The AAPS Journal (2019) 21: 53
DOI: 10.1208/s12248-019-0313-2

Research Article

Fetal Concentrations of Budesonide and Fluticasone Propionate: a Study in Mice

Syedsaoud Zaidi,[1] Mong-Jen Chen,[1] Daniel T. Lee,[1] Elsa Neubart,[1] Pär Ewing,[2] Anna Miller-Larsson,[2] and Günther Hochhaus[1,3]

Received 2 April 2018; accepted 27 February 2019; published online 16 April 2019

Abstract. The study goal was to evaluate the transplacental transfer of two corticosteroids, budesonide (BUD) and fluticasone propionate (FP), in pregnant mice and investigate whether P-glycoprotein (P-gp) might be involved in reducing BUD transplacental transfer. Pregnant mice ($N = 18$) received intravenously either low (104.9 μg/kg) or high (1049 μg/kg) dose of [^{3}H]-BUD or a high dose of [^{3}H]-FP (1590 μg/kg). In a separate experiment, pregnant mice ($N = 12$) received subcutaneously either the P-gp inhibitor zosuquidar (20 mg/kg) or vehicle, followed by an intravenous infusion of [^{3}H]-BUD (104.9 μg/kg). Total and free (protein unbound) corticosteroid concentrations were determined in plasma, brain, fetus, placenta, kidney, and liver. The ratios of free BUD concentrations in fetus *versus* plasma $K_{(\text{fetus, plasma, u, u})}$ 0.42 ± 0.17 (mean ± SD) for low-dose and 0.38 ± 0.18 for high-dose BUD were significantly different from $K = 1$ ($P < 0.05$), contrary to 0.87 ± 0.25 for FP, which was moreover significantly higher than that for matching high-dose BUD ($P < 0.01$). The BUD brain/plasma ratio was also significantly smaller than $K = 1$, while these ratios for other tissues were close to 1. In the presence of the P-gp inhibitor, $K_{(\text{fetus, plasma, u, u})}$ for BUD (0.59 ± 0.16) was significantly increased over vehicle treatment (0.31 ± 0.10; $P < 0.01$). This is the first *in vivo* study demonstrating that transplacental transfer of BUD is significantly lower than FP's transfer and that placental P-gp may be involved in reducing the fetal exposure to BUD. The study provides a mechanistic rationale for BUD's use in pregnancy.

KEY WORDS: placental drug transporters; P-gp; budesonide; fluticasone propionate.

INTRODUCTION

Inhaled corticosteroids (ICS) represent a cornerstone in the management of asthma. Asthma guidelines recommend that pregnant asthma patients should be treated with ICS to the same degree as non-pregnant women (1). The lack of toxicity of ICS in the treatment of asthma during pregnancy has been reported in systematic reviews (2–4). Recent studies showed that fetal adrenal activity and growth are unaffected by continued ICS treatment during pregnancy (5). Study results from the Swedish Medical Birth Register found that 2968 mothers who had used budesonide (BUD) during early pregnancy gave birth to normal infants (3). Because more data are available on BUD during pregnancy than for other ICS, the US Food and Drug Administration assigned inhaled and intranasal BUD a safety category B ranking (6) and the NAEPP (National Asthma Education Prevention Program) considered it to be the preferred ICS (7). However, even though BUD and fluticasone propionate (FP) are widely used in pregnant women with asthma, our understanding of placenta transfer of those ICS is limited.

During pregnancy, nutrient and drug transfer across the placenta can be mediated by passive or facilitated diffusion, active transport, and pinocytosis (8). A number of efflux drug transporters have been identified in the placenta. These include the breast cancer resistance protein (BCRP), P-glycoprotein (P-gp), and other multidrug resistance proteins (8,9). P-gp is expressed at the apical surface of the syncytiotrophoblast membrane of the human placenta (8), and its major role is believed to reduce the transplacental transfer of toxic xenobiotic compounds from the mother to

Syedsaoud Zaidi and Mong-Jen Chen contributed equally to this work.

[1] Departments of Pharmaceutics, JHMHC, P3-33, University of Florida, 100494, Gainesville, Florida 32610, USA.
[2] AstraZeneca Gothenburg, 431 83, Mölndal, Sweden.
[3] To whom correspondence should be addressed. (e–mail: hochhaus@cop.ufl.edu)

Abbreviations: *BCRP*, Breast cancer resistance protein; *BUD*, Budesonide; *CL*, Clearance; *GBq*, Giga becquerel; *FP*, Fluticasone propionate; f_u, Free, protein unbound fraction of drug in plasma or tissue; *HPLC*, High-performance liquid chromatography; *ICS*, Inhaled corticosteroid; $K_{(\text{tissue, plasma, u, u})}$, Free (protein unbound) drug concentration ratio of tissue to maternal blood plasma; K_0, Infusion rate; *MRP*, Multidrug resistance protein; *P-gp*, P-glycoprotein; *USP*, US Pharmacopeia; Vd_{ss}, Volume of distribution at steady state; *v*, Volume; *w*, Weight

the fetus. Not only endogenous but also some synthetic corticosteroids are substrates for placental metabolism (10). For example, the placental enzyme 11 beta-hydroxysteroid dehydrogenase-2 (11 beta-HSD2) converts cortisol and corticosterone to their inactive metabolites (10). Metabolism of synthetic corticosteroids such as dexamethasone, beclomethasone dipropionate, prednisolone, and betamethasone by 11 beta-HSD2 has also been shown, while BUD and FP were not metabolized by this placental enzyme (11).

Dexamethasone, methylprednisolone, cortisol, and aldosterone have been shown to be P-gp substrates with dexamethasone acting as P-gp substrate and inducer (12–15). Several *in vitro* studies have suggested that BUD, beclomethasone dipropionate, mometasone furoate, and ciclesonide, but not FP, are P-gp substrates (16,17). However, studies evaluating the placental P-gp-mediated efflux of ICS are missing.

To better understand the transplacental transfer of ICS, and its potential relevance for selecting effective and safe corticosteroids for treatment of women during pregnancy, this study evaluated the placental transfer of two corticosteroids, BUD and FP, which are used in inhalation therapy of asthma. We further assessed the potential role of the placental drug transporter P-gp in modulating the access of fetal exposure to BUD in pregnant mice.

MATERIALS AND METHODS

Chemicals

[^{3}H]-budesonide (BUD) (specific activity 540 GBq mmol^{-1}) and [^{3}H]-fluticasone propionate (FP) (specific activity 125 GBq mmol^{-1}) were provided by AstraZeneca (Mölndal, Sweden). Isoflurane USP was purchased from Piramal (Piramal Critical Care, Inc., Bethlehem, PA, USA). Zosuquidar 3HCL was purchased from MedKoo Biosciences (Morrisville, NC, USA), and ^{3}H-digoxin (specific activity 975 GBq/mmol) was obtained from PerkinElmer (Boston, MA, USA). Unlabeled BUD, FP, and digoxin were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of the Study Drugs

In order to prepare tritium-labeled, low-dose and high-dose BUD, and high-dose FP solutions for intravenous administration, adequate amounts of unlabeled BUD or FP were added to an ethanol stock solution of [^{3}H]-BUD or [^{3}H]-FP, respectively. Ethanol was evaporated under a stream of nitrogen. The residue was reconstituted in a mixture of phosphate-buffered saline (PBS) (85% (*v*/*v*)), ethanol (5% (*v*/*v*)), and Cremophore RH40 (10% (*v*/*v*)).

