Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 290 (2005) 101–108
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

# Preparation of prostaglandin $E_1$-hydroxypropyl-β-cyclodextrin complex and its nasal delivery in rats

Fu-gen Gu[a], Fu-de Cui[a,*], Yong-liang Gao[b]

[a] *Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China*
[b] *Department of Pharmaceutics, Beijing Institute of Pharmacology and Toxicology, Beijing 100850, PR China*

Received 18 September 2004; received in revised form 18 November 2004; accepted 21 November 2004

**Abstract**

The potential use of hydroxypropyl-β-cyclodextrin (HP-βCD) in the solubilization and stabilization of prostaglandin $E_1$ ($PGE_1$) was investigated. The solubility and chemical stability of $PGE_1$ were significantly improved upon complexation with HP-βCD. The nasal delivery of $PGE_1$ from the complex formulation was also studied in Wistar rats and compared with intravenous administration. $PGE_1$ complex after nasal administration caused a rapid decrease of blood pressure and exhibited an obvious dose-efficacy relationship, showing results nearly similar to those obtained for intravenous route. The time to reach the peak effect ($T_{max}$) was approximately 3–4 min. Except $T_{max}$, other pharmacodynamic parameter values such as the maximal percent of blood pressure decrease ($E_{max}$, %), the lasting time of effect ($T_d$), and the area under the curve (AUC, blood pressure decrease % min) were increased with increasing the administered doses. The $E_{max}$, $T_d$, and in particular AUC values between doses were significantly different ($P < 0.01$), but $T_{max}$ between doses were not significantly different ($P < 0.05$). The AUC values per unit dose of $PGE_1$ for nasal administration, however, were smaller than those for intravenous route, probably due to the incomplete absorption of nasally administered $PGE_1$. Besides, the in vitro effect of the $PGE_1$ complex on nasal mucociliary movement was also investigated with a toad palate model. The $PGE_1$ complex formulation exerted only minor effect on nasal mucociliary movement. These results indicate that the $PGE_1$–HP-βCD complex formulation for nasal delivery is a very promising preparation with advantages such as rapid and effective absorption, good chemical stability, ease of administration, and minor nasal ciliotoxicity.


*Keywords:* Prostaglandin $E_1$; HP-βCD; Complex; Solubility; Stability; Nasal delivery

## 1. Introduction

Prostaglandin $E_1$ ($PGE_1$) is known to have various physiological and pharmacological activities, such as vasodilation, reduction of blood pressure, angiogen-

* Corresponding author. Tel.: +86 24 23843711 3736; fax: +86 24 23843711 3736.
*E-mail address:* cuifude@163.com (F.-d. Cui).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.11.021

esis, and inhibition of platelet aggregation (Wallace, 1992; Igarashi et al., 2001). The drug has therefore been widely used in the treatment of severe peripheral arterial occlusive disease, adult respiratory distress syndrome, impotence, pulmonary hypertension, ischaemic heart disease and congestive cardiac failure, etc. (Mizushima et al., 1983; Virag and Adaikan, 1987; Wang, 2002). However, the chemical instability as well as the low aqueous solubility of the drug have limited the development of its new dosage forms and presented a substantial challenge to the pharmaceutical scientist (Yamamoto et al., 1992). Presently, cyclodextrins (CDs) such as αCD, βCD, γCD, carboxymethyl-ethyl-βCD (CME-βCD), and maltosyl-βCD ($G_2$-βCD) have been reported to be able to form complexes with $PGE_1$, resulting in the improvement in the solubility and stability of the drug (Uekama et al., 1984; Wiese et al., 1991; Adachi et al., 1992; Yamamoto et al., 1992). For example, a solid, lyophilized complex (Prostavasin) of $PGE_1$ and αCD (ratio 1:11.8) has been introduced into the market. Besides, $PGE_1$ are scarcely absorbed following oral administration as a result of extensive degradation in the gastrointestinal tract, so it is mainly administered by parenteral route. The most often used administration route of $PGE_1$ is by intravenous infusion, but this usually leads to high noncompliance in patients due to its adverse effects such as a redness of the upper arm vein, feeling of pressure and warmth in the lower arm, pain at the injection site, etc. (Trübestein et al., 1989; Cawello et al., 1995).

