Integrated pharmacokinetics and pharmacodynamics of epoprostenol in healthy subjects

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WHAT IS ALREADY KNOWN

- Continuous infusion with synthetic prostacyclin (epoprostenol) is generally regarded as the most effective treatment against severe cases of primary pulmonary arterial hypertension, associated with decreased pulmonary vascular resistance, increased cardiac index (CIn), and survival benefits. To date, the pharmacokinetics (PK) of epoprostenol have not been fully characterized due in part to the instability of epoprostenol.

WHAT THIS STUDY ADDS

- The present study provides the first characterization of the PK of epoprostenol in man via assessment of 6-keto-prostacyclin $F_{1\alpha}$ and another primary metabolite, 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$. Overall, PK/pharmacodynamic (PD) modelling showed that CIn relates proportionally and linearly to the plasma concentrations of 6-keto-prostacyclin $F_{1\alpha}$ suggesting that this major metabolite represents a suitable surrogate marker of plasma concentrations of epoprostenol.

AIM

The aim of the study was to report the first thorough characterization of the pharmacokinetics (PK) and pharmacodynamics (PD) of epoprostenol in an integrated manner.

METHOD

Twenty healthy male subjects received two formulations of i.v. epoprostenol, in a crossover design, in sequential infusions of 2, 4, 6 and 8 ng kg⁻¹ min⁻¹ for 2 h each. A sensitive assay was developed which allowed accurate PK characterization of epoprostenol via analysis of the concentration–time profiles of its two primary metabolites, 6-keto-prostacyclin $F_{1\alpha}$ and 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$. PD parameters included cardiac output (CO), cardiac index (CIn) and heart rate (HR).

RESULTS

The pharmacokinetics of epoprostenol deviated slightly from dose-proportionality, probably due to a food effect. After infusion of the two formulations of epoprostenol, the $t_{1/2}$ values expressed as geometric mean (95% confidence interval) were 0.25 h (0.14, 0.46) and 0.22 h (0.13, 0.38) for 6-keto-prostacyclin $F_{1\alpha}$ and 0.32 h (0.22, 0.45) and 0.34 h (0.26, 0.46) for 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$. A single compartment infusion model with first order elimination adequately described the PK of 6-keto-prostacyclin $F_{1\alpha}$. This model also characterized the food effect. Stepwise infusions with epoprostenol resulted in a progressive increase in CO, CIn and HR.

CONCLUSION

Of the two metabolites analyzed, the appearance of 6-keto-prostacyclin $F_{1\alpha}$ in plasma was more closely associated with the haemodynamic effects of i.v. epoprostenol. PK and PD profiles showed that CIn relates proportionally and linearly to the plasma concentrations of 6-keto-prostacyclin $F_{1\alpha}$. These results suggest that 6-keto-prostacyclin $F_{1\alpha}$ is a suitable surrogate marker of plasma concentrations of epoprostenol.

Introduction

Primary pulmonary arterial hypertension (PAH) is a fatal haemodynamic and pathophysiological condition affecting the pulmonary vasculature, characterized by sustained elevation of pulmonary vascular resistance with normal pulmonary capillary wedge pressure that leads ultimately to right ventricular failure and death. The histopathological profile including vasoconstriction, vascular proliferation, remodelling of the vascular wall and *in situ* thrombosis [1–5], has a multifactorial origin [6].

Indeed, when compared with healthy subjects, PAH patients show increased concentrations of endothelin-1 and thromboxane A_2 [6–11], whereas their production of nitric oxide (NO), vasoactive intestinal peptide, and prostacyclin are decreased [6, 10]. In healthy subjects, prostacyclin and NO counterbalance the vasoconstrictive, mitogenic and prothrombotic actions of endothelin-1 and thromboxane A_2 [5, 7, 11], whereas in patients with PAH the homeostatic activity of these mediators is deregulated [6, 10].

These findings have resulted in the development of therapeutic corrective approaches aimed at restoring the homeostatic balance between factors having vasodilatory/vasoconstrictive, growth inhibiting/mitogenic effects and antithrombotic/prothrombotic properties. Among the available medications, prostacyclin (also known as prostaglandin I_2 , PGI_2), a major metabolite of arachidonic acid produced by vascular endothelial cells, is a key natural short-acting vasoactive substance having vasodilatory, platelet inhibitory and anti-proliferative properties [3, 12]. Continuous infusion with synthetic prostacyclin (epoprostenol) is generally regarded as the most effective treatment against severe cases of PAH, associated with decreased pulmonary vascular resistance, increased cardiac output (CO) and cardiac index (CIn) [13–15] and survival benefits [13, 16].

