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Amniotic fluid disposition of cefazolin during pregnancy

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Data on amniotic fluid (AF) disposition of cefazolin and its co-variates during pregnancy are limited. We therefore collected AF samples during *in utero* surgery in the second and third trimester of pregnancy and compared these observations with available data on AF disposition in very early pregnancy and at term gestation. During 45 *in utero* surgical interventions, 57 AF samples were collected. The median AF cefazolin concentration was 0.62 mg/l. Significant correlations between cefazolin concentration in AF and time after initiation of intravenous administration ($r = 0.36$, $P < 0.01$) and gestational age ($r = 0.58$, $P < 0.01$) were observed. In two of these *in utero* interventions, fetal urine was simultaneously collected. Fetal urine cefazolin concentrations were significantly higher than in AF. It is therefore to be anticipated that the GA-dependent increase in cefazolin concentration in AF in part reflects fetal renal maturation. The current observations on cefazolin disposition hereby illustrate the need to consider pharmacokinetic alterations during pregnancy as a continuous instead of a dichotomous variable.

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Introduction

Cefazolin is a first generation cephalosporin only available for intravenous or intramuscular administration. Based on its spectrum, safety and tolerance, cefazolin is administered for prophylaxis during a variety of surgical interventions, including during pregnancy[1]. Based on these characteristics, we routinely administer this drug intravenously to mothers for prophylaxis during *in utero* surgery. The physiological adaptations of pregnancy result in changes in drug disposition, and both clearance as well as distribution volume of cefazolin are higher during pregnancy while observations on cefazolin disposition in amniotic fluid (AF) throughout pregnancy are limited[2-4]. This is of specific importance for prophylaxis during *in utero* surgery, since next to routine surgical compartments (e.g. blood, abdomen, skin and subcutaneous tissues), the fetus, placenta and amniotic cavity are surgical fields of interest[5-7].

Previous studies of cefazolin ($n = 40$) concentrations in AF in the first trimester of pregnancy have been reported[8]. Cefazolin concentration in AF remained <0.3 mg/l in the first 15 weeks and <1 mg/l between 15 and 20 weeks of gestational age (GA), strongly suggesting that passive diffusion through the membranes is very limited[8]. Cefazolin AF concentrations in pregnancies,

complicated by Rh isoimmunisation, have been reported following intravenous administration of 2 g cefazolin in the second and third trimester of pregnancy[9]. During 10 procedures at 27 (SD 3) weeks GA, median AF concentration was 0.9 (SD 0.4) mg/l. Mean AF concentration (n = 20) was 2 (SD 1.1) mg/l following administration of 1 g cefazolin in 26 full term pregnancies during elective Caesarean section[10].

We recently reported on characteristics of cefazolin AF disposition during *in utero* surgery in the second and third trimester of pregnancy[11]. Based on 42 AF samples collected during 30 interventions, we concluded that GA in part explained the inter-individual variability in cefazolin AF concentrations observed since cefazolin concentrations in AF increased with increasing GA[11]. We therefore decided to further collect AF samples during *in utero* surgery to enlarge the number of observations to describe the co-variates of cefazolin AF disposition. In addition, simultaneous sampling of AF and fetal urine were done in selective cases when urine bladder puncture was performed for lower urinary tract obstruction (LUTO).

Methods

Patients

The study was approved by the ethical board of the University Hospitals Leuven. Patients were included after informed written consent. Eligible *in utero* surgical interventions were fetoscopic laser ablation of placental anastomoses for twin to twin transfusion syndrome (TTTS), fetal endoscopic tracheal occlusion (FETO) and its reversal for severe congenital diaphragmatic hernia (CDH) and derivative shunting [pericardio-amniotic shunt for pericardial effusion, vesico-amniotic shunting for LUTO, thoraco-amniotic shunt for pleural effusion]. An extensive technical description of these interventions is available in the literature[12-14].