Recently, intranasal administration of drug has received considerable attention because of the noninvasive route, rapid absorption, circumvention of gastrointestinal tract and liver first-pass metabolism, ease of administration, and self-medication (Behl et al., 1998). Thus, the nasal route may be an ideal alternative to the parenteral administration of $PGE_1$.

HP-βCD, a new derivative of βCD, has a greater aqueous solubility and higher safety than the aforementioned CDs (Carpenter et al., 1995). Therefore, the objective of present study was to investigate the effect of HP-βCD on the solubility and stability of $PGE_1$ and to further explore the nasal absorption of the drug from its HP-βCD complex formulation in rats. Since a prerequisite for nasal drug delivery is that drugs and additives should not disturb normal nasal functioning, the effect of $PGE_1$–HP-βCD complex on nasal mucociliary movement were also studied in this study.

## 2. Materials and methods

### 2.1. Materials

$PGE_1$ with a purity of 99.0% was kindly supplied by Nanyang Pukang Group Chemical Pharmaceutical Factory (Nanyang, China). HP-βCD with an average degree of substitution of 5.0 was purchased from Shanxi Liquan Chemical Industrial Company (Shanxi, China). Acetonitrile was of HPLC grade from Fisher Scientific (New Jersey, USA). All other chemicals and solvents were of analytical reagent grade obtained from commercial sources and used as received without further purification. Deionized double-distilled water was used throughout.

### 2.2. HPLC analysis

The concentration of $PGE_1$ was determined by HPLC according to the reported method (Gatti et al., 1995) with minor modification. A HPLC system consisted of a pump (Model P580, Dionex, USA) and a UV detector (Model 170S, Dionex, USA). The HPLC analyses were performed at ambient temperature on a Kromasil $C_{18}$ column (5 μm, 150 mm × 4.6 mm, Zirchrom, Sweden) using a mobile phase of 0.01 M $KH_2PO_4$ solution (pH 3.5) acetonitrile (58:42, v/v). The flow rate was 1.0 ml/min and detection wavelength was set at 205 nm. An injection volume of 20 μl was used and peak areas were measured to determine the concentration of $PGE_1$.

### 2.3. Solubility studies

Solubility determination was carried out according to Higuchi and Connors' method (Dollo et al., 1999). Excess amounts of $PGE_1$ were added to aqueous solutions containing increasing concentrations of HP-βCD ranging from 0 to 10 mM and were then shaken at 25 °C for 48 h (equilibrium and absence of drug degradation were confirmed in preliminary studies). After equilibrium, an aliquot was filtered through a 0.45 μm membrane filter and analyzed by HPLC. The apparent stability constant ($K_c$) for complex was calculated from

the slope and intercept of the straight portion of the phase solubility diagram.

### 2.4. Preparation of $PGE_1$ complex

$PGE_1$–HP-βCD complex was prepared by the freeze-drying method in a 1:10 molar ratio, which was reported to be a suitable ratio used in $PGE_1$ complex formulations (Wiese et al., 1991; Yamamoto et al., 1992; Gu et al., 2004). Accurately weighted $PGE_1$ was dissolved in minimal volume of absolute ethanol, while required amount of HP-βCD dissolved in distilled water, after which, the two solutions were mixed together and the resultant solution frozen by immersion into liquid nitrogen and freeze dried for 24 h. The solid complex was kept under vacuum in a desiccator for 48 h.

### 2.5. Stability studies

Appropriate amount of $PGE_1$ and its HP-βCD complex (equivalent to 100 μg $PGE_1$) were put in test tubes, sealed tightly with glass-stoppers, and stored in an incubator at 60 °C. At suitable time intervals, the samples were taken and intact $PGE_1$ in the samples was assayed by HPLC method described above.

