TERATOGENESIS

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Objectives

- Process of teratogenesis
- Characteristics of teratogenic agent
- Critical period of teratogenesis
- Etiology of teratogenesis
- Famous examples of teratogenic drugs
- Molecular Mechanisms
Birth defects are known to occur in 3-5% of all newborns.

10% of all birth defects are caused by a prenatal exposure or teratogen.

50% of pregnant women have been exposed to at least one medication during gestation, half of them were exposed to a potential teratogen.

3% of all live-born infants have a major birth defect, whereas single minor defects are present in about 14% of newborns.
Definitions

- **Teratology** is the science that studies the causes, mechanisms, and patterns of birth defect.

- **Teratogenesis** is a process with threshold-level effect which are of clinically significant types: malformation, disruption, and deformation.

- **Teratogenicity** is a manifestation of developmental toxicity representing a particular case of embryo/fetotoxicity, by the induction or the increase of frequency of structural disorders.
Definitions

A **teratogen** is an exogenous agent that can produce a permanent alteration of structure or function in an organism exposed during embryonic or fetal life.
Types of Birth Defects

- **Malformation** is a primary structural defect resulting from a localized error of morphogenesis – may be gross or microscopic, on the surface of the body or within it, single or multiple.

- **Disruption** is specific abnormality that results from disruption of normal developmental processes. It depends on time not on agent.

- **Deformation** is an alteration in shape / structure of previously normally formed organ/tissue/part.
Periods of Teratogenesis

- **Fertilization-to-postimplantation Period**: low susceptibility to malformations
- **Organogenesis Period** (3rd – 8th week of gestation in humans): greatest sensitivity to teratogenic agent and peak susceptibility for malformation
- **Fetal Period**: exposure to teratogenic agent after organ and tissue differentiation leads to functional mutations and fetal death
Embryonic/Fetal Critical Periods

- Origination of organs
- Implant.
- Fert
- Sensitivity
- Formation of tissues from undifferentiated cells
- Organogenesis
- Histogenesis
- Functional Maturation
- Embryonic
- Fetal
- Birth
Etiology of Human Birth Defect

Unknown Genetic Environmental

- Unknown etiology: 50-60%
- Multifactorial inheritance: 7-10%
- Chromosomal abnormalities: 7.8%
- Mutant genes: 6-7%
- Environmental agents: 20-25%
# Etiology of Human Maldevelopment

<table>
<thead>
<tr>
<th>Suspected cause</th>
<th>Percent of total</th>
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<tbody>
<tr>
<td><strong>Genetic</strong></td>
<td></td>
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<tr>
<td>Autosomal genetic disease</td>
<td>15–20%</td>
</tr>
<tr>
<td>Cytogenetic (chromosomal abnormalities)</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
<td></td>
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<tr>
<td>Polygenic</td>
<td>65%</td>
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<tr>
<td>Multifactorial (genetic-environmental interactions)</td>
<td></td>
</tr>
<tr>
<td>Spontaneous errors of development</td>
<td></td>
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<tr>
<td>Synergistic interactions of teratogens</td>
<td></td>
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<tr>
<td><strong>Environmental</strong></td>
<td></td>
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<tr>
<td>Maternal conditions: diabetes, endocrinopathies, nutritional deficiencies, drug and substance addictions</td>
<td>4%</td>
</tr>
<tr>
<td>Maternal infections: rubella, toxoplasmosis, syphilis, herpes, cytomegaloctic inclusion disease</td>
<td>3%</td>
</tr>
<tr>
<td>Mechanical (deformations): abnormal uterus, amniotic bands, umbilical cord constrictions, disparity in uterine size and uterine contents</td>
<td>1–2%</td>
</tr>
<tr>
<td>Chemicals, drugs, radiation, hyperthermia</td>
<td>&lt;1%</td>
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FDA Pregnancy Category

A - Adequate, well-controlled studies in pregnant women fail to demonstrate a risk to the fetus in any or all trimester(s).

B - Animal studies do not indicate a risk to the fetus; however, there are no adequate, well-controlled studies in pregnant women. OR Animal studies have shown an adverse effect on the fetus but adequate, well-controlled studies in pregnant women have failed to demonstrate a risk to the fetus.

