

Teratology: General considerations and principles

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Approximately 3% to 5% of children born in the United States manifest developmental defects. Between 2% and 3% of these birth defects can be classified as teratogen-induced malformations, which result from environmental or iatrogenic exposures during pregnancy. The final manifestations of drug-induced abnormal development can be death, malformation, growth retardation, or functional disorder. In considering the potential teratogenic effects of drugs, it is important to remember that susceptibility to a drug-induced malformation depends on: (1) the genotype of the conceptus, (2) the developmental stage at which exposure occurs, (3) the mechanisms of action of the drug, (4) the access of the drug to developing tissues, and (5) the dose of the drug. Nearly all teratogen-induced birth defects are preventable if the dose-response relationship for teratogen exposure and the teratogenic activity of the drug can be clearly defined. (*J Allergy Clin Immunol* 1999;103:S337-42.)

Adverse effects resulting from maternal drug use during pregnancy bring to mind visions of babies born with serious birth defects, such as limb reductions associated with use of thalidomide or spina bifida associated with use of valproic acid (an anti-epileptic drug [AED]). However, despite the emotion and drama surrounding drug-induced birth defects, congenital defects are actually rare events. Approximately 3% to 5% of children born in the United States have developmental defects, ranging in severity from relatively benign (eg, depressed nasal bridge) to severe (eg, heart lesions or mental retardation). Nonetheless, birth defects do occur and have a staggering impact on our society, including enormous medical costs. The estimated lifetime medical costs for a child with a birth defect can easily exceed \$500,000. The combined lifetime cost of 18 common congenital defects is approximately \$8 billion dollars annually.

CAUSES OF BIRTH DEFECTS

Birth defects can be attributed to a variety of causes (Table I). Sixty-five percent to seventy percent of all birth defects are classified as having an unknown etiology. Approximately 20% are genetic in nature; and chromosomal, or cytogenetic, defects account for 3% to 5% of all congenital malformations. These include problems

Abbreviations used

AED: Anti-epileptic drug

EH: Epoxide hydroxylase

such as trisomy (the inheritance of a complete extra chromosome) or the 4p-syndrome (alteration of a small part of the chromosomal complement). Two percent to three percent of developmental defects are due to maternal infection with viral agents such as rubella, cytomegalovirus, or HIV. A small percentage of birth defects are caused by imbalances in maternal metabolism, such as that related to diabetes. Between 2% and 3% of defects are classified as teratogen-induced malformations. These are considered to be the result of environmental or iatrogenic exposures during pregnancy.

HISTORY OF TERATOLOGY

Teratology, the study of environment-induced malformations, began as a modern science in the 1930s, with the publication of a set of experiments in which pregnant pigs were fed a diet deficient in vitamin A.¹ The resulting abnormalities in the offspring demonstrated that mammalian development, by its residence within the mother, was not as protected as was previously believed. In fact, the results of these experiments showed that relatively simple alterations in the environment could have devastating effects on the embryo. The susceptibility of mammalian embryos to toxicity from xenobiotic agents was demonstrated in a series of studies in experimental animals with congeners of biologically important molecules, such as the amino acid mimic azaserine.² A human counterpart to these experiments was reported in the 1950s, when aminopterin was used in some human pregnancies to produce abortion. Several malformed children were born after the drug failed to terminate the pregnancies.³

The thalidomide episode of the early 1960s increased our understanding of developmental toxicology by providing an example of an agent that produced minimal toxicity to adults but a high degree of toxicity for the embryo. Such selective embryotoxicity remains the key element of many experiments evaluating the toxic potential of drugs used during pregnancy. It was during the 1960s that regulatory agencies, including the Food and Drug Administration in the United States, developed requirements for testing drugs in animals before approval for marketing. Drug studies were required to use doses high enough to cause maternal toxicity in order to add confidence that a biologically relevant dose for that

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TABLE I. Causes of birth defects

Cause	%
Teratogen-induced malformations	2-3
Maternal infection with viral agents	2-3
Chromosomal defects	3-5
Genetic factors	20
Unknown	65-70

species was included in the test protocol. Extrapolation of these results to human beings was initially viewed with skepticism because of the frequent finding of adverse effects in animals with drugs believed to be acceptable for use in human beings.

Part of the solution to the difficulties of extrapolating results from animals to human beings is the recognition that maternal disposition (metabolism and distribution) of the drug influences the effect that the drug has on the fetus. Thalidomide is not well absorbed when given orally to rats. Therefore administration of the drug in typical oral teratology studies does not result in a significant dose reaching the embryo. When thalidomide is made soluble in dimethyl sulfoxide and given parenterally, its ability to adversely affect the fetus increases nearly to that observed in rabbits, a species that is very sensitive to thalidomide effects. Modern developmental toxicity studies in animals are performed with an understanding of how each species handles the drug. With the use of current animal testing protocols, drugs that could produce birth defects in human beings should be able to be identified.