### 2.6. Nasal absorption studies

For the convenience of nasal administration, the $PGE_1$ complex was dissolved in 0.9% (w/v) saline to the desired concentrations (1000, 500, 250 μg/ml as $PGE_1$, respectively), and $PGE_1$ solutions for intravenous injection was prepared by dissolving the marketed $PGE_1$ lyophilized preparation for injection in 0.9% (w/v) saline to the concentrations (20, 10, 5 μg/ml, respectively) prior to the experiment. Nasal absorption studies were performed as reported earlier (Schipper et al., 1990). Briefly, 15 male Wistar rats of approximately 200 g were randomly divided into three groups containing five rats each, i.e. the low, middle and high dose groups. They were anesthetized with 50 mg/kg of pentobarbital intraperitoneally and were placed on a warming plate maintained at 37 °C, after which, the trachea was canulated with a polyethylene tube to prevent nose respiration, and the oesophagus was tied to this cannula to prevent peroral absorption. For intravenous administration, however, the trachea cannula and the oesophagus ligation were omitted. Finally, a polyethylene catheter was inserted into the left carotid artery to measure the blood pressure using a pressure transducer (Medlab-U/4CS Biological Signal Collection and Processing System, Nanjing Meiyi Scientific and Technological Company, China). The $PGE_1$ complex were administered at 25, 50, and 100 μg/kg (10 μl/100 g body weight) unilaterally through the nares using PVC tubing connected to a microliter syringe, while the intravenous $PGE_1$ solution were given at 2.5, 5.0, and 10 μg/kg (50 μl/100g body weight) via the femoral vein. Blood pressure changes were recorded immediately after administration, and the blood pressure change percent (%) were plotted versus time. The pharmacodynamic parameters such as the time to reach peak effect ($T_{max}$), the maximal percent of blood pressure decrease ($E_{max}$, %) and the duration of effect ($T_d$) were directly obtained from the blood pressure change percent (%) time curves, while the area under the curve (AUC, blood pressure decrease % min) from the start of hypotensive action to the end was calculated using the trapezoidal rule. The AUC was used to show the total efficacy of each dose or each administration route.

### 2.7. Nasal ciliotoxicity studies

The $PGE_1$ complex was dissolved in 0.9% (w/v) saline to the $PGE_1$ concentration of 500 μg/ml as test solution, while 0.9% (w/v) normal saline and 1% (w/v) sodium deoxycholate (a known nasal ciliotoxicity agent) were used as a negative and positive control, respectively. Nasal ciliotoxicity studies were carried out using toad palate model, which was reported to a good method for studying the ciliotoxicity of nasal formulation (Puchelle and Tournier, 1983; Jiang et al., 1995). In brief, the upper palate mucosa of toads (30–40 g, Experimental Animal Center of Beijing Capital Medical University, China) was dissected into the small patches of the same size (3 mm × 3 mm), then rinsed with saline, after which, the mucosa samples were spread on the glass slide and immediately treated with 0.2 ml of test solution. They were then examined under an optical microscope (Nikon Fx-35A, Japan) and the duration of ciliary movement was recorded.

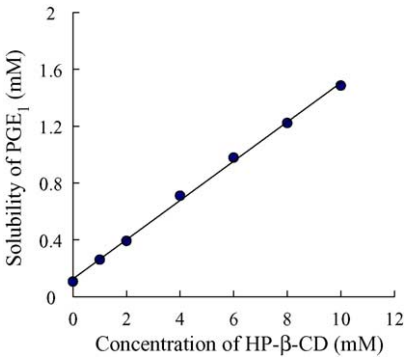


Fig. 1. Phase solubility diagram of $PGE_1$–HP-β-CD system in water at 25 °C.

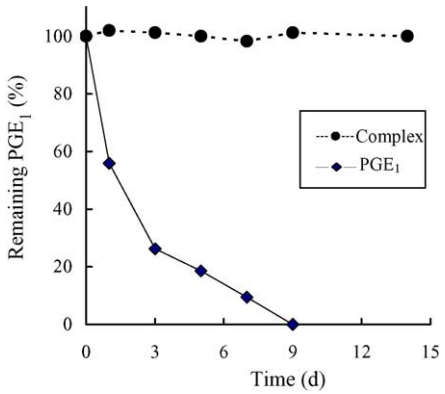


Fig. 2. The stability of $PGE_1$ and its complex with HP-β-CD at 60 °C.

