The placental transfer of erythromycin in human pregnancies with group B streptococcal infection

Przepływ erytromycyny przez łożysko w ciąży powikłanej zakażeniem paciorkowcami grupy B

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Abstract

Objectives: *The aim of this study was to investigate the effectiveness of erythromycin in preventing fetal and intrauterine group B streptococcal (GBS) infections. The study evaluated the penetration of erythromycin through the placenta, by comparing umbilical vein and maternal serum erythromycin concentrations.*

Material and methods: *The study subjects were 42 pregnant women, with GBS-positive screening or whose laboratory screening was not available, who delivered between 17th April 2013 and 22nd July 2013. The women were given 600 mg of erythromycin intravenously. After delivery, blood was drawn from the mother's antecubital vein and umbilical cord vein. Serum erythromycin concentrations were evaluated using enzyme-linked immunosorbent assay (ELISA) kit. The percentage and correlation between umbilical vein and maternal serum erythromycin concentration were calculated. Based on regression function parameters selected factors: maternal age, maternal body weight, gestational age at delivery, related to the umbilical vein serum erythromycin concentration, were investigated.*

Results: *A total of 42 umbilical vein-maternal serum pairs were included in the analysis. The mean umbilical vein-maternal serum erythromycin concentration percentage was 2,64±1,55%. There was a moderate correlation between umbilical vein serum and maternal serum erythromycin concentration. Pregnancy complications and selected variables of mothers in control group had no effect on the serum erythromycin concentration in the umbilical vein .*

Conclusions: *Intravenous application of erythromycin at a dose of 600 mg, allowed to achieve therapeutic concentration in maternal serum. However, when it comes to placental transfer of erythromycin, the lack of therapeutic concentration in umbilical vein serum was observed. The limited transplacental transfer of erythromycin, which was approximately 2,6%, suggests compromised efficacy in the treatment of intrauterine fetal infections. On the other hand, the placenta seems to produce an effective barrier reducing the fetal exposure when erythromycin is used exclusively to treat maternal infections.*

Key words: **erythromycin** / **group B streptococcal infection** / **placental transfer** /

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Streszczenie

Cel: Celem pracy była ocena skuteczności erytromycyny w zapobieganiu infekcjom wewnątrzmacicznym oraz zakażeniem płodu paciorkowcami grupy B (GBS). Dokonano oceny penetracji erytromycyny przez łożysko, poprzez porównanie stężenia leku w surowicy krwi matki i żyle pępowinowej.

Materiał i metody: Badaniem objęto 42 ciężarne, z pozytywnym wynikiem badań na nosicielsto GBS lub dla których wyniki badań nie były znane. Kobiety rodziły w okresie od 17 kwietnia 2013 roku do 22 lipca 2013 roku. Pacjentkom podawano dożylnie 600 mg erytromycyny. Po porodzie pobierano próbki krwi z żyły pępowinowej oraz od matki z żyły łokciowej. Poziom stężenia erytromycyny oceniano z użyciem testu immunoenzymatycznego (ELISA). Obliczono zależność procentową oraz korelację między poziomem stężenia erytromycyny w surowicy krwi matki i w żyle pępowinowej. Na podstawie parametrów funkcji regresji, zbadano zależność między poziomem stężenia erytromycyny w żyle pępowinowej a wybranymi zmiennymi: wiekiem matki, masą ciała matki, wiekiem ciąży .

Wyniki: Przezłożyskowy transport erytromycyny w badanej grupie wynosił średnio 2,64±1,55%. Zaobserwowano umiarkowaną korelację między poziomem stężenia erytromycyny w surowicy krwi matki i w żyle pępowinowej. Wybrane zmienne opisujące matkę oraz choroby towarzyszące ciąży nie miały wpływu na poziom stężenia erytromycyny w żyle pępowinowej.

