Serum levels of valproic acid during delivery in mothers and in umbilical cord – correlation with birth length and weight

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Aims. The data on the valproic acid transplacental transfer and risk to the fetus of exposure, remain sparse and only a limited number of studies have reported umbilical cord blood levels.
Materials and Methods. Maternal and umbilical cord serum levels were analyzed at delivery in a cohort of 58 women, between the years 1991 - 2013. The request forms for routine therapeutic drug monitoring were used as the data source. Maternal levels and dosing information were used for estimating the maternal apparent oral clearance and the paired umbilical cord and maternal levels for estimation of umbilical cord/maternal level ratios.
Results. The levels varied from 5.3 - 59.5 mg/L in maternal and 5.4 - 72.1 mg/L in umbilical cord serum. The umbilical cord/maternal level ratios ranged from 0.64 - 2.49. Significant correlation was found between maternal and umbilical cord levels. Significant inverse correlations were found between birth length, and both maternal and umbilical cord levels in monotherapy.
Conclusions. There were large individual variations in umbilical cord/maternal level ratios of valproic acid. Neonatal length and weight were inversely related to maternal and umbilical cord levels, but not to dose. Therefore, therapeutic drug monitoring in mothers is more useful than the given dose for the estimation of fetal exposure and minimization of the risk of fetal effects.

Key words: delivery, epilepsy, birth length, therapeutic drug monitoring, valproic acid

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INTRODUCTION

Valproic acid (VPA) is a monocarboxylic acid effective in patients with all types of seizures, and especially in those with genetic generalized epilepsy. It has become one of the three major drugs in the treatment of pregnant women with epilepsy[1-3]. However, data on valproic acid transplacental transfer and the risk to the fetus of exposure remain sparse, and only a limited number of studies have actually reported umbilical cord blood levels. In cord blood, the concentrations of valproic acid had been reported to be either lower (or identical to) or much higher than maternal concentrations in various studies each with a small number of patients or in individual case reports[4,6-16]. Therefore, regular therapeutic drug monitoring (TDM) of VPA during pregnancy and the postpartum period has been recommended[5]. Valproic acid is a known human teratogen. Exposure in pregnancy is associated with approximately a three-fold increase in the rate of major malformations, mainly spina bifida, and only rarely, in increased risk for anencephaly, cardiac, craniofacial, skeletal and limb defects, and a possible set of dysmorphic features called the "valproate syndrome" associated with decreased intrauterine growth[17-23]. Intrauterine growth restriction associated with the maternal use of both VPA monotherapy and combination therapy have been described[24-29]. However, normal birth weights, lengths, and head circumferences have been observed in infants born to mothers receiving VPA monotherapy[30-33]. Analytical models should also take into account not only antiepileptic drug (AED) type and dose, but AED levels in the mother and the neonate at birth as a closer surrogate marker for fetal exposure, especially given that AED clearance can have substantial intraindividual and interindividual variability during pregnancy[34]. Correlation of maternal and umbilical cord serum VPA levels with birth length and weight has not yet been reported. The purpose of this retrospective study was to examine VPA transfer through the placenta in a larger cohort than in all previous published studies, to analyze maternal and umbilical cord serum levels and their ratio at delivery, to estimate maternal apparent oral clearance of VPA and the influence of co-medication with enzyme inducers (carbamazepine - CBZ, phenytoin - PHT and phenobarbital - PB) and lamotrigine (LTG). We also analyzed the relationship between birth length and weight and both the dose of VPA as well as the maternal and umbilical cord VPA concentrations.