## 3. Results and discussion

### 3.1. Solubility studies

As shown in Fig. 1, the solubility of $PGE_1$ increased linearly as a function of HP-βCD concentration. The phase solubility diagram follows an $A_L$-type according to Higuchi and Connors' classification, suggesting the formation of a soluble complex of 1:1 molar ratio (Higuchi and Connors, 1965). The apparent stability constant ($K_c$) for the complex was calculated to be 1282 $M^{-1}$, while $K_c$ values for $PGE_1$ complexes with αCD, βCD, γCD, and $G_2$-βCD were reported to be 1430, 1700, 530, and 1060 $M^{-1}$, respectively (Uekama et al., 1984; Yamamoto et al., 1992). The $K_c$ value for HP-βCD complex was smaller than that for βCD complex, probably due to the steric hindrance of the hydroxypropyl group in HP-βCD, whereas the $K_c$ value for HP-βCD complex was greater than that for $G_2$-βCD complex, probably due to the steric hindrance effect of the maltosyl group being stronger than that of hydroxypropyl group as a result of the larger group size. On the other hand, since αCD, βCD, and γCD consist of six, seven and eight glucose units, respectively, leading to the differences in the hydrophobic cavity size of three CDs, the host–guest interactions, the complex conformations, and subsequent $K_c$ values were obvious different between the three CDs complexes. Additionally, since HP-βCD has greater aqueous solubility (>75%, w/v) and higher safety than the aforementioned CDs, it should be a better solubilizer of $PGE_1$.

### 3.2. Stability studies

The β-hydroxylketone moiety of $PGE_1$ is known to be extremely susceptible to dehydration in relatively high acidic and alkaline conditions, giving $PGA_1$, which is then isomerized consecutively to $PGB_1$ under alkaline conditions with loss of the pharmacological activity (Monkhouse et al., 1973).

In this study, an attempt was made to evaluate the effect of HP-βCD on the stability of $PGE_1$ in the solid state. The formation of the $PGE_1$–HP-β CD complex has already been confirmed by UV, circular dichroism, IR, and X-ray diffraction methods in our earlier experiment (Gu et al., 2004). As can be seen in Fig. 2, no degradation of $PGE_1$ in the complex occurred within 14 days at 60 °C, whereas free $PGE_1$ totally degraded in 9 days under the same conditions. Above results indicate that HP-βCD produced very significant stabilizing effect on $PGE_1$ by the formation of complex. The significant improvement of the stability of $PGE_1$ may be attributed to the inclusion of the β-hydroxylketone moiety of the drug molecule into the hydrophobic cavity of HP-βCD (Adachi et al., 1992).

### 3.3. Nasal absorption studies

As plasma levels of $PGE_1$ are in the low pg/ml range, a highly sensitive and specific analytical method, generally GC–MS have to be used to determine the plasma concentration of $PGE_1$ following administration (Schweer et al., 1994). Furthermore, prior to GC–MS determination, blood samples require com-

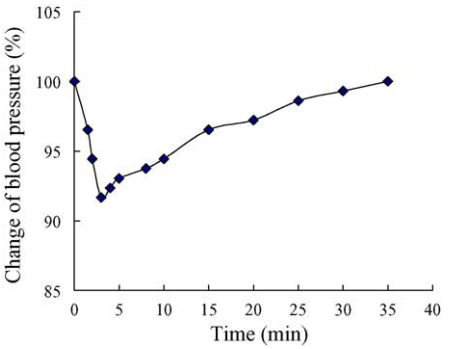


Fig. 3. The mean hypotensive effect after nasal administration of $PGE_1$ complex of 50 μg/kg to Wistar rats ($n = 5$).

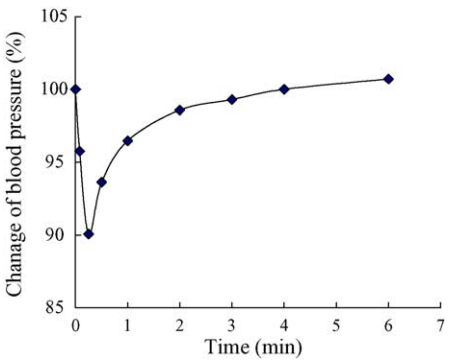


Fig. 4. The mean hypotensive effect after intravenous administration of $PGE_1$ of 5.0 μg/kg to Wistar rats ($n = 5$).

plicated and laborious pretreatment procedures. As an example, $PGE_1$ in blood sample is first extracted and purified, then undergoes derivatization reactions such as esterification, methoximation, and silylation consecutively, and lastly subjected to GC–MS (Schweer et al., 1985).

