

VALPROIC ACID TRANSFER ACROSS HUMAN PLACENTAL COTYLEDON DURING DUAL PERFUSION *IN VITRO*

Anna Semczuk-Sikora¹, Stanisław Czuczwar², Andrzej Semczuk³,
Anna Kwaśniewska¹, Marian Semczuk¹

¹Department of Obstetrics and Pathology of Pregnancy, Medical University of Lublin, Poland

²Department of Pathophysiology, Medical University of Lublin, Poland

³II Department of Gynecology, Medical University of Lublin, Poland

Semczuk-Sikora A, Czuczwar S, Semczuk A, Kwaśniewska A, Semczuk M: Valproic acid transfer across human placental cotyledon during dual perfusion *in vitro*. *Ann Agric Environ Med* 2010, **17**, 153–157.

Abstract: Valproic acid (VPA) is a well-known antiepileptic drug with a significant teratogenic effect when administered during pregnancy. To investigate the transplacental transport of VPA, we used an *in vitro* experiment of dual perfusion of a human placental cotyledon. Eighteen normal placentas at term were investigated; ten were treated with a therapeutic dose of VPA (initial level at maternal circulation 75 µg/ml), while the remaining eight were supplied with toxic VPA doses (initial level at maternal circulation 225 µg/ml). VPA concentrations in fetal compartment were lower than those in the maternal compartment at all timepoints with both doses applied. The maternal and foetal VPA concentrations were stable at 60 min and 120 min for the therapeutic dose of VPA (transfer percentages from the maternal to the fetal circulation were $22.7 \pm 9.1\%$ and $22.7 \pm 7.1\%$, respectively). Interestingly, a significant decrease of VPA level in the maternal perfusate was observed after 120 min due to the slightly higher transfer of the drug to the foetal compartment. In conclusion, our data confirmed an easy and rapid transfer of VPA across the placental barrier. Since the incidence of congenital malformations in infants correlates positively with VPA concentrations in maternal serum, monitoring of VPA should be mandatory due to possible harmful effects on the foetus.

Address for correspondence: Anna Semczuk-Sikora, MD, Department of Obstetrics and Pathology of Pregnancy, Medical University of Lublin, ul. Staszica 16, 20-081 Lublin, Poland. E-mail: annsemcz@poczta.onet.pl

Key words: valproic acid, placental transfer, human pregnancy.

INTRODUCTION

Valproic acid (VPA) is widely used as an anticonvulsant drug with a well-known efficiency directed against different types of epilepsy. Moreover, as a potent stabilizer of a mood it is used in the treatment of bipolar disorders (predominantly in mania), schizophrenia, various forms of personality disturbances, as well as in migraine. Its pharmacological effects are associated with the central inhibition of GABA (Gamma Amino Butyric Acid) transaminase that increases GABA content in the brain [32].

In general, this drug is well-tolerated and possible adverse effects are relatively uncommon and mild. In contrast, the

administration of VPA during pregnancy is associated with the possible risk of foetal teratogenicity. It was shown that up to 2% of foetuses exposed to VPA during the first trimester of pregnancy develop neural tube defects (predominantly “spina bifida”), compared to 0.006–0.012% in the general population [33]. Furthermore, the increased incidence of malformations of other organs, such as cardiovascular, urogenital, cranio-facial (foetal valproate syndrome), digital and skeletal anomalies, as well as intrauterine growth restriction syndrome were previously reported [2, 15, 19, 38]. The exposure to VPA *in utero* is also suspected of lowering the verbal IQ of children in their postnatal life [1, 3]. This problem is of paramount importance since the number

of women at childbearing age with epilepsy and psychiatric disorders treated with VPA is on the increase [20, 29]. Nowadays, epilepsy affects 0.5% of pregnant women, thus being the most common neurological disorder in this group [35].

