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Do Teratogenic Exposures Act through Common Pathways or Mechanisms of Action?

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The term “mechanisms of action” refers to the chemical interactions of an agent with the organism that lead to an adverse effect. Mechanisms of action are diverse: agents can interact with a receptor, bind to DNA or protein, degrade cell membranes or proteins, inhibit an enzyme, or modify proteins. If enough of these interactions between exogenous agent and the organism occur at a biochemical level, changes can occur at the cell and tissue level and can lead to changes in cell function, cell fate, or result in cell death. If the magnitude of the response is extensive enough, abnormal development results. The string of events leading from initial mechanism to adverse effect is termed a pathway, and there has been a significant effort over the past several years to map out these adverse outcome pathways, not just for teratogenicity but for any disease state. Different agents can act through the same mechanism of action, producing similar effects.

Receptor interactions
Receptors are proteins within or on the surfaces of cells that are targeted by hormones or other signaling molecules. Receptors perform the same function for cells as our senses perform for our bodies: they inform the cell about its environment and, when activated, bring about changes in cell function. Some teratogens act by interacting with receptors, either mimicking the endogenous hormone or signaling molecule or by interfering with the hormone’s ability to interact with its receptor. Examples include retinoic acid (the biologically active form of vitamin A) and DES (diethylstilbestrol, a potent estrogen that was once given to pregnant women in an effort to prevent miscarriage). Retinoic acid, the active form of vitamin A, is essential for normal development and has a family of receptors that are expressed in certain embryonic structures; too much retinoic acid causes defects in those structures. DES binds to estrogen receptors and causes defects in male and female reproductive organs, as well as a rare form of vaginal cancer in about one of every thousand women whose mothers took DES during pregnancy. The retinoic acid receptor and the estrogen receptor are part of a family of receptors called nuclear receptors; many other receptors in this family are known or suspected to be targets for teratogenic exposures, such as the androgen receptor and the thyroid hormone receptor. These receptors function by binding to specific DNA response elements that elicit a number of changes in gene expression that change cell function for hours or days.

Covalent binding to DNA or protein
Some agents are chemically reactive or are metabolized by the body to chemically reactive forms. These reactive forms create covalent bonds to important biomolecules, changing the function of these molecules. For example, cyclophosphamide, a drug used to treat cancer, is metabolized to phosphoramide mustard, a reactive intermediate that covalently binds DNA and other important molecules in the cell, impairing the function of these cells. While this property makes cyclophosphamide valuable in treating rapidly dividing cancer cells, it also makes the drug risky during pregnancy because it harms rapidly dividing cells in the embryo.

Peroxidation and oxidative stress
Chemicals that generate highly reactive substances like hydrogen peroxide can oxidize molecules, particularly the lipids that form the foundation for cell membranes. Other agents can also produce oxidation within cells, damaging macromolecules and organelles.

Enzyme inhibition, interference with sulfhydryl groups
Enzymes are proteins that catalyze chemical reactions, such as the reactions that break down sugars to produce energy for the cell or that synthesize the large molecules needed for cell structure and function. Inhibiting the function of an enzyme may have teratogenic consequences. For example, methotrexate, an inhibitor of dihydrofolate reductase, an enzyme in the folic acid synthesis pathway, interferes with metabolic processes that require folic acid, including the synthesis of nucleotides needed to make DNA.

Sulfhydryl groups, which contain sulfur and hydrogen and are found on the amino acid cysteine, are important in creating the three-dimensional structure of proteins: two sulfur atoms that are distant from each other link together to form a disulfide bridge, creating a loop in the protein. Sulfhydryl groups are also used to hold essential minerals like zinc in place in proteins. Sulfhydryl groups are also important in caspases and other enzymes involved in programmed cell death, a normal developmental process. Cadmium, mercury, or other heavy metals can interact with sulfhydryl groups, disrupting the function of the proteins that contain them.

Modification of Proteins
Some proteins require modification in order to carry out their function, and these modifications can be another target of teratogenic exposures. For example, a signaling protein called sonic hedgehog (Shh) must first be clipped into two fragments, with the signaling fragment having a cholesterol molecule added to it in order for it to function normally. Shh functions to delineate the ventral portion of the central nervous system. Defects of the central nervous system in which the ventral portion is poorly defined, such as holoprosencephaly or cyclopia, arise when Shh does not function correctly. A number of different agents have been shown to interfere with Shh function, including cyclopamine (an alkaloid in certain range plants in the Western U.S.) and some but not all inhibitors of cholesterol synthesis. These agents appear to act by interfering with the cholesterol modification of Shh. Mutations of one particular gene in the cholesterol synthesis pathway can cause identical abnormalities, as does mutation of Shh itself, another example of how different mechanisms at a biochemical or molecular level can have common outcomes.

Progression of Mechanistic Events to Pathology
If the mechanistic events are sufficiently widespread, they may result in changes at the cellular and tissue level. Different exposures can cause the same cascade of events that result in abnormal development. For example, the edema syndrome results when embryos are exposed to low oxygen levels. Heart rate and blood pressure drop, sodium and potassium concentrations in the plasma change, and fluid seeping out of blood vessels causes hollow organs to swell and blisters to form in solid structures. The distortions caused by fluid accumulation disrupt development. But other agents can cause the edema syndrome as well; trypan blue (a biological stain) and other agents that affect the nutrition of the early embryo cause similar effects.

Toxicologists have begun the process of mapping out these adverse outcome pathways as a way of codifying the chain of events that leads from initial molecular interaction to adverse effect. These adverse outcome pathways also provide a basis for understanding how insults that do not have the same target initially can converge on the same pathway and produce the same adverse response. To build on the Shh example introduced above, holoprosencephaly via altered Shh signaling can be produced by alkaloids that alter the post-translational modification of the Shh protein; by agents that inhibit specific enzymes in the cholesterol synthesis pathway, thereby affecting the modification of

Shh; or by mutations in the genes for those enzymes or Shh itself. Each of these agents is different at the molecular level but converges on the same pathway by altering Shh function.

Research into teratogenic mechanisms and pathogenesis is advancing as progress is made in our understanding of the molecular processes that control embryonic development, and this field is the subject of considerable research activity. As more becomes known about the underlying molecular mechanism of teratogenicity, testing for developmental toxicity may include screening using in vitro assays that cover a broad range of mechanisms, either by large batteries of tests for a single mechanism, conducted in high-throughput fashion, or by global evaluation of gene expression, which can be diagnostic for mechanism.

Suggested Reading

Knudsen, T. B., and Daston, G. P. Developmental Toxicology, vol. 12 in the 2nd Edition of Comprehensive Toxicology, Elsevier Publishing, 2010.
Adverse outcome pathways, molecular screening and toxicogenomics. OECD. 2017 http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm