Enhanced elimination of betamethasone in dichorionic twin pregnancies

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Abstract

Aims: No study has evaluated the BET pharmacokinetics in twin pregnancies separated by chorionicity. The aim this study is to describe and compare the BET pharmacokinetic parameters in singleton, dichorionic (DC) and monochorionic (MC) twin pregnancies in the third trimester of pregnancy. Methods: Twenty-six pregnant women received an intramuscular dose of 6 mg of BET sodium phosphate plus 6 mg BET acetate. Serial blood samples were collected for 24 hours after the first intramuscular BET esters dose. BET plasma concentrations were quantified using a validated HPLC analytical method. BET pharmacokinetic parameters were obtained employing a non-compartment model, and were compared using ANOVA's test with Tukey's multiple comparisons test. Correlations between clinical features and pharmacokinetic parameters were analyzed using Pearson's correlation. Preliminary data on the BET placental transfer were also presented. Results: The geometric mean (IC 95%) of AUC0-[?] 670.0 (504.3-805.2) vs 434.9 (311.2-539.6) ng.h/mL and the CL/F 18.38 (13.84-22.65) vs 29.40 (21.17-36.69) were significantly lower and higher, respectively, in DC twin pregnancies compared to singleton. Others pharmacokinetic parameters did not differ among the groups. Conclusions: Data from this study suggest that the presence of two fetoplacental units may increase the BET metabolism by CYP3A4 enzyme and increase its elimination. Pharmacokinetic-pharmacodynamic clinical studies are needed to investigate whether this BET pharmacokinetic changes have clinical repercussions for the newborns and require dose adjustment in DC twin pregnancies.

Introduction

Antenatal corticosteroids administration to pregnant women at imminent risk of premature birth is strongly recommended in clinical practice worldwide.[1,2] It reduces the relative risks for respiratory distress syndrome, intraventricular hemorrhage, necrotizing enterocolitis, and systemic infections in the first 48 hours of life, which are common causes of morbidity and mortality in preterm newborns.[3] Current data demonstrate that corticosteroids administration is most beneficial within one week of therapy. Based on the scientific evidence, the World Health Organization, the American College of Obstetricians and Gynecologists, the Royal College of Obstetricians and Gynaecologists, and the Society of Obstetricians and Gynaecologists of Canada, all recommend antenatal corticosteroids administration to women at risk for preterm delivery between 24–34 weeks of gestation.[4–7]

Preterm birth is the most common perinatal morbidity associated with twin pregnancies, present in 30 to 50% of cases regardless of chorionicity.[8,9] Therefore, the current guidelines also recommend administration of antenatal corticosteroids to women with multiple pregnancy and threatened preterm delivery. However, it is uncertain whether the benefits of antenatal corticosteroids observed in singleton pregnancies are similar in twin pregnancies because of the small number of twin pregnancies included in the studies.[10,11] Also, the effi-

cacy of corticosteroids in promoting lung maturation in twins may be reduced because of the more remarkable maternal physiological changes in multiple pregnancies. These changes could affect the pharmacokinetics of many drugs, including half-life, volume of distribution, and clearance in singleton pregnancy differently from that in twin pregnancy.[12–14]Consequently, that efficacy of corticosteroids in inducing maturation could be reduced due to the lower plasma exposure of twin fetuses to these drugs.

Betamethasone (BET) has been more commonly used in studies evaluating the effect of antenatal corticosteroid therapy on neonatal outcomes. BET is formulated as a fast releasing BET phosphate ester prodrug or a dual-acting formulation containing BET phosphate plus BET acetate esters. Both esters are fast and extensively metabolized to the active glucocorticoid BET.[15,16] As far as we know, there are no bioavailability data after intramuscular BET esters administration. Ke and Milad (2019) have assumed that 94% of BET elimination is dependent on CYP3A4 based on the BET renal clearance in healthy adults of approximately 0.49 L/ h.[17] Considering that CYP3A is induced by 86-124% in late pregnancy compared to postpartum period,[18] BET total clearance is probably enhanced in pregnancy according to studies in healthy adults and pregnant women.[19–22,11,15,23] BET total clearance has also been reported to be increased in twin pregnancies compared to singleton ones.[19] Although there are studies that have evaluated the BET pharmacokinetics in twin pregnancies, they are few, and none of them studied multiple pregnancies separated by chorionicity.

