DEVELOPMENTAL BIOLOGY

Teratology

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CONTENTS

Key words:

Terata, teratogen, congenital malformation, phocomelia, anencephaly microcephaly, hydrocephaly, Microphthalmia, Cleft lip, Cleft palate

INTRODUCTION

TERATOLOGY is the study of structural abnormalities that arise during embryonic development.

Embryonic development is a complicated process involving cell division, migration, interaction and differentiation. Any interference in these processes can result in a deviation from the normal which leads to chaotic development resulting in the formation of an abnormal embryo called 'a terata'or 'monster'. The word monster which comes from *Latin monstrate*, was earlier used frequently to describe malformed infants.

Monsters exhibit various abnormalities like disproportionate development of some parts, lack of certain parts like missing limbs or missing digit or chaotic arrangement of the body parts. Thus, monster or terata results due to congenital malformations. Formation of terata is called teratogenesis and the study of terata is known as teratology.

The study of teratology has interested both developmental biologists and physicians alike. To developmental biologists, the birth defects elucidate the normal mechanisms involved in embryogenesis.

Physicians became interested in this science because of its importance to human welfare. It has been estimated that about half the total number of human conceptions do not survive to be born. In many cases, the embryo fails to implant in the uterus because of early expression of abnormality. About 2 % of the human infants are born with some observable anatomical abnormality. Certain abnormalities like defect in lungs, digestive system or limbs and mouth, etc. are not deleterious to the fetus because while developing, the fetus does not depend on these but after the birth of the baby these malformations can threaten its life.

Therefore, to investigate the causes of the birth defects, and counseling the parents of the risk of having malformed infants becomes an important task of the physician. The field of teratology is now moving to a more molecular level seeking mechanisms of action by which the various factors cause abnormal development.

CAUSATION OF ABNORMAL DEVELOPMENT

Abnormal development may be caused by errors in genetic programming, from environmental agents / factors or from unknown causes that interfere with development. About 7 % of all live birth defects are due to prenatal exposure to radiation, environmental factors, chemicals and drugs. Abnormalities caused by genetic events, e.g. mutation in genes, structural changes in chromosomes and aneuploidies, etc. are called malformations. Some of the malformations occurring in human populations are Down's syndrome,

Anencephaly (absence of fore brain), Hydrocephaly (enlarged head), Aniridia (absence of iris), Cardiac abnormalities, Cleft lip, Cleft palate and so forth.

Aniridia is caused by a mutation of the PAX 6 Gene (Grindley *et al* , 1995) whereas Down's syndrome is caused by trisomy of chromosome 21. Another human malformation called piebaldism is due to dominant mutation in a gene (KIT) on the long arm of the chromosome 4 (Halliban & Moellmann, 1993). The KIT gene encodes a protein that is expressed in the neural crest cells and in the precursors of the blood cells, and germ cells enable these cells to proliferate. In the absence of this protein, proliferation and migration of these cells is inhibited resulting in various abnormalities, like under pigmentation, deafness, gut malformations, anemia and sterility. In most cases, death of the early embryo is because of chromosomal abnormalities interfering with normal development.

Abnormalities caused by environmental agents are called disruptions. The environmental factors may be either biological (e.g. viruses and parasites) or non biological such as physical factors (e.g. temperature, radiation) and chemical factors (e.g. drug, chemicals and nutritional imbalances). The agents responsible for the disruptions are called teratogens. Mutagens and carcinogens also are the causes of abnormal development but their mode of action differ. Teratogens are agents that affect the embryo at dose levels. They are harmless to adult organisms and do not permanently damage the genetic material. On the other hand, mutagens are agents that alter the genes, whereas carcinogens are agents that lead to excessive growth and loss of differentiation, generally in adult tissue.

