



# International Journal of Pharmacology

ISSN 1811-7775

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## Tissue Distribution, Metabolism and Excretion of PGE<sub>1</sub> Following Prolonged High-Dose Inhalation in Neonatal Pigs

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**Abstract:** Inhaled prostaglandin E<sub>1</sub> (IPGE<sub>1</sub>) is a potential selective pulmonary vasodilator in neonatal pulmonary hypertension. The objective of this study was to evaluate the bioavailability and metabolism of IPGE<sub>1</sub>. Anesthetized ventilated piglets received either high dose IPGE<sub>1</sub> (1200 ng/kg/min) [Study group] or nebulized saline [Control group] using a jet nebulizer. PGE<sub>1</sub> and its metabolite, 15-keto-PGE<sub>1</sub>, were quantified in blood, urine and lung tissue using high performance liquid chromatography-mass spectrometry. Fourteen piglets underwent the experimental protocol (age 1-9 days). Of these, nine received IPGE<sub>1</sub> and five received nebulized saline. Among control pigs, two died of complications at 3-4 h, one at 12-13 h and the remaining two were euthanized at 24 h after start of aerosol. In the study group, three animals died after 14-15 h of aerosol treatment of iatrogenic complications and six animals received aerosol for 24 h. Plasma and urine PGE<sub>1</sub> levels increased significantly over time in study (p<0.05) but not control animals. Plasma and urinary 15-keto-PGE<sub>1</sub> levels and lung tissue prostaglandin profile were comparable in study and control animals. In conclusion, this is the first report of the tissue distribution, metabolism and excretion of prolonged high dose IPGE<sub>1</sub>. The increase in PGE<sub>1</sub> levels in plasma and urine over time without accumulation in lung tissue or systemic side effects suggests effective aerosol delivery, extensive pulmonary metabolism and efficient excretory mechanisms.

**Key words:** Neonatal hypoxemic respiratory failure, persistent pulmonary hypertension of the newborn, aerosolized, selective pulmonary vasodilator, prostaglandin E<sub>1</sub>/alprostadil, newborn

### INTRODUCTION

Neonatal Hypoxemic Respiratory Failure (NHRF) in term/near-term infants is often associated with persistent Pulmonary Hypertension of the Newborn (PPHN). Intravenous PGE<sub>1</sub> (ivPGE<sub>1</sub>) has been used empirically in NHRF as a pulmonary vasodilator to improve oxygenation (Walsh-Sukys *et al.*, 2000). However, its use has been reported to be associated with systemic hypotension and worsening of oxygenation due to increased venous admixture (Awad *et al.*, 1996). This had led investigators to explore the delivery of PGE<sub>1</sub> directly to the lungs as an inhalation, thus minimizing systemic effects and achieving selective pulmonary vasodilation (Meyer *et al.*, 1998; Putensen *et al.*, 1998; Walmrath *et al.*, 1997). In previous studies, we have reported on the feasibility, effectiveness of delivery, emitted dose, aerosol particle size distribution and safety of IPGE<sub>1</sub>, with minimal pulmonary toxicity (Sood *et al.*, 2004, 2006, 2007, 2008a, b, 2009). The

objective of this study is to extend our prior work by evaluating the bioavailability of IPGE<sub>1</sub> following prolonged high dose administration in an *in vivo* neonatal animal model. A secondary objective is to evaluate the effect of the administration of exogenous vasodilator i.e., IPGE<sub>1</sub> on the synthesis of the endogenous vasodilator, PGE<sub>2</sub>.

PGE<sub>1</sub> (11 $\alpha$ ,15 $\alpha$ -dihydroxy-9-oxoprost-13-enoic acid, alprostadil) is a stable compound at neutral pH, but is rapidly metabolized to the inactive 15-keto-PGE<sub>1</sub> and 13,14-dihydro-15-keto-PGE<sub>1</sub> upon contact with the pulmonary endothelium (Leonhardt *et al.*, 1992). The metabolites of PGE<sub>1</sub> are excreted primarily by the kidney, with ~90% of an administered intravenous dose excreted in urine within 24 h. Aqueous solutions of PGE<sub>1</sub> show a small, spontaneous dehydration to the significantly less active PGA<sub>1</sub> within 2 h following dilution from an ethanolic solution at room temperature (RT) (Sood *et al.*, 2007).