Because of limitation of experimental conditions, a simple and reliable pharmacodynamic method was employed to study the nasal absorption of $PGE_1$ from the complex formulation in this study. The in vivo pharmacodynamic investigation can provide direct data regarding kinetic parameters and estimate whether the drug has reached the target site, which would result in a pharmacological effect. Presently, this method has been used in the evaluation of the absorption of $PGE_1$ from various transcutaneous formulations (Foldvari et al., 1998). $PGE_1$ can relax vascular smooth muscle and would cause decreased blood pressure. Hence, the relative efficacy of nasal $PGE_1$ delivery from its complex formulation can be assessed by monitoring hypotensive effect of the drug.

Fig. 3 presents the blood pressure change after nasal administration of 50 μg/kg of $PGE_1$ complex to Wistar rats. $PGE_1$ complex after administration caused a rapid and significant decrease of blood pressure, followed by a slow return to the initial pressure. The peak effect appeared about 3 min after administration. All the pharmacodynamic parameters such as $T_{max}$, $E_{max}$, $T_d$, and AUC are summarized in Table 1. Except $T_{max}$, other pharmacodynamic parameters, $E_{max}$, $T_d$, and AUC values were increased with increasing doses. The $T_{max}$ values between doses were not significantly different ($P > 0.05$), but the $E_{max}$, $T_d$, and in particular AUC values between doses were significantly different ($P < 0.01$). Therefore, the above results revealed that $PGE_1$ in the complex was rapidly absorbed by the nasal mucosa of rats and exhibited an obvious dose–efficacy relationship.

For the purpose of comparison, different doses of $PGE_1$ were given intravenously to Wistar rats. Fig. 4 shows the blood response after intravenous injection of 5.0 μg/kg of $PGE_1$. Just like nasal administration, $PGE_1$ after intravenous administration caused a significant decrease of blood pressure, followed by a relatively rapid return to the initial level. The peak effect appeared about 20 s after administration. The pharma-

Table 1
Pharmacodynamic parameters of nasally administered $PGE_1$ complex solution to Wistar rats[a]

| Dose (μg/kg) | $T_{max}$ (min) | $E_{max}$ (%) | $T_d$ (min) | AUC (% min) |
|---|---|---|---|---|
| 25 | $3.00 \pm 1.27^{*}$ | $4.52 \pm 1.44^{**}$ | $19.00 \pm 7.42^{**}$ | $24.22 \pm 7.23^{**}$ |
| 50 | $3.40 \pm 0.55$ | $6.87 \pm 1.30$ | $44.00 \pm 14.75$ | $140.70 \pm 74.58$ |
| 100 | $4.10 \pm 0.55$ | $10.28 \pm 3.92$ | $56.00 \pm 8.94$ | $236.03 \pm 50.19$ |

[a] Mean ± S.D. ($n = 5$).
* No significant difference between doses, $P > 0.05$.
** Significant difference between doses, $P < 0.01$.

Table 2
Pharmacodynamic parameters of intravenously administered $PGE_1$ solution to Wistar rats[a]

| Dose (μg/kg) | $T_{max}$ (min) | $E_{max}$ (%) | $T_d$ (min) | AUC (% min) |
|---|---|---|---|---|
| 2.5 | 0.43 ± 0.33* | 5.03 ± 2.19** | 4.30 ± 3.23** | 8.17 ± 6.51** |
| 5.0 | 0.28 ± 0.04 | 11.53 ± 2.65 | 5.70 ± 5.33 | 16.14 ± 15.10 |
| 10.0 | 0.46 ± 0.09 | 21.30 ± 4.87 | 18.20 ± 10.06 | 100.60 ± 49.77 |

[a] Mean ± S.D. ($n = 5$).
* No significant difference between doses, $P > 0.05$.
** Significant difference between doses, $P < 0.01$.

