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## An opportunistic study evaluating pharmacokinetics of sildenafil for the treatment of pulmonary hypertension in infants

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### Abstract

**Objective**—To assess sildenafil and *N*-desmethyl sildenafil (DMS) exposure in infants receiving sildenafil for the treatment of pulmonary hypertension (PH).

**Study design**—Data were collected from 6 infants receiving sildenafil for the treatment of PH, and plasma samples were collected at the time of routine laboratory blood draws. The echocardiography results were assessed for improvement in right ventricular (RV) hypertension following sildenafil treatment.

**Result**—The median (range) sildenafil and DMS concentrations were 27.4 ng/mL (2.6–434.0) and 105.5 ng/mL (3.6–314.0), respectively. The median metabolite-to-parent ratio was higher in infants receiving co-medications that can induce cytochrome P450 (CYP) enzymes (5.2 vs. 0.7). The echocardiography results showed improvement in RV hypertension for the majority of infants (5/6).

**Conclusion**—The concentrations of sildenafil and DMS were within the previously observed ranges. Our results suggest that caution may be warranted when CYP-related co-medications are administered during sildenafil treatment for PH.

### Introduction

Sildenafil is a phosphodiesterase type-5 inhibitor approved in adults for the treatment of pulmonary hypertension (PH). Sildenafil-mediated inhibition of phosphodiesterase type-5

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leads to an increase in intracellular cyclic guanosine monophosphate, which results in pulmonary vasodilation.<sup>1,2</sup> In infants, PH affects term infants (often called persistent pulmonary hypertension of the newborn) and premature infants with bronchopulmonary dysplasia and associated pulmonary arterial hypertension.<sup>3-5</sup> Few clinical studies have evaluated the pharmacokinetics (PK) and use of sildenafil in infants, yet the use in premature infants is increasing.<sup>6</sup> In 2012, the Food and Drug Administration issued a black box warning for sildenafil use in children (ages 1 through 17 years). Despite this warning, sildenafil is still used in infants with bronchopulmonary dysplasia and pulmonary arterial hypertension. The relationship between sildenafil dosing, safety, and efficacy in infants is currently unclear, and further studies are required to better understand sildenafil disposition in infants.

In adults, the PK of sildenafil have been extensively investigated. Sildenafil is highly protein bound (96%) and is distributed throughout the body (volume of distribution=1-2 L/kg).<sup>7,8</sup> After oral administration, the apparent terminal elimination half-life of sildenafil in adults is 3-5 hours,<sup>8-10</sup> and it undergoes almost complete hepatic metabolism.<sup>11,12</sup> The major circulating metabolite of sildenafil is *N*-desmethyl sildenafil (DMS), which is active and is reported to have 50% *in vitro* potency of sildenafil.<sup>11</sup> Previously published studies have reported that the major pathways of sildenafil biotransformation are cytochrome P450 (CYP) enzymes 3A4 (CYP3A4) (~80%) and 2C9 (CYP2C9) (~20%).<sup>11,12</sup> Currently, limited information exists about the impact of ontogeny of CYP enzyme on the PK of sildenafil in infants.

Few studies have evaluated the PK of sildenafil in term infants and none in premature infants.<sup>13,14</sup> This is due to the challenges associated with conducting clinical trials in infants, including limited vascular access for PK sampling and blood volumes. Opportunistic study designs can help overcome these challenges by capitalizing on blood sampling from standard medical care procedures.<sup>15-18</sup>

In this opportunistic study, we collected data from six infants (five premature and one full-term) to evaluate the disposition of sildenafil and DMS.

## Methods

### Patient population

From 2010-2012, plasma samples were collected from infants receiving sildenafil enterally (by nasogastric tube) for the treatment of PH at the time of routine blood draws as part of standard of care labs. All infants were enrolled at the Newborn Critical Care Center at The University of North Carolina at Chapel Hill Children's Hospital. The following information was collected for each infant enrolled: gender, gestational age, postnatal age, and weight at first dose and concomitant medications (only systemically administered drugs with known interactions with the CYP450 pathways). The study protocol was reviewed and approved by the University of North Carolina at Chapel Hill Institutional Review Board (IRB number: 10-0865). Written informed consent was obtained from the parent/guardian of each infant for use of the opportunistic data for research purposes.

## Dosing and sample collection

Dosing information was obtained for at least five doses prior to the plasma samples collected for PK analyses. Because this was an opportunistic study design, the dosing varied and was determined by the treating clinician, not by protocol. In addition, PK sample collection was performed during time of routine blood draws as ordered by the treating physician, and thus occurred at differing time intervals. Samples were collected and stored at -80°C until further analysis. Echocardiography data were collected in all six infants.