Wnioski: Podanie dożylne erytromycyny w dawce 600 mg pozwala na osiągnięcie stężenia terapeutycznego w surowicy krwi matki, jednocześnie obserwuje się słabą penetrację leku do płodu i brak stężenia terapeutycznego w żyle pępowinowej. Ograniczony transport przezłożyskowy erytromycyny, który wynosił średnio 2,6%, sugeruje wątpliwą skuteczność leczenia infekcji wewnątrzmacicznych. Z drugiej strony łożysko stanowi barierę skutecznie chroniącą płód w sytuacji, gdy erytromycyna jest stosowana wyłącznie w celu leczenia matki.

Słowa kluczowe: **Erytromycyna** / **zakażenie paciorkowcami grupy B** / / **transport przezłożyskowy** /

Introduction

Erythromycin is a broad spectrum macrolide antibiotic. It can be administered orally, intravenously or topically. It is well absorbed from a digestive system. The alkaline form of the antibiotic – erythromycin base – is decomposed under the influence of hydrochloric acid in the stomach. Thanks to different pharmaceutical forms coated by acid resistant compounds, it can reach the small intestine in its unchanged form. Esters and salts of erythromycin are more stable in acid environment because they form a stable suspension in water. The absorption of erythromycin is inhibited by food (except erythromycin estolate). Furthermore, erythromycin is characterized by good tissue penetration, except the brain tissue and the cerebrospinal fluid [1]. It can also penetrate through the placenta and is excreted into breast milk [2, 3]. The half-time of erythromycin in human serum varies from 1 to 2 hours and the therapeutic concentration is maintained for 6 hours. The recommended oral dose for adult patients is 250-1000mg every 6 hours, while the intravenous dose is 500-1000mg every 6 hours [1, 4, 5]. Erythromycin is excreted primarily in bile; only 2-5% is excreted in the urine [1].

The appropriateness of erythromycin for use during pregnancy has been internationally accepted [7, 8]. Food and Drug Administration (FDA) classifies all drugs according to their potential to harm the fetus when used during pregnancy. According to this classification, erythromycin has been assigned to pregnancy category B, which means that research conducted on pregnant animals has not shown an increased risk for fetuses or it has indicated an unfavorable effect but it has not been proven in pregnant women in the first trimester of pregnancy [6].

Erythromycin is an antibiotic which is used most frequently when treating pregnant women due to its broad spectrum of activity and favourable safety profile. It is usually given in cases of intrauterine or genital tract infections, which can be caused by bacteria such as Group B *Streptococcus* (GBS) and also as a prophylaxis in cases of premature rupture of membranes [1, 9, 10]. Group B *Streptococcus*, also known as *Streptococcus agalactiae* (*S. agalactiae*), is an encapsulated gram-positive bacterium that is a common inhabitant of the human gastrointestinal and genitourinary tracts. GBS can cause urinary tract infections as well as serious maternal and fetal complications. Intrauterine infection of the fetus may result from ascending spread of GBS from the vagina or during passage through the birth canal, of a colonized woman who is typically asymptomatic. Nevertheless, the transmission of GBS from mother to neonate occurs primarily after the onset of labor or membrane rupture. Infants can become ill after passage through a colonized birth canal, via aspiration of contaminated amniotic fluid, via ascending infection through ruptured membranes, or, very rarely, when GBS is transmitted via the maternal bloodstream [10]. Approximately 10% to 30% of pregnant women are colonized with GBS in the vagina or rectum. Maternal intrapartum GBS colonization is a common and major risk factor for early-onset disease (EOD) among infants. EOD appears within the first 7 days after birth, usually as bacteremia, pneumonia, meningitis, sepsis or infection of urinary tract, osteomyelitis and/or arthritis. In the absence of prevention, approximately 50% of infants born to colonized mothers, become colonized with GBS during delivery, and about 2% of those go on to develop invasive GBS disease. Intrapartum antimicrobial chemoprophylaxis with erythromycin is said to reduce infant colonization and early-onset disease, if administered more than 4 hours prior to delivery [10, 11]. GBS late-onset disease (LOD) occurs at an incidence of 0,3 to 0,5 cases/1000 live birth and appears between 7-89 days after birth. LOD presents primarily with sepsis, meningitis or pneumonia and more rarely with arthritis or cellulitis. Maternal vertical transmission may be responsible for only 50% of this late onset disease; in other cases LOD results from postpartum infections [10, 12].