codynamic parameters $T_{max}$, $E_{max}$, $T_d$, and AUC are summarized in Table 2. Except $T_{max}$, other pharmacodynamic parameters, $E_{max}$, $T_d$, and AUC values were increased with increasing doses. The $T_{max}$ values between doses were not significantly different ($P > 0.05$), but the $E_{max}$, $T_d$, and, especially AUC values between doses were significantly different ($P < 0.01$), also showing an evident dose–efficacy relationship. As AUC here comprehensively represents the magnitude of drug efficacy, hence it can be used as a tentative measure of drug absorption extent. It is obvious that AUC value per unit dose of $PGE_1$ for nasal administration was smaller than that for intravenous route. For example, the AUC value for the nasal administration of 50 μg/kg of $PGE_1$ complex was approximately equivalent to that for intravenous route of 10 μg/kg of $PGE_1$. Therefore, the nasal absorption of $PGE_1$ in the complex appeared to be incomplete. There can be several reasons for this decreased nasal absorption. Firstly, one possible reason may be that the metabolism of $PGE_1$ occurs in the nasal cavity due to the presence of a very active cytochrome P450-dependent drug metabolizing system in rodent olfactory epithelia (Moromoto et al., 1991). Another possible reason may be that since it is the $PGE_1$ complex solution, not the solid complex powders that was used in this experiment, the drainage of the drug solution from the nasal cavity to the mouth also occurs after nasal administration, resulting in the loss of the administered $PGE_1$. Thus, it is reasonable to predict that the $PGE_1$ complex powders following nasal administration have a greater extent of absorption than the complex solution due to the longer-time contact with the nasal mucosa (Marttin et al., 1997). Lastly, the experimental animal conditions may also affect nasal drug absorption. For instance, in rats lying on their back, it is likely that the upper part of the nasal cavity functions as the penetration barrier for the nasal formulations, which is lined with olfactory epithelium (Hermens et al., 1990).

In addition, among cyclodextrin derivatives, dimethyl-βCD (DM-βCD) has been reported to be a potent nasal absorption enhancer for some drugs such as insulin, 17β-estradiol, and progesterone, etc. (Hermens et al., 1990; Schipper et al., 1990; Merkus et al., 1991), however, presently few data are available on the absorption enhancing effect of HP-βCD in nasal drug delivery. Therefore, in this study whether the nasal absorption of $PGE_1$ is promoted by HP-βCD remains unknown, a further investigation is now under investigation.

### 3.4. Nasal ciliotoxicity studies

As the nasal ciliary movement is mainly responsible for the body's non-specific defensive mechanism by removing dust, allergens and bacteria, a prerequisite in nasal formulation development is that drugs and additives should not adversely affect the nasal ciliary function (Hermens and Merkus, 1987). As shown in Table 3, the lasting time for the mucociliary movement of the toad palate treated with the $PGE_1$ complex solution of high concentration (500 μg/ml, far greater than the aqueous solubility of the drug) was found to be about 7 h, which was nearly equivalent to the duration for the negative control 0.9% (w/v) saline. However,

Table 3
Effect of the $PGE_1$ complex on the toad palate cilia movement[a]

| Solution (w/v) | Duration of ciliary movement (min) | Relative % |
|---|---|---|
| 0.9% Normal saline | 423.6 ± 59.8 | 100 |
| 0.05% $PGE_1$ complex | 398.4 ± 59.2* | 94.1** |
| 1% Sodium deoxycholate | 0 | 0 |

[a] Mean ± S.D. ($n = 5$).
* Not significantly different compared with normal saline group, $P > 0.05$.
** Relative to the normal saline group.

the mucociliary movement of toad palate ceased immediately after the ciliary epithelium was treated with 1% (w/v) sodium deoxycholate solution. Although the $PGE_1$ complex solution, not the solid complex powders was used in this study, the effect of the $PGE_1$ complex on the ciliary movement in vivo is probably less pronounced than that in vitro, because in vitro, the ciliated tissue is directly exposed to the $PGE_1$ complex solutions of high concentration, whereas in vivo the cilia are protected by the mucus layer. Moreover, under in vivo conditions, since very limited amount of the complex powders are dispersed on a larger-area nasal mucosa surface, the concentration of dissolved $PGE_1$ may be far lower than that of the above tested $PGE_1$ solution. On the other hand, the administered drug will be diluted by the nasal mucus layer and subsequently eliminated by the nasal mucociliary clearance. Hence, the observed results indicate that the $PGE_1$ complex formulation has only little nasal ciliotoxicity.