SENSITIVE PERIOD OF TERATOGENS

The effect of teratogens on the embryo is dependent upon the dose as well as the period of embryonic development in which the fetus encounters the teratogens. Most teratogens produce their effects only during certain critical period of development. The most critical time for any organ is when it is growing and forming its structures. For many human organs, the sensitive period lies between day 15 to day 60 of gestation.

Critical period of different organs is different, e.g. heart is sensitive during weeks 3 & 4 whereas the critical period of external genitalia is during weeks 8 & 9. There are certain organs, which are always sensitive like brain and skeleton for which the critical period is from week 3 to the end of pregnancy.

TERATOGENIC AGENTS

Exposure of developing fetus to teratogens causes congenital abnormalities. Understanding how a teratogen causes its effect is not only important in preventing congenital abnormalities but also has the potential for developing new therapeutic drugs safe for the use of pregnant mothers. Teratogenic agents occur naturally in the environment as well as due to agricultural, pharmaceutical, and industrial manufacturing. The largest category of teratogens being the drugs and chemicals. A list of teratogenic agents causing congenital malformations is given in table 1.

Table 1 : A list of some teratogenic agents causing birth defects

Natural Teratogens
Some poisonous plants like Skunk cabbage veratrum Ionizing radiations
Pharmaceutical Teratogens
Thalidoamide Tetracycline Streptomycin Valproic acid Warfarin Diethylstilbestrol Retionic acid Pencillin

Industrial Teratogens
Lead Methyl mercury Cadmium Arsenic
Microbial Teratogens
Toxoplasma gondii (toxoplasmosis) *Treponema pallidum* (syphilis) Coxsackie virus Herpes simplex Rubella (German measles) *Cytomeagalovirus (CMV)*
Recreational Teratgoens
Alcohol Tabacco (cigarette smoke) Cocaine Heroin
Metabolic conditions in the mother
Diabetes Auto immune disease (including Rh incompatibility) Phenylketonuria Dietary deficiencies, malnutrition

NATURAL TERATOGENS

1. Chemicals

Certain chemicals naturally occuring in some plants can be highly teratogenic. Skunk cabbage (*Veratrum californicum*) growing in the pristine alpine meadows of the rocky mountains can cause severe abnormalities in the fetus of sheep or cattle feeding on this plant. Birth defects include neurological damage and cyclopia (the fusion of two eyes in the centre of the face). Two chemicals produced by this plant jervine and cyclopamine inhibit cholesterol synthesis in the developing fetus and prevent sonic hedgehog from functioning , thereby causing severe brain defects including the lack of Pituitary gland. Another plant product quinine can also cause congenital malformation such as deafness.

2. Ionizing radiation

Excessive amount of radiation are teratogenic. They cause chromosomal fragmentation, alter DNA structure leading to mutations. Exposure of the fetus to radiation also causes impaired cell division, cell death and malignancy. A small dose of radiation (10 rads) will most likely kill preimplantation embryos.

X – ray radiation was used to induce abortions in early 1900's. A single dose of 360 rads is enough to kill a fetus before the 14^{th} week of gestation. Some of the teratogenic effects of radiation include defects in the brain and eyes like microcephaly, hydrocephaly, microphthalmia, optic atrophy and cataracts. Skeletal, visceral and genital abnormalities are less frequent. Small doses of radiation may induce mutations of germ cells.

PHARMACEUTICAL TERATOGENS (Therapeutic drugs as teratogens)

Drugs that are used to control diseases in adults may have deleterious effects on fetuses.

1. Thalidomide

Thalidomide was prescribed as mild sedative to many pregnant women during late 1950's. In early 1960's approximately 7000 abnormal infants were born to pregnant women in Scotland who took thalidomide during pregnancy. In 1961 Lenz & McBride independently discovered that thalidomide was a human teratogen. It was then withdrawn from the market in Nov 1961. The most noticeable feature of fetuses exposed to this drug was phocomelia, a condition in which the long bones of limbs are either greatly reduced in length or absent. Even a single tablet could cause severe damage to the developing limbs. Other abnormalities in the affected children include malformed intestine, hearing defects, absence of external ears and renal anomalies. The drug was found to be teratogenic only during 20 – 36 days post conception (34 – 50 days after last menstruation) , see Table 9.2. (Nowack,1965). Thalidomide affects different structures at different times of development. From day 34 to day 38 thalidomide causes the absence of ears.