Therefore, the objectives of the present study were to describe and compare the BET pharmacokinetic parameters in singleton, dichorionic (DC) and monochorionic (MC) twin pregnancies in order to evaluate, in the future trials, the need for adjusted doses of BET according to the chorionicity of multiple pregnancies.

Methods

Patients A cross-sectional pharmacokinetic study of singleton and twin pregnancies was performed, including pregnant women underwent tocolysis in the local hospital because of preterm labour from August 2018 to September 2019. This study was approved by the local Ethics Research Committee (protocol number 8676/2017) of University Hospital of the Ribeirão Preto Medical School (São Paulo, Brazil), and patients gave signed informed consent. The study protocol was registered on the Brazilian Registry of Clinical Trials (ReBEC, www.ensaiosclinicos.gov.br) under ID number RBR-2v2mwkz. Participation in the study was limited to the inpatient population to ensure compliance with serial blood sampling. Thirty-five pregnant women, between 24 and 34 weeks of gestation, and >18 years old were clinically eligible for the antenatal corticosteroid treatment and were approached for enrollment. The following exclusion criteria were applied: use of tobacco, alcohol or illegal substances; maternal diseases with vasculopathy identified by laboratory tests or signals of placental insufficiency; the use of drugs that interact with BET; or maternal desire to withdraw. Finally, the data from 26 pregnant women, divided into three groups (9 singleton pregnancies, 9 DC and 8 MC twin pregnancies) were analyzed.

Clinical protocol

A maternal blood sample (5 mL) was obtained from each patient before administering the first dose of BET esters to assess biochemical, renal, hepatic, metabolic and haematological parameters. Laboratory tests were carried out to assess the functional normality of the organ systems. All pregnant women received a standard regimen of BET esters intramuscularly (Celestone Soluspan®, Mantecorp, Anápolis, GO, Brazil – equal amounts of BET sodium phosphate and BET acetate at a dose of 12 mg every, two times 24h apart). Serial blood samples (5 mL) were collected immediately before and 5, 10, 15, 35 minutes and 1, 3, 6, 8, 10, 16 and 24 hours after the first intramuscular BET esters dose to determine BET plasma concentrations. In some cases, there was a failure to stop labour and delivery occurred (n=5). Simultaneous samplings of maternal blood, umbilical cord (for each fetus if twin pregnancy), and intervillous space (the space near the umbilical cord insertion if twin pregnancy) were performed to assess the placental transfer and distribution of BET in these compartments. Blood was collected from the umbilical cord after the cord was clamped and sectioned and the placenta expelled through the birth canal, with no risk for the mother or neonate. The technique described by Camelo Júnior et al. was adopted to collect blood samples from the umbilical vein and the intervillous space.[24]

All blood samples for BET pharmacokinetics and transplacental transfer studies were transferred to heparinized tubes protected from light and containing 10 µL of stabilizing solution of 2 M of sodium arsenate dibasic heptahydrate. The blood samples were then centrifuged within a maximum period of 15 minutes at 21.500 [?] g for 5 minutes at 4°C. The plasma was separated and transferred to cryogenic tubes containing 10 µL of a stabilizing solution of potassium fluoride 50% and were stored at -70°C until analysis.[25,16]

Betamethasone assay and analytical validation

BET determination in plasma was performed by an LC-MS/MS system according to a method previously validated by the research group (unpublished data – Rodrigues et al., manuscript accepted by The Brazilian Journal of Pharmaceutical Sciences). Briefly, 500 µL of plasma were added to an internal standard solution (IS; deuterated betamethasone-acetate; 1 µg/mL in methanol) and submitted to the extraction process with 6 mL of diisopropyl ether.