Our hypothesis was that following prolonged high-dose inhalation, there would be no significant changes in systemic PGE<sub>1</sub> levels because of direct delivery to the lungs and extensive first pass pulmonary metabolism; however, levels of the inactive metabolite, 15-keto-PGE<sub>1</sub> will be increased reflecting the enhanced metabolism of the exogenously administered PGE<sub>1</sub>. In addition, the local levels of PGE<sub>1</sub> and its metabolite 15-keto-PGE<sub>1</sub> will be elevated in lung tissue reflecting direct delivery to the lung and pulmonary metabolism. We also hypothesized that exogenously administered IPGE<sub>1</sub> will downregulate the synthesis of the endogenous vasodilator, PGE<sub>2</sub>.

## MATERIALS AND METHODS

**Subjects:** Healthy specific pathogen free domestic piglets underwent the experimental procedures after approval by the Institutional Animal Care and Use Committee at Wayne State University from March 2006 to March 2007. All animals received care in compliance with NIH guidelines.

**Animal preparation:** The animal preparation, ventilator circuit, aerosol administration and monitoring have been described previously and are briefly summarized below (Sood *et al.*, 2008a). Animals were intubated oro-tracheally and the carotid artery cannulated for continuous blood pressure monitoring and arterial blood sampling. Anesthesia was maintained by intravenous infusion of ketamine, midazolam and fentanyl. Blood pressure was monitored continuously and recorded every 2 h.

**Mechanical ventilation:** Time cycled, pressure limited assisted ventilation (Sechrist) was initiated at a rate of 15 bpm, FiO<sub>2</sub> of 1.0, peak inspiratory pressure of 18 cm H<sub>2</sub>O and positive end expiratory pressure of 4 cm H<sub>2</sub>O. Gas flow in the heated humidified ventilator circuit was 8 LPM. Ventilator parameters were adjusted to maintain normocapnia. Oxygen saturations were monitored continuously and arterial blood gas analyses performed every 6 h and as needed.

**Drug preparation:** Animals were randomly assigned to receive IPGE<sub>1</sub> (Study group) or nebulized saline (Control group) continuously for 24 h. PGE<sub>1</sub>, supplied as 500 µg of Alprostadil in 1 mL of ethanol (Gensia Sior Pharmaceuticals, Irvine, California), was diluted in normal saline to yield a dose of 1200 ng/kg/min when infused into the nebulizer chamber at a rate of 4 mL h<sup>-1</sup>.

**Administration of continuous aerosol:** The low flow MiniHeart jet nebulizer (Westmed Inc., Lakewood,

Colorado) was used to generate continuous aerosols in the inspiratory limb of the ventilator circuit ~20 inches from the endotracheal tube (Sood *et al.*, 2004). At the start of aerosol therapy, the nebulizer chamber was primed with 2 mL of the study medication followed by continuous delivery into the nebulizer chamber at a rate of 4 mL h<sup>-1</sup>. During the inhalation period, the ventilator flow was adapted according to the additional flow from the nebulizer.

**Blood sampling:** Blood for quantification of PGE<sub>1</sub>, 15-keto-PGE<sub>1</sub>, PGA<sub>1</sub> and PGE<sub>2</sub> was obtained prior to administration of aerosol (baseline) and at 2, 4, 12, 18 and 24 h after start of aerosol treatment. The blood was obtained in indomethacin-EDTA bottles, centrifuged immediately, the plasma separated and frozen at -80°C till the time of analysis.

**Urine samples:** A 20 G intracath was placed percutaneously once the urinary bladder became palpable. Urine could be obtained by this method only in some animals and at limited time points. Urine was frozen immediately after collection at -80°C till the time of analysis.

**Lung tissue samples:** At the end of the experimental protocol, the piglets were euthanized. At autopsy, sections were taken from upper and lower lobes of right and left lungs and flash frozen for analysis of PGE<sub>1</sub>, 15-keto-PGE<sub>1</sub>, PGA<sub>1</sub> and PGE<sub>2</sub>. Lung tissue was homogenized in 50 mM phosphate buffered (pH 7.2) saline at a ratio of 9 mL g<sup>-1</sup> of tissue using a Teflon homogenizer at 0°C. The homogenate was centrifuged at 2,000x g for 5 min and the supernatant was collected for prostaglandin analysis.

