml samples of the compatibility solution of prostol preparations Kaishi and Youdier were taken at 0, 1, 2, 3, 4, 6, and 8h, after prepration and the changes in average particle size and 90% cumulative particle size were measured with the Malvern Zetasizernano particle size analyzer (Malvin Instruments, UK). Three parallel tests were conducted for each rinse solution. Particles greater than 5 μm were detected by Accusizer 780/ APS (PSS particle size analyzer company, USA). Three parallel tests were conducted for each punch.

For measurement of encapsulation rate at different times, 1ml of each rinse solution was sampled on the gel column (Hitrap gel column, 5ml), and then eluted with acetic acid buffer at pH 4.5 (see Chinese pharmacopeia buffer). 5 ml was dissolved by isopropanol into 25 ml. After shaking, the sample solution 1 was obtained, and the enveloped PGE1 was determined by LC-MS/MS (Agilent 6460 LC -MS/MS mass spectrometer, USA). Another 1 ml sample was dissolved by isopropanol into 25 ml. After shaking, the sample solution 2 was obtained, and the enveloped PGE1 was also determined by LC-MS/MS. The encapsulation rate is calculated according to the following formula:

Encapsulation rate %= encapsulation PGE1/ total PGE1×100

For determination of alprostadil and its degradation product PGA1 at different times,1 ml of each rinse solution was dissolved by isopropanol into 10 ml. After shaking, the sample solution was obtained. LC-MS/MS was used to determine alprostadil and PGA1 in the sample solution.

For LC-MS/MS chromatographic conditions column were Agilent XDB C18 (2.1 × 50mm, 1.8μm); mobile phase: A is 0.05% glacial acetic acid aqueous solution, B is 0.05% glacial acetic acid methanol solution; flow rate: 0.3ml / min; injection volume: 20μl; with gradient wash in LC-MS/MS as shown below.

Time (min): 0, 4, 4.01, 6; A% 35, 0, 35, 35. MS conditions ESI ion source; dry gas flow rate: 8 L/min, dry gas temperature: 350 °C, atomizing gas pressure: 25 psi, capillary voltage: -3500 V; negative ion detection. Scanning method is Multiple Reaction Monitoring (MRM). The ions are: parent ion 353.2, daughter ion 317.2, fragmentation voltage 110 V, and collision energy 16 V.

Results and discussion

Comparing the pH and average particle size difference of two different dosage forms of alprostadil at different diluent volumes in two different solvents, the results showed that the difference of solvent type and placement time was not statistically significant. There were differences in pH and average particle size between dosage forms, and the pH value of lipo-PGE1 was lower after punching and the mean particle size of the lipo-PGE1 was larger after punching. The influence of dilution volume on pH and the mean particle size were different. The pH of 5μg:100ml was lower and the mean particle size was greated than 10μg:10ml, and the difference between 5μg:50ml and 10μg:10ml was not statistically significant. There was no difference in dosage, solvent, and volume

over time (Table I).

Comparing the $PFAT_5$ and encapsulation rateschanges of two different dosage forms of alprostadil in two different solvents with different dilution volume, the results showed that the difference of dosage type, solvent type, dilution volume and placement time was not statistically significant. There was no difference in the type of solvent over time. Compared with DN-PGE1, there were more particles larger than 5μmin lipo-PGE1 with the extension of the placement time. 5μg:50ml had the least number of particles greater than 5 μm over time, followed by 5μg:100ml and 10μg:10ml (Table I).

Table I. Analysis results of pH mean particle size, particles greater than 5μm and encapsulation rate variation mixed linear model.

Effect	Num DF	Den DF	F value	Pr > F
pH				
Dosage	1	62	17.23	0.0001
Solvent	1	62	2.19	0.1440
Dilution volume	2	62	5.01	0.0096
Time of placement	1	62	0.00	0.9768
Time* Dosage	1	62	0.12	0.7265
Time* Solvent	1	62	0.00	0.9920
Time* Dilution volume	2	62	0.03	0.9731
Mean particle size				
Dosage	1	62	199.49	<.0001
Solvent	1	62	2.12	0.1504
Dilution volume	2	62	4.17	0.0200
Time of placement	1	62	0.01	0.9324
Time * Dosage	1	62	6.45	0.0136
Time * Solvent	1	62	0.05	0.8280
Time * Dilution volume	2	62	0.45	0.6419
Particles greater than 5μm				
Dosage	1	62	0.95	0.3331
Solvent	1	62	0.06	0.8003
Dilution volume	2	62	0.11	0.8927
Time of placement	1	62	0.06	0.8053
Time * Dosage	1	62	7.13	0.0097
Time * Solvent	1	62	0.38	0.5373
Time * Dilution volume	2	62	8.87	0.0004
Encapsulation rate				
Dosage	1	62	0.64	0.4282
Solvent	1	62	0.46	0.4982
Dilution volume	2	62	7.50	0.0012
Time of placement	1	62	0.06	0.8152
Time * Dosage	1	62	3.29	0.0747
Time * Solvent	1	62	3.57	0.0635
Time * Dilution volume	2	62	13.08	<.0001