For each mouse, an individual drug solution was prepared, where the amount of unlabeled drug was adjusted to the mouse body weight, whereas the amount of tritiated drug was constant (each mouse received equal radioactive dose independent of mouse body weight). Thus, in the drug solutions prepared for different mice, the total drug concentrations were different and dependent on mouse body weight while radioactive concentrations were independent of mouse body weight. For a mouse weighing 30 g, BUD solution was prepared at 13.6 μg/mL for the low-dose administration and at 136 μg/mL for the high-dose administration. Radioactive concentration in all BUD solutions was 250 μCi/mL. In BUD solutions prepared for mice weighing 30 g, [^{3}H]-BUD made 54.4% of the total BUD content in the 13.6 μg/mL solution and 5.4% in the 136 μg/mL solution.

For each mouse, two concentrations of FP solution were prepared: one for intravenous bolus dose and another for intravenous infusion. For a mouse weighing 30 g, concentrations of bolus and infusion solutions were 253 μg/mL and 145 μg/mL, respectively. Radioactive concentrations for all FP bolus and infusion solutions were 318 μCi/mL and 182 μCi/mL, respectively. The percentage of [^{3}H]-FP in the solutions was the same in the bolus and infusion solutions; in FP solutions prepared for a mouse weighing 30 g, [^{3}H]-FP made 18.6% of the total FP content.

The solution of P-gp inhibitor, zosuquidar, was formulated in a mixture of ethanol (20% (*v*/*v*)), polyethylene glycol 200 (60% (*v*/*v*)), and 5% glucose (20% (*v*/*v*)) to the final concentration of 5 mg/mL.

Animal Studies

All animals were housed in an animal facility of the University of Florida and provided with standard chow *ad libitum*. Studies were performed using protocol 201207548 approved by the Institutional Animal Care and Use Committee at the University of Florida, Gainesville, FL, USA. C57BL/6J mice with weight between 25 and 36 g (The Jackson Laboratory, Bar Harbor, ME, USA) were allowed to breed. After male and female mice were caged together, female mice were checked daily and pregnant mice were identified by the presence of a vaginal plug. The day on which the vaginal plug was detected was designated as day E0.5 (half a day pregnant). Studies were performed with mice averaging a gestation period of 14.5 days.

Animal Studies with Either BUD or FP Administered

Pregnant C57BL/6J mice ($N = 18$, average gestation period ~ 14.5 days) were divided into three groups ($N = 6$ per group). Animals in a given group received either a low dose of [^{3}H]-BUD (group 1), high dose of [^{3}H]-BUD (group 2), or a high dose of [^{3}H]-FP (group 3). The drugs were administered through the tail vein employing a loading bolus dose of 50.5 μg/kg BUD (in 112 μL volume), 505 μg/kg BUD (in 112 μL volume), or 1010 μg/kg FP (in 120 μL volume) for groups 1, 2, and 3, respectively. This was followed by a constant rate infusion of 54.4 μg/kg BUD, 544 μg/kg BUD, or 580 μg/kg FP for groups 1, 2, and 3, respectively, each in 120 μL volume infused over a period of 2 h at a flow rate of 1 μL/min. Thus, mice in low-dose BUD group, high-dose BUD group, and high-dose FP group received total doses of 104.9 μg/kg BUD, 1049 μg/kg BUD, and 1590 μg/kg FP, respectively. The radioactive dose was independent of mouse body weight, with all mice getting either low-dose or high-dose BUD received 58 μCi per mouse and all mice getting FP received 60 μCi per mouse.

At the end of infusion, blood was collected through cardiac puncture. Mice were sacrificed by cervical dislocation after anesthesia with isoflurane. Blood plasma was prepared, while brain, placenta, fetus, kidney, and liver were collected,

washed in PBS to remove blood, and then homogenized after transfer into fresh 3 volumes of PBS (volume of PBS adjusted to the volume of actual organ) using an ULTRA-TURRAX three times over 10-s burst with 10 s under cooling on ice. Total and free (protein unbound) concentrations of BUD and FP in homogenates were determined immediately as described below.

Animal Studies with P-gp Inhibitor

Zosuquidar was chosen as P-gp inhibitor because it is highly specific in blocking P-gp—but not BCRP—or MRP-mediated processes (18,19). The dose of 20 mg/kg was selected in preliminary experiments using [^{3}H]-digoxin as the P-gp substrate (20). To investigate the role of P-gp in modulating fetal exposure of BUD, pregnant mice ($N = 12$) were randomized to receive subcutaneous zosuquidar ($N = 6$) or vehicle ($N = 6$). After 30 min, this was followed by tail vein administration of [^{3}H]-BUD (104.9 μg/kg, consisting of loading bolus dose of 50.5 μg/kg and infusion of 54.4 μg/kg BUD over 2 h). Maternal plasma and tissue homogenates were prepared as described above, and the total and free (protein unbound) drug concentrations were determined immediately as described below.

Drug Analysis

Plasma and tissue homogenates prepared for the determination of total drug concentrations, or samples taken from the donor and acceptor compartment of the dialysis unit for the determination of the fraction unbound to protein, were mixed after addition of 10 volumes of a mixture of methanol/acetone (50:50 (*v*/*v*)). After centrifugation, the supernatant was removed, dried over a stream of nitrogen, and reconstituted in mobile phase (methanol/water 70:30 (*v*/*v*)), to be further analyzed by high-performance liquid chromatography (HPLC). The recovery during this precipitation step was investigated for plasma and all tissues included in this study over a drug concentration range of 0.2 to 200 ng/mL for BUD and FP. The overall recovery across all biological fluids and drug concentrations (0.2–200 ng/mL in plasma and tissue homogenates) was 93.2 ± 6.9% for BUD and 94.2 ± 5.0% for FP with no concentration or tissue dependency observed.

The HPLC system consisted of an LC 20AD Shimadzu pump, a SIL 10AF auto injector, a SPD-M10 A VP controller, and a FRC 10A fraction collector (Shimadzu, Columbia, MD, USA). UV detection was performed at 254 nm. The C18 reversed phase column (150 × 4.6 mm; 5-mm particle size) (Waters, Milford, MA, USA) was used. The mobile phase containing methanol and water (70:30 (*v*/*v*)) was used at a flow rate of 1 mL/min. The fractions containing intact drug, indicated by the appearance of the unlabeled drug peak, were collected using the HPLC fraction collector mixed with 10 mL of scintillation fluid (Ultima Flo M; PerkinElmer, Boston, MA, USA), and radioactivity (disintegrations per minute (dpm)) was determined using a Beckman Scintillation Counter LS 6500 (Beckman Inst., Fullerton, CA, USA). The drug concentrations were calculated from dpm estimates considering the specific activity of the analytes. The HPLC method (21) was previously shown to separate BUD from its main metabolites. In preliminary experiment, it was ensured that both BUD and FP labeled and unlabeled material co-eluted. In addition, radioactivity was only eluting together with unlabeled BUD or FP (no additional radioactive peaks), with the exception of radioactivity eluting in the solvent front (radioactive water). BUD and FP's calibration curves were linear over the concentration range of 0.8–80 ng/mL ($r^2 > 0.993$ for BUD; $r^2 > 0.996$ for FP).