**Liquid Chromatography-Mass Spectrometric (LC-MS) analysis of PGE<sub>1</sub> and its metabolites:** The levels of PGE<sub>1</sub> and its metabolites 15-keto PGE<sub>1</sub> and PGA<sub>1</sub> as well as the endogenous PGE<sub>2</sub> were quantified by LC-MS using tetradeuterated PGE<sub>1</sub> (PGE<sub>1</sub>-d<sub>4</sub>) as internal standard. PGE<sub>1</sub>-d<sub>4</sub> (10 ng) was added to 1 mL of the sample (plasma, urine, or the supernatant of the lung homogenates) and mixed thoroughly. The sample was applied to an Empore, SD C18 membrane cartridge (3 M Corporation, Minneapolis, MN) preconditioned with 1 mL each of methanol followed by water. After all the sample was passed through, the cartridge was washed with 5 mL each of distilled water and hexane. The cartridge was dried under suction and eluted with 0.5 mL of ethyl acetate. The eluate was evaporated under a stream of nitrogen and the residue was redissolved in 0.1 mL of the HPLC mobile phase (acetonitrile: water; Acetic acid; 45:55:0.1). The

extracted samples were analyzed by LC-MS/MS using Micromass Quattro LC coupled to Waters Alliance HPLC system as described before (Sood *et al.*, 2007). Under the standard conditions the limit of detection (LOD) for both PGE<sub>1</sub> and its potential metabolites (PGA<sub>1</sub> and 15-keto PGE<sub>1</sub>) is 0.0025 µg mL<sup>-1</sup> and the Limit of Quantitation (LOQ) is 0.03 µg mL<sup>-1</sup>.

**Statistical analysis:** Differences in PGE<sub>1</sub> concentrations in blood, urine and lung tissue between animals in the study and control groups were evaluated by the student t-test. Paired Student t-test was conducted to compare baseline PGE<sub>1</sub> levels to each of the subsequent time points. The results were further verified using the General Estimation Equation (GEE) to take into account the correlation of repeated measures (Walker, 2002). Tissue concentrations of the PGs in fresh frozen samples obtained from the four lung lobes were averaged to obtain a single value for each animal (ng g<sup>-1</sup>). Two-tailed significance level was set at 0.05. Statistical analyses were performed using the SPSS® statistical package, version 15.0.1 (SPSS Inc., Chicago, IL, USA) and SAS/STAT® software, Version [9.1.3] (SAS Institute Inc., Cary, NC, USA).

## RESULTS

Fourteen piglets underwent the experimental protocol. Of these, nine received IPGE<sub>1</sub> (study group) and five received nebulized saline (control group). In the control pigs, two died of iatrogenic complications at 3-4 h, one at 12-13 h and the remaining two were euthanized at 24 h after start of aerosol treatment. In the study group, three animals died after 14-15 h of aerosol treatment of iatrogenic complications and six animals received aerosol for 24 h. Data for all animals was included in the analyses presented in this report. Two values of arterial PGE<sub>2</sub> that were outliers (measured at 0 (n = 1) and 2 h (n = 1)) and one value of arterial 15-keto-PGE<sub>1</sub> were excluded as they were >3 SD beyond the mean (Winsorizing). Results of statistical analysis were similar with and without winsorizing.

**Baseline characteristics:** The mean birth weight of the piglets was 2.1 kg (range 1.4 to 2.5 kg) and the mean age was 4.7 days (range 1 to 9 days). Majority of the piglets were male (86%).

**Hemodynamic parameters:** The mean heart rate, systolic and diastolic blood pressures were not significantly different between control and study animals, although the values were lower in the study animals for all three