Despite the inevitable harmful effects on the foetus, treatment with antiepileptic drugs (AEDs) cannot be discontinued in pregnant woman with epilepsy since uncontrolled seizures *per se* may cause foetal distress or even miscarriage [31]. Thus, the benefits of the antiepileptic treatment clearly predominate the possible disadvantages. To improve the safety of the therapy for epilepsy in pregnancy, it is necessary to explain all the aspects of pharmacokinetics of AEDs in relation either to the mother or the foetus. A crucial event determining the foetal exposure to the maternal drug is its ability to pass across the placenta. However, for ethical reasons and methodological limitations, studies assessing this process could not be performed *in vivo*. Due to interspecies variations, the results from animal studies can not be directly extrapolated to humans. Therefore, several experimental methods *in vitro* have been developed to investigate this aspect regarding drugs and toxins [9, 23, 30, 36]. Currently, we investigated the VPA transfer cross the placenta, applying the experimental model of dual perfusion of a single human placental cotyledon.

MATERIALS AND METHODS

The current study was carried out on an experimental model of the dual recirculating perfusion system for an isolated placental lobule, originally described by Schneider *et al.* [37], with slight modifications by Miller *et al.* [22]. The study was approved by the Ethics Committee of Medical University of Lublin and written consent obtained from all participant patients. Altogether, eighteen term placentas were collected from healthy women who have had uncomplicated pregnancies at the Department of Obstetrics and Pathology of Pregnancy, Medical University of Lublin, Poland, from 2006–2007. Placentas were immediately submitted to the laboratory to perform the perfusion of the suitable placental lobule within 7–10 minutes after birth. The “foetal and maternal circuits” were established by cannulation of the foetal artery and vein, as well as by the placement of two needles into the intervillous space. The lobule was placed in a plexiglass perfusion chamber filled with buffered Krebs solution (500 ml 0.9% NaCl and 70.3 mg Na₂HPO₄), the composition of which resembles that of amniotic fluid. The foetal (100 ml) and maternal (250 ml) circuits were perfused with a fluid based upon tissue medium (Medium 199 W/HBSS, Life Technologies, Paisley, Scotland). Both circulations were maintained by peristaltic pumps. The foetal and the maternal flow rates were set at 3.8 ± 0.1 ml/min and 14 ml/min, respectively. The maternal perfusate was equilibrated with the mixture of carbogene (95% O₂ and 5% CO₂) and the foetal perfusate

was gassed with 95% N₂ and 5% CO₂. The created gas terms outlined above gave conditions close to those found in umbilical arteries *in vivo*.

The temperature of both the reservoirs and inside the perfusion chamber was kept at 37°C, and pH of perfusate was maintained between 7.2–7.4. Each perfusion lasted 150 min and the first 30 min was considered as an adaptation period. The physical integrity and the function of each lobule was monitored (foetal arterial pressure ≤ 60 mm Hg, foetal to maternal leakage of perfusate ≤ 2 ml/hr, foetal artery to vein oxygen gradient ≥ 90 mm Hg), according to Cannell *et al.* [6].

The experiment was performed in two groups: A and B. Group A included perfusions of 10 placental lobules, when a therapeutic dose of VPA (Depakine I.V., Sanofi Biocom, Warsaw, Poland) was added to the maternal circuit resulting in the final concentration of 75 µg/ml. This dose corresponds with an accepted therapeutic concentration of VPA in human plasma ranging from 40–100 µg/ml [6]. To test the saturability of transfer, eight placental lobules (group B) were perfused with 3-fold higher concentration of VPA (toxic dose) amounting to 225 µg/ml at the maternal circuit. During the study, the samples of perfusate from both maternal and foetal circuits were checked for VPA concentration every 60 min. The samples were subsequently centrifuged and stored at -20°C, until determinations were performed. The concentration of VPA was measured by immunofluorescent method (Abbott Laboratories, Abbott Park, IL, USA). The transfer percentage of the drug from maternal to foetal circulation was calculated using the following equation:

$$100 \times C_F \times V_F / [(C_F \times V_F) + (C_M \times V_M)],$$

where C_F is the foetal concentration of the drug, V_F is foetal perfusate volume, C_M is the maternal concentration of the drug, and V_M is the maternal perfusate volume [13].

Statistical analysis was performed by the non-parametric Mann-Whitney U test since values of quantitative variables did not show the normal distribution in the Shapiro-Wilk test. The statistical package used was SPSS-software package (version 14.0 PL for Windows; SPSS Inc., Chicago, IL, USA); p value less than 0.05 was considered statistically significant.