Analytes were separated in a LiChrospher reversed-phase C-8 column with a LiChrospher® 100 RP-8 C-8 guard column, using a mixture of methanol and ammonium formate 0.05 mM (90:10 v/v) as a mobile phase.[25,16] The MS/MS detection system was a Quattro Micro LC triple quadrupole (Micromass, Manchester, United Kingdom) equipped with an electrospray interface (ESI) operating in positive ion mode. The method revealed linearity in the range of 2-250 ng/mL plasma for BET. Coefficients of variation and inaccuracy percentage were [?]15%, indicating that the method was precise and accurate. Moreover, the method showed selectivity and did not presented matrix or carry-over effects. Stability tests also presented the coefficient of variation and relative standard errors [?] 15%. Therefore, the method for BET pharmacokinetics studies had adequate confidence limits, ensuring the results' reproducibility and repeatability.

Pharmacokinetic analysis

The BET pharmacokinetics was calculated based on the plasma concentration versus time using PhoenixWinNonLin software, version 6.3 (Certara, Princeton, New Jersey, USA), employing a noncompartmental analysis. Maximum plasma concentration (Cmax) and time to reach Cmax (Tmax) were obtained from data of the individual patients. Areas under the plasma concentration-time curves were calculated by the trapezoidal rule and extrapolated to the infinity. Apparent total clearance (CL/F) was evaluated by the equation CL/F = Dose/$AUC^{0\text{-}[?]}$ and apparent volume of distribution (Vd/F) was calculated by the equation of the software. The BET placental transfer was evaluated by the umbilical cord to maternal vein concentration ratio and intervillous space to maternal vein concentration ratio.

Statistical analysis

Data was analyzed by GraphPad Prism software (CA, USA). The BET pharmacokinetics data are presented as median and interquartile range. Pharmacokinetic parameters presented a normal distribution (Shapiro-Wilk test; p-value$<$0.05) so they were compared using ANOVA's test with Tukey's multiple comparisons test (p-value$<$0.05).[26,27] Correlations between clinical features and pharmacokinetic parameters were analyzed using Pearson's correlation (p-value $<$0.05).

Results

The 26 pregnant women were divided into three groups: singleton pregnancies (n=9), DC twin pregnancies (n=9) and MC twin pregnancies (n=8). The groups of pregnant women were compared regarding maternal age, body mass index and gestational age at childbirth. There were no significant differences among these features for the three groups. The demographic and laboratory test results of the pregnant women are presented in Table 1. Serum albumin concentration was significantly higher in MC twin pregnancies compared to singleton and DC twin pregnancies. On the other hand, the plasmatic urea concentration was significantly lower in MC twin pregnancies.

The median curves of plasma BET concentration versus time for the three groups were determined during the 24h after a single intramuscular dose of 12mg of BET sodium phosphate and BET acetate (Figure 1). Table 2 lists the pharmacokinetic parameters, demonstrating differences among the groups of pregnant women.

The $AUC^{0-[?]}$ and CL/F were significantly lower and higher, respectively, in DC twin pregnancies compared to singleton and MC twin pregnancies. On the other hand, Cmax, Tmax and Vd/F were not significatively different among the groups. Vd/F (p=0.02; r=0.4530) was correlated to body mass index, but not to any other demographic or biochemical data (Figure 2).

Figure 3 shows preliminary data on the BET placental transfer, expressed by the umbilical cord to maternal vein concentration ratio and intervillous space to maternal vein concentration ratio.

Discussion

The present study demonstrated significant differences in the BET pharmacokinetics in DC twin pregnancies. The $AUC^{0-[?]}$and CL/F were significantly lower and higher, respectively, in DC twin pregnancies compared to singleton and MC twin pregnancies while there were no differences in the other pharmacokinetic parameters. The understanding of the pharmacokinetics of drugs during pregnancy is crucial for the development of appropriate dosage regimens. The clinical BET pharmacokinetics have been investigated in healthy adults,[20,21,23] as well as in pregnant women.[19,22,11,15]

Previous studies developed BET population pharmacokinetic models[19,22] or classical pharmacokinetic approaches[20,21] in both singleton and twin pregnancies. However, none of these studies evaluated the BET pharmacokinetics or BET placental transfer in twin pregnancies discriminated by chorionicity. This study is unprecedented regarding the BET pharmacokinetic studies in singleton, DC and MC twin pregnancies.