MATERIALS AND METHODS

This study analysed retrospectively data from 58 pregnant women with epilepsy receiving either VPA mono-

therapy (n=36), VPA combined with LTG (n=7), or VPA with enzyme inducers (n=15). The study was carried out between January 1991 and December 2013 using blood samples collected from mothers and umbilical cords at delivery and the samples were analysed for VPA and other AEDs concentrations. Request forms for routine TDM were used as the source of data on drug dosing. The demographics of the mothers and their infants are given in Table 1 and treatment characteristics in Tables 2 and 3 (total daily dose and daily dose related to the body weight of both valproic acid and other currently used antiepileptic drugs). Total VPA concentrations were measured by gas chromatography[35] using a gas chromatograph (Chrom 5, Czech Republic) with a glass packed column 1 200 x 3mm filled with 10% SP-1000 on 80/100 Supelcoport (Supelco, USA). To caprylic acid (internal standard) in Eppendorf vials 50 μL of serum, 50 μL acetone and a small amount (approximately 30 mg) of solid ammonium sulphate was added and vortex-mixed. After centrifugation 2 μL of the acetone layer was injected directly on column for analysis using FID detection. Performance characteristics of the method were as follows: linearity was found between 5 and 125 mg/L, recovery 97.2 - 103.5% at these concentrations, and coefficient of variations were 3.5-5.5 %, respectively. The method was quality controlled in external quality control (EQC) RfB (Bonn, Germany) twice a year. Levels of other antiepileptic drugs were measured by high-performance liquid chromatography[36]. Apparent oral clearance (Cl) was calculated for VPA: VPA Cl = daily dose (mg/kg) / serum VPA concentration (mg/L) (ref.[37]). Paired umbilical cord and maternal serum VPA levels were utilized for estimation of the umbilical cord/maternal serum concentration ratio. The D'Agostino and Pearson omnibus normality test, the unpaired t test, the Mann-Whitney test, the Pearson and the Spearman correlation tests were performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. A value of $P < 0.05$ was considered statistically significant.

RESULTS

No significant differences were found in either maternal VPA levels or umbilical cord/maternal serum concentration ratios for all three groups (VPA monotherapy, VPA+LTG, and VPA+enzyme inducers). The VPA concentrations varied from 5.3 to 59.5 mg/L in the maternal serum and between 5.4 and 72.1 mg/L in the umbilical cord serum. Only 9% of the maternal VPA concentrations were in the reference range (50.0 - 100.0 mg/L), and 91% concentrations were lower. Similarly, concentrations of concomitant added AEDs, with the exception of LTG, were below the recommended reference ranges[38]. The umbilical cord /maternal serum concentration ratios ranged from 0.64 to 2.49 (Fig. 1). Concomitant medication with enzyme inducers, but not with LTG, significantly increased ($P = 0.0311$) the maternal apparent oral clearance of VPA by about 30% (Table 2). Highly significant correlation was found between the maternal and the umbilical cord VPA serum levels in women receiving both VPA monotherapy and polytherapy ($P < 0.0001$, Fig. 2). The umbilical cord /maternal serum VPA concentration ratios correlated inversely with the maternal VPA levels,

Table 1. Demographics of the mothers and their infants.

		Number	mean ± SD (range)
maternal:	age (years)	58	27 ± 5 (19; 39)
	weight* (kg)	54	76 ± 11 (60; 104)
maternal seizures:	genetic generalized epilepsy	41	
	psychomotoric	5	
	focal-motoric	4	
	myoclonic	1	
	not done	7	
maternal VPA therapy:	Depakine Chrono	25	
	Orfiril Long	23	
	Convulex CR	6	
	Everiden	4	
concomitant AEDs therapy:			
phenytoin	Sodanton	4	
carbamazepine	Timonil Retard	4	
	Tegretol CR	4	
	Biston	1	
phenobarbital	Phenaemal	4	
lamotrigine	Lamictal	7	
infant:	birth weight (kg)	53	3.3 ± 0.6 (1.8; 4.5)
	birth length* (cm)	50	49 ± 2 (43; 53)
	female	23	
	male	30	

*values have not been recorded in all cases

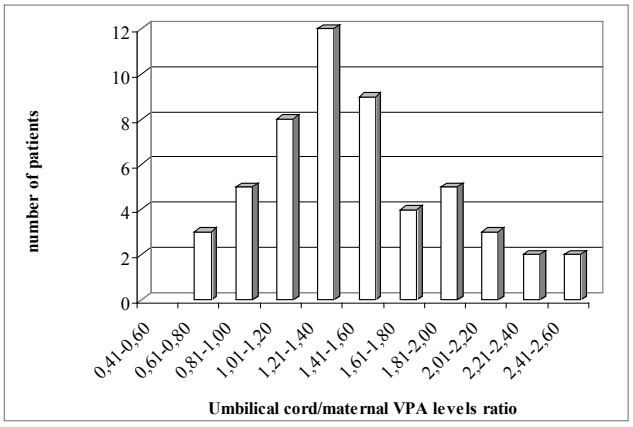

Fig. 1. Distribution of umbilical cord/maternal serum level ratios of valproic acid (VPA).