RESULTS

In group A, the VPA concentration in the maternal circuit after 60 and 120 min of the experiment were 30.8 ± 10.8 µg/ml and 30.2 ± 8.5 µg/ml ($p = \text{NS}$), respectively. VPA concentrations in the foetal circuit were 24.9 ± 9.9 µg/ml and 25.5 ± 6.5 µg/ml ($p = \text{NS}$), respectively (after 60 and 120 min). Concentrations of VPA in foetal circuit at both time-points were significantly lower than those in the maternal circuit, amounting to 81.3% and 84.3%. In group B, 60 and 120 min after the addition of VPA, concentrations of the drug in the maternal circuit were 97.4 ± 37.0 µg/ml and 83.5 ± 23.6 µg/ml

Table 1. VPA transfer percentage (\pm SD) after the administration of therapeutic (group A) and toxic (group B) dose of VPA to the maternal perfusate.

| Group | Timepoints (min) | |
|------------|------------------|----------------|
| | 60 | 120 |
| A (n = 10) | 22.7 \pm 9.1 | 22.7 \pm 7.1 |
| B (n = 8) | 21.9 \pm 5.6 | 23.9 \pm 5.3 |

($p < 0.05$), respectively, whereas these concentrations in the foetal circuit were $74.8 \pm 13.0 \mu\text{g/ml}$ and $81.0 \pm 16.1 \mu\text{g/ml}$ ($p = \text{NS}$), respectively (Fig. 1B). Similarly to group A, the concentration of VPA in the foetal circuit at 60 min was significantly lower than that in the maternal circuit, amounting to 76.8%, whereas at 120 min the concentration of the drug in the foetal circuit displayed a similar value (97%) to that at the maternal circuit.

The transfer percentage of VPA from the maternal to the foetal reservoir at 60 min was similar with both concentrations applied ($22.7 \pm 9.1\%$ with $75 \mu\text{g/ml}$ and $21.9 \pm 5.6\%$ with $225 \mu\text{g/ml}$).

There were no significant differences in VPA transfer percentage after 120 min of the experiment when different drug doses were compared ($22.7 \pm 7.1\%$ with $75 \mu\text{g/ml}$ and $23.9 \pm 5.3\%$ with $225 \mu\text{g/ml}$) (Tab. 1).

DISCUSSION

A limited number of studies elucidated the mechanisms of VPA transport across the human placenta but their results are conflicting [4, 5, 10, 11, 14, 16, 17]. Initial information was based on the measurement of the VPA concentration ratio between cord and maternal serum at the time of delivery. In this indirect method, it was found that VPA crosses the placenta and reaches the foetus, at least at the later part of pregnancy. Although a previous report suggested a lower concentration of VPA in the cord blood than in maternal serum (ratio 0.1–0.5) [5], further studies showed reverse tendencies [4, 10, 14, 16, 17]. They revealed that the VPA level in foetal (cord) serum was equal or exceeded that in the maternal serum (ratio 1–3) in the majority of cases. This phenomenon was also found in a pregnant *Rhesus* monkey treated with VPA [10]. In contrast, the average steady-state foetal/maternal unbound VPA plasma concentration ratio was 0.81 ± 0.09 during maternal drug administration in pregnant sheep [21]. However, these results could be influenced by the fact that this experiment was performed 1–2 weeks before term [21].

Previous data indicated a high degree of foetal exposure to VPA after its administration to the gravida. Furthermore, it was shown that the mean half-life of VPA in neonates of epileptic mothers was several times longer than those in adults, $47 \pm 15 \text{ hr}$ and $8\text{--}17 \text{ hr}$, respectively [12, 17, 28]. Although *in vivo* studies revealed the fact of rapid transfer of VPA across the placental barrier, only *in vitro* experiments