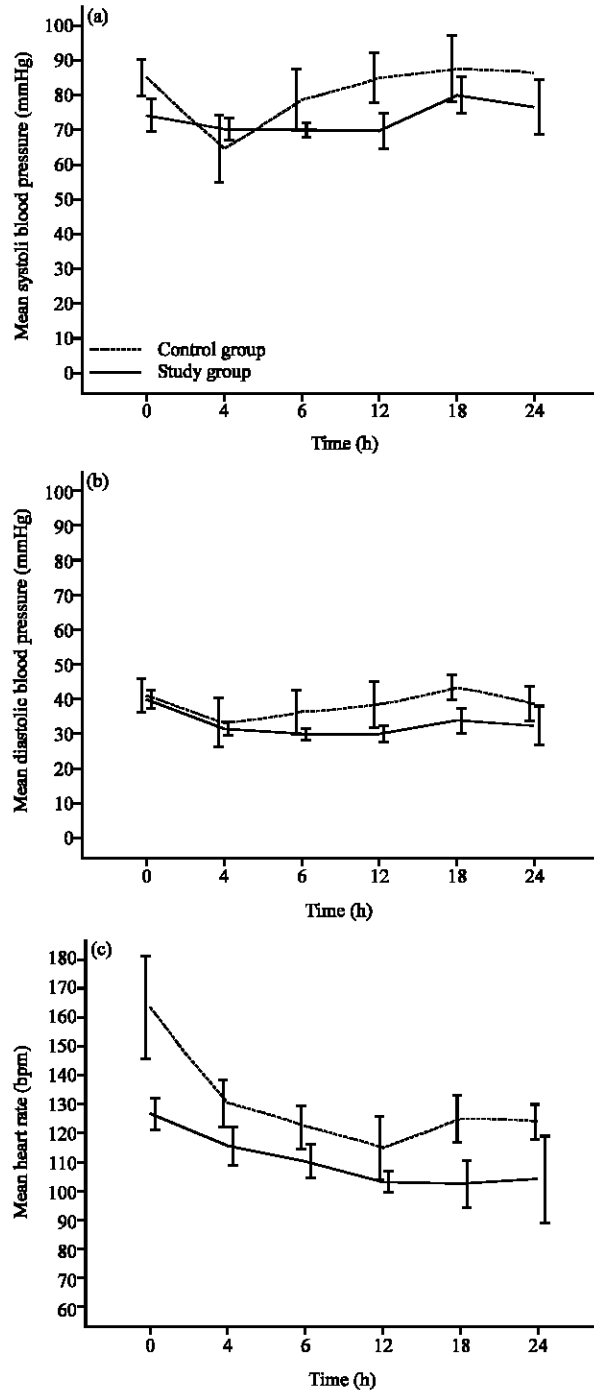


Fig. 1: (a-c) Hemodynamic parameters following IPGE<sub>1</sub>. Error Bars represent ±SE

hemodynamic parameters. No significant group differences or time trends in heart rate or blood pressure were observed during the aerosol administration in either group (Fig. 1a-c). The mean systolic blood pressure was above 60 mmHg, the mean diastolic blood pressure greater

Table 1: Plasma prostaglandin profile

Time (h)	N		PGE <sub>1</sub>		15-keto-PGE <sub>1</sub>		PGA <sub>1</sub>		PGE <sub>2</sub>	
	Control	Study	Control	Study	Control	Study	Control	Study	Control	Study
0	5	9	100±62	083±60	286±88	311±101	18±18	9±6	3±3	138±81
2	5	9	154±60	160±63	115±115	198±75	10±10	9±6	58±58	200±81
6	3	9	247±89	360±153	123±83	167±65	0	14±10	30±30	89±53
12	3	9	133±133	567±116**	227±114	196±74	20±20	14±11	40±20	158±82
18	2	6	340±340	648±152*	350±20	382±107	0	13±13	15±115	115±80
24	2	6	250±220	242±159	280±70	458±156	10±10	18±12	110±20	54±50

Numbers represent Mean±SD of PG levels in pg mL<sup>-1</sup>, \*\*p<0.005 compared to baseline (time 0), \*p<0.05 compared to baseline (time 0)

than 30 mmHg and the mean heart rate above 100 bpm in both groups at all time points. None of the piglets in either group required vasopressors.

**Plasma prostaglandin profile:** There was marked variability in the concentration of PGE<sub>1</sub>, 15-keto-PGE<sub>1</sub>, PGA<sub>1</sub> and PGE<sub>2</sub>, in study and control animals (Table 1). There was no difference in baseline plasma PGE<sub>1</sub> levels between study and control animals (Fig. 2a, b). On bivariate analysis, study animals had higher mean PGE<sub>1</sub> levels over the course of the experiment as compared to the control animals. However, these differences were not statistically significant. There was a significant increase in PGE<sub>1</sub> levels at 12 (p = 0.003) and 18 (p = 0.013) h compared to baseline (paired samples t-test) in the study animals but not in control animals. At 24 h, PGE<sub>1</sub> levels were not significantly different from baseline (p >0.1). Longitudinal data analysis revealed significant increase of PGE<sub>1</sub> levels (t-value = 2.50, p = 0.0171) with time in the study group but not for the control animals. Age, gender and duration in study were not important determinants of plasma PGE<sub>1</sub> levels in either group. There were no group differences or time trends noted in plasma levels of 15-keto-PGE<sub>1</sub> (Fig. 2), PGA<sub>1</sub> and PGE<sub>2</sub> in the control and study animals. Notably, study animals had higher levels of PGE<sub>2</sub> at baseline as compared to control animals; however this difference was not statistically significant.