Many demographic and clinical variables such as body composition, blood volume, hormonal levels, hepatic and placenta metabolism could interfere with BET pharmacokinetics and explain the findings of the present study.[28,29,11] Furthermore, the influence of the maternal physiological changes on the kinetics disposition of drugs is more remarkable in the third trimester of pregnancy and multiple pregnancies.[28–30] It is worth mentioning that all pregnant women included in this clinical trial were in the last trimester of their pregnancies.

Few studies that investigated BET elimination in unchanged form in urine evidenced that the contribution of renal clearance in total clearance is not significant, supposing that BET is eliminated mainly by its metabolism.[17,23,31] Although pathways that mediate the BET hepatic metabolism have not been fully established, some studies have proposed a primary and fundamental role for the cytochrome P450 enzymes, mainly CYP3A4.[17,32] Even though some authors have suggested that BET placental metabolism seems to be low or negligible,[33] others have highlighted the possibility of increased metabolism due to two or more fetoplacental units in twin pregnancies.[11]

Data on the impact of twin pregnancies on BET pharmacokinetics appear to be contradictory to date. Our data show BET $AUC^{0-[?]}$lower in DC twin pregnancies than in singleton and MC twin pregnancies. The presence of two fetoplacental units in DC twin pregnancies could result in a higher metabolism mediated by CYP3A enzymes. Besides, potentially higher hormone levels in DC pregnancy could induce CYP3A enzymes in both the placenta and maternal liver and explain these changes. Also, the increased hepatic flow during pregnancy could rise the BET clearance and reduce the AUC.

Although some studies have shown significant differences in CL/F or $t_{1/2}$ between singleton and twin pregnancies,[19,11] other research groups have not found similar results.[22] Foissac and collaborators found a 28% increase in BET CL/F in twin pregnancies without chorionicity discrimination compared to singleton pregnancies.[19] In the present study, the BET CL/F values did not differ between MC twin and singleton pregnancies, but DC twin pregnancies presented a 40% increase in this pharmacokinetics parameter compared other pregnancies. Enhanced hepatic metabolism can also explain the pregnancy-related increase in CL/F.

Our study showed a positive correlation between maternal body mass index and BET Vd/F. This finding is similar to the Della Torre et al. study, which showed that maternal lean body weight was a covariate for the Vd/F using a population pharmacokinetic model.[22] Also, the magnitude of the values of the dose-independent parameters such as CL/F and Vd/F in the present study were similar to those of previous

studies conducted with pregnant women (Table 2).[19,22,11]Betamethasone is a lipophilic drug and can bind significantly to tissue.[34] The significant change in pregnant women's body size with twin fetuses provides one more possible explanation for the lower AUC in these pregnancies than singleton ones.

Our study has some strengths. First, few studies have analyzed BET pharmacokinetics in twin pregnancies, and no study to date has analyzed BET pharmacokinetic parameters in twin pregnancies separated by chorionicity. Second, there was only one researcher conducting the clinical protocol. This ensured the correct interval between the BET administration and the first blood sample and the serial blood sampling intervals. Third, before BET pharmacokinetic analysis in the present study, a BET assay and analytical validation were performed, and the method showed suitable sensibility, linearity, accuracy and precision for pharmacokinetics and placental transfer studies in a twin pregnancy.

Our study was not without limitations. Preliminary data on BET placental transfer can be considered limited mainly due to the low number of patients in each group and different intervals between drug administration and delivery. On the other hand, a previous study showed that BET concentration in cord blood decreases within days.[35] The analysis of umbilical vein/maternal vein and intervillous space/maternal vein ratios showed that BET concentration in intervillous space is higher than the fetal compartments. The inclusion of a more significant number of pregnant women and applying a protocol with greater precision in the intervals between the BET administration and the collection of maternal and fetal blood samples are being carried out to confirm this finding.