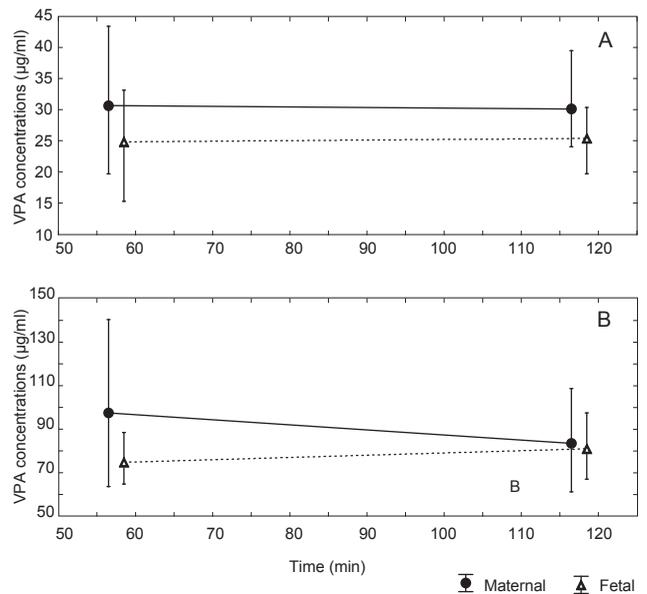


Figure 1. Time course of VPA concentrations (mean \pm SD) in maternal and foetal circulations after administration of therapeutic (group A) and toxic (group B) dose of VPA to the maternal perfusate. Group A included perfusions of placental lobules, when a therapeutic dose was added to the maternal circuit resulting in the final concentration of $75 \mu\text{g/ml}$. Eight placental lobules (group B) were perfused with 3-fold higher concentration of VPA amounting to $225 \mu\text{g/ml}$ in the maternal circuit. Samples of perfusate from maternal and foetal circuits were checked every 60 min for drug concentrations.

were able to provide more precise data. Currently, we used the dual recirculation human placental system and confirmed a rapid transplacental passage of VPA. We found that for the therapeutic dose of VPA, the system was in an equilibrium state, i.e. maternal and foetal VPA concentrations were stable after one and two hours of the experiment. However, we observed a significant decrease of the VPA level in maternal perfusate at 2 hr due to a slightly higher transfer into the foetal compartment. This was generally consistent with the results by Fowler *et al.* and Barzago *et al.* who also did not find any accumulation of the drug in placental tissue [4, 11].

In contrast to *in vivo* studies, in our and other *in vitro* experiments [4, 11], VPA concentrations in the foetal compartment were lower than in the maternal compartment at all timepoints, and with different concentrations applied. This phenomenon could be explained by a higher binding of VPA to the foetal than to the maternal protein, especially during the last few weeks of pregnancy [26]. The reduction of maternal plasma protein binding of VPA was probably aggravated by the increased plasma free acids level at this period [26, 27]. A similar diversity between results of *in vivo* and *in vitro* studies was found recently for diazepam metabolism [24].

Only a few data explain the mechanism by which VPA crosses through the biological membranes, and the detailed mechanisms of the transplacental passage of VPA are still

unknown. The animal model suggested that VPA pass the placental barrier via passive diffusion, as with most chemical agents [21]. However, VPA as a low-molecular weight (144 Da) fatty acid with pKa of 4.9 is almost completely ionized at physiological pH that may theoretically alter its placental permeability [7, 34]. Recently, it was shown that the transplacental passage of VPA may be mediated by a proton-linked saturable transport system, including the family of monocarboxylic acid transporters (MCT). The action of this system could facilitate the transplacental transport of VPA and additionally increase its foetal concentration [25].

It was confirmed that the incidence of congenital malformations in infants correlates positively with VPA concentrations in maternal serum [15, 19]. Therefore, all efforts should be undertaken to reduce foetal exposure to this drug. One option could be a reduction of the placental drug transfer. In fact, Barzago *et al.* showed that this is possible by encapsulation of VPA in liposomes which serve as drug carriers [4]. However, it should be proved whether such a modification does not influence the anticonvulsant activity of VPA before it becomes a routine therapeutic option. To date, the prescription of the lowest effective dose of VPA is recommended, and divided into 2–3 doses. Additionally, a close monitoring of maternal serum drug level is mandatory to avoid its dangerous peak concentration [18].

Despite the introduction of several new AEDs in recent decades, no agreement has been reached about the safest AED in relation to pregnancy; all of them cross the placenta, at least to some extent [23, 29]. Therefore, further studies are needed to improve the safety of antiepileptic therapy in pregnancy. There is no doubt that the placental perfusion method will be helpful in this respect.

Acknowledgements

The authors would like to acknowledge Prof. Maciej Józwick, Department of Obstetrics and Gynecology, Białystok Medical University, Poland, for careful reading of the manuscript.

