

## VALPROATE-INDUCED DEVELOPMENTAL MODIFICATIONS MAYBE PARTIALLY PREVENTED BY COADMINISTRATION OF FOLINIC ACID AND S-ADENOSYLMETHIONINE

Natalia ÚBEDA<sup>1</sup>, Elena ALONSO<sup>2</sup>, Juan Carlos MARTÍN-RODRÍGUEZ<sup>1</sup>, Gregorio VARELA-MOREIRAS<sup>2</sup>,  
Javier PUERTA<sup>1</sup>, Julia PÉREZ-MIGUELSANZ<sup>1</sup>.

<sup>1</sup> Departamento de Ciencias Morfológicas I. Facultad de Medicina. Universidad Complutense. 28040-Madrid.

<sup>2</sup> Departamento de Ciencias Biomédicas. Facultad de Ciencias Experimentales y Técnicas. Universidad San Pablo - C.E.U. Boadilla del Monte. 28668-Madrid. Spain

Neural Tube Defects (NTD) are congenital anomalies caused by a wide range of different factors, including both genetic and environmental effects, which include severe lesions such as anencephaly and microcephaly and anomalies commonly known as spines bifidas.

Folic acid seems to be involved in the prevention of this type of defects. The most conclusive studies showed that periconceptional administration of multivitamins, specially folate, reduces the recurrence risk of NTD in a 50 to 66% of the cases (MRC Vitamin Study Research Group, 1991).

Antiepileptic agents such as Valproic acid (VPA) are known teratogenic agents highly specific for the induction of NTD. Furthermore, it also induce a wide range of skull malformations and tissues alterations. This agent are also recognized as interferer of folate metabolism. Folate concentrations decrease during normal pregnancy and treatment with antiepileptic drugs, including VPA, intensifies this effect. Furthermore, several reports suggest that epileptic patients with malformed infants had particularly low levels of folate in their plasma (Ogawa et al., 1991). The molecular mechanism of the teratogenicity of VPA is not well known, but one of the hypothesis proposed is that VPA interacts with embryo folate metabolism (Nau, 1994).

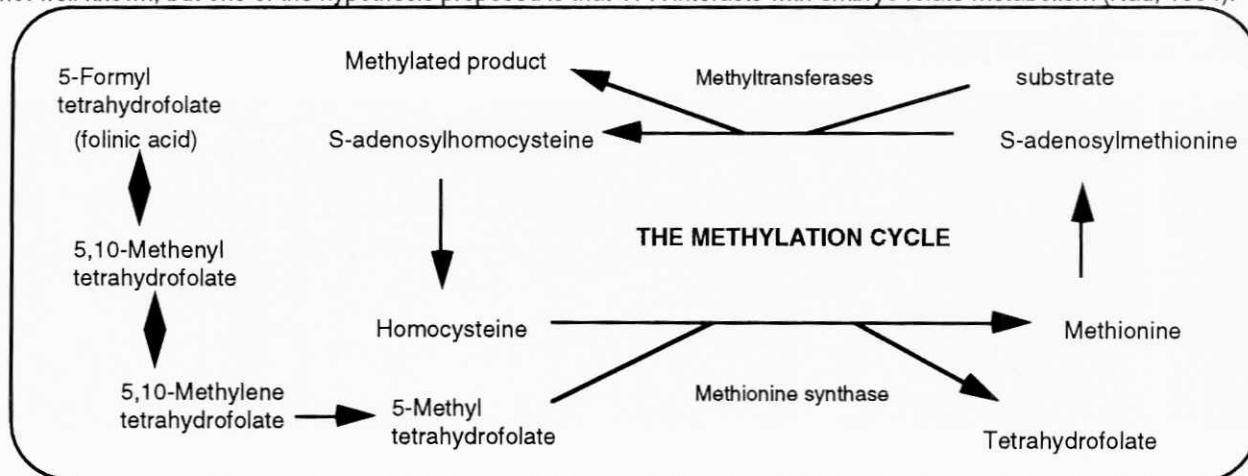


Figure 1: Methylation cycle modified of Scott et al. (1994).