Note: SAS 9.4 is adopted to take time as a random effect; dose, solvent, volume as fixed effect.

The encapsulation rate of 5μg:100ml was significantly lower than that of 10μg:10ml in different diluted volumes, and the difference between 5μg:50ml and 10μg:10ml was not statistically significant (Table I).

The instructions limit the solvent to 10ml and require immediate use. This is mainly because the use of large amounts of solvent will destroy the structure of lipid microspheres in the targeted drugs of alprosidil injection, which will dissolve the drug into the solvent. On the one hand, it will increase the incidence of adverse drug reactions (phlebitis); on the other hand, it will also destroy the targeting of the drug. However, other clinical studies on the treatment of different diseases by intravenous infusion of 50-100ml 0.9% sodium chloride after infusion of proprodil targeting preparations and achieved good therapeutic effects (Liang *et al*., 2018; Hao *et al*., 2017; Wang *et al*., 2016). Tian *et al*. (2017) conducted a systematic evaluation of phlebitis caused by different administration methods of alprostadil injection. Intravenous infusion of alprostadil was more recommended by the conclusion.

However, from the perspective of infection control and infusion safety, the safety of intravenous drug dispensing centers for treatment infusion is much higher than that of nurses in an exposed environment. Intravenous injection not only increases the difficulty of clinical nursing but also increases the risk of infection. Therefore, the clinical application of alprostadil targeted injection by intravenous drip is relatively common, and the treatment is effective (Liang *et al*., 2018; Yi-na *et al*., 2014).

Since the inner diameter of the venous capillaries is 4~9 μm, in a clinical continuous infusion, excessive particles with a diameter of > 5 μm may block the capillaries after entering the systemic circulation, leading to the inflammatory response. Particle retention in the lungs will cause pulmonary embolism and liver function impairment (Yi-na *et al*., 2014). Therefore, the size and size distribution of lipo-particles are important indexes to evaluate the stability and safety of injection. The United States Pharmacopoeia (USP) (Kelley *et al*., 2012) stipulates that MDD < 500 nm and $PFAT_5 \leq 0.05\%$. The national drug standard (Hutt, 2008) stipulates that MDD should be 120~280 nm. In this study, the dynamic light scattering method was used to measure MDD, which has a low sensitivity to the ions with a diameter of >1 μm. Therefore, light shading-single particle optical sensing technology (LO/SPOS) was used to detect $PFAT_5$. LO/SPOS technology is highly sensitive to large liposomes. It can accurately measure the size and number of large liposomes by detecting and counting the large size particles one by one, instead of obtaining an approximate value based on mathematical transformation. Encapsulation rate is an important index to evaluate the preparation

technology and quality of lipid microspheres, and it is also the key to improve drug treatment index, reduce adverse drug reactions, and reduce drug dose. In this study, gel chromatography was used to separate lipid microspheres from free drugs by the difference in molecular weight and particle size. The larger size of the lipid microspheres was eluted first, and the smaller size of the free drug was eluted to achieve the separation effect. After separation, the enriched samples were completely dissolved with 4 times the volume of isopropanol. The content of PGE1 was determined by LC-MS /MS and the encapsulation rate was calculated.

According to the experimental results, the 5μg: 50ml group maintained good targeting of the preparation within 6h. The encapsulation rate of 5 μg:100ml group decreased significantly after 6h. The percentage of $PFAT_5$ in 10 μg:10ml group was higher. The risk of embolization is easily caused by push injection or small pot infusion, which may be related to the insufficient amount of solvent and the inability to completely disperse the lipid microspheres. As for lipo-PGE1 injection and DN-PGE1 injection, the average particle size and $PFAT_5$ percentage of DN-PGE1 are in line with USP standards, and the stability indicators are more dominant.