## 4. Conclusions

Effect of HP-βCD on the solubility and chemical stability of $PGE_1$ was investigated in this study. Solubility studies revealed that the aqueous solubility of $PGE_1$ increased linearly with an increase in the concentration of HP-βCD, suggesting the formation of a 1:1 molar ratio complex. Furthermore, stability study also showed that the solid $PGE_1$ complex with a 1:10 molar ratio of the drug to HP-βCD had quite good chemical stability, which is a very important advantage for $PGE_1$ preparations. Thus, the $PGE_1$-HP-βCD complex may have great utility in the development of fast-action solid dosage forms of $PGE_1$ with good storage properties. Moreover, the present approach may be applicable to other PGEs having the same undesirable physicochemical properties.

Both the nasal absorption of $PGE_1$ from the complex formulation in rats and the in vitro effect of the complex preparation on the toad palate ciliary movement were also investigated. Nasal absorption studies demonstrated that $PGE_1$ was readily released from the complex formulation and effectively absorbed by the nasal mucosa of Wistar rats. The in vitro effect of the $PGE_1$ complex on the nasal cilia movement seemed to be negligible.

In conclusion, the $PGE_1$–HP-βCD complex formulation may be a very promising novel preparation for the nasal delivery of $PGE_1$ for clinical application. A further study is necessary to determine the pharmacokinetics and bioavailability of the $PGE_1$–HP-βCD complex formulation in humans after nasal administration.

## References


Adachi, H., Irie, T., Hirayama, F., Uekama, K., 1992. Stabilization of prostaglandin $E_1$ in fatty alcohol propylene glycol ointment by acidic cyclodextrin derivative, *O*-carboxymethyl-*O*-ethyl-β-cyclodextrin. Chem. Pharm. Bul1. 40, 1586–1591.

Behl, C.R., Pimplaskar, H.K., Sileno, A.P., Demeireles, J., Romeo, V.D., 1998. Effects of physicochemical properties and other factors on systemic nasal drug delivery. Adv. Drug. Del. Rev. 29, 89–116.

Carpenter, T.O., Gerloczy, A., Pitha, J., 1995. Safety of parenteral hydroxypropyl β-cyclodextrin. J. Pharm. Sci. 84, 222–225.

Cawello, W., Leonhardt, A., Schweer, H., Seyberth, H.W., Bonn, R., Leonlomeli, A., 1995. Dose proportional pharmacokinetics of alprostadil (prostaglandin $E_1$) in healthy volunteers following intravenous infusion. Br. J. Clin. Pharmacol. 40, 273–276.

Dollo, G., Corre, P.L., Chollet, M., Chevanne, F., Bertault, M., Burgot, J.L., Verge, R.L., 1999. Improvement in solubility and dissolution rate of 1, 2-dithiole-3-thiones upon complexation with β-cyclodextrin and its hydroxypropyl and sulfobutyl ether-7 derivatives. J. Pharm. Sci. 88, 889–895.

Foldvari, M., Oguejiofor, C.J.N., Wilson, T.W., Afridi, S.K., Kudel, T.A., 1998. Transcutaneous delivery of prostaglandin $E_1$: in vitro and laser doppler flowmetry study. J. Pharm. Sci. 87, 721–725.

Gatti, R., Gotti, R., Cavrini, V., Soli, M., Bertaccini, A., Carparelli, F., 1995. Stability study of prostaglandin $E_1$ ($PGE_1$) in physiological solutions by liquid chromatography (HPLC). Int. J. Pharm. 115, 113–117.

Gu, F.G., Cui, F.D., Gao, Y.L., 2004. Effect of complexation with hydroxylpropyl-β-cyclodextrin on the solubility, dissolution rate and chemical stability of prostaglandin $E_1$. J. Chin. Pharm. Sci. 13, 158–165.

Hermens, W.A.J.J., Merkus, F.W.H.M., 1987. The influence of drugs on nasal ciliary movement. Pharm. Res. 4, 445–449.

Hermens, W.A.J.J., Deurloo, M.J.M., Romeyn, S.G., Verhoef, J.C., Merkus, F.W.H.M., 1990. Nasal absorption enhancement of 17β-estradiol by dimethyl-β-cyclodextrin in rabbits and rats. Pharm. Res. 7, 500–503.

Higuchi, T., Connors, K.A., 1965. Phase solubility techniques. Adv. Anal. Chem. Instrum. 4, 117–212.