Objectives

The aim of this study was to evaluate the effectiveness of erythromycin in preventing fetal and intrauterine GBS infection. To this end, we investigated the penetration of erythromycin through the placenta by comparing umbilical vein and maternal serum erythromycin concentrations after intravenous drug administration.

Meterials and methods

Subjects

The study was conducted in the Department of Biopharmacy, Faculty of Pharmacy, Medical University of Lodz with the collaboration of Perinatology and Gynecology Clinic at Polish Mother's Memorial Hospital-Research Institute in Lodz.

The approval of Bioethic Board of Scientific Research at Polish Mother's Memorial Hospital-Research Institute in Lodz was obtained. Before each hospitalization patients signed consent to personal data processing as well as the agreement for the provision of medical services and procedures undertaken. The study covered 42 women who delivered naturally or by elective Caesarean section between 17th April 2013 and 22nd July 2013. The inclusion criteria were as follows: mothers with GBS-positive screening and mothers whose laboratory screening was not available.

The women were given 600 mg of erythromycin lactobionate intravenously (Erythromycin Intravenosum TZF 300 mg, Polfa Tarchomin S.A.). After completion of delivery, average of 2 hours after drug administration the material from all patients was taken for analysis. Approximately 5-8 ml of maternal blood from antecubital vein and 5-8 ml of cord blood from umbilical vein were taken. Maternal and cord blood samples were obtained in parallel. Cord blood fully reflects the composition of the fetal blood. Nevertheless cord blood is the residual material, because the whole blood remaining in the placenta after birth is intented for utilization.

Serum preparation

The maternal and umbilical vein blood was centrifuged at 4000 rpm for 10 min., serum was separated, samples were frozen in -70°C and sent weekly in dry ice to the Department of Biopharmacy. Samples were collected and stored in -70°C until assayed.

Measurement of erythromycin concentration

Serum erythromycin concentrations were estimated using specific enzyme-linked immunosorbent assay (ELISA) kit (Max Signal, Erythromycin ELISA Test Kit, Bioo Scientific Corp.), based on competitive colorimetric enzyme immunoassays.

Statistical analysis

The percentage between the umbilical vein and the maternal serum erythromycin levels was calculated. Using correlation and regression analysis, the relationships between umbilical vein and maternal serum erythromycin concentration were found. Finally, the correlation between the umbilical vein serum erythromycin concentration and certain variables, such as maternal age, maternal body weight and gestational age at delivery, was estimated.

Statistical analysis was performed using Statistica software version 10.0. Figures were plotted using Microsoft Excel 2010, version 14.0. A *p-value* of <0.05 was considered significant.

Results

A total of 42 umbilical vein-maternal serum pairs were obtained from 42 patients during the study period. The basic variables characterizing the sample were: maternal age, maternal body weight, a number of pregnancies, number of childbirths and gestational age at delivery. For these variables basic descriptive statistics were calculated, including the arithmetic mean, median, standard deviation, coefficient of variation and minimum and maximum value obtained in the study (Table I).

The mean age of the study group was slightly less than 32 years (median 32 years) and was characterized by a moderate differentiation (CV = 14,2%). The average weight was about 78.2 kg (median 78.5 kg) and the low value of standard deviation and the coefficient of variation indicate the homogeneity of the research group. The mean gestational age at delivery was 38.1 weeks (median 38.0 weeks). For half of the women it was a second pregnancy and second delivery as well. However, for a few of them (10 women), the mean of number of childbirths (1.8) was lower than the number of pregnancies (2.0), which indicates that several previous pregnancies ended in miscarriage.

As shown in the collected data, 8 patients gave birth vaginally and 34 by a Ceasarean section. Among 42 births, 3 were twin births. 27 female and 18 male neonates were delivered. The mean±standard deviation (SD) birth weight was 3164±606g. All of the children were born in a good condition; the lowest Apgar score – recorded in a single case – was 6. In 3 cases fetal heart defect was observed, in other 3, fetal macrosomia and in the next 3, threatening intrauterine asphyxia.