C - Animal studies have shown that the drug exerts teratogenic effects, and there are no adequate, well-controlled studies in human, OR No studies are available in either animals or humans.

D - Positive evidence of human fetal risk exists, but benefits in certain situations (eg, life-threatening situations or serious diseases) may make use of the drug acceptable despite its risks.

X - Studies in animals or humans have demonstrated fetal abnormalities. Contraindicated in pregnant women.
Placental transfer of drugs dependant upon:

- **Protein binding**: Protein-bound medications cross with difficulty - e.g. bupivicaine

- **Lipid solubility**: highly lipid-soluble drugs cross with ease; water-soluble do not cross unless actively transported – e.g. vitamins

- **Degree of ionization**: highly polar or ionized substances do not cross the placenta readily e.g. succinylcholine
1. Thalidomide

EXAMPLES

◆ Sedative/hypnotic introduced in 1960 for treatment of nausea during 1st trimester.

◆ Appearance of newborns in West Germany with phocomelia.

◆ Drug withdrawn in mid-1962; 5850 cases of malformations
2. Alcohol

- Known as Fetal Alcohol Syndrome
- Craniofacial abnormalities, CNS dysfunction, postnatal growth retardation.
- Most severe effects in children born to alcoholic mothers (25 per 1000).
- Mechanism of toxicity poorly understood; thought to involve cell death and inhibition of cell migration during early pregnancy.
3. Tobacco Smoke

Examples

- Leading cause of environmentally induced developmental disease and morbidity
- Spontaneous abortions, behavioral and attention deficit disorders, and lower birth weight
- Nicotine can by itself produce many of the adverse effects of tobacco smoke
Vitamin A (retinol) and retinoids (used in treatment of acne) are long known to produce malformations.

Effects include malformations of face, limbs, heart, CNS and skeleton.

Mechanism of toxicity mediated:
- Activation of nuclear receptors, retinoic acid receptors (RARs) & retinoid X receptors (RXRs)
- Activation of homeobox (Hox) genes which direct embryonic pattern development by retinoids at inappropriate times
4. Retinoids

RXR Signaling Activation
5. Radiation

- Radiation represents a possible teratogen for the fetus.
- Such as x-rays, γ-rays, and UV.
- Growth retardation, eye malformation, and CNS defect are reported at Hiroshima.
- The risk is dependent on dosage and gestational stage.
UV light is absorbed by DNA adjacent thymine bases on the same DNA strand to covalently bond together → thymine-thymine dimers → nucleotides do not complementary base pair with the thymine dimers → terminates the replication of that DNA strand.
Ionizing radiations (x-rays and $\gamma$-rays)

- High energy & powerful penetration
- Ionize water within all aqueous solution to generate highly reactive radicals ($\cdot$OH) $\rightarrow$ abstracting $\cdot$H at various places on the helix $\rightarrow$ break DNA strands.
5. Radiation

RADIATION DAMAGE TO DNA

- H2-Bond Breakage
- Double-Strand Break
- Pyrimidine Dimer
- Base Loss
- DNA Cross-Linkage
- Base Change
- Cross-Linkage
- Protein Cross-Linkage
- Single-Strand Break
Characteristics of teratogenic agents

- **Stage Sensitivity**

- **Dose-response relationship**
  - ↑ dosage, frequency, severity, and duration

- **Genetic differences**
  - Placental transport, metabolism, distribution
Placental transfer of drugs dependant upon:

- **pKa of substance**: most compounds are weak bases. pKa ~ 8.0 thus more ionized in fetal blood stream [pH fetal blood 7.3 pH maternal blood 7.4] predisposing to trapping of drug onto fetal side of circulation.

- **Molecular weight of substance**: <600 cross placenta; >1000 do not cross – *heparin*.

- **metabolism**: Maternal, placental and fetal.
Wilson’s General Principles of Teratology

- Susceptibility to teratogenesis depends on genotype of offspring and the manner in which it interacts with adverse environmental factors.
- Susceptibility varies with the developmental stage at the time of exposure.
- Teratogenic agents act via specific mechanisms on developing cells/tissues.
- Access of teratogen to developing tissues depends on the nature of the agent.
- Four manifestations: death, malformation, growth retardation and functional deficit.
- Manifestations increase in frequency and degree in direct proportion to the dose of the agent.