## Determination of sildenafil and DMS plasma concentrations

A liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis was performed by Covance Laboratories Inc. (Madison, WI). Plasma samples were analyzed for concentrations of sildenafil and DMS using an LC/MS/MS detection system comprising a Shimadzu HPLC and API 4000 QTRAP mass spectrometer (Applied Biosystems, Carlsbad, CA). Sildenafil-*d*<sub>8</sub> and *N*-desmethyl sildenafil-*d*<sub>8</sub> were used as internal standards for sildenafil and DMS, respectively. The standards were prepared in plasma. The assay accuracy ranged from 100.2% to 107.3% for sildenafil and -96.1% to 110% for DMS. Method imprecision for plasma quality control samples at concentrations of 3, 30, and 350 ng/mL was 0.9, 1.5, and 9.81% coefficient of variation (CV), respectively, for sildenafil, and 7.95, 3.78, and 0.02% CV, respectively, for DMS. The lower limit of quantification for sildenafil and DMS in human plasma was 1 ng/mL for both analytes (range: 1-500 ng/mL for sildenafil and DMS). Peak areas were integrated and analyzed using Analyst software (version 1.5.1; Applied Biosystems, Carlsbad, CA).

## Data analysis

The sildenafil and DMS concentrations and the metabolite-to-parent ratios (calculated from individual data points) for each infant were analyzed.

## Results

### Patient characteristics

Six infants were enrolled; the median (range) gestational age was 25.6 weeks (23.6-38.7, Table 1). The median (range) body weight and postnatal age at the start of first dose were 3.2 kg (2.0-5.9) and 145 days (10-209), respectively. Sildenafil dose varied with time in different infants, with a median dose of 3 mg (1.5-9.2) or 0.92 mg/kg (0.5-2.1). Overall, five samples for each infant were collected and analyzed for sildenafil and DMS concentrations. The sample collection times varied between infants, ranging from 0-11 hours after the last dose.

During sildenafil treatment, all infants were receiving concomitant medications, and four out of six infants were administered inducers of CYP3A4 and CYP2C9 (dexamethasone and phenobarbital) (Table 1).

The echocardiography data were obtained and the right ventricular (RV) pressure was evaluated in the infants. The majority of infants (five out of six) showed improvement in RV hypertension with time following sildenafil treatment.

## Pharmacokinetics

A total of 30 samples were collected from all infants, and all but one sample were above the lower limit of quantification. The median (range) of concentration values obtained for sildenafil and DMS in all infants were 27.4 ng/mL (2.6-434.0) and 105.5 ng/mL (3.6-314.0), respectively. A high metabolite-to-parent ratio was observed in infants who were receiving additional drugs known to induce CYP enzymes (Table 2). The median (range) metabolite-to-parent ratio for infants receiving no CYP-related co-medications (infants 3 and 5) was 0.7 (0.3-1.0) versus 5.2 (1.1-14.5) for those receiving CYP-related co-medications.

## Discussion

In this opportunistic study, we assessed the concentrations of sildenafil and DMS in infants who were undergoing treatment for PH. The observed concentrations of sildenafil and DMS were within the range of values that were previously reported.<sup>14</sup> Our results suggest that potential CYP-mediated drug interactions may play an important role in the disposition of sildenafil.

PH is a devastating cardiopulmonary complication in premature infants with bronchopulmonary dysplasia and full-term infants with persistent pulmonary hypertension of the newborn. Sildenafil is frequently prescribed to both of these populations without adequate safety and efficacy information. Neonatologists generally use echocardiograms to diagnose and monitor PH in infants, as there are no validated tests to evaluate the severity of PH in infants (such as the walking test in adults).<sup>19,20</sup> Regardless, the echocardiography results from our study demonstrated that the majority of infants (five out of six) had improvement in RV hypertension, which is consistent with a recent study in premature infants treated with sildenafil for bronchopulmonary dysplasia and PH.<sup>21</sup> These authors reported that although an echocardiographic improvement occurred in the majority of infants, fewer infants had clinical improvement.<sup>21</sup> Taken together, these data support the idea that sildenafil may be of therapeutic benefit, and future studies should focus on the optimal dose, schedule, and duration in this vulnerable population. Catheterization studies sometimes show large differences between measured RV pressure and that estimated from a tricuspid regurgitant jet velocity from echocardiogram. Finally, there is a need to validate the use of echocardiography as an appropriate pharmacodynamic endpoint to diagnose and monitor PH in infants and assess the adequacy of PH treatment in infants.