The current study was designed to perform the first thorough characterization of the pharmacokinetics (PK) of epoprostenol following administration of either epoprostenol (Flolan®, GlaxoSmithKline, Brentford United Kingdom) or epoprostenol with expanded stability (epoprostenol ES) (Veletri®, Actelion Pharmaceuticals, Allschwil, Switzerland), the latter differing in terms of excipients. Due to the very short half-life of epoprostenol (approximately 6 min in human blood [17]), the PK were characterized via analysis of the concentration–time profiles of two primary metabolites, 6-keto-prostacyclin $F_{1\alpha}$ (spontaneously formed by hydration) and 6,15-diketo-13,14-dihydroprostacyclin $F_{1\alpha}$ (formed by enzymatic degradation [17]). In addition, the effects of the two formulations of epoprostenol on selected pharmacodynamic (PD) cardiovascular markers CO, CIn, heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and platelet aggregation were evaluated. Finally, a PK model was developed to characterize the rela-

tionship between the infused dose of epoprostenol and the plasma concentrations of the primary metabolite 6-keto-prostacyclin $F_{1\alpha}$. A subsequent PK/PD model was developed to quantify the relation between 6-keto-prostacyclin $F_{1\alpha}$ concentration and CIn.

Methods

Subjects

The protocol and the subject information and consent form were submitted to and approved by a duly constituted Institutional Review Board (IRB) prior to study initiation and conduct at Cetero Research, Miami, FL, USA. The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice (E6) guideline and principles of the Declaration of Helsinki.

Following written informed consent, healthy male subjects aged ≥ 18 years were enrolled on the basis of inclusion and exclusion criteria, including medication during a 3 month period prior to treatment and at screening. Physical examination, clinical laboratory (haematology and serum chemistry) determinations, electrocardiographic assessments were performed and vital signs recorded. Because epoprostenol is a known vasodilator and anticoagulant, subjects with a history of fainting, collapses, syncope, orthostatic hypotension, vasovagal reactions, abnormal coagulation screen or history/clinical evidence of any haemostasis disorder were excluded.

Study design

The study was a single centre, open label, two period, two treatment, randomized, crossover, ascending dose study in which PK, PD and tolerability of epoprostenol ES and the reference product (epoprostenol), were assessed.

Epoprostenol ES and epoprostenol (1.5 mg vials) were reconstituted and diluted, and cassettes containing 100 ml of epoprostenol ES or epoprostenol solution for i.v. infusions with a final concentration of 6000 ng ml^{-1} in water for injection or specific diluent, respectively, were prepared. Epoprostenol ES was administered via an i.v. catheter (on the dominant arm) at room temperature using an ambulatory infusion pump (CADD-Legacy® 1, ambulatory infusion pump Model 6400 for continuous delivery). As recommended in the prescribing information, epoprostenol was administered with the pump contained in a special pouch using frozen gel packs to maintain its stability. For both formulations, infusion delivery rates to achieve doses of 2, 4, 6 and 8 ng $kg^{-1} min^{-1}$ were a function of the dose and the body weight of the subject.

For each treatment period, subjects entered the clinic on day –1. On day 1, epoprostenol ES or epoprostenol, according to the randomization scheme, was administered as sequential i.v. infusions of 2, 4, 6 and 8 ng $kg^{-1} min^{-1}$ for 2 h each, followed by an observation period of 40 h. The

infusion schedule applied in the present study was in accordance with clinical practice [17].

In the morning of day 2, 24 h after the infusion start, the subjects left the clinic after completion of the scheduled assessments. On day 3, 48 h after the infusion start, the subjects returned to the clinic to complete the assessments of the end of period visit. The end of period visit of the last treatment period was considered the end of study (EOS) visit.

On day 1, a light breakfast between 0.5 and 1 h before start of drug infusion was given followed by a standardized lunch after the 4 h blood sample and an evening meal after the 10 h assessments were completed.

Physical examinations, clinical laboratory tests (haematology, including coagulation screen and serum chemistry), electrocardiograms, vital signs, PK and PD measurements were performed at various time points throughout the study. Concomitant medications and adverse events (AEs) occurring at any time during each treatment period were recorded.

Blood sampling for pharmacokinetic assessments

During both treatment periods, serial samples of 4 ml were collected into EDTA-containing tubes, immediately before and at pre-defined time points up to 24 h after start of drug infusion. Plasma was separated within 15 min of collection and stored at -20°C pending analysis.

Bioanalytical method

Plasma concentrations of the two metabolites were measured using a validated high pressure liquid chromatography coupled to mass spectrometry method (HPLC-MS/MS).

Plasma samples (500 μl each) were combined with 10 μl of phosphoric acid (8.5% in water) and 20 μl of the internal standard solution (10.0 ng ml^{-1} of 6-keto-prostacyclin $\text{F}_{1\alpha}$ -D4 and 250 ng ml^{-1} of 2,3-dinor-6-keto-prostacyclin $\text{F}_{1\alpha}$ -D9). Samples were incubated at 5°C for approximately 15 min, combined with 500 μl of water, and mixed. Two aliquots of 400 μl were transferred onto a solid phase extraction well plate previously conditioned with 450 μl of methanol and 450 μl of water. After the wells were washed, the analytes were eluted using 100 μl methanol containing 0.1% ammonia solution (24.5%), combined with 100 μl of formic acid (0.5% in water), and mixed. For each sample, 50 μl was injected onto the HPLC-MS/MS system for chromatographic separation and quantification of the three metabolites.