Conclusion

The clinical treatment of alprostadil targeted injection can be dispensed by pharmacy intravenous admixture service. The concentration of the drug should over 0.05 μg /ml, and the concentration of the drug 0.1 μg /ml is the most appropriate. The drug can be administered by intravenous infusion, and the finished infusion should be used within 6 h after deployment.

Acknowldegments

The research is supported by the program of training young and middle-aged academic and technical leaders in Yunnan Province (No. 2017HB052).

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20200722050704

Statement of conflict of interest

The authors have declared no conflict of interest.

References

Driscoll, D.F., Ling, P.-R., Silvestri, A. and Bistrian, B., 2008. *Clin. Nutr.,* **27**: 889-894. https://doi.org/10.1016/j.clnu.2008.08.011

Fan, Q., Zhang, L., Zhong, M. and Dong, L., 2019. *Interventional effect of off-label use of alprostadil lipid microsphere injection in neurosurgical patients: A before-after study*. Preprint, https://doi.org/10.21203/rs.2.12797/v1

Hao, Y., Zhu, W., Yuhe, K., Zhihua, Y., Tian, L., Duan, G., Wen, F. and Zheng, Q., 2017. *Chinese J. Cardiovas. Rehab. Med.,* **26**: 402-405.

Hutt, P.B., 2008. *Admin. L. Rev.,* **60**: 431.

Ishihara, T., Takahashi, M., Higaki, M., Takenaga, M., Mizushima, T. and Mizushima, Y., 2008. *Pharm. Res.,* **25**: 1686-1695. https://doi.org/10.1007/s11095-008-9549-8

Kelley, K.E., Hernández-Díaz, S., Chaplin, E.L., Hauser, R. and Mitchell, A.A., 2012. *Environ. Hlth. Perspect.,* **120**: 379-384. https://doi.org/10.1289/ehp.1103998

Lewis, A.B., Freed, M.D., Heymann, M.A., Roehl, S.L. and Kensey, R.C., 1981. *Circulation*, **64**: 893-898. https://doi.org/10.1161/01.CIR.64.5.893

Liang, M., Yang, S., Fu, N., Lu, C., Tian, F., Xing, X., Lin, W. and Liu, J., 2018. *Catheter. Cardiovasc. Interv.,* **91**: 742-750. https://doi.org/10.1002/ccd.27353

Low, P., Schmelzer, J., Ward, K. and Yao, J., 1986. *Am. J. Physiol. Endocrinol. Metab.,* **250**: E94-E99. https://doi.org/10.1152/ajpendo.1986.250.1.E94

Martin, M. and Tooke, J., 1982. *Br. med. J. (Clin. Res. Ed.)*, **285**: 1688-1690. https://doi.org/10.1136/bmj.285.6356.1688

Mizushima, Y., Yanagawa, A. and Hoshi, K., 1983. *J. Pharm. Pharmacol.,* **35**: 666-667. https://doi.org/10.1111/j.2042-7158.1983.tb02862.x

Okuno, T., Morimoto, S., Nishikawa, H., Haraguchi, T., Kojima, H., Tsujino, H., Arisawa, M., Yamashita, T., Nishikawa, J. and Yoshida, M., 2020. *Chem. Pharm. Bull.,* **68**: 234-243. https://doi.org/10.1248/cpb.c19-00736

Peled, N., Dagan, O., Babyn, P., Silver, M. M., Barker, G., Hellmann, J., Scolnik, D. and Koren, G., 1992. *N. Engl. J. Med.,* **327**: 505-510. https://doi.org/10.1056/NEJM199208203270801

Preece, K.E., 2017. *Protein extraction during soybase production: Enhancing yields using cavitational techniques.* University of Birmingham.

Tian, F., Zou, M., Bin, W., Ting, X. and Jiang, X., 2017. *China Pharm.,* **28**: 2955-2958.

Wang, W., Lu, Z.-Y., Ren, Z. and Zhang, G.-Z., 2016. *J. Hainan med. Univ.,* **22**: 40-43.

Yabek, S.M. and Mann, J.S., 1979. *Chest,* 76: 330-331. https://doi.org/10.1378/chest.76.3.330

Yi-na, L., Shi-cheng, Y. and Nai-kuan, F., 2014. *Chinese J. Geriat. Heart Brain, Vessel Dis.,* 580-583.