Determination of the Percent Protein Unbound Drug in Plasma and Tissue Homogenate

Equilibrium Dialysis

Experiments were performed using a commercial equilibrium dialysis cell (Pierce Biotechnology, Thermo Fisher Scientific, Waltham, MA, USA) using a cellulose membrane with 12-kDA cutoff under conditions suggested by the manufacturer and validated by Waters *et al.* for a diverse set of compounds (22). Under these conditions, our *in vivo* results in mice (percent of unbound BUD in plasma and tissues; Table I) agreed well with those obtained in rats (plasma in mice: % unbound = 8.7–9.7, in rat: % unbound = 7.4; brain in mice: % unbound = 3.3–3.5, in rat: % unbound = 5.8; kidney in mice: % unbound = 1.2–1.5, in rat: % unbound = 2.6; liver in mice: % unbound = 2.7–3.2, in rat: % unbound = 2.0) (23). The comparison between rat and mice data seems feasible, as plasma binding of BUD was shown to be constant across a wide range of species (24).

In Vitro Experiments

To estimate the percent unbound of BUD and FP in plasma and tissues, control *in vitro* experiments were performed where freshly prepared plasma and tissue homogenate, obtained from untreated animals, were spiked with BUD or FP: 60 ng/mL into plasma and 50–500 ng/mL into tissue homogenates, to mimic results of the *in vivo* experiments (for details, see Table I). Experiments were performed in triplicate.

In Vivo Experiments

To evaluate the *in vivo* binding of BUD and FP, plasma and tissue homogenate samples from six mice per group were investigated. For plasma samples, 200 μL of plasma was added to the donor compartment and 350 μL of PBS was added to the receiver compartment. For tissue samples, 500 μL of homogenate was added to donor compartment and 750 μL of PBS was added to the receiver compartment. The rapid equilibrium dialysis (RED) (Thermo Fisher Scientific, Waltham, MA, USA) device was then placed onto a shaker (30 rpm) and incubated at 37°C in a temperature-controlled incubator for 4 h until equilibration of the drug concentrations in the two compartments. Samples were taken from donor and receiver compartments (plasma 150 μL donor, 200 μL receiver; tissues including brain, fetus, placenta, kidney 300 μL donor, 500 μL receiver; liver tissue 150 μL donor, 200 μL receiver), matched with the equal volume of opposite matrix and precipitated with 10 parts (*v*/*v*) of methanol/acetone (50:50 (*v*/*v*)), vortexed, and then centrifuged. The supernatant was collected, dried, reconstituted in HPLC mobile phase (methanol/water (70:30 (*v*/*v*)), and analyzed by HPLC (as described above).

Table I. Percent Unbound (%) of BUD and FP (Mean (SD)) Determined in *In Vitro* and *In Vivo* Experiments in Maternal Plasma and Selected Tissues from Pregnant Mice (N = Number of Mice per Group)

	Plasma	Brain	Fetus	Placenta	Kidney	Liver
BUD						
In vitro ($N = 3$)	10.2 (1.9)	3.0 (1.3)	5.1 (2.3)	3.8 (1.8)	1.4 (0.3)	2.9 (1.7)
In vivo						
104.9 μg/kg ($N = 6$)	8.7 (1.2)	3.3 (2.0)	4.8 (1.9)	4.7 (2.1)	1.2 (0.5)	2.7 (0.7)
1049 μg/kg ($N = 6$)	9.7 (1.7)	3.5 (1.6)	5.5 (1.9)	4.3 (1.1)	1.5 (0.4)	3.2 (1.0)
FP						
In vitro ($N = 3$)	1.4 (0.3)	0.80 (0.04)	2.1 (0.4)	1.2 (0.2)	0.40 (0.04)	0.40 (0.04)
In vivo						
1590 μg/kg ($N = 6$)	1.1 (0.2)	1.2 (0.2)	1.5 (0.5)	0.9 (0.2)	0.5 (0.1)	0.5 (0.1)

For BUD *in vitro* study: plasma, brain, and fetus were spiked with 60 ng/mL BUD, placenta and liver with 200 ng/mL, and kidney with 500 ng/mL to mimic the *in vivo* conditions. For FP *in vitro* study: plasma, brain, and fetus were spiked with 60 ng/mL FP, placenta, kidney, and liver with 200 ng/mL FP. A density of 1 g/mL was assumed for the tissue preparations

The protein unbound fraction of drug was calculated using the ratio of the concentration in the receiver compartment (PBS) and the concentration in the donor compartment (spiked tissues) at equilibrium obtained after dialysis according to Eq. 1:

$$fu = \frac{C\ \text{receiver compartment}}{C\ \text{donor compartment}} \tag{1}$$

where f_u is the fraction of unbound drug in the tissues, $C_{\text{receiver compartment}}$ is the measured drug concentration in the receiver compartment, and $C_{\text{donor compartment}}$ is the measured drug concentration in the donor compartment after dialysis. The percentage of protein bound drug was then calculated as $(1 - f_u) \times 100$. Assuming linear binding, the fraction of unbound drug in tissues was calculated from the estimates in diluted tissue homogenate according to Eq. 2:

$$fu, \text{tissue} = \frac{fu, \text{homogenate}}{(1 - fu, \text{homogenate}) \times D + fu, \text{homogenate}} \tag{2}$$

where $f_{u,\text{tissue}}$ is the free fraction of drug in the tissue, $f_{u,\text{homogenate}}$ is the free fraction of drug in the tissue homogenate, and D is the volume dilution factor of the tissue homogenate (4-fold for all tissues). The fraction bound to protein in the tissue was then calculated as $1 - f_{u,\text{tissue}}$.

Free drug concentration was derived from Eq. 3:

$$Cu = C\text{total} \times fu \tag{3}$$

where C_u is the free concentration of study drug, C_{total} is the total concentration of study drug, and f_u is the free, unbound fraction of drug.

The ratio of free drug tissue concentration to free drug maternal plasma concentration was calculated according to Eq. 4:

$$K\ (\text{tissue}, \text{plasma}, u, u\ ) = \frac{Cu, \text{tissue}}{Cu, \text{plasma}} \tag{4}$$

where $C_{u,\text{tissue}}$ is the free drug concentration in tissues and $C_{u,\text{plasma}}$ is the free drug concentration in maternal plasma.

Corrections for blood contamination of the tissue samples were performed as described by Khor and Mayersohn (25) with tissue contamination fractions (VF_b, fraction of removed tissue representing blood) taken from Brown *et al.* (26). VF_b for brain, kidney, and liver were reported to be 0.03, 0.24, and 0.31, respectively, from the Table 30 in Brown *et al.* paper. Briefly, plasma concentrations were converted into blood concentrations (CT_b) by considering blood/plasma ratio of 0.8 for BUD (27) and 0.7 for FP (28). The ratios of corrected to uncorrected (C_{tot}) drug tissue concentrations were derived from Equation 18 in Khor and Mayersohn paper, and calculated as $(C_{\text{tot}} - VF_b \times CT_b)\ /\ C_{\text{tot}} \times (1 - VF_b)$, which is equivalent to R/R' ratio in Khor and Mayersohn, assuming estimated blood concentrations to be relevant for all tissues, and where R is tissue-to-blood distribution coefficient when a correction is made for residual blood but not for the interstitial fluid while R' is apparent tissue-to-blood distribution coefficient when no correction is made.

Preliminary experiments in non-pregnant mice were performed to ensure that the combination of loading dose and 2-h infusion would be sufficient to reach steady state. Theoretical steady-state concentrations calculated from the relationship between steady-state drug concentrations in plasma Cp_{ss}, infusion rate K_0, and systemic clearance ($Cp_{ss} = K_0$ /CL) were compared for these two high extraction drugs with experimentally observed plasma concentrations considering the mouse liver blood flow as clearance (5.4 L/h/kg) (29). The good agreement between predicted Cp_{ss} concentrations (53.7 ng/mL for high-dose BUD and FP infusions) with measured plasma concentrations (54.2 ± 6.8 ng/mL for BUD and 63.4 ± 2.3 ng/mL for FP) strongly suggests that steady state was reached in the animals.