The HPLC-MS/MS system consisted of an HPLC 1200 binary pump (Agilent Technologies Inc, Santa Clara, CA, USA), coupled with a TSQ Vantage mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). For chromatographic separation, a reversed phase column (ReproSil Gold 120 C18; $2 \times 50 \text{ mm}$, $3 \mu\text{m}$; Dr Maisch HPLC GmbH, Ammerbuch, Germany) was used. The mobile phase con-

sisted of water containing 0.2% ammonia solution (24.5%) and acetonitrile. The running time was 7 min with a constant flow rate of 0.3 ml min^{-1} .

The method was precise and accurate for all three metabolites. For 6-keto-prostacyclin $\text{F}_{1\alpha}$, mean values for the inter-batch coefficient of variation (CV) ranged from 4.4–5.6%, with mean inter-batch accuracy ranging from 93.1–94.0%. For 6,15-diketo-13,14-dihydro-prostacyclin $\text{F}_{1\alpha}$ the mean inter-batch CV ranged from 8.6–11.5%, with mean inter-batch accuracy of 91.3–95.0%. For 2,3-dinor-6-keto-prostacyclin $\text{F}_{1\alpha}$ the mean inter-batch CV and the mean inter-batch accuracy were 5.6–5.9% and 94.0–95.5%, respectively. The calibration range was 50.0–5000 pg ml^{-1} for 6-keto-prostacyclin $\text{F}_{1\alpha}$, 100–10 000 pg ml^{-1} for 2,3-dinor-6-keto-prostacyclin $\text{F}_{1\alpha}$ and 50.0–5000.0 pg ml^{-1} for 6,15-diketo-13,14-dihydro-prostacyclin $\text{F}_{1\alpha}$ using 500 μl of human plasma.

Pharmacokinetic and statistical analysis

The measured individual plasma concentrations of both metabolites were used to obtain C_{2h} , C_{4h} , C_{6h} , C_{8h} , i.e. concentrations measured at the end of each 2 h infusion step. PK parameters were calculated by non-compartmental analysis from individual plasma concentration–time data using a constant rate i.v. infusion model (model 202, Professional WinNonlin, Version 5.2.1, Pharsight Corporation, Mountain View, CA, USA). These parameters included the area under the curve (AUC) during each infusion step ($\text{AUC}(0,2 \text{ h})$, $\text{AUC}(2,4 \text{ h})$, $\text{AUC}(4,6 \text{ h})$, $\text{AUC}(6,8 \text{ h})$) and the AUC from time point zero to infinity ($\text{AUC}(0,\infty)$), and the half-life ($t_{1/2}$) of the terminal phase. Area under the plasma concentration–time curve (AUC) was estimated using the linear trapezoidal rule. $\text{AUC}(0,\infty)$, estimated from $\text{AUC}(0,t)$ (i.e. the AUC from time point zero to the last sampling time with a concentration above the limit of quantification (LOQ)) and $\text{AUC}(\text{extra})$ were also determined. $\text{AUC}(\text{extra})$ is defined as C_t/λ_z , where t is the last sampling time with a concentration above the LOQ and λ_z is the terminal elimination rate constant, estimated by log-linear least squares regression of the plasma concentration vs. time data in the terminal phase. The half-life ($t_{1/2}$) of the terminal phase was calculated using the equation $t_{1/2} = \ln 2/\lambda_z$.

At each 2 h infusion step, attainment of steady-state conditions was assessed by visual inspection of the mean concentration–time profiles obtained for the two metabolites.

The assessment of bioequivalence of the two formulations was based on the 90% confidence intervals (CI) for the ratio of geometric means (epoprostenol ES : epoprostenol, using epoprostenol as reference treatment) for concentration, AUC, and $t_{1/2}$ values. The effect of the formulation on metabolite PK was also estimated by means of a linear mixed effects model.

Dose-proportionality of log-transformed concentration and AUC values for both metabolites was explored using the Gough power model [18]. Using this model, dose

proportionality is concluded when β , the dose-proportionality coefficient, is close to 1 and its 95% CI includes 1.

Pharmacodynamic assessments

Cardiovascular markers were assessed immediately before and at pre-defined time points up to 24 h after start of drug infusion. HR, SBP and DBP were measured. Mean arterial pressure ($MAP = DBP + 1/3 [SBP - DBP]$), pulse pressure ($PP = SBP - DBP$), cardiac output ($CO = HR \times \text{stroke volume}$) and cardiac index ($CI_n = CO / (0.007184 \times \text{weight}^{0.425} \times \text{height}^{0.725})$) were calculated. HR and stroke volume were determined by echocardiography and SBP and DBP were measured using an automated oscillometric device.

Pharmacodynamic and statistical analysis

For CO, CI_n and HR, i.e. the cardiovascular markers for which epoprostenol induced the most pronounced effect, time (t_{max}) to reach the maximum response (R_{max}) during the 8 h of infusion, ratios between cardiovascular response obtained 8 h after the infusion start and response at baseline ($R_{8h} : R_{0h}$) and drug response over the 8 h of infusion (using the area under the effect-time curve, $AUE(0,8h)$) were obtained by non-compartmental analyses of individual time profiles using WinNonlin model 220 for PD data.

