Workshop 1: Developmental toxicology. Different Models, different endpoints

W1-1
In vitro and *in vivo* models to study teratogenicity. Misoprostol as an example

M.F. Cavieres

Escuela de Química y Farmacia, Facultad de Farmacia, Universidad de Valparaíso, Valparaíso, Chile

Introduction: Prostaglandins of the E-series play an important role on SNC and bone and limb development. Misoprostol is a prostaglandin E1 analog and is registered in Chile for the prevention and treatment of non-steroidal anti-inflammatory peptic ulcers. It is also used to induce abortions. Preclinical teratology studies in rats did not show induction of defects, although reports in the literature have shown teratogenicity in mice. In utero exposure to misoprostol in humans, after failed abortion attempts, has been associated to cerebral palsy and joint and skeletal defects. The mechanism of action which mediates this teratogenicity is unknown. Since misoprostol acts on prostanoid receptors, we hypothesize that this interaction can explain the induction of defects.

Objective: The aim of this study was (1) To establish an appropriate model for the study of misoprostol induced birth defect; (2) To study if misoprostol developmental toxicity is due to its binding to prostanoid receptors.

Methods: We have used in vivo and in vitro assays to study misoprostol induced-teratogenicity and embryo and fetal toxicity in the rat. The assays include in vivo teratology and in vitro micromass, whole embryo culture at early (GD 9.5) and late (GD13.5) organogenesis. We have cultured the embryos in the presence of increasing doses of misoprostol as well as prostanoid receptor antagonists.

Results: In vivo rat teratology showed no defect induction. Similarly, misoprostol was not cytotoxic in the micromass assay; neither did it affect cell differentiation. On the other hand early and late organogenesis whole embryo culture successfully showed embryotoxicity. These defects could be mediated by interaction to the EP3 prostanoid receptor in the GD9.5 rat embryo. Limb defects are not mediated by EP1 or EP2 receptors.

Conclusions: In vitro whole embryo culture studies are better and appropriate models to study misoprostol teratogenicity. EP3 receptor may be mediating the induction of defects in the rat.

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W1-2
Developmental origins of reproductive disorders in the adult: The example of glucocorticoids and statins

W.G. Kempinas

Department of Morphology, Institute of Biosciences of Botucatu, UNESP – Univ Estadual Paulista, Botucatu, SP, Brazil

It has been shown that developmental exposure to certain chemicals can lead to reproductive disorders later in life. In this presentation we will talk about two classes of pharmaceuticals widely used in human clinic: glucocorticoids and statins. Bethametasone is the glucocorticoid of choice for antenatal treatment in the promotion of lung maturation. Previous studies reported that prenatal bethametasone treatment reduces sperm quality and can negatively impact the pituitary-adrenal-axis in the second generation of treated rats. More recently we observed delay in the onset of puberty and decrease in sperm production, motility and fertility in rats whose dams received betamethasone during pregnancy. The histopathology of testis and epididymis revealed uncommon features. Dyslipidemias are occurring earlier in the population due to the increase of obesity and bad eating habits. Statins are used to reduce cholesterol. Taking this into consideration we have been investigating the short and long-term reproductive effects of statin exposure in prepubertal rats. The administration of rosuvastatin at this age provoked, in adult rats, decreased sperm motility and sperm counts and increased tail sperm abnormalities and post-implantation losses. These results raise concern for humans, considering the widespread use of these drugs. In this presentation we also plan to discuss the use of alternative endpoints for the determination of fertility in rats, such as biomarkers of fertility and intrauterine insemination, since natural mating is not a sensitive method for rodents, due to their high reproductive efficiency.

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W1-3
Computer simulation of developmental processes and toxicities

T.B. Knudsen

National Center for Computational Toxicology, US Environmental Protection Agency, Research Triangle Park, NC 27711, USA

Introduction: Standard practice for assessing developmental toxicity is the observation of apical endpoints (intrauterine death, fetal growth retardation, structural malformations) in pregnant rats/rabbits following exposure during organogenesis. EPA's computational toxicology research program (ToxCast) generated vast in vitro cellular and molecular effects data on >1858 chemicals in >600 high-throughput screening (HTS) assays. Translating these data into higher order-predictions of tissue dysfunction is a significant challenge for complex adaptive systems such as the embryo.

Objective: This talk will address the use of computational systems models to recapitulate the kinematics of dynamical cell signaling networks, and their application for translating in vitro data (ToxCast/Tox21) on human cell-based assays into predictive models of developmental toxicity.

Methods: An open source CompuCell3D.org modeling environment was used to build embryologically-inspired computer models that recapitulate cell signaling networks for: VEGF-mediated angiogenesis (angiodysplasia), androgen-mediated urethral closure (hypospadias), TGFb-mediated tissue fusion (cleft palate), and retinoid-mediated limb outgrowth (ectrodactyly).

Results: Being numerically responsive to perturbation, the models are amenable to various computational methods of data processing and simulation from ToxCast. For example, a newly added metabolomics-based platform that predicts the exposure-based potential for developmental toxicity (pDT) in a human system with ~83% accuracy yielded a hit on 138 ToxCast chemicals (~13% of the 1065 chemicals tested to date). Computer simulation models that integrate in vitro perturbations (ToxCast) with embryological signaling networks output systems-specific predictions of dose-response for gestational susceptibilities.

Conclusions: A heuristic computational intelligence framework that recapitulates the kinematics of dynamical cell signaling networks in the embryo can be used to translate in vitro data