Statistical Analysis

Data are presented as arithmetic means ± standard deviation (SD). For two groups of samples with equal variances, unpaired two-tailed Student's t test was performed, while for two groups of samples with unequal variances, two-tailed Welch's t test was used. To test whether unbound partition coefficients ($K_{(\text{tissue, plasma, u, u})}$) were significantly different from $K_{(\text{tissue, plasma, u, u})} = 1$, the 95% confidence intervals (CIs) were calculated and one-sample t test was

used. The analyses were performed using R-3.2.4 and Prism (GraphPad Software Inc., San Diego, CA, USA). Differences were considered statistically significant at $P < 0.05$.

RESULTS

The protein unbound of BUD and FP (percent unbound) in plasma and selected tissue homogenates (brain, fetus, placenta, kidney, and liver) were determined both in *in vitro* and *ex vivo* experiments (for details, see "Materials and Methods" section). Both methods resulted in similar percent unbound values (Table I). The percent unbound of FP was nearly one order of magnitude lower than that of BUD in plasma and liver, two to four times lower in fetus, three to four times lower in brain, and three to five times lower in placenta and kidney, which is consistent with a more pronounced protein binding of FP compared to BUD. Moreover, the observed plasma protein binding for BUD and FP in experimental mice was in agreement of those in humans. The observed plasma protein binding in mice *in vivo* was 90.3–91.3% for BUD and 98.9% for FP (Table I), while the literature reported values for BUD and FP were 91.4% and 99.3%, respectively, in humans (30). Table II indicates that for the selected infusion time of 2 h, steady state was reached as free concentrations of BUD and FP in plasma were similar to those in the control organs (liver and kidney). This was further supported by comparison of observed and predicted steady-state ($Cp_{ss} = K_0$ / CL) concentrations. To calculate infusion dose and intravenous loading dose, we have used the mouse liver blood flow of 5.4 L/h/kg (29) as systemic clearance for BUD and FP, and literature values for volume of distribution at steady state (Vd_{ss}) 9.4 L/kg for BUD (24) and 2-fold higher (18.8 L/kg) for FP. The results indicated that the measured concentrations agreed with predicted total drug plasma concentration at steady-state (Cp_{ss}) values (Table II).

The total and free (not bound to protein) drug concentrations in plasma and tissues obtained in the *in vivo* studies for the low dose of BUD (104.9 μg/kg) are summarized in Table II. Increasing the BUD dose by a factor of 10 resulted in approximately 10-fold higher total and free BUD concentrations in plasma and tissues, which suggests linear pharmacokinetics of BUD in this dose range. Total concentrations observed after low and high BUD doses in plasma and selected tissues were in the range of 10^{-8} M and 10^{-7} M with higher concentrations observed in kidney and liver. The total BUD concentrations in plasma and tissues after the high dose of BUD (1049 μg/kg) were similar to concentrations found after administration of FP (1590 μg/kg; Table II), with the exception of plasma and kidney concentrations for which statistically significant differences between FP and BUD were detected. Although the total BUD plasma concentration was 30% lower, the BUD kidney concentration was 2.4-fold higher than those of FP (Table II). In contrast, the free FP concentrations in plasma and all investigated tissues were significantly lower than those of BUD: 6-fold in plasma, 3-fold in brain and fetus, 5-fold in placenta and liver, and 8-fold in kidney (Table II), which is consistent with the several-fold lower percent unbound of FP compared to BUD (Table I).

BUD and FP high-dose groups revealed total drug concentrations which were in average 35–40% lower in fetus than in plasma (Table II). More importantly, however, the free BUD concentrations in fetus and brain were more than 60% lower than in plasma while other tissues showed similar or higher (kidney) free concentrations when compared with free plasma concentrations (Table II). Differences or agreement between free concentrations in plasma and tissues can be evaluated by calculating the partition coefficients ($K_{(\text{tissue, plasma, u, u})}$) between drug free concentrations in the selected tissues and plasma (Table III); e.g., the $K_{(\text{fetus, plasma, u, u})}$ of 0.38 and $K_{(\text{brain, plasma, u, u})}$ of 0.39 for high-dose BUD were significantly different from $K = 1$, in contrast to K values for the other tissues. A very similar pattern was observed in the low-dose BUD group with free BUD concentrations in fetus and brain being 58% and 67% lower than that in plasma, while other tissue concentrations of free BUD were similar to or higher (kidney) than plasma concentrations (Table II). This resulted in BUD's $K_{(\text{fetus, plasma, u, u})}$ estimate of 0.42 and $K_{(\text{brain, plasma, u, u})}$ estimate of 0.33, both being significantly different from $K = 1$, contrary to K values for the other tissues (Table III). Compared to BUD, the distribution of free FP in plasma, fetus, and brain was much more uniform (Tables II and III); free FP concentrations in fetus and brain were only 13% and 25% lower than that in plasma, respectively, and concentrations of free FP in other tissues were similar to or higher (liver) than that in plasma. This is reflected by an $K_{(\text{fetus, plasma, u u})}$ ratio of 0.87 and a $K_{(\text{brain, plasma, u, u})}$ ratio of 0.75 which are not significantly different from $K = 1$ and similar to K values for other tissues (Table III). Furthermore, the $K_{(\text{fetus, plasma, u, u})}$ and $K_{(\text{brain, plasma, u, u})}$ estimates were significantly larger for FP than those for high-dose BUD (Table III, Fig. 1), indicating that BUD transport from plasma to fetus and brain is significantly reduced as compared to FP.

To evaluate the role of P-gp in modulating the placental transfer of BUD, the effect of the P-gp inhibitor, zosuquidar, on BUD plasma-tissue distribution was investigated after administration of the low dose of BUD (104.9 μg/kg; Table IV). After maternal subcutaneous administration of zosuquidar (20 mg/kg), the increase in total BUD concentrations was observed in all tissues and plasma; however, this increase was statistically significant only for the liver tissue (Table IV). The free concentrations of BUD in all the tissues and plasma were also increased after inhibitor administration, and the 2.7-fold increase in fetus and the 1.9-fold increase in brain were statistically significant as compared to vehicle control, while the increases for the other tissues and plasma were not statistically significant (Table IV). The resulting $K_{(\text{fetus, plasma, u, u})}$ increased nearly 2-fold in the presence of the inhibitor as compared to vehicle control ($K_{(\text{fetus, plasma, u, u})}$ 0.59 ± 0.16 *versus* 0.31 ± 0.10; $P < 0.05$), whereas K values for other tissues were not significantly increased. The 1.4-fold increase in $K_{(\text{brain, plasma, u, u})}$ was close to statistical significance ($K_{(\text{brain, plasma, u, u})}$ 0.61 ± 0.19 *versus* 0.44 ± 0.13; $P = 0.09$; Table V and Fig. 2) while for other tissues, the resulting P values were far from statistical significance.

DISCUSSION

This is the first study in a pregnant mouse model to determine the total and free drug concentrations of BUD and FP in maternal plasma, placenta, and fetus. This study was

Table II. Total and Free BUD and FP Concentrations (Mean (SD)) in Maternal Plasma and Selected Tissues in Pregnant Mice ($N = 6$ Mice per Group) After Intravenous Bolus and Infused Dose (Total Drug Dose is Shown)

	Plasma (ng/mL)	Brain (ng/g)	Fetus (ng/g)	Placenta (ng/g)	Kidney (ng/g)	Liver (ng/g)
Total concentration						
BUD (104.9 μg/kg)	4.91 (0.79)	4.67 (1.35)	3.77 (0.85)	8.43 (3.29)	48.0 (5.9)	13 (3)
BUD (1049 μg/kg)	50.7 (8.0)	52.2 (6.2)	32.9 (14.3)	97.7 (37.5)	447 (78)	170 (38)
FP (1590 μg/kg)	72.3 (16.5)*	48.1 (20.2)	43.9 (7.7)	97.5 (24.2)	185 (41)**	204 (37)
Free concentration						
BUD (104.9 μg/kg)	0.43 (0.09)	0.14 (0.08)	0.18 (0.08)	0.37 (0.18)	0.56 (0.25)	0.35 (0.13)
BUD (1049 μg/kg)	4.90 (1.30)	1.81 (0.92)	1.92 (1.08)	4.26 (2.32)	6.98 (2.76)	5.25 (1.32)
FP (1590 μg/kg)	0.77 (0.21)**	0.58 (0.29)*	0.66 (0.22)*	0.88 (0.32)*	0.83 (0.21)**	1.06 (0.31)**

A density of 1 g/mL was assumed for the tissue preparations. When adjusting total concentrations in brain, kidney, and liver for potential blood contamination (0.03, 0.24, and 0.31 as volume fraction of blood in brain, kidney, and liver, respectively (26)), total drug concentrations in brain, kidney, and liver for low BUD dose were calculated to be 0.5%, 29.0%, and 31.4% higher, respectively, when the method by Khor and Mayersohn was applied (25). Similar estimates were obtained for the high-dose studies (BUD 0.7%, 28.7%, 34.2%; FP − 0.2%, 22.9%, 33.8% for brain, kidney, and liver, respectively). It was assumed that fetus was not contaminated with maternal blood and that the very small differences between corrected and uncorrected brain concentrations were of no relevance for the conclusions of this study. Therefore, uncorrected values were continued to be used, as we were mainly interested in fetus and brain concentrations, while liver and kidney data are only shown for completeness

*$P < 0.05$; **$P < 0.01$ for the comparison between FP and high BUD dose (1049 μg/kg); this was the only statistical comparison done

performed to assess potential differences between BUD and FP in their placental transfer.

P-gp expression in mouse placenta has been demonstrated in a number of studies (e.g., (31)). As a further example, placental P-gp deficiency has been shown to enhance susceptibility to chemically induced birth defects in mice (32).

BUD has been identified as substrate for intestinal P-gp in human cell lines *in vitro* (16). It has also been associated with murine P-gp at the blood-brain barrier in rodents (33) as we demonstrated that the brain glucocorticoid receptor occupancy of BUD and triamcinolone acetonide (a murine P-gp substrate (33)) is directly correlated to the degree of P-gp expression in adult (high P-gp expression, low receptor occupancy) and neonatal rats (low P-gp expression, higher receptor occupancy) (33–35). In addition, BUD lacks affinity for other main transporters (36). Further, murine and human P-gp show similar substrate specificity for glucocorticoids (37). All together, based on literature data, it is likely that BUD is a substrate for murine P-gp, although direct evidence to support this is lacking. This further suggests that the mouse model might be a good model for assessing the exposure of the fetus to ICS and potential control of this exposure by P-gp. We were therefore interested in investigating whether the distribution of BUD and other ICS into the fetus of mice might be restricted and whether P-gp might be involved.

We decided to deliver the drugs through the intravenous route. As we were only interested in evaluating the systemic fate of the two ICS, conclusions drawn from systemic, intravenous drug administration are valid also for pulmonary delivery, while intravenous dosing is more reproducible and results in smaller variability in exposure than pulmonary delivery. During initial setup and selection of the animal model, drug recovery, length of the infusion time, and protein binding were evaluated. Recovery of BUD and FP during the purification step was judged to be sufficient as generally more than 90% of the drugs were recovered. Further, an agreement was shown between *in vitro* and *in vivo* maternal plasma and tissue protein–binding estimates (percent unbound) for each of the drugs in the matrices tested (Table I), validating further the *in vivo* and analytical procedures used. While a direct comparison with literature data was not possible because of the lack of such data for mice, plasma protein–binding

Table III. The Free Drug Concentration Ratios of Selected Tissue to Plasma ($K_{(\text{tissue, plasma, u, u})}$) for BUD and FP (Mean (SD)) in Pregnant Mice ($N = 6$ Mice per Group) After Intravenous Bolus and Infused Dose (Total Drug Dose Is Shown)

	$K_{(\text{brain, plasma, u, u})}$	$K_{(\text{fetus, plasma, u, u})}$	$K_{(\text{placenta, plasma, u, u})}$	$K_{(\text{kidney, plasma, u, u})}$	$K_{(\text{liver, plasma, u, u})}$
Low dose					
BUD (104.9 μg/kg)	0.33 (0.16)#	0.42 (0.17)#	0.82 (0.28)	1.30 (0.41)	0.84 (0.28)
95% CI	0.17–0.49	0.25–0.59	0.53–1.11	0.87–1.72	0.55–1.14
High dose					
BUD (1049 μg/kg)	0.39 (0.20)#	0.38 (0.18)#	0.84 (0.24)	1.43 (0.47)	1.10 (0.30)
95% CI	0.18–0.60	0.18–0.57	0.59–1.09	0.94–1.92	0.78–1.41
FP (1590 μg/kg)	0.75 (0.30)*	0.87 (0.25)**	1.14 (0.32)	1.13 (0.35)	1.39 (0.24)
95% CI	0.44–1.06	0.61–1.12	0.81–1.47	0.76–1.49	0.13–1.64

Data are based on results of Table II. A density of 1 g/mL was assumed for the tissue preparations

95% CI 95% confidence interval

*$P < 0.05$; **$P < 0.01$ for the comparison between FP and high BUD dose (1049 μg/kg); #$P < 0.05$ for the comparison between the actual K values and $K = 1$ (the same results obtained by 95% CI analysis and one-sample t test)

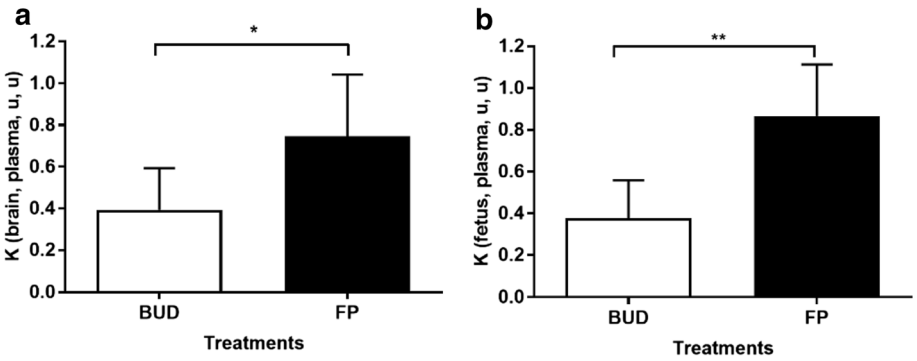

Fig. 1. The bar plot of free drug concentration ratios of brain (**a**) and fetus (**b**) to maternal plasma in pregnant mice after high dose of BUD (1049 μg/kg) or FP (1590 μg/kg). Mean ± SD ($N = 6$ per group); *$P < 0.05$; **$P < 0.01$

estimates (Table I) agreed with literature values for other rodents and humans.