Malformation of arms is seen earlier (from day 38 to 47) than legs (from day 41 to 47).

The mechanism by which thalidomide causes abnormalities in human fetus is still unknown. Many theories have been proposed. One theory suggests that neural crest cells are affected, another possibility includes a reduced size of the ganglia and their neurons as normal development of limbs needs proper nerve supply. Inhibited cell – cell interactions and reduced quantities of cell adhesion molecules may also affect proper limb growth. Thalidomide has again come in the market as potential anti- tumor and anti- auto immunity drug (Raje & Anderson, 1999).

Table 2 : Effect of thalidomide on different structures at different times of human fetus development.

Days after last menstruation	Defect
34 – 38	Absence of ear
38 – 42	Deformed / Absent thumbs
38 – 43	Absence of arms
38 – 47	Severe shortening of arms

38 – 48	Hip dislocation
39 – 43	Deformed ears
41 – 45	Absence of legs
42 – 47	Severe shortening of legs
46 – 50	Deformed thumbs

2. Retionic Acid

Retinoic acid is the active compound of drug Accutane that is used to treat severe cystic acne. This drug was introduced in the market in Sep 1982. Exposure to retionic acid during pregnancy results in malformed infants having craniofacial alterations, cleft palate, neural tube defects, cardiovascular malformations, absent or defected ears, thymic deficiencies, small jaw, kidney alterations and low I Q. The critical exposure time is between 3 – 5 weeks of pregnancy. The drug carries a label warning that it should not be used by pregnant women.

A study was conducted by Lammer and his co-workers in 1985 on a group of pregnant women who were exposed to this drug. Out of 59 fetuses 26 had no observable anomalies, spontaneous abortion took place in 12 cases and 21 malformed infants were born with hydrocephaly, ear malformations, cardiovascular defects and decreased IQ.

Retinoic acid is biologically active in two forms: all *trans* retionic acid and 9 – *cis* retionic acid. Vitamin A and 13 – *cis* retionic acid are converted to these biologically active forms. A proposed mode of its action is that biologically active retionic acid binds retionic acid receptors which in turn binds DNA enhancer elements like the retionic acid response elements. Several Hox genes responsible for early patterning of the embryo contain this enhancer element in their promoters. Thus Hox signaling may be altered due to increased retionic acid concentrations resulting in various congenital abnormalities. It has been shown that radioactively labeled retionic acid binds to the cranial neural crest cells and arrests both their proliferation and migration (Johnston *et al.* 1985). The critical exposure time of the drug is confined to a specific developmental period from days 20 to 35 in humans and days 8 to 10 in mice.

3. Diethylstilbesterol (DES)

DES increases estrogen and progesterone synthesis by placenta. This drug was used to prevent miscarriages in high-risk pregnancies. If DES is ingested early in pregnancy it results female children having vaginal and cervical carcinomas and uterine anomalies.

4. Valproic acid

Valproic acid is an anticonvulsant drug to control epilepsy. It was marketed in Europe in 1967 and in United States in 1978. If this drug is taken in higher doses during pregnancy, it acts as a teratogen causing major and minor bone defects. The affected children are born with lumbosacral spina bifida, mid facial hypoplacia, deficient orbital ridge, prominent forehead and decreased postnatal growth. Valproic acid is known to influence folate metabolism thereby affecting the spinal column closure resulting in spina bifida condition.

Barns and colleagues (1996) have shown that Valproic acid decreases the level of Pax 1 transcription in chick somites causing malformations of vertebrae and ribs.

