The ENDpoiNTs project ¬- Novel Testing Strategies for Endocrine Disruptors linked to Developmental Neurotoxicity

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INTRODUCTION

Clear evidence supports associations between exposure to endocrine disrupting chemicals (EDCs) and impaired neurodevelopment. Yet, current hazard assessments of EDCs barely address developmental neurotoxicity. This is due to a lack of scientific knowledge on how endocrine disruption is linked to developmental neurotoxicity (DNT). Thus, there is an urgent need for novel testing and screening tools to address endocrine disruption (ED-) induced DNT, based on new scientific knowledge. ENDpoiNTs is a H2020 research and innovation action that involves 17 participants in Europe, USA and Australia and which addresses this issue. It integrates expertise in endocrine disruption and DNT and combines state-of-the-art in silico and in vitro tools and advanced biostatistics on human epidemiological and biomonitoring data. The project began on 1 January 2019 and is running for 5 years. Here, we present the results of the establishment of endocrine-sensitive DNT key events in vitro that ultimately have sufficient throughput to test chemicals in a timely and cost-effective manner.

METHODOLOGY

An array of in vitro models was employed to assess the responsiveness of neurodevelopmental key events (KE) to endocrine signalling pathways. To address differences in species, both rodent and human cell models were included, ranging from 2D- and 3D rodent neur(on)al primary cultures and cell lines to primary human neural progenitor cells (NPCs) and human induced pluripotent stem cell (hiPSC)-derived neurons as well as neuro-glia co-cultures. The models covered different levels of complexity, developmental stages and neurodevelopmental KE –NPC proliferation, differentiation, migration, myelination, network formation and maturation, including synaptogenesis, dendritic spine formation, neuronal apoptosis and astrocyte and oligodendrocyte maturation. The responsiveness of the in vitro models and endpoints to endocrine signalling was tested using endocrine model compounds, i.e. agonists and antagonists of relevant hormone receptors. These receptors had been identified as potential targets of EDCs associated with neurodevelopmental outcomes in humans, based on computational predictions.

RESULTS

Agonists and antagonists for the estrogen receptors (ER), androgen receptor (AR), thyroid hormone receptors (TR), glucocorticoid receptor (GR), retinoic acid receptor (RAR), retinoic X receptor (RXR), progesterone receptor (PR), aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptors (PPARα, PPARγ, PPARδ), vitamin D3 receptor (VDR), liver X receptor (LXR), and prostaglandin E2 receptors were tested in physiologically relevant concentrations. In several model systems, the previously known role for TRs for oligodendrocyte differentiation and myelination as well as for RAR in neuronal differentiation could be confirmed. Furthermore, new roles were identified for LXR in oligodendrocyte differentiation and neural induction, for PPARs in neuronal and oligodendrocyte differentiation and neural induction, for GR in NPC proliferation, and for ER and AhR in oligodendrocyte differentiation. Both differences and similarities between human and rodent models were observed. Human models tended to be more sensitive than rodent models.

DISCUSSION

Our results demonstrate that cellular processes crucial for proper brain development are influenced by endocrine signalling, whereby oligodendrocyte differentiation was one of the endpoints most sensitive to different endocrine interference. The endocrine systems involved in regulating these key events included not only thyroid hormone signalling, which has a well-established role in neurodevelopment, but also others that are significantly less studied in the context of endocrine disruption, e.g. LXR signalling. In the next step, the assays showing endocrine responsiveness will be tested with known EDCs to assess their sensitivity to ED-induced DNT. Furthermore, the in vitro results will be integrated with in vivo data, linking these key events to adverse outcomes in whole organisms. As hormone responsiveness was added to DNT models and assays that are already established and partly included in regulatory (pre-)validation efforts, it is likely that these assays can be developed quickly for regulatory use and brought into such use. Together with in silico models and in vivo endpoints, they will ultimately be incorporated into the ENDpoiNTs integrated testing approach for ED-induced DNT.