**Urine prostaglandin profile:** Urine prostaglandin levels were determined at 2 h (4 control and 2 study animals) and 6, 12, 18 and 24 h (two control and two study animals each) after aerosol administration. Urinary PGE<sub>1</sub> levels were higher in study animals at 12, 18 and 24 h. Study animals had 8-fold higher levels of 15-keto-PGE<sub>1</sub> at 2 and 6 h after aerosol administration. By 12 h, the urinary 15-keto-PGE<sub>1</sub> levels were comparable in the two groups. Statistical tests of significance using paired t-tests were not performed because of the small numbers of urine samples at each time point. Longitudinal data analysis revealed significant increase of urinary PGE<sub>1</sub> (t-value = 2.77, p = 0.0324) levels with time for animals in the study group but not for control animals. Age, gender and duration in study were not important determinants of

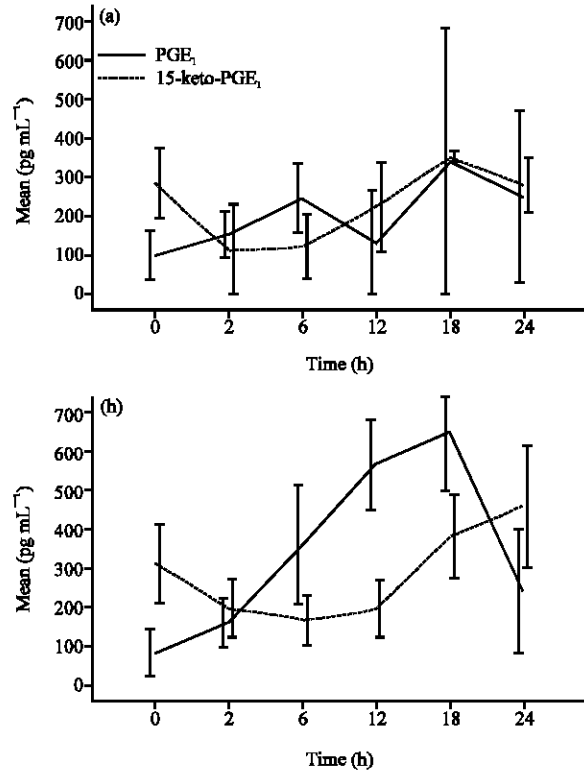


Fig. 2: Plasma PGE<sub>1</sub> and 15-Keto-PGE<sub>1</sub> Levels Following IPGE<sub>1</sub>. (a) control group, (b) study group. Error bars represent ±SE

urine PGE<sub>1</sub> levels in either group. There were no group differences or time trends noted in urine levels of 15-keto-PGE<sub>1</sub>, PGA<sub>1</sub> and PGE<sub>2</sub> in the control and study animals.

**Lung tissue prostaglandin profile:** Pulmonary concentrations of PGE<sub>1</sub>, its metabolites and PGE<sub>2</sub> were much higher than in lung tissue than in blood (Table 2). Most of the PGE<sub>1</sub> detected in the lung tissue was in the form of its primary metabolite, 15-keto PGE<sub>1</sub> (>97%) Although, the lung tissue concentrations of PGE<sub>1</sub>, 15-keto-PGE<sub>1</sub>, PGA<sub>1</sub> and PGE<sub>2</sub> were higher in the study animals, these differences were not statistically significant. There were no regional differences in

Table 2: Lung prostaglandin profile

PG Type	Control (n = 5)	IPGE <sub>1</sub> (n = 9)
PGE <sub>1</sub>	128±54	139±95
15-Keto-PGE	3388±2686	4976±3001
PGA <sub>1</sub>	3.75±4.41	6.92±6.44
PGE <sub>2</sub>	2525±619	2791±1378

Numbers represent Mean±SD of PG levels in ng mL<sup>-1</sup>

pulmonary PGE<sub>1</sub> and metabolite concentrations in the control or study animals.