5. Warfarin

Warfarin is an anticoagulant used by patients with artificial heart valves. If the drug is used during early pregnancy it results in malformed infants having hypoplastic nose, eyes abnormalities, mental retardation and brachydactyly. If taken at a later stage in pregnancy, affected children show central nervous system disorders. The mode of action of Warfarin is not known. It appears that an alteration in post translational carboxylation of proteins may result in skeletal disorders.

INDUSTRIAL TERATOGENS

Industrial teratogens include lead, mercury, arsenic, zinc and cadmium. Lead, mercury and zinc are found in high concentrations in drinking water, vegetables and air in certain parts of Kazakhstan. Half the population tested of these places has extensive chromosomal breakage. Since 1980, the incidence of birth defects have doubled in some areas (Edwards, 1994). Lead is found almost every where in the natural environment. It is a cumulative poison. The critical period of exposure to lead is 12 – 14 weeks of gestation in humans. Fetal exposure of lead is through maternal ingestion of food and beverages containing the metal. Lead is not easily excreted and it continues to accumulate in fetal brain causing neurological defects.

Lead impairs cell adhesion during neurological development and alters neuronal migration. Exposure to lead is linked with premature birth, decreased postnatal growth and increased neonatal deaths.

Another teratogen, methyl mercury is extremely toxic to humans. It accumulates in the fetal tissues through placenta. High levels of mercury results in altered neuronal migration and binds directly to DNA and RNA, altering their conformation.

MICROBIAL TERATOGENS

This class of teratogens includes infectious microorganisms. These microbes affect 1-5% of all live births and are among the leading causes of neonatal morbidity and mortality. General symptoms include premature birth, growth retardation, neurological abnormalities, damage of the eye, liver, heart and ear along with bone lesions.

1. Rubella

Abnormal babies are born to women suffering from Rubella (German measles) during the first five weeks of pregnancy. The abnormalities include, microphthalmia, cataracts, glaucoma, cardiac malformations, hearing loss and mental retardation. Rubella syndrome was the first evidence that the placental barrier between mother and fetus does not fully protect the fetus from teratogens. It was found that infected fetuses during their first trimester of gestation have one in six chances of eye cataracts, heart malformations and deafness (Gregg, 1941). The Rubella epidemic of 1963 – 1965 resulted in thousands of fetal deaths and infants born with birth defects in United States. In 1969, Rubella vaccine was introduced. Since then the cases of congenital Rubella syndrome have decreased significantly.

The mode of action of the pathogen can be direct viral effects or damage to immune response. Virus infection stops the cells from replicating, as a result organogenesis is inhibited causing malformations of eye and heart. An immune response may induce cell lysis, tissue destruction, and disruption of lining of blood vessels leading to brain defects, cataracts, and hearing loss.

2. Cytomegalovirus (CMV) & Herpes simplex

Cytomegalovirus infection early in gestation is fatal while infection of later embryos might lead to blindness, deafness, cerebral palsy and mental retardation. 90 % of the time inutero infection is without any clinical symptoms. However, 5 % do exhibit atypical illness and the other 5% with CMV damage including hepatitis, gestational prematurity, microcephaly, anemia and intracranial calcifications. Mode of action of CMV is similar to that of rubella virus, i.e. cell lysis and immune response.

Herpes simplex virus is also teratogenic but in the past few years in utero infection with herpes is an uncommon event.

3. Toxoplasma

Toxoplasma goondii is a protozoan parasite carried by rabbits and cats. It can cross the placenta causing hydrocephaly, microphthalmia, chorioretinitis, brain lesions and multiple organ damage and dysfunction in the fetus. Fetal infection occurs approximately one in four thousand pregnancies. The fetal damage caused by the teratogen is maxmium if the mother is infected in third trimester resulting in 60% of the infected new born.