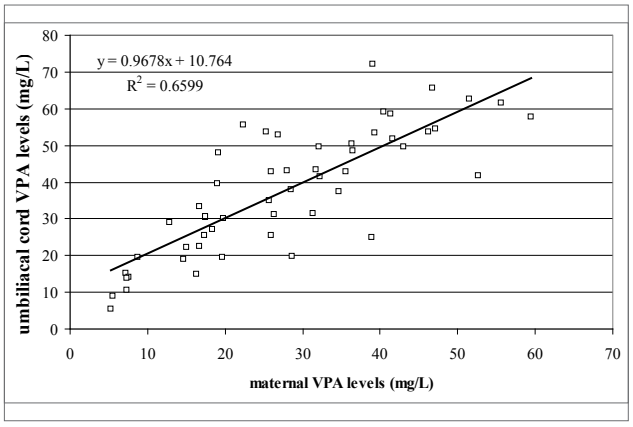

Fig. 2. Correlation between paired maternal and umbilical cord serum levels of valproic acid (VPA); $P < 0.0001$; correlation coefficient = 0.8124

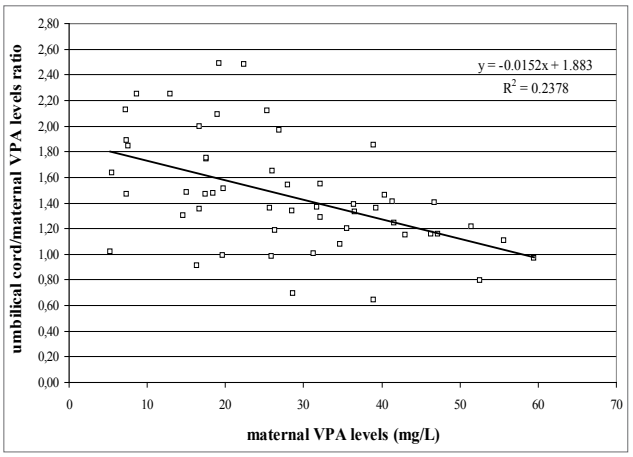

Fig. 3. Relationship between umbilical cord/maternal serum levels ratio of valproic acid (VPA) and all maternal valproic acid levels; $P = 0.0002$; correlation coefficient = -0.4878

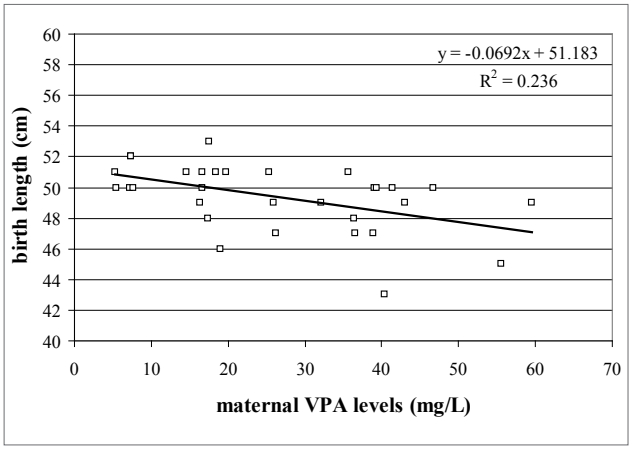

Fig. 4. Correlation between birth length and maternal valproic acid (VPA) levels in monotherapy; $P = 0.0032$; correlation coefficient = -0.5123