REFERENCES

- Adab N, Kini U, Vinten J, Ayres J, Baker G, Clayton-Smith J, Coyle H, Fryer A, Gorry J, Gregg J, Mawer G, Nicolaidis P, Pickering L, Tunnicliffe L, Chadwick DW: The longer term outcome of children born to mothers with epilepsy. *J Neurol Neurosurg Psychiatry* 2004, **75**, 1575–1583.
- Alexander FW: Sodium valproate and pregnancy. *Arch Dis Child* 1979, **54**, 240–245.
- Banach R, Boskovic R, Einarson T, Koren G: Long-term developmental outcome of children of women with epilepsy, unexposed or exposed prenatally to antiepileptic drugs: a meta-analysis of cohort studies. *Drug Saf* 2010, **33**, 73–79.
- Barzago MM, Bortolotti A, Stellari FF, Diomede L, Algeri M, Efrati S, Salmons M, Bonati M: Placental transfer of valproic acid after liposome encapsulation during *in vitro* human placenta perfusion. *J Pharmacol Exp Ther* 1996, **277**, 79–86.
- Branchet-Liermain A, Demarquez JL: Pharmacokinetics of diopryl acetate in infants and young children. *Pharm Weekbl* 1997, **112**, 293–297.
- Cannell RG, Kluck RM, Hamilton SE, Mortimer RE, Hooper WD, Dickinson RG: Markers of physical integrity and metabolic viability of the perfused human placental lobule. *Clin Exp Pharmacol Physiol* 1988, **15**, 837–844.
- Davis R, Peters DH, Mc Tarvish D: Valproic acid. A reappraisal of its pharmacological properties and clinical efficacy in epilepsy. *Drugs* 1994, **47**, 332–372.
- Diav-Citrin O, Shechtman S, Bar-Oz B, Cantrell D, Arnon J, Ornoy A: Pregnancy outcome after in utero exposure to valproate: evidence of dose relationship in teratogenic effect. *Drugs* 2008, **22**, 325–334.
- Dickinson RG, Harland RC, Lynn RK, Smith WB, Gerber N: Transmission of valproic acid (Depakene) across the placenta: half-life of the drug in mother and baby. *J Pediatr* 1979, **94**, 832–835.
- Dickinson RG, Lawyer CH, Kaufman SN, Lynn RK, Gerber N, Novy MJ, Cook MJ: Materno-fetal pharmacokinetics and fetal distribution of valproic acid in a pregnant rhesus monkey. *Pediatr Pharmacol* 1980, **1**, 71–83.
- Fowler DW, Eadie MJ, Dickinson RG: Transplacental transfer and biotransformation studies of valproic acid and its glucuronide(s) in the perfused human placenta. *J Pharmacol Exp Ther* 1989, **249**, 318–323.
- Gugler R, von Unruh GE: Clinical pharmacokinetics of valproic acid. *Clin Pharmacokinet* 1980, **5**, 67–83.
- Henderson GI, Hu ZQ, Johnson RF, Perez AB, Yang Y, Schenker S: Acyclovir transport by the human placenta. *J Lab Clin Med* 1992, **120**, 885–892.
- Ishizak T, Yokochi K, Chiba K, Tabuchi T, Wagatsuma T: Placental transfer of anticonvulsants (phenobarbital, phenytoin, valproic acid) and the elimination from neonates. *Pediatr Pharmacol* 1981, **1**, 291–303.
- Jager-Roman E, Deichl A, Jacob S, Hartman AM, Koch S, Rating D, Steldinger R, Nau H, Helge H: Fetal growth, major malformations, and minor anomalies in infants born to women receiving valproic acid. *J Pediatr* 1986, **108**, 997–1004.
- Johannessen SI: Pharmacokinetics of valproate in pregnancy: Mother-foetus-newborn. *Pharmaceutisch Weekblad Scientific Ed* 1992, **14**, 114–117.
- Kaneko S, Otani K, Fukushima Y, Sato T, Nomura Y, Ogawa Y: Transplacental passage and half-life of sodium valproate in infants born to epileptic mothers. *Br J Clin Pharm* 1983, **15**, 503–505.
- Kaneko S: Epilepsy, pregnancy and the child. *Epilepsia* 2000, **41** (Suppl. 9), 8–13.
- Kaneko S, Battino D, Andermann E, Wada K, Kan R, Takeda A, Nakane Y, Ogawa Y, Avanzini G, Fumarola C, Granata T, Molteni F, Pardi G, Minotti L, Canger R, Dansky L, Oguni M, Lopes-Cendes I, Sherwin A, Andermann F, Seni MH, Okada M, Teranishi T: Congenital malformations due to antiepileptic drugs. *Epilepsy Res* 1999, **33**, 145–158.
- Kennedy D, Koren G: Valproic acid use in psychiatry: issues in treating women of reproductive age. *J Psychiatry Neurosci* 1998, **23**, 223–228.
- Kumar S, Wong H, Yeung SA, Riggs KW, Abbot FS, Rurak DW: Disposition of valproic acid in maternal, fetal, and newborn sheep. I: placental transfer, plasma protein binding, and clearance. *Drug Metab Disp* 2000, **28**, 845–856.
- Miller RK, Wier PJ, Maulik D, di Sant'Agnese PA: Human placenta *in vitro*: characterization during 12 h of dual perfusion. *Contrib Gynecol Obstet* 1985, **13**, 77–84.
- Myllynen P, Pienimäki P, Vähäkangas K: Human placental perfusion method in the assessment of transplacental passage of antiepileptic drugs. *Toxicol Appl Pharmacol* 2005, **207** (Suppl. 2), 489–494.
- Myllynen P, Vähäkangas K: An examination of whether human placental perfusion allows accurate prediction of placental drug transport: Studies with diazepam. *J Pharmacol Toxicol Methods* 2002, **48**, 131–138.
- Nakamura H, Ushigome F, Koyabu N, Satoh S, Tsukimori K, Nakano H, Ohtani H, Sawada Y: Proton gradient-dependent transport of valproic acid in human placental brush-border membrane vesicles. *Pharmaceutical Res* 2002, **1**, 154–161.
- Nau H, Krauer B: Serum protein binding of valproic acid in fetus-mother pairs throughout pregnancy: Correlation with oxytocin administration and albumin and free fatty acid concentrations. *J Clin Pharmacol* 1986, **26**, 215–221.