## DISCUSSION

This study is the first report of the tissue distribution, metabolism and excretion of high-dose IPGE<sub>1</sub> following prolonged administration (12-24 h) in healthy anesthetized ventilated neonatal pigs using the highly sensitive and selective HPLC-MS. Contrary to our hypothesis, there was a statistically significant increase in PGE<sub>1</sub> levels in plasma and urine in Study animals over time ( $p < 0.05$ ) but not in the control animals. Study animals had higher urinary 15-keto-PGE<sub>1</sub> levels at 2 and 6 h after start of IPGE<sub>1</sub>; however these differences were not statistically significant. There were no differences in lung tissue prostaglandin profile between study and control animals. None of the animals had hypotension requiring treatment with vasopressors. These observations signify effective aerosol delivery, extensive pulmonary metabolism and efficient excretory mechanisms for PGE<sub>1</sub> and its metabolites resulting in lack of local lung accumulation, rise in plasma and urine levels over time without associated systemic effects followed by return to baseline levels at 24 h. The PGE<sub>2</sub> levels in blood and lung tissue were similar in control and study animals suggesting that inhalation of high dose PGE<sub>1</sub> over 12-24 h does not downregulate the endogenous production of PGE<sub>2</sub>.

IPGE<sub>1</sub> differs from the other commonly used inhaled medications in undergoing extensive first pass pulmonary metabolism. There are only two studies that have investigated the pharmacokinetics of inhaled prostaglandins following short-term administration (Borok *et al.*, 1991; Sood *et al.*, 2006). Analytical methods with high sensitivity have to be used to describe the pharmacokinetics of PGE<sub>1</sub> because of low endogenous plasma levels (Sood *et al.*, 2006). Additionally, the analytical methods have to be selective and specific to distinguish the parent compound from its metabolites. In this report we have used an efficient HPLC resolution of PGE<sub>1</sub> and its potential degradation products combined with mass spectrometric identification and quantitation which offers excellent selectivity and sensitivity.

Several investigators have reported PGE<sub>1</sub> levels in a small number of adults, pregnant women and newborns

following ivPGE<sub>1</sub> (Awad *et al.*, 1996; Cawello *et al.*, 1994, 1995; Hammes *et al.*, 1999; Kunimoto *et al.*, 1997; Leonhardt *et al.*, 1992; Schweer *et al.*, 1994a, b; Shimizu *et al.*, 1992; Sood *et al.*, 2006). Previous reports have highlighted the marked variability of PGE<sub>1</sub> concentrations in blood and pulmonary tissue between individuals as also noted in the present study (Awad *et al.*, 1996; Sood *et al.*, 2006). These reflect the variance in individual clearances of PGE<sub>1</sub>.

We have previously reported levels of PGE<sub>1</sub> ranging from 440±239 to 538±228 pg mL<sup>-1</sup> when escalating and weaning doses of IPGE<sub>1</sub> (50 to 300 ng/kg/min) were administered to neonates with NHRF for a maximum duration of 3 h (Sood *et al.*, 2006). In the present study, we used high dose IPGE<sub>1</sub>, 1200 ng/kg/min, a dose four times the maximal dose reported in humans, to be able to evaluate drug toxicity; these results have been previously published (Sood *et al.*, 2008a). The range of plasma PGE<sub>1</sub> levels noted in the present study, 160±63 to 648±152 pg mL<sup>-1</sup>, when higher doses of IPGE<sub>1</sub> (1200 ng/kg/min) were administered continuously to healthy anesthetized neonatal pigs for a maximum duration of 24 h are comparable to the results of our previous study. In the present study, plasma and urine PGE<sub>1</sub> levels showed a significant increase over time in Study animals without systemic hemodynamic effects suggesting effective aerosol delivery and efficient metabolic and excretory mechanisms that prevent cumulative toxicity. An early rise in the levels of 15-keto-PGE<sub>1</sub> in the urine (2 and 6 h) without an increase in plasma PGE<sub>1</sub> or 15-keto-PGE<sub>1</sub> levels can be explained by the existence of efficient metabolic and excretory pathways. An alternative explanation could be that the timing of blood collection in the experimental protocol described may have missed an early rise in plasma PGE<sub>1</sub> or 15-keto-PGE<sub>1</sub> levels. An increase in plasma PGE<sub>1</sub> levels at 12 and 18 h together with elevated urine PGE<sub>1</sub> levels at 12, 18 and 24 h may indicate a critical point at which the metabolic pathway is unable to immediately metabolize the continuing exogenous load of IPGE<sub>1</sub> to 15-keto-PGE<sub>1</sub> resulting in higher plasma and urine levels. The subsequent trend of falling PGE<sub>1</sub> levels by 24 h with increasing urinary excretion despite continuing administration of IPGE<sub>1</sub> may reflect either up regulation of the endogenous excretory pathways in response to the exogenous load or be a spurious observation because of the small number of animals surviving to 24 h.