Wilson’s General Principles of Teratology
Factors That Influence Teratogenicity

- Gestational timing
- Concurrent exposures
- Concurrent illness
- Genetic susceptibility
  - Mother
  - Fetus
Principal Mechanisms of Teratogenesis

- Cell growth or proliferation
- Cell death
- Cell migration
- Cell and tissue interactions
- Disruptions
Principal Mechanisms of Teratogenesis

- Mutagenesis
- Mitotic Interference
- Nucleic Acid Alteration
- Nutritional Deficiency
- Enzyme Inhibition
- Osmolar Imbalance
- Others
Birth Defects in Childhood

- Teratogens: 10%
- Multifactorial: 42%
- Unknown: 37%
- Monogenic: 8%
- Chromosomal: 3%

Baird et al. AJHG 42:677, 1988
Mutagenesis

◆ Principal mechanisms
  – Gene mutation
  – Chromosomal abnormalities
Mutation

- **Mutation** is the mechanism by which nucleotide sequence in DNA strands is altered.

- An error during DNA replication that results in a change in the sequence of deoxyribonucleotide bases in the DNA. For example, substitution of the nucleotide pair AT by GC, CG, or TA.

- There are two main types of mutation:
  - **Spontaneous**: occurs naturally in 1:10^6
  - **Induced**: occurs in higher rate and caused by mutagens.
Mutation

- **Mutagens** are more likely teratogenic due to their cytotoxic effects, which are related to cell destruction, and to genetic changes that persist and affect embryonic development for many cell cycles in the developing embryo.

- may cause cell death, retardation of differentiation, or mitotic delay.

- If germ cell mutation $\rightarrow$ heritable, whereas for somatic cell mutation $\rightarrow$ not heritable $\rightarrow$ developmental defect
Chemical mutagens generally work in one of three ways:

1. causing chemical modifications of purine and pyrimidine bases.
   - alter their hydrogen-bonding properties.
   - For example, nitrous acid converts cytosine to uracil which then forms hydrogen bonds with adenine rather than guanine.
Mutation

2. Working as a base analog.
   - resemble a nucleotide base closely enough that during DNA replication, they can be incorporated into the DNA in place of the natural base.
   
   - Examples: 2-amino purine, a compound that resembles adenine, and 5-bromouracil, a compound that resembles thymine.
   
   - However, do not have the hydrogen-bonding properties of the natural base.
Mutation

3. intercalating between adjacent base pairs.

- thus pushing the nucleotides far enough apart that an extra nucleotide is often added to the growing chain during DNA replication.

- they are planar three-ringed molecules that are about the same size as a nucleotide base pair.

- An example is ethidium bromide.
Correction of a mutation by gene repair

- corrective vector carrying genomic DNA with wild-type sequence undergoes homologous recombination with the mutant gene, resulting in the correction of the genetic mutation.
Chromosomal Aberrations

- Chromosomal aberrations are disruptions (gain or loss) in the normal chromosomal content of a cell.

- Chromosomal abnormalities are a major cause of genetic conditions in humans.

- Some chromosome abnormalities do not cause disease, such as chromosomal inversions, however, abnormal numbers of chromosomes may be lethal or cause genetic disorders.
Types of Chromosomal Aberrations

1. Numerical chromosomal abnormalities
   - Usually non-disjunction- error in cell division
   - Examples: Down syndrome, Edwards syndrome.

2. Structural chromosomal abnormalities
   - chromosomal breaks: deletion, inversion, duplication, translocation, and insertion.
   - Ex. Cri du chat syndrome, Wolf-Hirschhorn syndrome
Numerical Chromosomal Abn.

1. Down syndrome

- It is one of the most frequently occurring chromosomal abnormalities found in humans occurring 1: 800 to 1,000 live births.

- Is a numerical type of chromosomal abnormalities

- is caused by an extra copy of chromosome 21 (trisomy 21).
Numerical Chromosomal Abn.

1. Down syndrome

– Characterized by:
  » decreased muscle tone, stockier build.
  » asymmetrical skull
  » slanting eyes
  » mild to moderate mental retardation
Numerical Chromosomal Abn.

2. Edwards syndrome

- is the second most common numerical chromosomal abnormalities after Down syndrome, defect in chromosome 18.