Pharmacokinetic/pharmacodynamic modelling

The PK were characterized by a single compartment infusion model with first order elimination. A noticeable effect of food on the PK of epoprostenol was determined at 4 h after start of treatment, at the time of food intake. The postprandial increase in systemic clearance of i.v. drugs having high hepatic clearance is well known and is mainly explained by an increased hepatic blood flow [19]. However, because the effect of food on hepatic blood flow was not assessed in the present trial, the PK model estimates the food effect on the clearance based on the observed concentration data. The corresponding clearance change is characterized by the sum of two exponential terms of the form $\exp(-A \times \text{ftime}) - \exp(-(A+B) \times \text{ftime})$ with a lag time until occurrence of the food effect. The term ftime denotes the time after food intake (here: $\text{ftime} = 0$ at 4 h after start of treatment). The population estimates are given in Table 1.

Since the precise time of food intake and amount of food was not recorded, the food effect was characterized by correlated random effects parameters with inter-occasion variation, capturing apparent differences in concentration within subjects between epoprostenol and epoprostenol ES after food intake.

The parameters were estimated sequentially: clearance and volume of distribution were estimated based on the PK data up to 4 h before food intake. In a subsequent step, clearance and volume were kept fixed to estimate the food effect parameters and the corresponding random effects

Table 1

Pharmacokinetic parameter estimates

Parameter	Estimate	SD.	% CV
CL ($l\ h^{-1}$)	84.92	3.07	3.61
V (l)	23.74	2.88	12.12
A (food effect)	1.69	0.06	3.41
B (food effect)	3.57	1.82	50.98
Lag time food effect (h)	0.22	0.02	11.33

(for parameters identifiability). The PK model was based on all available concentration data up to 10 h after start of treatment.

The PD model links the fitted 6-keto-prostacyclin $F_{1\alpha}$ concentrations to the change in CI_n . A linear regression model without intercept was employed for characterization of the relationship.

NONMEM version 7.1.2 [20] was used for the population PK modelling, Berkeley Madonna version 8.3.18 [21] for model visualization and R version 2.11.1 [22] for data preparation, processing of results, data exploration, CI_n (PD) modelling and model diagnostics.

Results

Subject demographics

In total, 20 eligible male subjects were enrolled in the study, 18 of whom completed the study as per-protocol and were evaluable for PK and PD analysis. One drop-out was due to treatment-emergent adverse events (nausea and vomiting) and another subject was withdrawn due to a positive ethanol test. All 20 subjects received epoprostenol and 18 received epoprostenol ES.

Fifteen of the 20 subjects were Caucasians and five were African Americans, the mean ($\pm$ SD) age was 33.7 ($\pm$ 8.1) years (range 18–45) with a body mass index of 19.3–27.9 $kg\ m^{-2}$.

Tolerability

Treatment with epoprostenol ES or epoprostenol was generally well tolerated. Both formulations showed similar safety and tolerability profiles. No deaths or serious adverse events (SAEs) were reported.

A total of 100 treatment-emergent AEs (TEAEs) were reported. Most AEs were mild in intensity and none was rated as severe. Nineteen subjects experienced 55 TEAEs after administration of epoprostenol and 16 subjects experienced 45 TEAEs after administration of epoprostenol ES. The majority of these TEAEs were judged to be related to study drug. The most frequently reported TEAEs were headache, nausea, muscle tightness, diarrhoea, vomiting and pain in jaw (in descending order of occurrence).

Pharmacokinetics

The plasma concentration vs. time curves of epoprostenol ES and epoprostenol with respect to 6-keto-prostacyclin $F_{1\alpha}$ and 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$ were essentially superimposable (Figure 1A,B), which translated into comparable PK parameters. The 90% CIs of the geometric mean ratios for the PK parameters of the two formulations were contained within the 0.8–1.25 equivalence range for all assessed variables except half-life (Tables 2 and 3). Irrespective of the formulation administered, there was no measurable plasma concentration of 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$ in the first 2 h of infusion with epoprostenol (Figure 1B).

For 6-keto-prostacyclin $F_{1\alpha}$ steady-state concentrations were attained within 2 h after the start of each sequential infusion step except during the 6 ng kg⁻¹ min⁻¹ infusion

rate period, i.e. from 4–6 h after infusion start (Figure 1A). In contrast, steady-state levels of 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$ were not achieved within the 2 h of each infusion step (Figure 1B). Although the effect of the formulation on the half-life of either metabolite was not significant ($P > 0.05$, as assessed by a linear mixed-effect model), the 90% CIs of the geometric mean ratios were outside the 0.8–1.25 range (Tables 2 and 3).