Igarashi, R., Takenaga, M., Takeuchi, J., Kitagawa, A., Matsumoto, K., Mizushima, Y., 2001. Marked hypotensive and blood flow-increasing effects of a new lipo-$PGE_1$ (lipo-AS013) due to vascular wall targeting. J. Control Release 71, 157–164.

Jiang, X.G., Cui, J.B., Fang, X.L., Wei, Y., Xi, N.Z., 1995. Toxicity of drugs on nasal mucocilia and the method of its evaluation. Acta Pharm. Sin. 30, 848–853.

Marttin, E., Romeijn, S.G., Verhoef, J.C., 1997. Nasal absorption of dihydroergotamine from liquid and powder formulations in rabbits. J. Pharm. Sci. 86, 802–807.

Merkus, F.W.H.M., Verhoef, J.C., Romeijn, S.G., Schipper, N.G.M., 1991. Absorption enhancing effect of cyclodextrins on intranasally administered insulin in rats. Pharm. Res. 8, 588–591.

Mizushima, Y., Yanagawa, A., Hoshi, K., 1983. Prostaglandin $E_1$ is more effective, when incorporated in lipid microspheres, for treatment of peripheral vascular diseases in man. J. Pharm. Pharmacol. 35, 666–667.

Monkhouse, D.C., Campen, L.V., Aguiar, A.J., 1973. Kinetics of dehydration and isomerization of $PGE_1$ and $E_2$. J. Pharm. Sci. 62, 576–580.

Moromoto, K., Yamaguchi, H., Iwakura, Y., Miyazaki, M., Nakatani, E., Iwamoto, T., Ohashi, Y., Nakai, Y., 1991. Effects of proteolytic enzyme inhibitors on the nasal absorption of vasopressin and an analogue. Pharm. Res. 8, 1175–1179.

Puchelle, E., Tournier, J.M., 1983. The frog palate for studying mucus transport velocity and mucociliary frequency. J. Respir. Dis. 64, 293–296.

Schipper, N.G.M., Hermens, W.A.J.J., Romeyn, S.G., Verhoef, J., Merkus, F.W.H.M., 1990. Nasal absorption of 17-beta-estradiol and progesterone from a dimethyl-cyclodextrin inclusion formulation in rats. Int. J. Pharm. 64, 61–66.

Schweer, H., Kammer, J., Seyberth, H.W., 1985. Simultaneous determination of prostanoids in plasma by gas chromatography-negative-ion chemical-ionization mass spectrometry. J. Chromatogr. 338, 273–280.

Schweer, H., Meese, C.O., Watzer, B., Seyberth, H.W., 1994. Determination of prostaglandin $E_1$ and its main plasma metabolites 15-keto-prostaglandin $E_0$ and prostaglandin $E_0$ by gas chromatography/negative ion chemical ionization triple stage quadrupole mass spectrometry. Bio. Mass. Spectrom. 23, 221–227.

Trübestein, G., Bary, S., Breddin, K., 1989. Intravenous prostaglandin $E_1$ versus pentoxifylline therapy in chronic arterial occlusive disease—a controlled randomized multicenter study. VASA 28, 44–49.

Uekama, K., Fujise, A., Hirayama, F., Otagiri, M., Inaba, K., 1984. Improvement of dissolution characteristics and chemical stability of prostaglandin $E_1$ by γ-cyclodextrin complexation. Chem. Pharm. Bull. 32, 275–279.

Virag, R., Adaikan, P.G., 1987. Effects of prostaglandin $E_1$ on penile erection and erectile failure. J. Urol. 137, 1010.

Wallace, J.L., 1992. Prostaglandins, NSAIDS and Cytoprotection. Gastroenterrol. Clin. North Am. 21, 631–634.

Wang, H., 2002. Clinical applications of prostaglandin $E_1$. Tianjin. Pharm. 14, 20–21.

Wiese, M., Cordes, H.P., Chi, H., Seydel, J.K., Backensfeld, T., Müller, B.W., 1991. Interaction of prostagladin $E_1$ with α-cyclodextrin in aqueous systems: Stability of the inclusion complex. J. Pharm. Sci. 80, 153–156.

Yamamoto, M., Hirayama, F., Uekama, K., 1992. Improvement of stability and dissolution of prostaglandin $E_1$ by maltosyl-β-cyclodextrin in lyophilized formulation. Chem. Pharm. Bull. 40, 747–751.