- 90% die in infancy; however, those who live past their first birthday usually are quite healthy thereafter.

- Characterized by:
  » mental and motor retardation
  » numerous congenital anomalies.
  » clenched hands and overlapping fingers.
Structural Chromosomal Aberrations

1. Deletion

- A deletion mutation is where genetic material is deleted from a gene, or entire genes are deleted.

2. Inversion

- rearrangement in which a segment of a chromosome is reversed end to end within itself.
- lead to a higher chance of having a child with a chromosome disorder.

3. Duplication

- an extra chromosomal segment within the same homologous chromosome or on another.
- Clinical findings are variable depending on chromosomal segments involved.
Structural Chromosomal Aberrations

4. Translocations
- two chromosomes swap pieces of their arms.
- a segment of chromosome 4 becomes attached to chromosome 20 and vice versa.
- estimated that 1 in 500 individuals
5. Insertion

- An insertion is when one portion of a chromosome is inserted into another.

- Genetic material is not swapped, it is just moved to another chromosome.

- A segment of chromosome 4 becomes attached to chromosome 20 and vice versa.
Structural Chromosomal Abn.
1. Cri du chat syndrome

– Cri du chat means "cry of the cat", babies make high-pitched cries that sound like a cat.

– caused by the deletion of part of the short arm of chromosome 5.

– Characterized by:
  » wide-set eyes.
  » small head and jaw
  » Moderate-to-severe mental retardation.
Numerical Chromosomal Abn.

2. Wolf-Hirschhorn syndrome

- caused by partial deletion of the short arm of chromosome 4.

- characterized by:
  » severe growth retardation
  » severe to profound mental retardation.
Detection

- Karyotyping can be done from blood, hair, or any other tissue.

- Most karyotyping for medical diagnostic purposes is done on embryonic or fetal cells from unborn babies still in the uterus.

- The cells are usually collected by one of two methods:
  - amniocentesis
  - chorionic villi sampling
Detection

1. Amniocentesis

- is a procedure used to diagnose fetal defects in the early second trimester of pregnancy

- involves sampling the liquid immediately surrounding a fetus (amniotic fluid).

- The amniotic fluid contains fetal urine and millions of fetal skin cells that can be cultured to produce a karyotype and many types of genetic disorders
Detection

1. Amniocentesis

- discover the presence of about 400 specific genetic abnormalities in a fetus

- usually is done in the 2nd trimester, because only at this point in a pregnancy there is sufficient amniotic fluid to allow some of it (about 20 cc) to be drawn off without significant danger to the fetus

- **Example:** $\alpha$-fetoprotein blood levels may indicate fetal defects.
Detection

3. Chorionic villus sampling

- CVS is a form of prenatal diagnosis to determine chromosomal or genetic disorders in the fetus.

- The advantage of CVS is that it can be carried out 10-13 weeks after the last period, earlier than amniocentesis.

- Limitations:
  » Miscarriage
  » Oligohydramnios
  » Maternal Cell Contamination
Detection

3. Blood Tests

– testing pregnant’s blood for α-fetoprotein

– significantly less expensive and has no risk of causing a miscarriage

– information gained is less reliable in predicting a chromosomal abnormality

– Results should be confirmed with other Amniocentesis.

- environmental agent may directly alter embryonic gene transcription.

- by acting as ligands for several transcription factors.

- These proteins mediate their effects by binding specific DNA elements located in promoter and enhancer elements of target genes, and so they modulate the actions of RNA polymerase II.

- Examples: 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (TCDD)
TCDD-Gene Expression Abn.

- Carcinogenesis
- Teratogenesis
- Poisoning of Dioxin
- Immunosuppression
- Endocrine disruption
- Lipid peroxidation

AhR ligands (TCDD)

Nucleus

Cytoplasm

Hepatocyte

AhR

ARNT

Cyp1a1 gene

mRNA

Activity

Cyp1a1 protein

Ribosome
Treatment of murine embryonic stem cells (ES) with RA resulted in a significant expression of Oct-4 at day 6–10, however the gene expression from day 0–4 did not demonstrate a significant alteration in comparison to the control. No significant modification in Oct-4 expression was observed for LiCl treatment with respect to the control.
3. Gene Expression Abnorm

Tratogens could modulate gene expression through interfering with:

– nucleic acid replication,
– translation.
– Translation
4. Oxidative Stress

- Recent studies showed that several developmental defect may result from endogenous or exogenous sources of reactive oxygen species (ROS).