Irrespective of the formulation administered, the concentrations of 6-keto-prostacyclin $F_{1\alpha}$ observed at 2, 4, 6 and 8 h increased slightly less than dose-proportionally whereas the observed increase in AUC(0,2, 2,4, 4,6, 6,8 h) was slightly more than dose-proportional. Indeed, when epoprostenol ES was administered, values of β (90% CI) calculated for C₂, 4, 6, 8 h and AUC(0,2, 2,4, 4,6, 6,8 h) of 6-keto-prostacyclin $F_{1\alpha}$ were 0.91 (0.84, 0.98) and 1.12 (1.07, 1.17), respectively. When epoprostenol was infused, values of β (90% CI) were 0.91 (0.84, 0.97) for C₂, 4, 6, 8 h and 1.15 (1.10, 1.20) for AUC(0,2, 2,4, 4,6, 6,8 h). Concentrations of 6, 15 diketo-13, 14-dihydro-prostacyclin $F_{1\alpha}$ observed at 4, 6, and 8 h and the AUC(2,4, 4,6, 6,8 h) increased dose-proportionally and more than dose-proportionally, respectively, irrespective of the formulation administered. Concentration and AUC values at 2 ng kg⁻¹ min⁻¹ were excluded from this analysis as more than 50% of the values were zero. In subjects treated with epoprostenol ES, values of β (90% CI) for C₄, 6, 8 h and AUC(2,4, 4,6, 6,8 h) of 6, 15 diketo-13, 14-dihydro-prostacyclin $F_{1\alpha}$ were 1.02 (0.83, 1.21) and 1.33 (1.19, 1.48), respectively. When epoprostenol was infused, values of β (90% CI) were 0.96 (0.77, 1.14) and 1.32 (1.17, 1.46) for C₄, 6, 8 h and AUC(2,4, 4,6, 6,8 h), respectively.

Figure 1

Arithmetic mean plasma concentration vs. time profiles on linear and semilogarithmic (insert) scales (A) for 6-keto-prostacyclin $F_{1\alpha}$ and (B) for 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$ during and after infusions of 2,4,6 and 8 ng kg⁻¹ min⁻¹ of epoprostenol ES (●, $n = 18$) or epoprostenol (□, $n = 18$), each for a period of 2 h

Pharmacodynamics

Overall, the average time profiles of cardiovascular markers observed with both formulations of epoprostenol were superimposable (Figure 2).

CO, Cln and HR, the cardiovascular markers for which epoprostenol induced the most pronounced effect, progressively increased over the 8 h of infusion with epoprostenol ES and epoprostenol (Figure 2). Maximum values were attained between 6 and 8 h after infusion start. After termination of infusion, CO and Cln returned rapidly to baseline values, whereas HR returned to baseline within 4–16 h. For these markers, t_{max} , R_{max} , ratios between cardiovascular response obtained 8 h after the infusion start and response at baseline ($R_{8h} : R_{0h}$) and AUE(0,8 h) values were comparable between epoprostenol ES and epoprostenol (Table 4). When compared with baseline values, maximum mean increases in CO, Cln and HR of approximately 1 l min⁻¹, 0.6 l min⁻¹ m⁻² and 12–16 beats min⁻¹, respectively, were observed during infusion with both formulations of epoprostenol. This translated into increases of approximately 30% in CO and Cln observed after 8 h of infusion with epoprostenol ES and epoprostenol, when compared with baseline ($R_{8h} : R_{0h}$

Table 2

Plasma pharmacokinetic parameters of 6-keto-prostacyclin $F_{1\alpha}$ after infusions of 2, 4, 6, and 8 ng kg⁻¹ min⁻¹ of epoprostenol ES ($n = 18$) or epoprostenol ($n = 18$), each for a period of 2 h

6-keto-prostacyclin $F_{1\alpha}$ PK parameters	Epoprostenol ES ($n = 18$)*	Epoprostenol ($n = 18$)*	Ratio epoprostenol ES : epoprostenol†
C2 h (pg ml ⁻¹)	101 (91.6, 111)	97.6 (85.6, 111)	1.03 (0.94, 1.14)
C4 h (pg ml ⁻¹)	201 (184, 218)	206 (192, 222)	0.97 (0.88, 1.07)
C6 h (pg ml ⁻¹)	275 (246, 307)	267 (236, 302)	1.03 (0.93, 1.13)
C8 h (pg ml ⁻¹)	358 (327, 392)‡	347 (308, 392)	1.03 (0.94, 1.14)
AUC(0,2 h) (pg ml ⁻¹ h)	157 (140, 176)	148 (133, 164)	1.06 (0.99, 1.13)
AUC(2,4 h) (pg ml ⁻¹ h)	385 (356, 417)	378 (354, 404)	1.01 (0.95, 1.08)
AUC(4,6 h) (pg ml ⁻¹ h)	561 (519, 606)	554 (516, 595)	1.01 (0.94, 1.08)
AUC(6,8 h) (pg ml ⁻¹ h)	735 (666, 812)	726 (668, 789)	1.01 (0.94, 1.08)
AUC(0,t) (pg ml ⁻¹ h)	1964 (1804, 2137)	1923 (1813, 2040)	1.02 (0.97, 1.07)
AUC(0,∞) (pg ml ⁻¹ h)	2021 (1833, 2227)	1972 (1840, 2114)	1.02 (0.98, 1.05)
t _{1/2} (h)	0.25 (0.14, 0.46)	0.22 (0.13, 0.38)	1.08 (0.64, 1.82)

*Data shown as geometric means (95% CI); †Data shown as ratio of geometric means (90% CI), with epoprostenol (Flolan®) as the reference; ‡ $n = 17$.