Folic acid is mainly involved in two metabolic processes: DNA synthesis and the methylation cycle (FIGURE 1). DNA synthesis is essential for cell proliferation and, therefore, for neural tube closure. The methylation cycle is also essential as it provides the methyl groups necessary for the normal activity of methyltransferases involved in various processes of cellular life. A reduction in one of the enzymes or an alteration in one or both cycles could lead to the development of an NTD (Scott et al., 1994).

The aim of this study was to analyse the possible alterations of the methionine cycle and morphology during gestation under conditions that could enhance the risk of NTD occurrence. VPA treatment was approached to create a high risk of NTD and as model of altered folate metabolism. Folinic acid, 5-formyl tetrahydrofolic acid (FOL), and S-adenosylmethionine (SAM) were administered to determine if they prevent the alterations induced by VPA. For this, pregnant Wistar rats were classified according to the treatment:

\*VPA: Valproic acid: 300 mg/Kg/day, s.c., on days 8, 9 and 10 of gestation.

\*VPA+FOL: Valproic acid: 300 mg/Kg/day, s.c., on days 8, 9 and 10 of gestation and Folinic acid: 4 mg/Kg/day, i.p., on days 8, 9 and 10 of gestation.

\*VPA+SAM: Valproic acid: 300 mg/Kg/day, s.c., on days 8, 9 and 10 of gestation and S-adenosylmethionine: 10 mg/kg/day i.m., during the first 10 days of gestation.

\*CONTROL: Untreated rats.

Rats were terminated on day 21 of gestation, implantation sites and resorptions were counted and both dams and fetuses were stored for further biochemical and morphological analyses. The parameters analysed were:

### +BIOCHEMICAL:

-Hepatic content of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH).

-Hepatic methionine synthase activity.

**+MORPHOLOGICAL:**

- Skeletal analysis after bone staining with alizarin red S and cartilaginous tissue staining with alcian blue.
- Immunohistochemical analysis: samples were freezing sectioned and staining with two different antibody:
  - Mouse Anti-Rat Macrophages, specific for hepatic Kupffer cells.
  - Mouse Monoclonal Antibody to Rat Thymocyte and T Cells.

**Table 1**

	VPA	VPA+FOL	VPA+SAM	CONTROL
FERT. IND.	75	90	90	100
WEIGHT(g)	3.7±0.05*	3.6±0.04*#	3.3±0.05*#	3.4±0.03
LENGTH(mm)	37.5±0.2	37.5±0.1	36.8±0.2*\$	37.4±0.1
SAM(nmol/g)	134.1±5.55	126.5±2.43	132.32±3.33*	119.6±3.76
SAH(nmol/g)	3.27±0.23*	3.03±0.30	3.40±0.25*	2.27±0.23
MET.SYN.	4.68±0.58*	6.09±0.55	4.70±0.44*	6.11±0.76
DEF1	0.18±0.38 $\emptyset$	0.12±0.33	0.18±0.39 $\infty$ †	0.08±0.28&
DEF2	0.40±0.49†	0.29±0.46†	0.62±0.49& $\infty$	0.29±0.46
DEF3	0.21±0.41 $\emptyset$	0.21±0.42	0.38±0.49	0.12±0.33& $\infty$

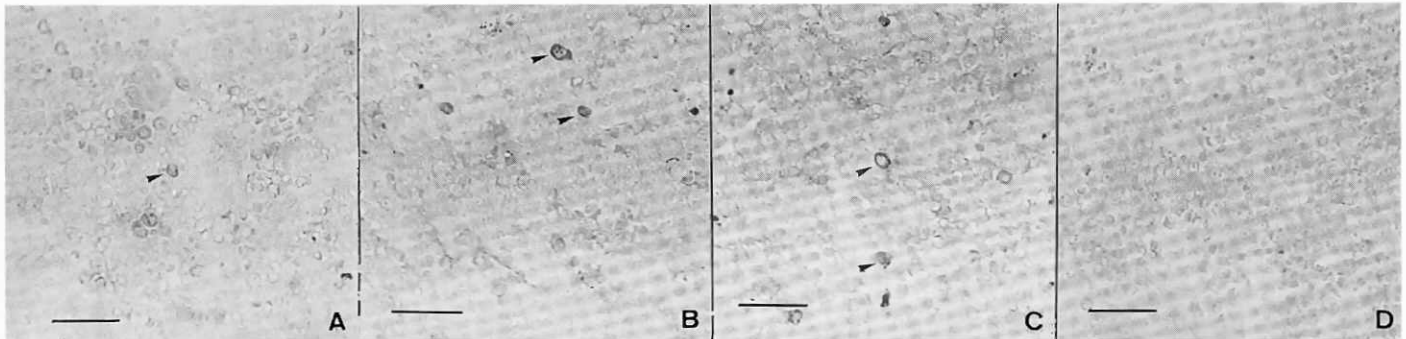
Results are expressed as means  $\pm$  SEM per group.

\* Values significantly different ( $p < 0.05$ ) from the CONTROL group. # Values significantly different ( $p < 0.01$ ) from the VPA-treated group.

\$ Values significantly different ( $p < 0.01$ ) from the VPA+FOL-treated group.  $\emptyset$  Values significantly different ( $p < 0.05$ ) from the DEF2:VPA-treated group. & Values significantly different ( $p < 0.05$ ) from the DEF2:CONTROL group.  $\infty$  Values significantly different ( $p < 0.05$ ) from the DEF3:VPA+SAM-treated group. † Values significantly different ( $p < 0.05$ ) from the DEF2:VPA+SAM-treated group.

ABBREVIATIONS: FERT. IND.: Fertility Index=  $\frac{\text{dams with live fetuses}}{\text{total pregnant rats}} \times 100$ .

MET.SYN.: Methionine syntase (nmol/ h/mg protein).  
DEF1:SKULL. DEF2:MIDDLE LINE BONES DEF3:LIMBS.



**Figure 2:** Fetal liver section stained with mouse anti-rat macrophages. A: VPA. B: VPA+FOL. C:VPA+SAM. D:CONTROL. Arrowheads show Kupffer cells. Scale marker:500  $\mu$ m

Table 1 shows that VPA is not able to induce NTD in Wistar rat using this dose, but reduces the fertility index and modifies significantly biochemical parameters, increasing SAM and SAH levels of fetal liver, and decreasing methionine synthase activity, compared to CONTROL. Also this drug induces skeletal modifications in the skull, appendicular bones, vertebrae and ribs. FOL can prevent these defects approaching the values to the CONTROL group, on the contrary, SAM does not have this protective role showing modifications of the treated fetus the ossification even higher than VPA group.

Preliminary results with antibodies shows that as Kupffer cells (FIGURE 2), as well T lymphocytes appear more frequently in VPA+FOL and VPA+SAM groups, meaning that drug treatment does not alter fetal liver haematopoiesis, but coadministration with the components of the methylation cycle enhances cell division. Stereological techniques will be applied to establish significant differences.

In conclusion, it is clear that VPA alters normal gestation inducing severe skeletal modifications, which could be prevented by FOL supplementation during gestation, but SAM does not have the same protective effect. Hepatic development is not modified by drug administration, increasing the haematopoietic activity when there is FOL and SAM supplementation.

**References**

- MRC Vitamin Study Research Group (1991). *Lancet*, 338: 131-137.
- Nau H. (1994). "Valproic acid induced neural tube defects". *Ciba Foundation Symposium*, 181:144-160.
- Ogawa Y, Kaneko S, Otani K, Fukusima Y. (1991). *Epilepsy Res*, 8:75-78.
- Scott JM, Weir DG, Molloy AM, McPartlin J, Daly L, Kirke P. (1994). "Folic acid metabolism and mechanisms of neural tube defects". *Ciba Foundation Symposium*, 181: 180-191.

**Acknowledgements**

This work has been supported by Proyecto Multidisciplinar de la U.C.M. n° PR 188/92-4129.