In conclusion, this study shows the influence of twin pregnancies on BET pharmacokinetics, suggesting that the presence of two fetoplacental units may increase the drug's metabolism by CYP3A4 enzyme and increase its elimination. Pharmacokinetic-pharmacodynamic clinical studies are needed to investigate whether this BET pharmacokinetic changes have clinical repercussions for the newborns and require dose adjustment in DC twin pregnancies.

Data availability : The data that support the findings of this study are available on request from the corresponding author.

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Table 1 – Demographic, clinical and laboratory data from singleton and twin pregnancies.

Variables (Reference value)	Singleton (n=9)	MC twin (n=8)	DC twin (n=9)	p value*
Age (years)	27 (21 – 31)	29 (25 – 33)	26 (22 – 30)	0.5199

Variables (Reference value)	Singleton (n=9)	MC twin (n=8)	DC twin (n=9)	p value*
Body mass index (kg/m^2)	26.82 (22.97 – 30.39)	29.10 (25.13 – 32.93)	27.64 (23.61 – 31.39)	0.6403
Gestational age (days)	209 (193 – 225)	216 (207 – 225)	200 (180 – 219)	0.2773
Glycemia (mg/dL) (70 – 100)	84 (61 – 104)	80 (68 – 92)	88 (60 – 112)	0.8488
α1-AGP (mg/dL) (50 – 120)	60.40 (54.17 – 66.36)	65.22 (52.76 – 76.94)	64.59 (46.15- 80.66)	0.8390
Albumin (g/dL) (3.5 – 4.8)	3.54 (3.47 – 3.61)	3.69 (3.45 – 3.93)	3.41 (3.28 – 3.55)	**0.0222**
Total proteins (g/dL) (6 – 8.5)	6.23 (6.01 – 6.44)	6.04 (5.62 – 6.47)	5.81 (5.42 – 6.18)	0.1203
Total bilirubin (mg/dL) (0.2 – 1.2)	0.40 (0.22 – 0.56)	0.33 (0.21 – 0.44)	0.82 (0.22 – 0.95)	0.3860
Alkaline phosphatase (U/L) (65 – 300)	245 (168 – 316)	353 (289 – 414)	278 (181 – 366)	0.0960
Gamma-GT (U/L) (11 – 50)	9.6 (7.2 – 12)	22 (12 – 30)	18 (9.8 – 26)	0.0734
AST (U/L) (< 32)	15.88 (12.05 – 19.25)	20.41 (10.89 – 28.27)	19.57 (15.81 – 22.87)	0.4880
ALT (U/L) (< 31)	12.06 (9.27 – 14.48)	18.17 (5.58 – 25.30)	18.01 (8.58 – 25.93)	0.5781
Creatinine clearance (75-129 mL/min/1.73)	203 (155 – 245)	213 (178 – 247)	203 (191 – 215)	0.8891
Creatinine (mg/dL) (0.7 – 1.5)	0.56 (0.48 – 0.64)	0.55 (0.45- 0.65)	0.58 (0.49 – 0.65)	0.8662
Urea (mg/dL) (10 – 50)	13.43 (9.21 – 17.13)	11.41 (7.88 – 14.76)	17.31 (13.79 – 20.49)	**0.0327**
Cholesterol (mg/dL) (< 200)	212 (188 – 235)	255 (212 – 296)	241 (188 – 291)	0.1500
Triglycerides (mg/dL) (< 150)	153.1 (86.87 – 205.1)	203.8 (114.2 – 286.2)	236.7 (175.6 – 293.2)	0.166
Hemoglobin (g/dL) (11,0 – 15,5)	12 (11 – 13)	12 (11 – 13)	12 (11 – 12)	0.620

Values are expressed as geometric mean (95% confidence interval). DC: dichorionic; MC: monochorionic; n: number of subjects; α1-AGP: α1-acid glycoprotein; Gama-GT: gamma-glutamyl transpeptidase; AST: aspartate aminotransferase; ALT: alanine transaminase. *ANOVA's test with Tukey's multiple comparisons test (p-value < 0.05).

Table 2 – Betamethasone (BET) pharmacokinetic parameters and placental transfer in singleton, monochorionic and dichorionic twin pregnancies after a single intramuscular dose of 6 mg of betamethasone acetate plus 6 mg betamethasone phosphate.

BET pharmacokinetic parameter	Singleton pregnancies (n=9)	MC twin pregnancies (n=8)	DC twin pregnancies (n=9)	*p*-value
C_{max} (ng/mL)	55.35 (36.48- 70.96)	52.34 (40.07 – 63.61)	44.87 (29.44 – 56.82)	0.5299
t_{max} (h)	2.06 (1.03 – 2.90)	1,5 (0.86 – 2.01)	1.80 (0.83 – 2.52)	0.5665
$AUC^{0-[?]}$ (ng.h/mL)	670.0 (504.3 – 825.2)	494.9 (355.4 – 624.1)	434.9 (311.2 -539.6)	**0.0345**
CL/F (L/h)	18.38 (13.84 – 22.65)	25.46 (18.30 – 32.13)	29.4 (21.17 – 36.69)	**0.0324**
Vd/F (L)	226.1 (112.7 – 316.9)	265.4 (172.0 – 353.6)	279.9 (159.6 – 389.6)	0.6746
BET transplacental transfer	**Singleton pregnancies (n=1)**	**MC twin (n=1)**	**DC twin (n=6[a])**	
Drug administration and delivery interval (h)	20.34	6.04	2.02 (1.53 – 2.62)	
Umbilical vein/Maternal vein ratio	0.33	0.20	0.15 (0.09 – 0.25)	
Intervillous space /Maternal vein ratio	0.54	0.31	0.22 (0.07 – 0.60)	

Values are expressed as geometric mean (95% confidence interval). BET: betamethasone; DC: dichorionic; MC: monochorionic; n: number of subjects; C_{max}: maximum plasma concentration; t_{max}: time to reach C_{max}; $AUC^{0-[?]}$: area under the plasma concentration vs time curve extrapolated to infinity; CL/F: apparent clearance; Vd/F: apparent volume of distribution. *ANOVA's test with Tukey's multiple comparisons test (p-value < 0.05).[a] Both twins were included, thus, 6 samples of umbilical vein and intervillous space were collected.

Figures legends:

Figure 1 – Plasma concentration versus time of betamethasone in singleton pregnancies (n=9; circles), monochorionic (MC) twin pregnancies (n=8; squares) and dichorionic (DC) twin pregnancies (n=9; triangles) after a single dose of 6mg of betamethasone acetate plus 6mg betamethasone phosphate. Values are expressed as geometric mean (95% confidence interval).

Figure 2 – Correlation between betamethasone apparent volume of distribution (Vd/F) and body mass index (BMI) (p=0.02; r=0.4530). Data are presented individually. Singleton pregnancies (n=8) are represented as full circles, monochorionic (MC) twin pregnancies (n=9) are represented as open circles and dichorionic (DC) twin pregnancies (n=9) are represented as grey circles.

Figure 3 – Betamethasone concentration in the transplacental transfer. Singleton pregnancies (n=1) are represented as circles, monochorionic (MC) twin pregnancies (n=1) are represented as squares and dichorionic (DC) twin pregnancies (n=3) are represented as hexagons. Values are expressed as individual data. Symbols with a dot inside represent maternal vein concentrations; Symbols with an "x" inside represent intervillous space concentrations; Semi-full symbols represent umbilical vein concentrations; Open symbols represent intervillous space to maternal vein ratios, and solid symbols represent umbilical vein to maternal vein ratios.

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Figure_16_03.pdf available at https://authorea.com/users/403302/articles/514826-enhanced-elimination-of-betamethasone-in-dichorionic-twin-pregnancies