Table 3

Plasma pharmacokinetic parameters of 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$ after infusions of 2, 4, 6 and 8 ng kg⁻¹ min⁻¹ of epoprostenol ES ($n = 18$) or epoprostenol ($n = 18$), each for a period of 2 h

6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$ PK parameters	Epoprostenol ES ($n = 18$)*	Epoprostenol ($n = 18$)*	Ratio epoprostenol ES : epoprostenol†
C2 h (pg ml ⁻¹)	n/a	n/a	n/a
C4 h (pg ml ⁻¹)	74.1 (61.9, 88.6)	76.6 (69.8, 84.0)	0.97 (0.87, 1.09)
C6 h (pg ml ⁻¹)	97.2 (87.3, 108)	88.7 (72.2, 109)	1.10 (0.98, 1.23)
C8 h (pg ml ⁻¹)	150 (130, 172)‡	153 (132, 178)	1.00 (0.89, 1.13)
AUC(0,2 h) (pg ml ⁻¹ h)	n/a	n/a	n/a
AUC(2,4 h) (pg ml ⁻¹ h)	106 (82.9, 134)	101 (85.6, 119)	1.05 (0.95, 1.16)
AUC(4,6 h) (pg ml ⁻¹ h)	193 (175, 214)	187 (164, 213)	1.04 (0.94, 1.14)
AUC(6,8 h) (pg ml ⁻¹ h)	264 (237, 295)	249 (222, 280)	1.06 (0.96, 1.17)
AUC(0,t) (pg ml ⁻¹ h)	629 (554, 714)	615 (537, 704)	1.02 (0.94, 1.11)
AUC(0,∞) (pg ml ⁻¹ h)	665 (582, 760)	654 (569, 752)	1.03 (0.96, 1.10)
t _{1/2} (h)	0.32 (0.22, 0.45)	0.34 (0.26, 0.46)	0.92 (0.67, 1.27)

*Data shown as geometric means (95% CI); †Data shown as ratio of geometric means (90% CI), with epoprostenol (Flolan®) as the reference; ‡ $n = 17$.

values). Over the same period, HR showed increases of 20% and 27% during infusion with epoprostenol ES and epoprostenol, respectively.

Mean maximum decreases in DBP of -3.6 and -4 mmHg were observed after 2 h of infusion with epoprostenol and epoprostenol ES, respectively (Figure 2). Regardless of formulation, SBP did not decrease. Mean increases of up to +3.5 and +6.3 mmHg were observed after the 4th hour of infusion with epoprostenol ES and epoprostenol, respectively. The composite MAP showed mean maximum decreases of -2.6 and -2.7 mmHg after 2 h of infusion with epoprostenol ES and epoprostenol, respectively, followed by slight mean increases of maximally +1.1 to +3.8 mmHg after the 4th hour of infusion. SBP, DBP, MAP and pulse pressure (PP) values returned to baseline when infusion was stopped after 8 h.

In addition, both epoprostenol ES and epoprostenol slightly decreased ADP-induced platelet aggregation (data not shown). Although the mean decrease was less pronounced with epoprostenol, the high inter-subject variability did not allow for differentiation of the effects between formulations.

Pharmacokinetic/pharmacodynamic modelling

The maximum increase in clearance is estimated to occur approximately 30 min after food intake with a clearance increase of 39% (from 85 l h⁻¹ to 119 l h⁻¹). The food effect is estimated to appear with a delay of 13 min after food intake and has almost entirely disappeared 2 h after food intake (Figure 3).

The pharmacokinetic model characterizes the 6-keto-prostacyclin $F_{1\alpha}$ data well (Figure 4A, top row). The

Figure 2

Arithmetic mean ($\pm$ SD) cardiac output, cardiac index, SBP, DBP, MAP and HR vs. time profiles measured during and after infusions of 2, 4, 6 and 8 ng kg⁻¹ min⁻¹ of epoprostenol ES ($\bullet$, $n = 18$) or epoprostenol ($\square$, $n = 18$), each for a period of 2 h

Table 4

Pharmacodynamic parameters for cardiac output, cardiac index and heart rate after infusions of 2, 4, 6 and 8 ng kg⁻¹ min⁻¹ of epoprostenol ES (*n* = 18) or epoprostenol (*n* = 18), each for a period of 2 h

Haemodynamic markers	Treatment	<i>t</i> _{max}	<i>R</i> _{max}	AUE(0,8 h)	<i>R</i> _{8 h} : <i>R</i> _{0 h}
Cardiac output	Epoprostenol ES	8 (0, 8) h	4.91 ± 0.81 l min ⁻¹	3.23 ± 4.13 l min ⁻¹ h	1.30 ± 0.27
	Epoprostenol	8 (0, 8) h	4.93 ± 0.67 l min ⁻¹	3.38 ± 4.28 l min ⁻¹ h	1.34 ± 0.27
Cardiac index	Epoprostenol ES	6 (0, 8) h	2.67 ± 0.47 l min ⁻¹ m ⁻²	1.80 ± 2.34 l min ⁻¹ m ⁻² h	1.30 ± 0.27
	Epoprostenol	8 (0, 8) h	2.67 ± 0.30 l min ⁻¹ m ⁻²	1.86 ± 2.39 l min ⁻¹ m ⁻² h	1.34 ± 0.28
Heart rate	Epoprostenol ES	6 (0, 8) h	81 ± 11 bpm	63 ± 49 beats min ⁻¹ h	1.20 ± 0.15
	Epoprostenol	6 (6, 8) h	81 ± 9.7 bpm	65 ± 44 beats min ⁻¹ h	1.27 ± 0.13

Data are arithmetic means (± SD) or for *t*_{max} the median (min, max). (*t*_{max}) time to reach the maximum response (*R*_{max}) during the 8 h of infusion; (*R*_{8 h} : *R*_{0 h}) ratio between cardiovascular response obtained 8 h after the infusion start and response at baseline; AUE(0,8 h)) area under the effect–time curve, i.e. drug response over the 8 h of infusion.

Figure 3

(A) Population-typical concentration–time profile of 6-keto-prostacyclin F_{1α} with and without food at 4 h after start of infusion at subsequent infusion rates of 2, 4, 6 and 8 ng kg⁻¹ min⁻¹, each for a period of 2 h. (B) Apparent drug clearance with food given at 4 h after start of infusion

introduction of individual random effects (Figure 4a, bottom row) improves the model fit overall while it emphasizes some individual values that would not be expected under the model (the individual predicted concentrations are

substantially higher than the observed concentrations). These observations arise from two subjects, one on epoprostenol and one on epoprostenol ES. The first subject shows an unusually large decrease in concentration after food intake while the second subject shows no decrease at all. The unexpected pharmacokinetic behaviour might thus be related to uncontrolled food intake.

Following start of infusion with epoprostenol, the haemodynamic effects appeared almost immediately (e.g. for HR, see Figure 2). For this reason, the relationship between Cln and concentrations of 6,15-diketo-13,14-dihydro-prostacyclin F_{1α}, for which a time lag of approximately 2 h for the appearance in the plasma was observed (Figure 1B), was not assessed. In contrast to 6,15-diketo-13,14-dihydro-prostacyclin F_{1α}, the appearance of 6-keto-prostacyclin F_{1α} in plasma occurred almost immediately following infusion start (Figure 1A), therefore, the relationship between Cln and 6-keto-prostacyclin F_{1α} considered the surrogate of epoprostenol, was examined. In agreement with the above, the data suggest that Cln responds instantaneously and in proportion to a change in concentration of 6-keto-prostacyclin F_{1α} returning to baseline levels immediately after ending infusion. The raw data indicate relatively large differences between subjects and a few outliers. For two subjects on epoprostenol, the Cln decreases during increasing infusion rates of epoprostenol. At low concentrations of 6-keto-prostacyclin F_{1α}, Cln decreases in some subjects whereas it increases consistently at higher concentrations.

The Cln change is shown against observed concentration of 6-keto-prostacyclin F_{1α} (Figure 4B) with linear regression (including an intercept) and nonlinear regression overlaid. Both indicate that a linear relationship without intercept is appropriate for characterization of the PK/PD effect.

The linear regression model was estimated as Cln change from baseline = 0.0011299 × 6-keto-prostacyclin F_{1α} concentration. An intercept proved statistically non-significant (*P* = 0.69). The standard error for the slope was estimated as 0.0001239, the coefficient of variation as 11.0% and the *t* statistic as 9.12, corresponding to a *P* value < 0.0001 and the residual standard error as 0.4296.

Figure 4

(A) Observed vs. model-predicted 6-keto-prostacyclin $F_{1\alpha}$ concentrations, (B) cardiac index change from baseline vs. 6-keto-prostacyclin $F_{1\alpha}$ concentrations with regression lines. ○ epoprostenol; + epoprostenol ES; — linear regression; - - - local regression (LOESS)

Discussion

To date, the PK of epoprostenol had not been fully characterized due in part to the instability of epoprostenol [23], with an *in vitro* half-life of 3–6 min in human blood [3]. In addition, no convenient validated bioanalytical method fulfilled the sensitivity and accuracy requirements because of the very low concentrations of epoprostenol (pg ml^{-1}) reported in human plasma [24]. Although some studies have suggested that 6-keto-prostacyclin $F_{1\alpha}$, the non-enzymatic hydrolysis and more stable product of epoprostenol, would represent a suitable surrogate marker of plasma concentrations of epoprostenol [25, 26], no robust PK characterization of this metabolite in humans has been performed. The present study provides the first characterization of the PK of epoprostenol via assessment of 6-keto-prostacyclin $F_{1\alpha}$ and another primary metabolite, 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$ [26].

Overall, the plasma concentration–time curves of epoprostenol ES and epoprostenol, with respect to the two metabolites 6-keto-prostacyclin $F_{1\alpha}$ and 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$, were virtually superimposable. This translated into comparable PK variables, not affected by the formulation, with 90% CIs of the geometric mean ratios contained within the 0.8–1.25 equivalence range [27] for all assessed parameters except half-life. Collectively, these results show that the PK of both formulations of epoprostenol are similar. Compared with 6-keto-prostacyclin $F_{1\alpha}$, there was a delay in the appearance of 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$ in plasma following start of infusion with epoprostenol. This is in agreement with the fact that 6-keto-prostacyclin $F_{1\alpha}$ is spontaneously and rapidly formed by hydration of epoprostenol whereas 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$ is formed by enzymatic degradation [17].