PRINCIPLES OF TERATOLOGY

Although teratogenic exposures account for a very small percentage of all birth defects, there are approximately 3 million persons in the United States today living with the consequences of developmental defects caused by in utero exposure to teratogens. It is important to remember that nearly all teratogen-induced birth defects are preventable, if we understand the 6 basic principles of teratology.⁴

Principle 1: Susceptibility to teratogenesis depends on the genotype of the conceptus and the manner in which it interacts with the environment

In considering what is known about the effects of exposure to established human teratogens, it is clear that 2 of the most defining features of teratogens are that they produce a *variable phenotype* in the exposed and affected infants and that not all exposed infants are affected (ie, *susceptibility is variable*).

Variable phenotype. The range of phenotypic expression in teratogen-affected infants is extremely broad. Over the past 3 decades, sufficient evidence has accumulated in the clinical literature to suggest that most teratogens cause a characteristic pattern of malformations,

which can vary among affected individuals. Because teratogens cause patterns of defects, it is misleading to only focus on single major malformations.

Variable susceptibility. Only a small percentage of infants who are exposed in utero to known or suspected teratogens display any adverse effects. It is not clear why some fetuses are at increased risk, but it is widely believed that susceptibility results from inheriting a set of genes that interacts with the teratogenic agent, altering normal morphogenetic pathways and resulting in the birth defect. Because relatively few infants present with birth defects despite a wide range of in utero exposures, it is often difficult for clinicians and families to believe that certain compounds have teratogenic potential. Additionally, given the low incidence of teratogen-induced birth defects, understanding the steps leading to susceptibility and development of a congenital defect is difficult and challenging.

Variable susceptibility to a teratogen can be best demonstrated by relating teratogenic exposures and birth defect rates to what has been observed in clinical practice. For example, less than 10% of the infants exposed in utero to hydantoins, the most commonly prescribed anticonvulsant drugs, have congenital defects. Similarly, only a small proportion of infants exposed to thalidomide were adversely affected. The same is true for fetal alcohol syndrome. Some mothers can apparently drink alcohol regularly during pregnancy with no adverse effects on their infants, whereas others can consume very little alcohol and find themselves in trouble. Understanding the mechanisms of variable susceptibility is key to identifying high-risk mother-infant pairs.

Susceptibility to phenytoin. Clinical experience with the anticonvulsant drug, phenytoin, provide examples of the interplay between variable phenotype and susceptibility. The pattern of anomalies observed with maternal exposure to phenytoin include craniofacial dysmorphism, growth deficiencies, limb defects, and developmental delay. The characteristic facial features associated with phenytoin exposure are a short nose with anteverted nostrils, a long philtrum, and a characteristically bowed upper lip. The nasal bridge is broad and depressed, giving rise to redundant innercanthal skinfolds. Women exposed to phenytoin during pregnancy have a twofold to threefold increased risk of having a child with a birth defect. This assessment of risk is based on thousands of births recorded in epidemiologic studies, both prospective and retrospective.^{5,6}

Genetic sensitivity is believed to play an important role in anticonvulsant drug-induced teratogenesis.⁷ For example, certain individuals who have mutations in specific biochemical pathways that alter the metabolism of the AEDs may be at increased risk after maternal exposure. These individuals may have abnormal forms of the genes that regulate enzyme activity, either by controlling the quantity of enzyme produced or by determining its functional activity.

AEDs are biotransformed to oxidative metabolites such as epoxides. These products are highly reactive and

capable of binding covalently to large molecules such as nucleic acids. If this occurs during critical periods of embryogenesis, it could result in problems in DNA repair mechanisms or in the preferential degradation of RNA and proteins. The resulting alterations in normal cellular housekeeping functions could lead to abnormal fetal development (as shown in Fig 1).