Borok *et al.* (1991) reported elevated PGE<sub>1</sub> levels in the broncho-alveolar lavage fluid in sheep following single dose of 5 mg IPGE<sub>1</sub>. These remained elevated above baseline for up to 2 h. Simultaneously obtained plasma PGE<sub>1</sub> levels did not change ( $p > 0.1$ ). The investigators concluded that aerosolization permitted

local delivery of the active drug to the alveolar epithelial surface with prolongation of its local half-life while avoiding systemic adverse effects. However, PGE<sub>1</sub> concentration in broncho-alveolar lavage fluid is more indicative of deposition in the central airways than in the alveolar compartment and lung tissue concentration may be more indicative of alveolar deposition (Goldstein *et al.*, 2002). In the present study, lung tissue levels of PGE<sub>1</sub> showed marked variability but were not significantly different between control and study animals. This is consistent with the extensive first-pass pulmonary inactivation of PGE<sub>1</sub> and suggests that this capacity is nonsaturable over the dose range and duration in the present study.

PGA<sub>1</sub> concentration were low in the plasma and lungs of both groups of animals suggesting that jet nebulization of PGE<sub>1</sub> over a 24 h period in a neonatal ventilator circuit with high FiO<sub>2</sub> does not lead to increased conversion of PGE<sub>1</sub> to PGA<sub>1</sub>. PGE<sub>2</sub> levels were much higher in lung tissue as compared to plasma; there were no differences between animals in the two groups suggesting that when administered in the doses and duration described in this report, IPGE<sub>1</sub> does not downregulate the endogenous production of PGE<sub>2</sub>.

Despite the important findings described in this study, there are potential deficiencies. We have reported the pharmacokinetics of IPGE<sub>1</sub> in a small number of mechanically ventilated healthy neonatal pigs. Mortality was high in the beginning of the study because of complications related to anesthesia, airway suctioning and failure of gas supply in the laboratory. Once the optimal dosing of anesthetic was determined and airway suctioning minimized as anesthetized piglets can develop profound bradycardia upon suctioning, these complications did not recur. The use of an animal model permitted evaluation of blood levels in repeated samples and in lung tissue, which may not have been ethical in human newborns. Another limitation is that urine samples were obtained in a small number of animals at limited time points because of inability to obtain urine consistently at various time points despite cannulation of the urinary bladder. Repeated measurements allowed each animal's baseline values to serve as control values and also permitted the use of longitudinal data analysis that provides more efficient estimators than cross-sectional design and in part compensated for the small sample size of this study. Species differences and the presence of underlying disease may influence alveolar drug delivery and metabolism. However, investigation of drug pharmacokinetics in a healthy animal model undergoing assisted ventilation was an important first step before proceeding to pharmacokinetic studies in the lung

injury model. Lastly, we did not measure further downstream metabolites of PGE<sub>1</sub> in plasma and urine (e.g., 13,14-dihydro-15-keto-PGE<sub>1</sub>), which may be detectable in higher amounts and yield statistically significant differences between the control and experimental groups.

In conclusion, this study is the first report of the bioavailability of high-dose IPGE<sub>1</sub> following prolonged administration in healthy anesthetized ventilated neonatal pigs using the highly sensitive and selective HPLC-MS. We have demonstrated increase in systemic and urinary PGE<sub>1</sub> levels following prolonged inhalation without systemic side effects suggesting effective delivery and pulmonary selectivity. Studies in patients with NHRF are necessary to determine optimal dose and to assess short and long-term tolerance in patients.

#### ACKNOWLEDGMENTS

The results presented in this manuscript were presented at the Pediatric Academic Societies' Annual Meeting, May 5, 2009, Baltimore, MD, USA.

We gratefully acknowledge the assistance of Cathy Eames, M.S.L.S. and Barbara Maynarich, Information Resources Technician, in searching the medical literature and preparing the bibliography.

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