Reference

[1] World report on hearing. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO

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New serum miRNA biomarkers to predict liver steatosis by valproic acid in paediatric epileptic patients

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Depakine (Valproate, VPA) has been the first line, most-frequently prescribed, anti-epileptic drug in children for the past 50 years. Idiosyncratic hepatotoxicity (iDILI) by VPA has been demonstrated in several case reports, where microvesicular liver steatosis was the most frequent feature. Moreover, more than half of VPA-treated patients could have silent fatty liver as demonstrated by ultrasounds. Extensive experimental studies support that VPA has a high potential to induce steatosis in hepatocytes. However, there is an apparent lack of significant hepatic problems in the Neuropediatric Units, despite transaminitis is not uncommon. One of the reasons could be that iDILI and liver steatosis diagnosis lack specific biomarkers. Thus, it is likely that a relevant number of children under VPA treatment may have a significant, but sub-clinical, hepatosteatosis.

The initial objective of this study was twofold: (1) to demonstrate VPA-induced triglyceride (TG) accumulation in cultured human upcyte hepatocytes, and (2) to identify miRNAs significantly deregulated by VPA in these cells. The long-term goal is to investigate if the identified miRNAs may become useful circulating biomarkers to find patients at risk of liver steatosis among paediatric epileptic patients on VPA therapy.

We first investigated TG accumulation (enzymatic-colorimetric assays) induced by subcytotoxic concentrations of VPA in human upcyte hepatocytes from different donors. Results demonstrated that only some donors had a significant response. Hepatocytes form the selected donor increased intracellular TG by 23% and 45% after 2 and 4 mM VPA for 24 h. The profiling of cellular miRNAs after 4 mM VPA by microarray analysis (GeneChip® miRNA 3.0 Array) identified 37 deregulated human miRNAs (fold-change >2 or <–2; $p < 0.05$). Some of them (n = 11), validated by RTqPCR, were selected as potential biomarker candidates: miR-127-3p, miR-500a-5p, miR-362-5p, miR-149-5p, miR-485-3p, miR-182-5p, miR-30a-3p, miR-212-3p, miR-432-5p, miR-183-5p and miR-675-5p. Next, we studied the expression level of these miRNAs in human serum and found that only 8 of the initial 11 miRNAs could be reliably quantified by RTqPCR: miR-127-3p, miR-485-3p, miR-182-5p, miR-30a-3p, miR-212-3p, miR-432-5p, miR-183-5p and miR-675-5p. Finally, a preliminary screening of this 8-miRNA signature in a cohort of 48 paediatric epileptic patients under VPA therapy identified 8 patients (17%) with simultaneous elevated levels (>2-fold) of 3-5 miRNAs.

In conclusion, VPA induces both TG accumulation and deregulation of a set of miRNAs in cultured human hepatocytes. A subset of 8 miRNAs has been established as a potential circulating biomarker signature to identify VPA-induced steatosis in epileptic patients. Those patients with a potential risk will need closer clinical follow-up to confirm drug-induced hepatic steatosis and, if so, to take measures to correct it.

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References

[1] Egunsola O., Choonara I. & Sammons H. M. (2017) Anti-epileptic drug utilisation in paediatrics: A systematic review. *BMJ Paediatr. Open* 1, 1–7.
[2] Balagura G., Iapadre G., Verrotti A. & Striano P. (2019) Moving beyond sodium valproate: choosing the right anti-epileptic drug in children. Expert Opin. Pharmacother. 20, 1449–1456.
[3] Gerstner T., Bell N. & König S. (2008) Oral valproic acid for epilepsy - Long-term experience in therapy and side effects. Expert Opin. Pharmacother. 9, 285–292.
[4] Koenig S. A. *et al.* (2006) Valproic acid-induced hepatopathy: Nine new fatalities in Germany from 1994 to 2003. *Epilepsia* 47, 2027–2031.
[5] Bryant A. E. & Dreifuss F. E. (1996) Valproic acid hepatic fatalities. III. U.S. experience since 1986. *Neurology* 46(2):465–9.
[6] Luef G. J. *et al.* (2004) Valproate Therapy and Nonalcoholic Fatty Liver Disease. Ann. Neurol. 55, 729–732.
[7] Farinelli E. (2015) Valproic acid and nonalcoholic fatty liver disease: A possible association? *World J. Hepatol.* 7, 1251.
[8] Powell-Jackson P. R., Tredger J. M. & Williams R. (1984) Hepatotoxicity to sodium valproate: A review. *Gut* 25, 673–681.

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S-28 | Putting the puzzle together: multiple lines of evidence to inform design and interpretation of long-term, repeated-dose animal studies to inform human health risk assessment

S-28-01
Characterising applicability domains of generic *in vitro* distribution kinetic and physiologically based kinetic models

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Next generation risk assessment encourages the use of *in silico* and/or *in vitro* tools as opposed to *in vivo* kinetic studies to parameterize physiologically based kinetic (PBK) models for quantitative *in vitro-in vivo* extrapolations (QIVIVE). In this study, we examine the applicability domains of quantitative structure property relationships (QSPR) for parameterizing both generic *in vitro* and *in vivo* kinetic models used in the EU H2020 ONTOX project. The aim is to assess to what extent these models sufficiently relate (1) nominal concentrations to cell-associated concentrations in a battery of *in vitro* assays screening for repeat-dose hepatotoxicity, developmental neurotoxicity and nephrotoxicity, and (2) external exposures to liver, fetal brain and kidney concentrations in humans. Using systematic review tools, a database of chemicals associated with steatosis, cholestasis, cognitive function defects, neural tube closure defects, crystallopathy and tubular necrosis and associated physicochemical and toxicokinetic (e.g., bioavailability, plasma protein binding, intrinsic clearance) properties was developed to analyse their chemical space and compare this with the applicablity domains of PK-Sim, the *in vitro* kinetics models and their associated QSPRs. In so doing, a prioritisation is made for the kinetic model compartments and QSPRs to adjust.

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