For 33% of women the course of pregnancy was uneventful. Among the rest, 3 suffered from gestational diabetes mellitus (GDM), 2 from cholestasis of pregnancy, 3 suffered from pregnancy-induced hypertension (PIH). Furthermore, 1 patient was thrombophilic and 2 developed pregnancy-induced low-platelet count. In individual cases the following conditions were observed: hypothyroidism, ulcerative colitis, ovarian cyst, urinary tract infection, herniated lumbar disc, sexually transmitted disease.

The average maternal drug concentration in the sample was 2373,98 ng/ml (median 2449,20 ng/ml) but the group was relatively highly diversified (CV = 39.0%). The mean umbilical vein serum concentration was 50,99 ng/ml (median 48,23 ng/ml) and this value did not significantly differ within the sample (CV = 16,8%). The mean umbilical vein-maternal serum erythromycin concentration percentage was 2,64%±1,55 and this feature was characterised by a high differentiation (CV = 58,6%) (Table II).

The distribution of erythromycin concentration in maternal and umbilical vein serum is consistent with the normal distribution at a significance level of 0,05 (Figure 1a-b).

On the basis of the statistical data obtained, it was verified whether there is any correlation between the level of drug concentration in maternal and umbilical vein serum. A positive and statistically significant correlation was found between these two variables. However, the value of Person's linear correlation coefficients (0,5295) indicates a relatively moderate relation between analyzed variables (Table III).

Moreover, using the regression analysis, the influence of individual changes in the drug level in maternal serum on changes in the drug level in the fetal serum, was shown. The results obtained indicate that the increase of drug concentration in maternal

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Table I. Descriptive statistics for the research sample.

Variable	Mean	Median	Standard deviation (σ)	Coefficient of variation (CV)	Minimum	Maximum
Maternal age [year]	31,8	32,0	4,5	14,2 %	23,0	43,0
Maternal body weight [kg]	78,2	78,5	12,2	15,6 %	56,0	118,0
Gestational age at delivery [week]	38,1	38,0	1,5	3,9 %	35,0	41,0
Number of pregnancies	2,0	2,0	1,1	55,0 %	1,0	6,0
Number of childbirths	1,8	2,0	0,94	52,2 %	1,0	5,0

Table II. Descriptive statistics for drug concentration levels in maternal and fetal serum.

Indicator	Mean	Median	Standard deviation (σ)	Coefficient of variation (CV)	Minimum	Maximum
Maternal serum erythromycin concentration [ng/ml]	2373,98	2449,20	926,47	39,0 %	563,60	4174,80
Umbilical vein serum erythromycin concentration [ng/ml]	50,99	48,23	8,56	16,8 %	32,12	65,21
Umbilical vein-maternal serum erythromycin concentration percentage [%]	2,64	2,05	1,55	58,6 %	1,39	7,98

Table III. Pearson's correlation coefficients between the level of erythromycin concentration in maternal and umbilical vein serum.

Variable	Marked correlations are significant at p < 0,05000 N=42 (Casewise deletion of missing data)	
	Maternal serum erythromycin concentration [ng/ml]	Umbilical vein serum erythromycin concentration [ng/ml]
Maternal serum erythromycin concentration [ng/ml]	1,0000 p=---	**0,5295 p=0,000**
Umbilical vein serum erythromycin concentration [ng/ml]	**0,5295 p=0,000**	1,0000 p=---

Table IV. Pearson's correlation coefficients for selected variables.