Intravenous administration of cefazolin (Cefazoline Sandoz®, Sandoz, Vilvoorde, Belgium), 2 g/day, eight hourly for two days is currently part of routine clinical care for scheduled *in utero* surgery in our unit. The first dose of cefazolin is administered one to two hours before surgery through a peripheral venous cannula after dilution in 50 ml of normal saline (Baxter, Lessines, Belgium). The intended duration of administration is 0.5 hour. Data on clinical characteristics [GA (weeks), weight, length, creatinaemia, proteinaemia, albuminaemia, type of *in utero* surgical intervention] of study patients were prospectively collected.

Sampling strategy

For reasons of ethical constraints and safety, AF sampling was only performed when puncture of the amniotic cavity and subsequent sampling of AF was part of the surgical intervention without additional burden for mother or fetus. AF samples were therefore collected at random times during puncture, at introduction of the trocar used for uterine access or at the end of the surgical intervention just before removal of the trocar. AF samples visually contaminated with blood were discarded and AF sampling was also discontinued when amniotic irrigation (with Hartmann solution) was performed during intervention. Time of sampling of AF (min) after initiation of intravenous cefazolin was recorded. Fetal urine sampling was performed at urine bladder puncture. Time of sampling of fetal urine after initiation of intravenous cefazolin was recorded.

Drug analysis

Cefazolin concentrations were determined by high-performance liquid chromatography (HPLC) after solid-phase column extraction, according to a novel method developed in our laboratory based on methods reported in literature[15,16]. Cefazolin concentration in AF was determined by adding 50 µl 5% of BSA, 50 µl of cefoxitin (100 µg/ml) and 0.5 ml of 5% trichloroacetic acid to 0.45 ml of AF. After vortexing for 15 seconds, and waiting for 10 min, samples were centrifuged for 8 min at 12000*g*. Standard curves were prepared in 0.5% BSA in 0.9% NaCl. After vortexing and centrifuging for 5 min at 1800*g*, samples were injected in solid-phase extraction columns (Oasis HLB 30 mg, 1 ml volume). Solid-phase extraction was performed with a Vac Elut SPS24 vacuum extraction system. These columns were activated twice with 1 ml methanol and 1 ml water, applying slight vacuum to the columns. Prepared standards, controls and samples were passed through the columns over a time period of 2–3 min. Then 1 ml water was applied and vacuum maintained for 2 min, followed by 1 ml methanol / water (80/20, v/v) and again vacuum maintained for 2 more min. Elution of the columns was performed with 0.5 ml of methanol (+ 0.2% triethylamine) twice. Eluates were evaporated with air stream in a water bath at 45° C and dried residues were dissolved in 400 µl mobile phase. Injection volumes varied between 20 and 50 µl. A Waters 600E pump was used in combination with a Waters 996 PDA detector and a Waters chromatographic data system Empower 2. The mobile phase was a mixture of acetonitrile and potassium phosphate buffer 15 mM pH 3.0 (+ 0.05 % triethylamine) (15/85, v/v) and the chromatographic separation performed on a

Hypersil BDS C18 5 µ column (250 x 4.6 mm I.D.). Column temperature was maintained at room temperature and the flow rate was 0.9 ml/min. UV-detection was set at 265 nm. Linearity of the calibration curve for cefazolin in plasma was found in the range of 0.1–100 µg/ml. A similar approach was used to quantify cefazolin fetal urine concentrations.

The lower limit of quantification for cefazolin was 0.1 µg/ml, being the lowest concentration of the standard curve with a coefficient of variation lower than 20 %. Analytical recovery (%) of cefazolin and internal standard cefoxitin was respectively 77.1 ± 9.2 and 82.2 ± 8.7 (mean ± SD). Coefficients of variation for intra- and interday precision and accuracy were below 15%.

Statistics

Cefazolin AF concentration (mg/l) were reported by median and range. Correlations (Spearman rank) between the cefazolin AF concentration and time after initiation of intravenous cefazolin administration (min) and gestational age (weeks) were investigated. Cefazolin concentrations in AF were compared with similar observations in fetal urine.

Results

During 45 *in utero* surgical interventions, 57 AF samples were collected. Clinical characteristics and type of surgical interventions are summarised in Table 1. The median AF cefazolin concentration was 0.62 (0.06–3.73) mg/l. Correlations between cefazolin concentration in AF and GA (r = 0.58, 95 % 0.38 to 0.73, $P < 0.01$) (Figure 1) and time after initiation of intravenous cefazolin administration (r = 0.36, 95 % CI 0.12 to 0.57, $P < 0.01$) (Figure 2) were observed.

In two cases, fetal urine was sampled 75 and 110 min after initiation of intravenous administration of cefazolin. The cefazolin concentration-time profiles in AF and fetal urine are presented in Figure 2. Both fetal urine concentrations were significantly higher compared to AF concentrations: 1.94 and 5.5 mg/l compared to 0.83 and 1.25 mg/l respectively (Figure 2).

Table 1 Clinical characteristics and indication for *in utero* surgery of 45 interventions in the second and third trimester of pregnancy

	Median	Range
Gestational age (weeks)	26	(17–34)
Maternal weight (kg)	72	(54–99)
Maternal length (cm)	165	(152–180)
Albuminaemia (g/l)	36.5	(33.9–39.2)
Indication for in utero surgery		
Laser ablation	24	
Endotracheal occlusion/reversal	17	
Derivative shunting, bladder	2	
Derivative shunting, cardiac	2	
Total	**45**	

Discussion

Based on observations in AF and fetal urine collected during 45 *in utero* surgical interventions, the cefazolin concentrations in AF were in part dependent on the time after administration and on the GA. In addition, cefazolin concentrations in fetal urine were higher compared to AF (Figure 2), hereby illustrating the contribution of fetal renal clearance on AF cefazolin disposition. This contribution of fetal renal clearance probably explains the significant correlation between cefazolin AF concentration and GA (Figure 1). The observations in AF during pregnancy illustrate the need to consider pharmacokinetic alterations during pregnancy as a continuous variable instead of a dichotomous variable[2,3].

The current observations on cefazolin concentration in AF in the second and third trimester of pregnancy combined with the above mentioned observations in the first trimester and at term gestation, strongly suggest a GA-dependent impact on AF disposition. This trend is the phenotypic result of GA-dependent alterations in maternal disposition, in placental permeability and transport capacity, in fetal protein binding capacity and in fetal renal elimination capacity while passive diffusion through the membranes seems to be very limited[8-10].

The clearance and volume of distribution of cefazolin are higher during pregnancy and as the drug is highly protein bound, it crosses the placenta only to a limited extent[4-6]. To the best of our knowledge, there are no studies on the impact of GA on placental transport capacity of cefazolin.

Taking these aspects of GA-dependent cefazolin disposition into account, the current observations on higher cefazolin fetal urine concentrations compared to AF support the contribution of fetal renal elimination to cefazolin AF disposition. Cefazolin fetal urine concentrations could be quantified in two cases in this cohort and in 10 of the 40 subjects in a previous study[9]. In all 12 cases, the cefazolin concentration in fetal urine was higher than in the AF, providing evidence for a relevant fetal renal contribution to the amniotic appearance of cefazolin during pregnancy[8]. Postnatal renal drug clearance matures with a postmenstrual age (PMA)-dependent sigmoid trend instead of a logarithmic trend[17,18]. The adaptation of the fetus to postnatal environment requires a pre-programmed change in renal

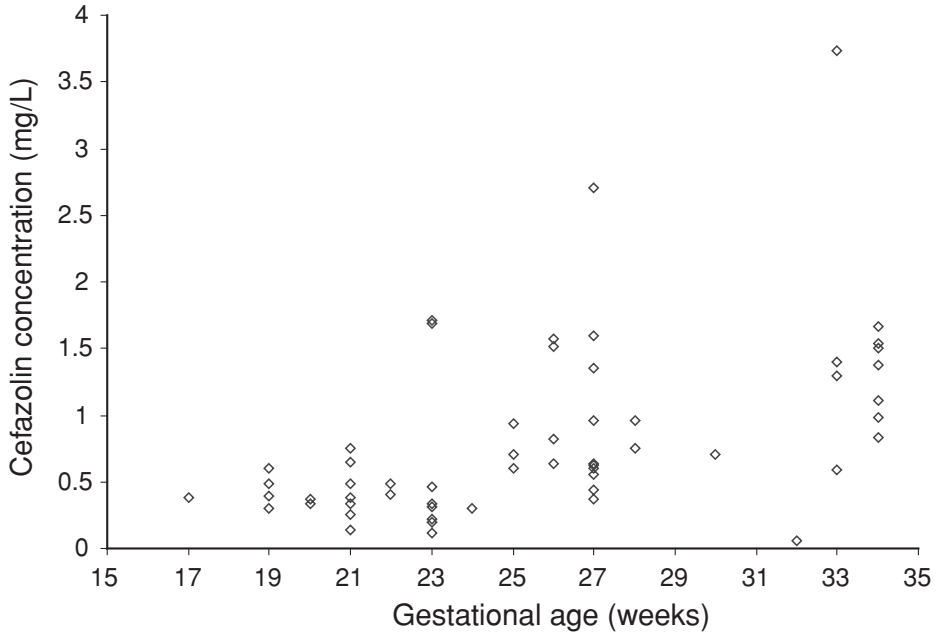

Figure 1 Correlation between cefazolin concentration in amniotic fluid and gestational age.

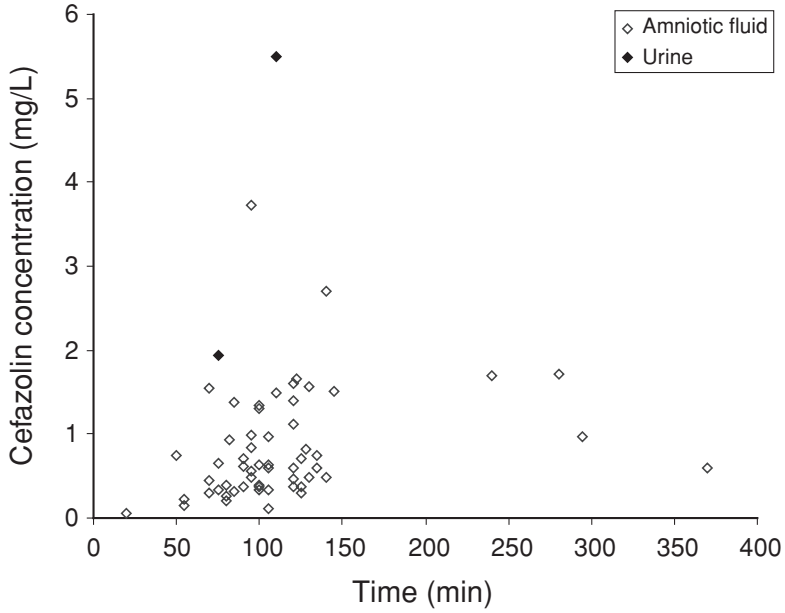

Figure 2 Correlation between cefazolin concentration in amniotic fluid and time.

clearance[19]. Glomerular filtration rate (GFR) is known to be low during fetal life and increases with increasing GA. It is therefore anticipated that maturation of fetal cefazolin drug clearance also depends on PMA but *in vivo* observations in human fetal life are limited because of obvious ethical constraints[19].

Observations on maturational changes in the GFR rate in animal experimental settings have been described in fetal sheep. GFR increased during the last third of gestation in fetal sheep[20]. However, there was no further increase in GFR when corrected for fetal weight or for kidney weight in the last third of gestation[20]. The subsequent impact of transition from fetal to newborn life in sheep has been previously described[21]. This transition was associated with a rapid rise in GFR and an important decrease in urinary sodium excretion and fractional excretion of sodium, independent of the changes in renal blood flow dynamics[21]. The current observations on cefazolin disposition in AF and fetal urine in human fetuses provide evidence in support of age-dependent, fetal renal maturation. An additional advantage of cefazolin compared to ampicillin or other penicillins is that fetal oral ingestion with subsequent re-absorption as described for ampicillin will be limited[6].

A more extensive, pooled population pharmacokinetics approach of cefazolin disposition in maternal and fetal compartments is warranted in order to provide clinicians with GA-dependent dosing regimes and to further document various co-variates of cefazolin disposition. This study has provided evidence that the cefazolin concentration in AF after intravenous administration to the mother in part depends on GA. Since fetal urine concentrations of cefazolin are higher compared to AF observations, it is to be anticipated that this GA-dependent effect in part reflects fetal renal maturation.

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