A previous study assessed the population PK of intravenously administered sildenafil in term infants up to 7 days of age.<sup>13</sup> Another report investigated the PK of sildenafil after enteral administration in neonates.<sup>14</sup> However, high interpatient variability was associated in both of these studies.<sup>13,14</sup> In our study, due to the small sample size, overall sparseness of the data, and poor reliability of sildenafil dosing times from the medical records, we were unable to perform a formal PK analysis. Thus, sildenafil and DMS exposure values were compared to previously reported values from a PK study of sildenafil administered enterally in neonates.<sup>14</sup> The concentrations of sildenafil and DMS observed in our study were within the approximate concentration ranges observed in the previous study.<sup>14</sup>

Our results show that higher metabolite-to-parent ratios were observed in infants receiving co-medications that are inducers of CYP3A4 and CYP2C9 as compared to infants who did not receive any CYP-related co-medications (Table 2). Little is currently known regarding the effect of ontogeny of CYP enzymes on the disposition of sildenafil, especially in premature infants. Previous *in vitro* studies have shown that the downregulation of CYP3A7 (a fetal CYP3A isoform) and concurrent upregulation of CYP3A4 and expression of CYP2C9 in fetal livers increase rapidly within 1 month after birth.<sup>22–24</sup> In line with these findings, one study reported an increase in sildenafil clearance in the first week of life after intravenous sildenafil administration in term infants.<sup>13</sup> Collectively, these findings support that sildenafil levels should be carefully monitored in infants with PH when CYP-related co-medications are administered.

Although sildenafil is widely used in clinic for the treatment of PH in infants, limited PK studies have been performed to inform its dosing. This may be due in part to the general challenges associated with performing clinical trials in infants and special population of interest. One way to overcome these challenges is the use of opportunistic study designs that can provide valuable information from standard medical care procedures. Such opportunistic clinical trial designs have been successfully implemented to evaluate the PK of several drugs in full-term and premature infants.<sup>15–18</sup> Our use of an opportunistic clinical trial design allowed us to gain a better understanding of sildenafil disposition in infants being treated for PH.

In summary, our observed concentrations of sildenafil and DMS were within the previously reported concentration ranges in older children.<sup>14</sup> Although echocardiography observations have suggested that sildenafil treatment leads to a therapeutic benefit, it is unclear whether this is the natural course of the disease or whether sildenafil was actually beneficial. Our findings suggest that caution may be warranted in infants receiving CYP-related concomitant medications with sildenafil for treatment of PH. Further PK, safety, and efficacy studies are needed to optimize the dose and indication for sildenafil in premature infants with PH.

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**Table 1****Summary of patient characteristics**

Infant	Gender	Gestational age (weeks)	Body weight (kg) <sup>*</sup>	Postnatal age (days) <sup>*</sup>	CYP co-medication <sup>†</sup>
1	F	25.4	2.00	110	Dexamethasone
2	F	27.6	4.12	124	Phenobarbital
3	F	25.1	5.94	209	-
4	F	23.6	5.07	193	Phenobarbital
5	F	38.7	2.68	10	-
6	M	24.1	2.67	104	Dexamethasone

<sup>\*</sup> At the start of first dose.

<sup>†</sup> Only systemically administered co-medications were considered.



**Table 2**  
**Doses, observed concentrations of sildenafil and *N*-desmethyl-sildenafil (DMS), and metabolite-to-parent ratios**

<b>Infant</b>	<b>Median dose (mg) (range)</b>	<b>Median sildenafil concentration (ng/mL) (range)</b>	<b>Median DMS concentration (ng/mL) (range)</b>	<b>Median metabolite-to- parent ratio (range)</b>
1	1.5 (1.5-5.0)	22.7 (2.6-37.6)	108.0 (10.4-171.0)	4.2 (3.1-7.5)
2	3.6	21.5 (7.1-30.0)	168.0 (103.0-247.0)	8.5 (7.7-14.5)
3	3.0	25.4 (5.5-35.4)	14.8 (3.6-28.0)	0.7 (0.3-0.8)
4	3.7 (2.5-8.0)	26.5 (9.4-40.7)	111 (10.2-249.0)	5.9 (1.1-8.8)
5	3.9 (2.6-3.9)	114.0 (35.6-434.0)	82.4 (32.0-120.0)	0.7 (0.3-1.0)
6	8.6 (6.4-9.2)	54.4 (21.7-162.0)	172 (102.0-314.0)	3.2 (1.9-5.1)

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