Variable	Marked correlations are significant at p<0,05000 N=42 (Casewise deletion of missing data)
	Umbilical vein serum erythromycin concentration [ng/ml]
Mother's age [year]	0,1745 p=0,269
Maternal body weight [kg]	-0,1204 p=0,448
Gestational age at delivery [week]	-0,1894 p=0,230

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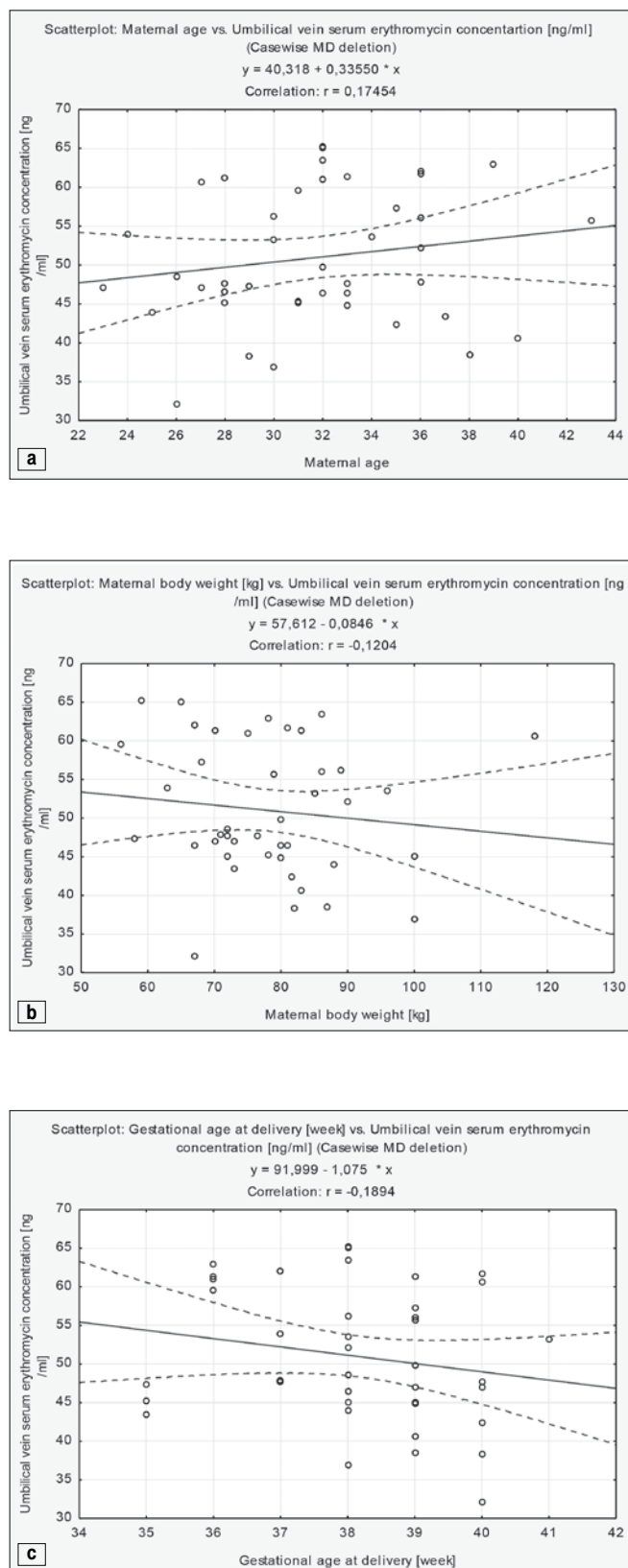**Table V.** Spearman rank correlation coefficient between maternal and umbilical vein serum erythromycin concentration [ng/ml] and selected variables.