The best evidence for genetic susceptibility to AEDs was obtained from a study investigating the use of the detoxification enzyme, epoxide hydroxylase (EH), as a biomarker to indicate which AED-exposed pregnancies are at increased risk for an adverse outcome.⁸ The study revealed a trimodal distribution of EH activity in the general population. For the study, normal EH levels were defined as 40% of control activity, intermediate levels were 40% to 75% of control activity, and high levels were more than 75% of control activity. Significantly lower levels of EH activity ($P < .05$) were observed in individuals with fetal hydantoin effects. In a random sample of 100 fetuses not exposed to AEDs, the average EH activity (mean $\pm$ SEM) was $51.7\% \pm 2.5\%$ of the standard, whereas the activity of this enzyme in AED-exposed individuals with the fetal hydantoin phenotype was $23.9\% \pm 1.6\%$ of the standard.⁷

An ongoing prospective study is monitoring the potential relationship of low EH levels (less than 40% of standardized control activity) to congenital malformations after in utero AED exposure. Microsomal EH activity is measured in amniocytes from mothers with epilepsy treated with AEDs. These patients had been referred for amniocentesis because of advanced maternal age and not because of drug use or EH status. Dysmorphology evaluations are performed after birth. Although the dysmorphologists are aware of the AED exposure, they are not aware of the patient's EH status.

At least 73 AED-exposed pregnancies have been monitored to date, with low EH levels determined for 7 fetuses. Of these, 6 infants were diagnosed with the features of fetal hydantoin syndrome. The remaining low EH pregnancy was terminated because of a cytogenetic defect. Intermediate EH activity has been determined for 43 pregnancies, and 25 infants born to date do not show any of the classic signs of fetal hydantoin syndrome. All infants born to date to mothers with normal EH activity are healthy.

Epoxide hydrolase may have a role in AED-induced congenital birth defects, but given the current understanding of teratogen-induced birth defects, EH is probably not solely responsible for determining susceptibility to all of these defects. Factors unknown at this time may determine individual differences in the disposition of the teratogenic agent. Because metabolic processes and pathways are ultimately directed by the individual's genotype, it is clear that genetic differences—maternal, fetal, or both—contribute to differential susceptibility to teratogen-induced anomalies and their ultimate phenotypic expression. In the future it may be possible to identify individuals who are genetically sensitive to AED-induced birth defects by charac-

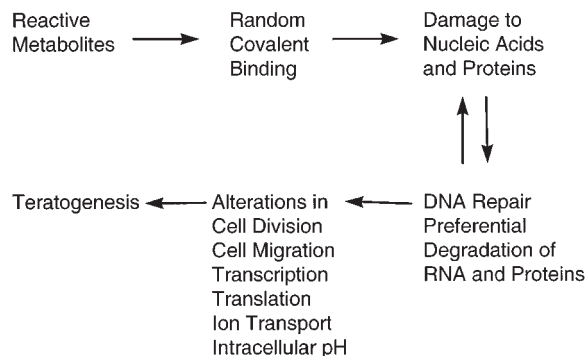


FIG 1. Pathway for teratogenesis with reactive metabolites.

terizing a genetically determined abnormality in some metabolic pathway.

Thus it is the interaction of the teratogen with a susceptible genotype that ultimately determines the degree of fetal sensitivity and the extent of the phenotypic expression. The importance of recognizing these differences in susceptibility is critical when it comes to interpreting the existing risk data.

Principle 2: Susceptibility to a teratogenic agent varies with the developmental stage at which the exposure occurs

It is a basic biologic precept that developing organisms are more sensitive to change than are mature, fully developed organisms. This implies that heightened susceptibility extends throughout the developmental span of the embryo, although not necessarily at a constant rate.

To relate this principle to human development, it is important to briefly review human embryology. After fertilization, but before implantation, there is a period during which the zygote divides but does not grow larger (the pre-implantation period). At this time, all of the cells of the embryo are totipotent (ie, all cells have the potential to differentiate into any tissue), so loss of certain cells will not induce any particular defect. The embryo implants in the uterus as a blastocyst with 2 layers of inner cell mass. Some cells will contribute to the embryo proper, whereas others will contribute to the development of the extraembryonic membranes. Neurulation, occurring from weeks 3 to 5, represents an important developmental landmark. Development and closure of the neural tube does not occur in isolation but with concurrent development of other systems. Once the neural tube closes, it continues to develop, undergoing vesiculation and ultimately producing the 5 regions of the brain and spinal cord. The period of organogenesis, occurring between weeks 3 and 8, is characterized by the segregation of cell groups into primordia that will form the future organs. Histogenesis begins before organogenesis is completed and continues essentially beyond parturition.