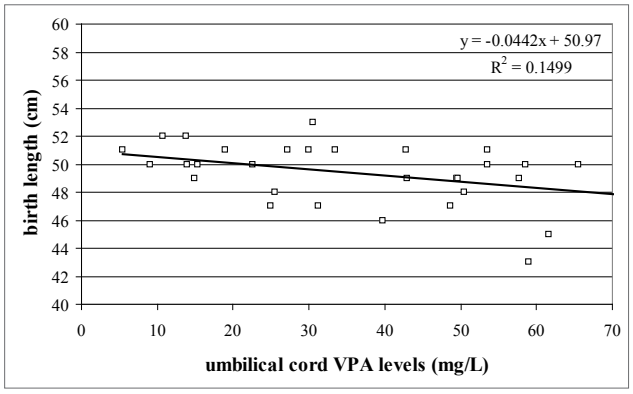

Fig. 5. Correlation between birth length and umbilical cord valproic acid (VPA) levels in monotherapy; $P = 0.0290$; correlation coefficient = -0.3925

$P = 0.0002$ (Fig. 3). The mean umbilical cord serum concentrations of CBZ, PHT, PB and LTG were found to be slightly lower than or equal to the maternal serum concentrations of these drugs and only the umbilical cord serum carbamazepine-epoxy concentrations were observed to be about 20% higher than the maternal serum concentrations of this metabolite (Table 3). Statistically significant inverse correlations were found between birth length and both maternal and umbilical cord VPA levels in all women whether or not they were receiving VPA monotherapy. Statistically significant inverse correlation between birth weight and maternal and umbilical cord VPA levels was demonstrated only in all mothers combined (monotherapy + polytherapy) (Table 4 and Fig. 4, 5).

DISCUSSION

The number of patients receiving VPA therapy at delivery was larger than all previously relevant studies (58 pregnant women in our report from only our center using consistent methodology compared with approximately 70 patients in 12 previous separate studies that used a variety

Table 2. Umbilical cord (UC)/maternal (M) serum level ratios of valproic acid (VPA) and the maternal apparent oral clearance (Cl) of VPA: in VPA monotherapy, combination with lamotrigine (LTG) and combination with enzyme-inducing antiepileptic drugs (AEDs) (carbamazepine, phenytoin, phenobarbital); values and umbilical cord VPA levels have not been recorded in all cases.

VPA monotherapy:	weight (kg)	dose (mg/day)	dose/kg (mg/kg)	Cl (L/kg)	M-level (mg/L)	UC-level (mg/L)	UC/M ratio
n	34	35	33	33	36	33	33
median	77	750	10.0	0.36	25.7	37.5	1.41
mean	78	712	9.3	0.38	27.5	37.1	1.45
SD	11.6	314	4.5	0.17	15.3	18.7	0.37
min	60	150	1.8	0.15	5.3	5.4	0.64
max	98	1250	17.9	0.79	59.5	72.1	2.13
VPA + LTG:							
n	6	7	6	6	7	6	6
median	68^{+}	600	9.9	0.39	28.5	39.9	1.36
mean	68	764	11.6	0.49	26.1	36.8	1.54
SD	5.0	431	6.4	0.31	7.1	10.3	0.53
min	61	300	4.5	0.22	17.5	19.4	0.99
max	76	1500	19.7	1.03	33.9	47.9	2.49
VPA + enzyme inducing AEDs:							
n	14	13	13	13	15	14	14
median	72	1000	14.1	0.51	26.9	38.4	1.31
mean	74	1000**	13.4**	0.50*	28.9	38.9	1.46
SD	11.3	367	5.1	0.15	13.7	14.7	0.57
min	63	300	4.4	0.26	8.7	19.6	0.70
max	104	1500	21.1	0.74	52.6	62.6	2.48

**P<0.01 - VPA monotherapy versus VPA + enzyme-inducing AEDs
*P=0.0311 - VPA monotherapy versus VPA + enzyme-inducing AEDs
^{+}P<0.04 - VPA monotherapy versus VPA + LTG

Table 3. Umbilical cord (UC)/maternal (M) serum level ratios of others antiepileptic drugs (carbamazepine - CBZ, carbamazepine-epoxy - EPO, phenytoin - PHT, phenobarbital - PB, lamotrigine - LTG).