27. Nau H, Helge H, Luck W: Valproic acid in the perinatal period: Decreased maternal serum protein binding results in fetal accumulation and neonatal displacement of the drug and some metabolites. *J Pediatr* 1984, **104**, 627–634.
28. Nau H, Rating D, Koch S, Hauser I, Helge H: Valproic acid and its metabolites: placental transfer, neonatal pharmacokinetics, transfer via mother's milk and clinical status in neonates of epileptic mothers. *J Pharmacol Exp Ther* 1981, **219**, 768–777.
29. Nulman I, Laslo D, Koren G: Treatment of epilepsy in pregnancy. *Drugs* 1999, **57**, 535–544.
30. Omarini D, Pistotti V, Bonati M: Placental perfusion. An overview of the literature. *J Pharmacol Toxicol Methods* 1992, **28**, 61–66.
31. Ornoy A: Neuroteratogens in man. An overview with special emphasis on antiepileptic drugs in pregnancy. *Reprod Toxicol* 2006, **22**, 214–226.
32. Ornoy A: Valproic acid in pregnancy: How much are endangering the embryo and fetus? *Reprod Toxicol* 2009, **28**, 1–10.
33. Padmanabhan R, Shafiullah MM: Amelioration of sodium valproate-induced neural tube defects in mouse fetuses by maternal folic acid supplementation during gestation. *Congenit Anom* 2003, **43**, 29–40.
34. Reynolds F, Knott C: Pharmacokinetics in pregnancy and placental drug transfer. *Oxford Rev Reprod Biol* 1989, **11**, 389–449.
35. Richmond JR, Krishnamoorthy P, Andermann E, Benjamin A: Epilepsy and pregnancy: an obstetric perspective. *Am J Obstet Gynecol* 2004, **190**, 371–379.
36. Sastry BV: Techniques to study human placental transport. *Adv Drug Deliv Rev* 1999, **38**, 17–39.
37. Schneider H, Panigel M, Dancis J: Transfer across the perfused human placenta of antipyrine, sodium and leucine. *Am J Obstet Gynecol* 1972, **114**, 822–828.
38. Tomson T, Battaino D: Teratogenic effects of antiepileptic medications. *Neurol Clin* 2009, **27**, 993–1002.