- ROS production, DNA damage, and ROS-mediated signal transduction are important determinants of teratogenesis.

- Down-regulation of some cytoprotective genes, NAD(P)H:quinone oxidoreductase (NQO1), glutathione transferase (GST) is another factor.
4. Oxidative Stress

ANTIEPILEPTIC DRUGS → Elimination
USP-glucuronosyltransferase

Bioactivation
PHS, LPO, P450

Reactive Intermediates:
Electrophiles
Free Radicals/ROS

Detoxification
GSH & Epoxide Hydrolase

Cytoprotection
GSH, SOD, Catalase, G6PD

Molecular Damage
DNA, RNA, Lipids, Proteins

Repair
p53

TERATOGENESIS
5. Enzyme Inhibition

- Inhibition of specific enzymes is thought to be primarily involved in initiating abnormal embryogenesis.

- It interferes with some essential metabolic activities results in immediate embryonic death.

- Examples of these enzymes:
  - Dihydrofolate reductase
  - Thymidylate synthetase (e.g. 5-fluorouracil)
  - Carbonic anhydrase
  - Histone deacetylase
  - glucose-6-phosphate dehydrogenase (e.g. 6-aminonicotinamide)
5. Enzyme Inhibition

- **Dihydrofolate reductase (DHFR)**
  - DHFR converts dihydrofolate into tetrahydrofolate, required for the de novo synthesis of purines, thymidylic acid, and certain amino acids.
  - DGFR deficiency has been linked to megaloblastic anemia and other fetal developmental defects.
  - Example of DHFR inhibitor is trimethoprim
5. Enzyme Inhibition

Histone deacetylase (HDAC)
- An enzyme responsible for reversible deacetylation processes of epsilon-amino groups of lysine residues present in the tail of core histones

- Inhibitors of histone deacetylase induce growth arrest, cell differentiation, and apoptosis of tumor cells
5. Enzyme Inhibition

- p53 is a transcription factor that regulates the cell cycle and hence functions as a tumor suppressor.
- p53 is acetylated HDAC and its cofactor CREB binding protein (CBP). Acetylation increases p53 stability and DNA binding activity. The end result is upregulated expression of p53-controlled genes, such as HTT.
6. Osmolar Imbalance

- Is a primary mechanism of teratogenesis by altering fluid pressures, viscosities, and composition in different compartments of the embryo.

- Embryos lack much of the homeostatic regulation available to fetus.

- Example: ethylenethioureia (ETU)
6. Osmolar Imbalance

◆ How:
- ETU exposure caused lower osmolality of the exocoelomic fluid (ECF) surrounding the embryo.
- Lowered osmolality would cause water to move out of the ECF
- Causing fluid accumulation in the embryo which leads to localized edema in the embryo.
- formation of birth defects
7. Lack of precursors

- Lack of precursors, substances, or coenzymes for biosynthesis leads to abnormal developments.

- This could be a result of:
  - Nutritional deficiency
  - Failure of absorption
  - Inadequate transport

- Insufficient supply of the anabolites needed for development.
7. Lack of precursors

- Certain teratogens can affect the energy supply for the metabolism of the fetal organisms by:
  - restricting the availability of substrates (e.g. dietary deficiencies)
  - presence of analogs or antagonists of vitamins, essential amino acids, and others.
7. Lack of precursors

- Hypoxia and agents inducing hypoxia (CO, CO2) are teratogenic by:
  - depriving the metabolic process of the required oxygen.
  - production of osmolar imbalances.
  - These can induce edema and hematomas, which in turn can cause mechanical distortion and tissue ischemia.
7. Lack of precursors

- **Zink Deficiency**
  - causes fetal malformation
  - may interfere with fatty acid metabolism, possibly by increasing the rate of lipid peroxidation.

- **Biotin Deficiency**
  - maternal biotin deficiency results in cleft palate, and limb hypoplasia.
  - Fetal biotin correlated significantly with maternal biotin status
  - biotin-dependent enzyme propionyl-CoA carboxylase is affected