One of the hypotheses of the study was that P-gp affects the transplacental transfer of corticosteroids. As the placental expression levels of P-gp vary during pregnancy, this study was performed at the highest level of expression during mid gestation in mice (pregnancy day 12.5 to day 15.5) (38). This allowed a better evaluation of the effects of placental P-pg on the transfer of BUD and FP as variability in P-gp levels across animals was reduced. Reaching steady state was important, as only then, a direct comparison between free concentrations in plasma and tissues was meaningful when the goal was probing for active transport phenomena between tissues and plasma. Free drug concentrations were used over total drug concentrations as only free drug is relevant for induction of pharmacological effects and relevant for distribution processes (39).

Kidney, liver, and brain were included in the study as control organs for which transporters are likely to not affect the free concentrations of corticosteroids (liver, kidney) or have been shown to affect the distribution of corticosteroids (brain) (33,34). It might be argued that kidney concentrations might be confounded by drug present in urine. However, BUD and most other ICS are predominantly cleared through hepatic metabolism, and metabolites are generally present in urine while intact corticosteroids are generally not found in urine. Indeed, intact BUD has been reported to be absent from urine (40). Similarly, FP is also cleared extensively through hepatic metabolism, and its intrinsic clearance is even higher than that of BUD. The high concentration of FP in the kidney samples requires, however, further investigations. It is recognized that in order to obtain a meaningful liver partition coefficient, e.g., for deriving correct volume of distribution terms, correction for liver metabolism has to be performed (41). We were, however, not interested in the determination of partition coefficients, but only in the comparison of free tissue concentrations in defined organs. These free tissue concentrations were determined experimentally and could have been compared directly across tissues. However, we used the free plasma concentrations as reference concentration by calculating the ratio of free tissue concentration to free plasma concentration, to easily capture differences in free tissue concentrations and compare them directly with free plasma concentrations. The lung was not included in the study to limit the experimental work, which had to be performed directly after the drug infusion, and as previous studies already showed that free concentrations of corticosteroids in lungs are similar to those in plasma (23).

As to be expected, total and free concentrations of BUD in plasma and all tissues investigated showed linear pharmacokinetics, with total concentrations in plasma, brain, fetus, and placenta in the order of 10^{-8} M after administration of low dose and 10^{-7} M after high dose of BUD (Table II). Importantly, free concentrations of BUD in brain and fetus, but not in the remaining tissues, were approximately 2.5–3 times smaller than free concentrations in maternal plasma after either dose of BUD. This suggests that for these tissues, active transporters might be modulating placental and brain distribution of BUD. Taking into account the low specific activity of [^{3}H]-FP as compared to [^{3}H]-BUD, and a greater chance for reliable results using higher doses and radioactivity, we have used a FP dose that matched the high

Table IV. The Total and Free BUD Concentrations (Mean (SD)) in Maternal Plasma and Selected Tissues After Low BUD Dose (104.9 μg/kg) Administered in Pregnant Mice ($N = 6$ Mice per Group) After Administration of P-gp Inhibitor or Its Vehicle

	Plasma (ng/mL)	Brain (ng/g)	Fetus (ng/g)	Placenta (ng/g)	Kidney (ng/g)	Liver (ng/g)
Total concentration						
BUD (+vehicle)	4.11 (0.81)	4.70 (1.55)	3.00 (0.83)	6.27 (1.59)	41.80 (11.19)	10.40 (2.00)
BUD (+inhibitor)	4.83 (1.47)	7.62 (4.57)	4.26 (1.26)	9.55 (3.63)	42.00 (16.24)	15.77 (5.24)*
Free concentration						
BUD (+vehicle)	0.34 (0.09)	0.15 (0.04)	0.10 (0.01)	0.24 (0.10)	0.36 (0.09)	0.30 (0.19)
BUD (+inhibitor)	0.44 (0.10)	0.27 (0.11)*	0.27 (0.12)*	0.37 (0.21)	0.70 (0.33)	0.49 (0.18)

*$P < 0.05$ for the comparison between the two groups (BUD + inhibitor *versus* BUD + vehicle) performed separately for the total and free concentrations of BUD

Table V. The Free BUD Concentration Ratios (Mean (SD)) of Selected Tissue to Plasma ($K_{(tissue,\ plasma,\ u,\ u)}$) After Low BUD Dose (104.9 μg/kg) Administered in Pregnant Mice ($N = 6$ Mice per Group) After Administration of P-gp Inhibitor or Its Vehicle

	$K_{(brain,\ plasma,\ u,\ u)}$	$K_{(fetus,\ plasma,\ u,\ u)}$	$K_{(placenta,\ plasma,\ u,\ u)}$	$K_{(kidney,\ plasma,\ u,\ u)}$	$K_{(liver,\ plasma,\ u,\ u)}$
BUD (+vehicle)	0.44 (0.13)	0.31 (0.10)	0.74 (0.29)	1.11 (0.32)	0.85 (0.36)
BUD (+inhibitor)	0.61 (0.19)	0.59 (0.16)**	0.81 (0.33)	1.54 (0.42)	1.10 (0.29)

**$P < 0.01$ for the comparison between the two groups (BUD + inhibitor *versus* BUD + vehicle)

dose of BUD. Similar to high-dose BUD, the total concentrations of FP in plasma, brain, fetus, and placenta were in the order of 10^{-7} M. Importantly, in contrast to BUD, the free concentrations of FP in brain and fetus were only slightly and non-significantly smaller than that in maternal plasma (Table II), although overall lower free concentrations of FP were observed in all tissues and plasma compared to BUD concentrations. This finding is in agreement with the higher protein binding of FP as compared to BUD. Noteworthy, a higher protein binding is not necessarily an advantage for an ICS as smaller free concentrations in lung and other organs will not improve therapeutic targeting by an ICS and can be adjusted for by optimizing the dose (23).

To adjust for differences in plasma concentrations, the ratio of steady-state free tissue to plasma concentrations (also known as unbound partition coefficient; i.e., $K_{(tissue,\ plasma,\ u,\ u)}$, Table III) was consequently used for comparison of BUD and FP results. The coefficient $K_{(tissue,\ plasma,\ u,\ u)}$ has been widely used in the literature to evaluate the transporter-mediated drug distribution in blood-brain barrier (42–44). In the present study, the smallest K values, in the range 0.33–0.42, were observed for BUD in brain and fetus (Table III), and they were significantly different from $K = 1$, demonstrating reduced transfer of BUD from plasma into brain and transplacental transfer into fetus, while K values for liver, kidney, and placenta were close to $K = 1$. The results for brain are in agreement with results of *ex vivo* glucocorticoid receptor binding studies for which BUD's receptor occupancy was smaller than for other organs (kidney, liver) (33). The rather high K value in placenta was surprising, as the placenta is thought to express transport proteins. Our results suggest that the functioning of the placental barrier does not result in lower placental drug concentrations, but a protection of the fetus. Contrary to BUD, estimates of the partition coefficient K observed for FP in brain (0.75) and fetus (0.87) were not significantly different from $K = 1$ and were significantly higher than the ones for BUD (0.39 for brain and 0.38 for fetus for high-dose BUD), indicating that brain and transplacental protection are lower for FP than those for BUD. Similar results for the brain, namely, a more efficient transfer of FP into the brain tissue, was also reported for *ex vivo* receptor binding studies (33).

The studies with BUD and a specific P-gp inhibitor, zosuquidar, showing statistically significantly increased free BUD concentrations in fetus (nearly three times) and brain (nearly two times) after administration of zosuguidar (Table IV) clearly indicate that P-gp is at least in part responsible for the reduction in transplacental and brain transfer of BUD. We observed that inhibition of P-gp with zosuquidar increased total BUD concentrations in fetus (1.42-fold; P=0.06), placenta (1.52-fold; P=0.07), and brain (1.62-fold; P=0.19). While these differences were not statistically significant because the studies were underpowered for total BUD concentrations, there was a clear trend towards increased total BUD concentrations in fetus and placenta. However, from a pharmacological point of view, free drug concentrations are of much greater relevance than the tissue total drug concentration. Table IV clearly shows that free BUD concentrations increased significantly in brain (1.80-fold) and fetus (2.70-fold) upon P-gp inhibition, despite the rather small group size ($N = 6$). In placenta, the increase of free BUD concentrations was not statistically significant (P=0.20), which can be explained by the fact that within the placenta, exchange occurs between maternal and fetal blood. Indeed, placental P-gp activity is generally quantified by deriving the fetus to maternal blood ratio, and placental drug concentrations are often not even reported (45).

As such, the differences found in our study between BUD and FP are also in agreement with *in vitro* transport studies, which indicated that BUD is a substrate and inducer of P-gp (13,16,46), while FP is not (17). The differences in the

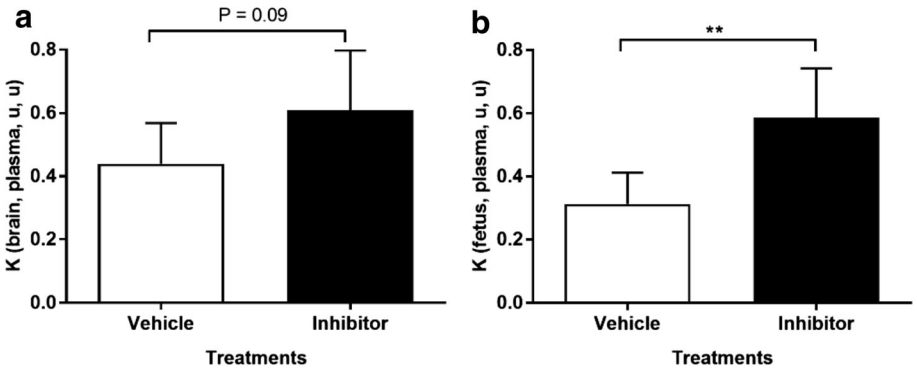

Fig. 2. The bar plot of free BUD concentration ratios of brain (**a**) and fetus (**b**) to maternal plasma in pregnant mice after low BUD dose (104.9 μg/kg) administered in pregnant mice after administration of P-gp inhibitor or its vehicle. Mean ± SD ($N = 6$ per group); **$P < 0.01$

partition coefficients of BUD observed between zosuquidar and vehicle control experiments were statistically significant for fetus, and almost achieved statistical significance for the brain (a larger number of animals probably would have resulted in statistical significance also for brain).

Although we demonstrated that P-gp is playing a critical role in placental transfer of BUD, the tissue partitioning of BUD or other corticosteroids may be influenced by other ATP-binding cassette (ABC) transporters or solute carrier (SLC) transporters. Placenta expresses a variety of influx and efflux transporters, and their expression levels are often dependent on gestational age. We thus do not rule out a possibility that other placental ABC or SLC transporters might also participate in regulating the exposure of fetus to BUD or other corticosteroids. This might also be the reason that inhibition of P-gp did not result in a complete elimination of the free drug concentration gradient between plasma and fetus.

It should be noted that for corticosteroids, effects and side effects are induced by the same glucocorticoid receptors. This means that for ICS, the concentration-effect and concentration-side effect relationships are superimposed. Safety and efficacy of an ICS are therefore only determined by corticosteroid dose, receptor affinity, and other pharmacokinetic factors. Since differences in the effect/safety profile of two ICS are solely based on differences in their pharmacokinetic profiles and dose used, differential tissue distribution between corticosteroids is likely to be of relevance.

Overall, it is important to emphasize that conclusions made in the present study are based on tissue-free drug concentration, making potential differences in tissue binding irrelevant. We strongly believe that the pregnant mouse model is providing meaningful information to further improve our understanding of placental transfer of corticosteroids. The fact that BUD is also a human P-gp substrate, as shown by Dilger *et al.* (16), argues for the relevance of our findings also for humans.

CONCLUSION

In conclusion, we demonstrated that transplacental passage of BUD, but not FP, is reduced and that P-gp is likely involved in this process. These results shed light on the mechanism by which placental P-gp modulates fetal exposure to BUD and improve our knowledge of placental transfer of ICS. If transferrable to humans, the reduction of free fetal BUD concentrations by zosuquidar-sensitive mechanisms might represent an additional argument for the current use of BUD in pregnant asthmatics, as suggested by epidemiological studies (3).

ACKNOWLEDGEMENTS

The financial support of AstraZeneca to the University of Florida is acknowledged.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest SZ, M-JC, DTL, and EN have no conflicts of interest to disclose. PE is an employee of AstraZeneca. AM-L was an employee of AstraZeneca at the time of the study. GH has no conflict of interest.

REFERENCES

1. Breton MC, Martel MJ, Vilain A, Blais L. Inhaled corticosteroids during pregnancy: a review of methodologic issues. Respir Med. 2008;102(6):862–75.
2. Smy L, Chan AC, Bozzo P, Koren G. Is it safe to use inhaled corticosteroids in pregnancy? Can Fam Physician. 2014;60(9):809–12 e433-5.
3. Norjavaara E, de Verdier MG. Normal pregnancy outcomes in a population-based study including 2,968 pregnant women exposed to budesonide. J Allergy Clin Immunol. 2003;111(4):736–42.
4. Namazy J, Schatz M, Long L, Lipkowitz M, Lillie MA, Voss M, et al. Use of inhaled steroids by pregnant asthmatic women does not reduce intrauterine growth. J Allergy Clin Immunol. 2004;113(3):427–32.
5. Hodyl NA, Stark MJ, Osei-Kumah A, Bowman M, Gibson P, Clifton VL. Fetal glucocorticoid-regulated pathways are not affected by inhaled corticosteroid use for asthma during pregnancy. Am J Respir Crit Care Med. 2011;183(6):716–22.
6. Blaiss MS. Are inhaled corticosteroids safe in pregnancy? Postgrad Med. 2004;116(6):57.
7. Expert Panel Report 3 (EPR-3). Guidelines for the diagnosis and management of asthma—summary report 2007. J Allergy Clin Immunol. 2007;120(5):S94–138.
8. Ni Z, Mao Q. ATP-binding cassette efflux transporters in human placenta. Curr Pharm Biotechnol. 2011;12(4):674–85.
9. Feghali M, Venkataramanan R, Caritis S. Pharmacokinetics of drugs in pregnancy. Semin Perinatol. 2015;39(7):512–9.
10. Plonait SL, Nau H. Physicochemical and Structural Properties Regulating Placental Drug Transfer. In: Polin RA, Fox WW, Abman SH, editors. Fetal and Neonatal Physiology. 3rd ed. Philadelphia, Pa: W.B. Saunders Co.; 2004; 197–211.
11. Murphy VE, Fittock RJ, Zarzycki PK, Delahunty MM, Smith R, Clifton VL. Metabolism of synthetic steroids by the human placenta. Placenta. 2007;28(1):39–46.
12. Mark PJ, Waddell BJ. P-glycoprotein restricts access of cortisol and dexamethasone to the glucocorticoid receptor in placental BeWo cells. Endocrinology. 2006;147(11):5147–52.
13. Crowe A, Tan AM. Oral and inhaled corticosteroids: differences in P-glycoprotein (ABCB1) mediated efflux. Toxicol Appl Pharmacol. 2012;260(3):294–302.
14. Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioka N, et al. Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. J Biol Chem. 1992;267(34):24248–52.
15. Lin JH, Chiba M, Chen IW, Nishime JA, deLuna FA, Yamazaki M, et al. Effect of dexamethasone on the intestinal first-pass metabolism of indinavir in rats: evidence of cytochrome P-450 3A [correction of P-450 a] and p-glycoprotein induction. Drug Metab Dispos. 1999;27(10):1187–93.
16. Dilger K, Schwab M, Fromm MF. Identification of budesonide and prednisone as substrates of the intestinal drug efflux pump P-glycoprotein. Inflamm Bowel Dis. 2004;10(5):578–83.
17. Cooray HC, Shahi S, Cahn AP, van Veen HW, Hladky SB, Barrand MA. Modulation of p-glycoprotein and breast cancer resistance protein by some prescribed corticosteroids. Eur J Pharmacol. 2006;531(1–3):25–33.
18. Chung FS, Santiago JS, Jesus MF, Trinidad CV, See MF. Disrupting P-glycoprotein function in clinical settings: what can we learn from the fundamental aspects of this transporter? Am J Cancer Res. 2016;6(8):1583–98.
19. Shepard RL, Cao J, Starling JJ, Dantzig AH. Modulation of P-glycoprotein but not MRP1- or BCRP-mediated drug resistance by LY335979. Int J Cancer. 2003;103(1):121–5.
20. Mayer U, Wagenaar E, Dorobek B, Beijnen JH, Borst P, Schinkel AH. Full blockade of intestinal P-glycoprotein and extensive inhibition of blood-brain barrier P-glycoprotein by oral treatment of mice with PSC833. J Clin Invest. 1997;100(10):2430–6.
21. Hochhaus G, Froehlich P, Hochhaus R, Mollmann A, Derendorf H, Mollmann HW. A selective HPLC/RIA for the