Variable	Maternal serum erythromycin concentration [ng/ml]	Umbilical vein serum erythromycin concentration [ng/ml]	Mother's age [year]	Maternal body weight [kg]	Gestational age at delivery [week]
Maternal serum erythromycin concentration [ng/ml]	1,000000	**0,452251**	-0,1460	-0,2582	-0,2864
Umbilical vein serum erythromycin concentration [ng/ml]	**0,452251**	1,000000	0,1615	-0,2152	-0,2174
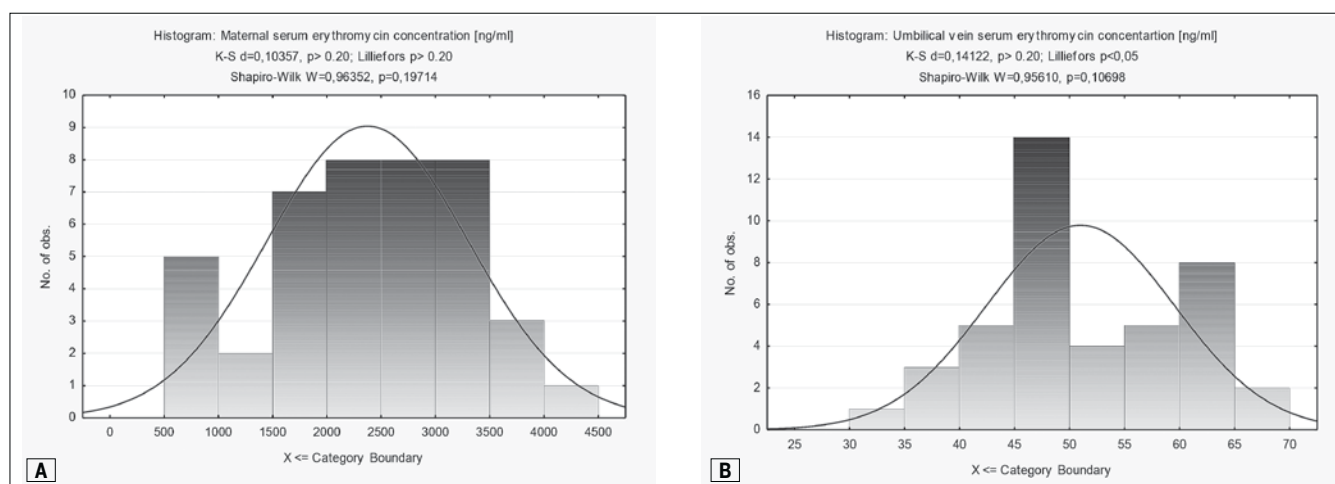
Figure 1. Rozkład stężenia erytromycyny [ng/ml] w (**A**) surowicy krwi matki (**B**) żyle pępowinowej.
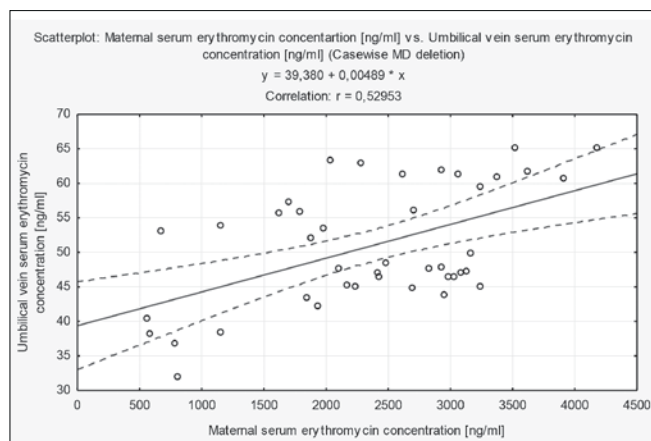
Figure 2. The influence of maternal serum erythromycin concentration on the umbilical vein serum concentration.

serum of every 100 ng/ml, results in the increase of drug concentration in umbilical vein serum of about 0,49 ng/ml (Figure 2).

Next, the correlations between umbilical vein serum erythromycin concentration and certain variables characterizing our sample, namely mother's age, maternal body weight and gestational age at delivery, were calculated. However, none of the results was statistically significant (Table IV, Figure 3a-c).

The lack of statistically significant relations between examined variables was confirmed by the results of the Spearman rank correlation analysis (Table V).

Discussion

The principal findings of this study were the following:
- the mean umbilical vein– maternal serum erythromycin concentration was 2,64%;
- there was a moderate correlation between umbilical vein and maternal serum erythromycin concentration;
- the complications of pregnancy and mother's characteristics (age, weight, etc.) did not have any impact on umbilical vein serum erythromycin concentration.

Macrolides are transferred across the placenta. However, according to the previous research, the placental transfer is reported to be low, from <2% to 6.1%.

Our results are in accordance with the clinical results of the previous studies on erythromycin [2, 3, 13].

In a study using abortus, the transplacental transfer of erythromycin was only about 2%. The concentration of erythromycin in the blood was measured after multiple oral doses [2]. In our study the concentration was measured after a single infusion; nevertheless, the results are similar. This may suggest the existence of placental mechanisms that restrict the flow of erythromycin from mother to fetus, and allow for the penetration of only a limited amount, irrespective of dose, route and multiple dosing.

Scatterplot: Maternal age vs. Umbilical vein serum erythromycin concentartion [ng/ml] (Casewise MD deletion)
$y = 40,318 + 0,33550 * x$
Correlation: $r = 0,17454$

(a)

Scatterplot: Maternal body weight [kg] vs. Umbilical vein serum erythromycin concentration [ng/ml] (Casewise MD deletion)
$y = 57,612 - 0,0846 * x$
Correlation: $r = -0,1204$

(b)

Scatterplot: Gestational age at delivery [week] vs. Umbilical vein serum erythromycin concentration [ng/ml] (Casewise MD deletion)
$y = 91,999 - 1,075 * x$
Correlation: $r = -0,1894$

(c)

Figure 3 a, b, c. Scatterplot correlation between the level of umbilical vein serum erythromycin concentration and (a) maternal age, (b) maternal body weight, (c) gestational age at delivery.

We also demonstrated a moderate correlation between umbilical vein and maternal serum erythromycin concentration. The regression analysis showed that the increase of drug concentration in maternal serum of every 100 ng/ml results in the increase of drug concentration in fetal serum of about 0,49 ng/ml. Based on this finding and knowing a minimal inhibitory concentration (MIC) value, we can presume that a desirable fetal blood erythromycin concentration, to prevent fetus from infections caused by *S. agalactiae,* can be expected at higher maternal doses. The effect of antibiotics depends on MIC of the target microorganism. As far as erythromycin is concerned, the recommended limit value for the sensitive strains is ≤500 ng/ml. For the Group B *Streptococcus*, which is in the scope of our interest, MIC_{50} has a value of 60 ng/ml while MIC_{90} 120 ng/ml [1]. Since the drug concentration in umbilical vein does not reach the MIC value, it may be reasonable to plan and carry out a further study using higher doses of erythromycin. It cannot be ruled out that the amount of erythromycin administered to pregnant women should be higher to obtain MIC_{90} or at least MIC_{50} in fetal serum and cover *S. agalactiae* or microorganisms with higher MICs. Such research may help us answer a crucial question: whether increasing mother's dose indeed results in the increase of fetal drug concentration and, hence, whether erythromycin may be used in the treatment or prevention of perinatal infections.

In order to explain the low umbilical vein serum erythromycin concentration, we examined its possible correlations with several variables, such as mother's age, maternal body weight and gestational age at delivery. However, our results showed that there was no a statistically significant correlation between these parameters. The possible impact of co-morbidities of pregnancy on umbilical vein serum drug concentration was also examined. However, such impact was not noticed either. Lack of correlation between analyzed variables may be a confirmation that placenta is the factor which inhibits the flow of the drug.

This study has also some limitations. First, the pharmacokinetics of erythromycin in pregnancy could not be clearly elucidated due to the limited sample size. Secondly, only one point of serum erythromycin concentration was measured, hence our findings may not reflect the entire complexity of human placental transfer. Although, it was not statistically significant, there was a moderate correlation between umbilical vein and maternal serum erythromycin concentration. Further research is needed to answer the questions – addressed in the present study. Erythromycin is used in the treatment of various maternal infections during pregnancy. Nevertheless, due to poor placental transfer this antibiotic seems not to be the drug of choice when treating the infected fetus.

Conclusions

In conclusion, a standard dose of erythromycin, used in our study, at least 4 hours before birth, allowed for a therapeutic concentration in maternal serum. However, erythromycin showed poor placental penetration and did not reach therapeutic concentration in umbilical vein serum. The limited transplacental transfer of erythromycin, which was approximately 2,6%, suggests a compromised efficacy in the treatment of intrauterine fetal infections. On the other hand, the placenta seems to produce an effective barrier reducing the fetal exposure when this macrolide is used to treat maternal infections.

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