The PK of both metabolites deviated slightly from dose proportionality regardless of the formulation administered. This may be partly explained by the decreases in plasma concentrations observed during infusion with epoprostenol ES or epoprostenol at $6 \text{ ng kg}^{-1} \text{ min}^{-1}$. These decreases coincided with a standardized meal given to every subject during the corresponding time interval (i.e. 4–6 h after start of the infusion). An increase in drug systemic clearance following food intake has been described in the literature, e.g. for propranolol [19], labetalol [28] or budesonide [29] administered via i.v. infusion. Indeed, for drugs having high hepatic clearance and administered i.v., the postprandial increase in liver blood flow results in an augmentation of the systemic clearance and a decrease in systemic concentrations when compared with fasted conditions. A change in the hepatic blood flow, although not assessed in the present study, may also explain the decrease in plasma concentrations of both metabolites observed after food intake.

The model-based estimates for clearance (85 l h^{-1}) and volume of distribution (23.7 l) are in line with animal

results ($93 \text{ ml kg}^{-1} \text{ min}^{-1}$ and 357 ml kg^{-1} , respectively) which translate into a clearance of 116 l h^{-1} and a volume of distribution of 26.8 l for a 75 kg adult (Actelion Pharmaceuticals Ltd, data on file). The apparent effect of food on the PK is characterized by an increase in clearance of up to 39% that disappears about 2 h after food intake, in agreement with previous publications reporting a systemic clearance increase of 38% after food intake [19, 30].

The postprandial decreases in plasma concentrations observed for 6-keto-prostacyclin $F_{1\alpha}$ and 6, 15-diketo-13, 14-dihydro-prostacyclin $F_{1\alpha}$ had no observable impact on the effect of epoprostenol ES and epoprostenol on haemodynamic measurements. This may reside in the fact that the haemodynamic effect of both formulations is driven by the parent compound epoprostenol, whose main metabolism pathway, i.e. leading to the formation of 6-keto-prostacyclin $F_{1\alpha}$, results from a spontaneous hydration and is therefore not a function of liver metabolism and liver blood flow.

In line with the PK results, epoprostenol ES and epoprostenol also produced comparable changes in the different cardiovascular parameters assessed in the present study. In accordance with the haemodynamic profile usually reported during infusion of epoprostenol in animals [31] and man [12, 13, 32–34], i.e. initial systemic vasodilatation activating a baroreflex which in turn reduces systemic vasodilatation, accelerates HR and leads to an increased CO, we found a transient decrease in DBP followed by a quick return to baseline and an increase in SBP, associated with a dose-dependent increase in CO, Cln and HR. Overall, the present observations are in agreement with the known vasodilator [35] and inotropic [36] properties of epoprostenol. In fact, these measured effects parallel some of the beneficial haemodynamic outcomes reported in PAH patients on continuous i.v. epoprostenol [10, 13, 14, 16, 36].

The PK/PD model shows that the PD, as reflected in Cln, are correlated in proportion to the change in concentration of 6-keto-prostacyclin $F_{1\alpha}$, suggesting that 6-keto-prostacyclin $F_{1\alpha}$ is a good surrogate to characterize the PK of epoprostenol.

At the doses administered ($2\text{--}8 \text{ ng kg}^{-1} \text{ min}^{-1}$), i.e. in the range of doses previously tested in studies in man without report of any safety and tolerability concerns [32, 33], the number of subjects experiencing AEs was similar for both formulations. The majority of the AEs was mild in intensity and observed with doses of 6 and $8 \text{ ng kg}^{-1} \text{ min}^{-1}$ for both epoprostenol ES and epoprostenol. As expected, the most commonly reported TEAEs included headache and nausea, which is consistent with the vasodilator properties of epoprostenol, the AEs described in the Flolan® [17] product monograph and previous studies in man [3, 13]. Clinical laboratory evaluations, vital signs, ECGs and physical examinations did not reveal any clinically relevant effect of the infusion with epoprostenol regardless of the formulation. Overall, both epoprostenol and epoprostenol ES were

well tolerated, with no difference between the profiles of both formulations.

In summary, this study characterizes for the first time the PK of epoprostenol in man via two major metabolites, 6-keto-prostacyclin $F_{1\alpha}$ and 6,15-diketo-13,14-dihydro-prostacyclin $F_{1\alpha}$. PK and PD profiles of i.v. epoprostenol showed that Cln relates proportionally and linearly to the plasma concentrations of 6-keto-prostacyclin $F_{1\alpha}$. These results suggest that 6-keto-prostacyclin $F_{1\alpha}$ is a suitable surrogate marker of plasma concentrations of epoprostenol. In addition, the present results further suggest that non-invasive haemodynamic measurements by echocardiography are suitable for assessing the potential haemodynamic effects of medications, e.g. against PAH, in early development studies in healthy subjects. Finally, the methodology established was applied in the context of a biocomparison study between two formulations of epoprostenol which showed comparable PK, PD, safety and tolerability characteristics.

Conflict of Interest

This study was sponsored by Actelion Pharmaceuticals Ltd (Allschwil, Switzerland). The authors Laurent B. Nicolas, Andreas Krause, Marcelo Gutierrez and Jasper Dingemans are full-time employees of Actelion Pharmaceuticals Ltd. Cetero Research Miami (Miami, FL, USA) received financial compensation for the costs associated with the clinical conduct.

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