The exposure of a developing fetus to a teratogenic insult at different gestational stages results in different patterns of malformation. During the pre-implantation

TABLE II. Proposed mechanisms for teratogenic pathogenesis

Excessive apoptosis
Reduced apoptosis
Reduced biosynthesis
Impeded morphogenetic movements
Mechanical disruption of tissues

period, the effects of teratogenic insult might result in an all-or-nothing effect: either the exposure is sufficient to kill the embryo (which is then lost before it was ever recognized) or the embryo survives, implants, and proceeds to normal (if not somewhat delayed) structural development. Organogenesis is the period of peak sensitivity to teratogenic insult. The more specialized needs of the differentiated cells make them more susceptible to deprivation or damage. As the more basic organizational patterns of organ formation are completed, the likelihood of a teratogen-induced structural change decreases, and gross malformations are no longer likely to be observed. Weeks 3 to 5 are most critical for central nervous system development, although the central nervous system remains sensitive throughout the remainder of development (as maturation occurs and neuronal synapses are formed). Similarly, weeks 7 to 9 are the critical period for urogenital development. Harmful influences during histogenesis would not result in gross malformations but instead would be at the finer, microscopic levels, which could compromise normal, functional development. Finally, during the fetal period (beyond week 8 after conception), effects are most likely to be on growth or functional maturation and may continue after birth (which is merely an interruption in the transition from fetal to infantile life). Myelination in the nervous system, for example, continues for at least 2 years after birth.

Having an appreciation for these developmental time frames is important when considering potential drug therapy during a given gestational period. Given the suspected target tissues and mechanisms of action, there may be better times to use one drug as opposed to another.

Principle 3: Teratogenic agents act in specific ways (mechanisms) on developing cells and tissues to initiate abnormal embryogenesis (pathogenesis)

The term *mechanism* refers to the early, and often the first, event in a series of intervening events between cause and an effect. The initial event is possibly the most important of the series, not only because it is the connecting link between the cause and the physiologic changes that follow but also because it very likely influences the nature of these latter changes.

Some of the proposed mechanisms for teratogenic agents are listed in Table II. In general, the changes that result from teratogenic exposures are not necessarily specific to the causative factors. For example, ionizing radiation may initiate mutation, chromosomal aberrations,

mitotic interference, or enzyme inhibition. Similarly, a nutritional deficiency most often leads to a lack of precursors and substrates for biosynthesis but could conceivably result in altered energy sources or osmolarity imbalances.

Teratogen-induced mutations result in abnormal structural and functional defects. Ionizing radiation is an example. Mitotic inhibition is better established as a mechanism of teratogenesis than most. It can occur in a variety of ways, including slowing or arresting DNA synthesis, and may explain the occurrence of neural tube defects associated with valproic acid treatment.

Altered nucleic acid integrity or function is another mechanism by which teratogens can exert their effects. Alteration of nucleic acid integrity or function during critical periods of development may induce congenital malformations. This mechanism may play a significant role in phenytoin-induced teratogenesis.

The teratogenic effects may be due to lack of precursors and substrates needed for biosynthesis. Nutritional deficiencies, especially of vitamins and minerals, have repeatedly been shown to be teratogenic. It is often wrongly believed that the embryo will receive ample supplies of scarce materials at the expense of the mother, and therefore signs of deprivation will be observed in the mother before fetal effects occur. However, the embryo has often been shown to manifest deficiency by death or abnormal development before the mother has shown signs of deprivation. Deficiency of essential materials in the embryo can also occur despite an apparent abundance in the maternal diet. The presence of analogs or antagonists to vitamins, essential amino acids, or purines may result in the utilization of these spurious metabolites instead of the normal precursors. Additionally, the normal precursors may not be absorbed from the maternal digestive tract, or they may fail to cross the placenta. Failure of placental transport of essential metabolites is now recognized as the cause of the teratogenic effects observed with azo dyes.⁴

Interruption of the supply of energy sources to the embryo is believed to be teratogenic. Inadequate energy supply may result from inadequate glucose sources, interference with glycolysis or the citric acid cycle, or even impairment of terminal electron transport, as in hypoxia-induced teratogenesis.

Enzymatic functions, which are ubiquitous in all aspects of differentiation and growth, are obvious targets for teratogenic effects. Examples of teratogens acting through this mechanism include folic acid antagonists such as phenytoin and valproic acid and drugs such as aminopterin or methotrexate that cause a deprivation of dihydrofolate reductase, which can produce developmental defects.

These primary mechanisms of teratogenesis ultimately result in a few pathogenetic changes: apoptosis, impeded morphogenetic movements, and mechanical disruption of tissues. These changes produce the abnormal phenotype through a process that is not fully understood or defined.

Principle 4: The final manifestations of abnormal development are death, malformation, growth retardation, and functional disorder

The possible consequences of abnormal development do not occur with equal probability and are most likely associated with the timing of exposure relative to fetal development. Although any one, or even all, of these outcomes could occur if sufficient quantities of an embryotoxic agent were introduced at times of high sensitivity, certain manifestations are more likely to occur at particular stages.