		dose (mg/day)	dose/kg (mg/kg)	M-level (mg/L)	UC-level (mg/L)	UC/M ratio	EPO-M-level (mg/L)	EPO-UC-level (mg/L)	EPO-UC/M ratio
CBZ	n	9	9	9	9	9	6	6	6
	median	600	8.8	3.1	3.0	0.80	1.0	0.9	1.17
	mean	567	7.5	2.9	2.7	0.86	0.9	1.2	1.23
	SD	155	2.4	1.1	1.0	0.24	0.3	0.6	0.31
	min	300	3.6	1.4	0.8	0.57	0.6	0.7	0.80
	max	750	9.7	5.2	4.0	1.39	1.3	2.2	1.69
PHT	n	4	4	4	4	4			
	median	280	4.1	2.9	2.3	0.70			
	mean	253	3.7	4.1	2.9	0.74			
	SD	97	1.4	3.0	1.8	0.10			
	min	100	1.6	1.4	1.0	0.66			
	max	350	5.1	9.0	5.9	0.91			
PB	n	2	2	4	4	4			
	median	65	1.0	6.5	7.0	0.92			
	mean	65	1.0	7.6	6.9	0.90			
	SD	9	0.2	4.9	4.1	0.19			
	min	56	0.8	2.2	1.4	0.64			
	max	74	1.2	15.1	12.0	1.11			
LTG	n	7	6	7	6	6			
	median	200	3.0	6.0	5.7	1.02			
	mean	221	2.9	7.0	7.0	0.92			
	SD	84	0.7	2.7	3.9	0.24			
	min	100	1.3	3.9	1.7	0.44			
	max	400	3.6	12.3	13.6	1.11			

Table 4. Correlations between birth weight and birth length versus maternal (M) and umbilical cord (UC) valproic acid levels.

	M-levels monotherapy	UC-levels monotherapy	M-levels mono + polytherapy	UC-levels mono + polytherapy
birth weight	NS	NS	$P = 0.0081$	$P = 0.0447$
			-0.3602*	-0.2770*
birth length	$P = 0.0032$	$P = 0.0290$	$P = 0.0016$	$P = 0.0114$
	-0.5123*	-0.3925*	-0.4345*	-0.3550*

* significant correlation coefficients; NS = non-significant correlation coefficients

of methods) (ref.[4,6-16]). We found highly significant correlation between the maternal and the umbilical cord VPA serum levels in women receiving both VPA monotherapy and polytherapy ($P < 0.0001$). Maternal and umbilical cord VPA levels are consistent with previously published studies (range 5.3 - 59.5 mg/L v.s. 5.5 - 74.2 mg/L maternal levels, 5.4 - 72.1 mg/L v.s. 5.2 - 123.0 mg/L umbilical cord levels) (ref.[4,6-16]). A wide range of umbilical cord/maternal serum concentration ratios (0.64 - 2.49) has been found. These findings are consistent with earlier reported data (0.32 - 2.95) (ref.[4,6-16]). No significant difference in the umbilical cord/maternal serum concentration ratios was found between the two groups (VPA monotherapy versus VPA in combination with enzyme-inducing AEDs) suggesting that the transplacental passage of VPA is not influenced by enzyme-inducing AEDs at the level of uridine 5´- diphosphate glucuronosyltransferase (UGT) 2B7 and placental transporters. We hypothesize that a genetic polymorphism of placental UGT2B7 and placental VPA transporters may explain the interindividual variability in the umbilical cord/maternal serum concentration ratio. The inverse correlation between the umbilical cord/maternal serum concentration ratios and the maternal VPA levels supports the saturable character of the placental transport system postulated by Nakamura et al.[39]. This result could explain the higher values of umbilical cord/maternal serum levels ratios of VPA in mothers with lower serum VPA levels. Also found was a significant inverse correlation between birth length and maternal and umbilical cord VPA levels in both mothers on VPA monotherapy and in all mothers combined (monotherapy + polytherapy). Kaneko et al.[40] reported the results of a prospective, multinational analysis which used a standardised method to collect data on major congenital malformations in 983 infants exposed in utero to AEDs. The first trimester mean VPA level in mothers who delivered malformed offspring (77.8 + 19.99 mg/L, n=8) was significantly higher than that in mothers who delivered normal offspring (46.86 + 21.22 mg/L, n=51, $P = 0.023$). Kaneko et al. proposed that the maximum VPA dose should not exceed 1 grm/day and/or plasma levels 70 mg/L in order to minimize the risk of malformations. Unfortunately, in this report plasma levels of selected AEDs were estimated only once during the first trimester, and neither the exact dates of sampling nor the method used for the quantification of the AEDs were described. The formulations used (sustained -released or not), their names, individual doses and dosage were also not reported but because recruitment of subjects began in April 1978, and was completed in December 1991, most preparations were not in sustained-released formulations. Kaneko et al. did not provide actual proof, but suggested that malformations could be prevented by improvements in drug regiments to avoid polypharmacy and higher levels of VPA (more than 70 mg/L) in the treatment of epileptic women of childbearing age. Our findings, although not a controlled study, suggest that growth restriction can be seen even when maternal VPA levels are lower than 70 mg/L. The significant correlation between maternal and umbilical cord serum levels supports the use of therapeutic drug monitoring as a way to minimize the fetal effects of VPA during pregnancy. This may be especially important for VPA since VPA exposure increases the frequency of congenital malformations more than other AEDs (ref.[41]). At least one prospective observational study suggested that higher doses of VPA may significantly increase the risk of major malformations[42]. The Australian Pregnancy Registry, the Finnish Birth Registry, the UK Behavioral Study and the Finnish Behavioral Study all support this conclusion, as they reported significant dose-response effects in women who took VPA during pregnancy[42]. VPA concentrations at birth have been correlated with the degree of neonatal hyperexcitability and later neurological dysfunction[16]. Our study represents the first evidence of a correlation between both maternal and umbilical cord VPA concentrations and birth length and weight. Most of the measured AED levels, with the exception of LTG, were below the usual therapeutic range for non-pregnant women; results that are in agreement with Sabers and Tomson[5] and our previous paper[3]. These findings can be explained by gestational alterations in the pharmacokinetics of AEDs (absorption, distribution, metabolism and/or elimination), by the use of lower doses of AEDs, or by non-compliance of pregnant women[3,5]. Even at these low serum VPA levels we also found an influence of comedication with enzyme-inducing AEDs on the maternal apparent oral clearance at the time of delivery. The daily dose of VPA or dose relative to body weight could be increased to achieve similar VPA concentrations in women with VPA monotherapy. The situation of the indication VPA for the treatment of girls and women of childbearing potencial dramatically changed this year after warnings from the Coordination Group for Mutual Recognition and Decentralised Procedures-Human of the European Medicines Agency, which highlight the risk of malformations and developmental problems in infants who are exposed to valproate in the womb and decreasing use of VPA is presumable in this group[43].

CONCLUSION

Our data from a large cohort showed significant correlation between maternal and umbilical cord levels of valproic acid with interindividual variability in umbilical cord/maternal serum concentration ratios. We found a significant inverse correlation between birth length and weight and maternal and umbilical cord VPA concentrations, but not dose. For this reason, TDM in mothers is more valuable than the given dose for estimating and possibly obviating the risk of VPA effects on the fetus.

ABBREVIATIONS

AED, Antiepileptic drug; CBZ, Carbamazepine; Cl, Apparent oral clearance; EQC, External quality control; EPO, Carbamazepine-epoxy; LTG, Lamotrigine; M, Maternal; NS, Non-significant; PB, Phenobarbital; PHT, Phenytoin; TDM, Therapeutic drug monitoring; UC, Umbilical cord; UGT, Uridine 5´- diphosphate glucuronosyltransferase; VPA, Valproic acid.

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Author contributions: IK: manuscript writing, statistical analysis, literature search; MG: conception and manuscript writing, final approval; HB: laboratory analyses.

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