determination of budesonide. J Pharm Biomed Anal. 1998;17(8):1235–42.
22. Waters NJ, Jones R, Williams G, Sohal B. Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding. J Pharm Sci. 2008;97(10):4586–95.
23. Wu K, Blomgren AL, Ekholm K, Weber B, Edsbaecker S, Hochhaus G. Budesonide and ciclesonide: effect of tissue binding on pulmonary receptor binding. Drug Metab Dispos. 2009;37(7):1421–6.
24. Andersson P, Appelgren LE, Ryrfeldt A. Tissue distribution and fate of budesonide in the mouse. Acta Pharmacol Toxicol (Copenh). 1986;59(5):392–402.
25. Khor SP, Mayersohn M. Potential error in the measurement of tissue to blood distribution coefficients in physiological pharmacokinetic modeling. Residual tissue blood. I. Theoretical considerations. Drug Metab Dispos. 1991;19(2):478–85.
26. Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP. Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health. 1997;13(4):407–84.
27. Kwapisz L, Jairath V, Khanna R, Feagan B. Pharmacokinetic drug evaluation of budesonide in the treatment of Crohn's disease. Expert Opin Drug Metab Toxicol. 2017;13(7):793–801.
28. Andersson P, Brattsand R, Dahlstrom K, Edsbacker S. Oral availability of fluticasone propionate. Br J Clin Pharmacol. 1993;36(2):135–6.
29. Davies B, Morris T. Physiological parameters in laboratory animals and humans. Pharm Res. 1993;10(7):1093–5.
30. Daley-Yates PT. Inhaled corticosteroids: potency, dose equivalence and therapeutic index. Br J Clin Pharmacol. 2015;80(3):372–80.
31. Zhang H, Wu X, Wang H, Mikheev AM, Mao Q, Unadkat JD. Effect of pregnancy on cytochrome P450 3a and P-glycoprotein expression and activity in the mouse: mechanisms, tissue specificity, and time course. Mol Pharmacol. 2008;74(3):714–23.
32. Lankas GR, Wise LD, Cartwright ME, Pippert T, Umbenhauer DR. Placental P-glycoprotein deficiency enhances susceptibility to chemically induced birth defects in mice. Reprod Toxicol. 1998;12(4):457–63.
33. Arya V, Issar M, Wang Y, Talton JD, Hochhaus G. Brain permeability of inhaled corticosteroids. J Pharm Pharmacol. 2005;57(9):1159–67.
34. Arya V, Demarco VG, Issar M, Hochhaus G. Contrary to adult, neonatal rats show pronounced brain uptake of corticosteroids. Drug Metab Dispos. 2006;34(6):939–42.
35. Arya V, Coowanitwong I, Brugos B, Kim WS, Singh R, Hochhaus G. Pulmonary targeting of sustained release formulation of budesonide in neonatal rats. J Drug Target. 2006;14(10):680–6.
36. Chen N, Cui D, Wang Q, Wen Z, Finkelman RD, Welty D. In vitro drug-drug interactions of budesonide: inhibition and induction of transporters and cytochrome P450 enzymes. Xenobiotica. 2018;48(6):637–46.
37. Karssen AM, Meijer OC, van der Sandt IC, Lucassen PJ, de Lange EC, de Boer AG, et al. Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain. Endocrinology. 2001;142(6):2686–94.
38. Kalabis GM, Kostaki A, Andrews MH, Petropoulos S, Gibb W, Matthews SG. Multidrug resistance phosphoglycoprotein (ABCB1) in the mouse placenta: fetal protection. Biol Reprod. 2005;73(4):591–7.
39. Bohnert T, Gan LS. Plasma protein binding: from discovery to development. J Pharm Sci. 2013;102(9):2953–94.
40. Szefler SJ. Pharmacodynamics and pharmacokinetics of budesonide: a new nebulized corticosteroid. J Allergy Clin Immunol. 1999;104(4 Pt 2):175–83.
41. Gueorguieva I, Rowland M. A comment on the "correction" of tissue-plasma partition coefficients for drug extraction in eliminating organs by Sven Bjorkman. J Pharmacokinet Pharmacodyn. 2005;32(5–6):659–62.
42. Kalvass JC, Maurer TS, Pollack GM. Use of plasma and brain unbound fractions to assess the extent of brain distribution of 34 drugs: comparison of unbound concentration ratios to in vivo p-glycoprotein efflux ratios. Drug Metab Dispos. 2007;35(4):660–6.
43. Edwards JE, Brouwer KR, McNamara PJ. GF120918, a P-glycoprotein modulator, increases the concentration of unbound amprenavir in the central nervous system in rats. Antimicrob Agents Chemother. 2002;46(7):2284–6.
44. Kodaira H, Kusuhara H, Fuse E, Ushiki J, Sugiyama Y. Quantitative investigation of the brain-to-cerebrospinal fluid unbound drug concentration ratio under steady-state conditions in rats using a pharmacokinetic model and scaling factors for active efflux transporters. Drug Metab Dispos. 2014;42(6):983–9.
45. Smit JW, Huisman MT, van Tellingen O, Wiltshire HR, Schinkel AH. Absence or pharmacological blocking of placental P-glycoprotein profoundly increases fetal drug exposure. J Clin Invest. 1999;104(10):1441–7.
46. Henriksson G, Norlander T, Zheng X, Stierna P, Westrin KM. Expression of P-glycoprotein 170 in nasal mucosa may be increased with topical steroids. Am J Rhinol. 1997;11(4):317–21.