As previously mentioned, the pre-implantation embryo is refractory to malformations. If the dosage is of sufficient strength, then the embryo will die before the pregnancy is recognized.

After organogenesis begins, malformations of a specific organ or system reflect the special sensitivities and needs of rapidly differentiating and growing tissues. This general elevation of sensitivity during organogenesis also makes the embryo susceptible to death. Functional deficits would not ordinarily be expected to result from teratogenic exposure during organogenesis. Teratogenic insult during histogenesis and functional maturation during the fetal period would be expected to result in structural defects at the tissue level, functional loss, or both. Furthermore, a general cell necrosis, at a level lower than that needed to cause death, could slow the overall growth proportionate to the time required to replenish the lost cells. Hence, growth retardation may occur as a result of late fetal exposures to teratogens.

Although these 3 manifestations of teratogenesis appear to be discrete, they may be interrelated to varying degrees. Death may occur with or as a result of severe malformation, growth retardation, or a generalized breakdown of essential functions.

The fourth principle of teratology is well demonstrated by the data obtained from pregnancies exposed to Accutane. Exposure to Accutane results in a 40% spontaneous abortion rate among exposed embryos. Of the remaining 60%, there is a 25% risk for a major malformation and an additional 52% risk for an impairment in cognition or development (E. J. Lammer, oral communication, September 9, 1995.) These are all valid, teratogenic end points.

Principle 5: Access of an adverse environmental agent to developing tissues depends on the nature of the agent (influences)

Not all teratogens reach their target (the embryo) in the same fashion. Physical agents such as x-rays, microwaves, and ultrasound pass unaltered through the mother into the uterus and have direct access to the fetus. Alternatively, agents that are consumed by the mother, such as drugs, are subject to her metabolism before having access secondarily to the fetus. As a result, chemicals or drugs usually reach the fetus in some fraction of their concentration in the maternal serum. Whether they reach

the fetus in sufficient quantity to cause problems is dependent on a variety of parameters.

Important factors to consider in determining whether a substance will reach the fetus include maternal dose, route of entry, physical properties (solid, liquid, or gas), and rate of absorption into the systemic circulation. The maternal system is equipped with detoxification enzymes for xenobiotic substances, which reduce the amount of the "parent compound" that actually reaches the fetus (although the metabolites themselves may have teratogenic properties).

Placental transfer is also an important factor. The placenta is not an absolute barrier, because virtually all unbound small molecules in the maternal plasma have access to the conceptus across the placenta. Molecules with molecular weights less than 600 kd and of low ionic charge cross the placenta by simple diffusion. Others cross by facilitated diffusion and active transport. The important question to consider is not whether the molecule will cross the placenta but rather at what rate the molecule will cross. The total dose of a chemical reaching the conceptus is a product of the interaction of many variables (eg, maternal functional capacity, chemical nature of the compound, and placental transport).

Principle 6: The manifestations of deviant development increase in degree as dosage increases from the no-effect to the lethal level

There is a dose-response relationship for teratogens and teratogenic activity, as there is for drugs and therapeutic effect. It is important to consider this principle as it relates to thresholds for various toxicologic effects.

For regulators attempting to define drug or product safety, the no-effect or threshold level for adverse effects is considered to be a critical benchmark. Is there a certain dose that does not cause adverse effects? If so, does this dose have therapeutic efficacy? If a certain dose produces adverse effects, an increase or decrease in the dose should increase or decrease the occurrence and risk of adverse outcomes.

In typical studies investigating teratogenicity, in which death and malformations were the end points for adverse effects, no-effect levels have usually been found when a suitable range of dosages has been used. The same is likely to be true for growth retardation. However, in terms of functional deficits caused by teratogen exposures, there is uncertainty related to a lack of information in the literature. Experience with teratogens indicates that all manifestations of abnormal development begin to be expressed only when the dosage exceeds a demonstrable threshold. Conversely, a lower range of dosage exists at which no embryotoxic effects occur.

SUMMARY

A teratogen is any plant, food, nutritional state, or physical agent that can compromise normal fetal development and result in the production of a congenital mal-

formation. Teratogenesis, in its simplest terms, is the destruction of a critical number of cells in excess of which the fetus is able to restore by later proliferation. In considering the effects of drugs on pregnancy, it is important to remember the 6 principles of teratology: genetic susceptibility, development stage, mechanisms, end points, access, and dose response. Keeping these principles in mind will facilitate critical review of the literature that is relevant to pregnancy management.

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