4. Syphilis

Treponema pallidum is the cause of syphilis. Several hundred children are born each year with syphilis. Early infection most often results in spontaneous abortion. New borns which survive are anemic having spleen and liver malformations. They also have skin lesions, nasal discharge, bone and joint pains.

Infection during late pregnancy results in deafness, dental and bony abnormalities, cardiovascular defects and skin lesions.

RECREATIONAL TERATOGENS

Alcohol, tobacco, cocaine, cigarette smoke and heroin can be grouped under recreational teratogens. These drugs significantly reduce fetal and postnatal growth and increase infant mortality. They usually disrupt fetal development before the mother knows that she is pregnant and has a chance to change her lifestyle.

1. Alcohol

Children of alcoholic mothers suffer from many birth defects referred to as fetal alcohol syndrome (FAS).

Alcohol permeates the placenta and enters fetal circulatory system causing developmental abnormalities.

Ethanol induces hypoxia and fetal malnutrition by impairing placental blood flow to fetus, thus constricting blood vessels. Fetal alcohol syndrome contains combinations of malformations such as microcephaly, small brain size, decreased philtrum size (the pair of ridges that runs between the nose and mouth above the centre of upper lip), a narrow upper lip, a low nose bridge, microphthalmia, cardiovascular disorders and maxillary hypoplasia. Children suffering from this syndrome are developmentally and mentally retarded with low IQ. They also exhibit behavioural abnormalities without any observable physical changes.

There are many proposed mechanisms of action by which alcohol can act as a teratogen. Experimental evidences for these mechanisms are mostly based on studies on mice embryos treated with alcohol.

Impairment of neural crest cell migration / increased neural cell death or general cell death by lysis of cells are some of the proposed mechanisms.

2. Tobacco and Cocaine

Women who smoke during pregnancy are 80% more likely to have spontaneous abortion as compared to a non smoker. Maternally inhaled nicotine can be detected in fetal lung. Nicotine restricts placental blood flow to fetus resulting in chronic hypoxia and malnutrition leading to birth defects. Children of smoker mothers are smaller and weigh less at birth as compared to a non-smoker's child. There is also reduction in overall fetal length, reduced head size and certain facial anomalies like cleft lip and palate. Smoking during pregnancy increases the risk for premature delivery.

Cocaine, which is an anesthetic and vasoconstrictor also, induces birth defects by disrupting placental vasculature, thereby inducing hypoxia and malnutrition. Fetuses exposed to cocaine show retarded growth, microcephaly, urinogenital anomalies and neuronal and behavioral abnormalities. There is also risk of premature labour, spontaneous abortion and fetal death.

METABOLIC CONDITIONS IN MOTHER

1. Diabetes

Multiple congenital malformations such as cardiac and skeletal malformations, central nervous system alterations and caudal dysgenesis have been linked to insulin dependent diabetes. The risk of malformations is 3 to 4 times that of the normal pregnancy. Diabetes enhances the risk of still birth and neonatal deaths.

These risks can be controlled by insulin therapy.

2. Nutritional stress / Malnutrition

Developing fetus depends solely on the mother for its nutritional requirements. Therefore, the nutritional stress to a pregnant mother may cause certain diseases when they become adults.

ABNORMAL DEVELOPMENT WITHOUT MUTATION

Although some teratogens are mutagenic or carcinogenic, most teratogens cause no obvious abnormalities of the chromosomes. If an embryo with normal genome is exposed to an abnormal environment, it can develop abnormally. Mouse blastocysts implanted ectopically develop into highly disorganised but well differentiated tissues. This disorganised mass of cells is invasive tumour and thus called teratocarcinoma. It has been shown that teratocarcinoma does not result from mutation. Most teratogenic induced abnormalities are limited to somatic cells and are not heritable.

GENETIC - ENVIRONMENTAL INTERACTIONS

Susceptibility to teratogenesis depends on the genotype of the conceptuous and the manner in which this interacts with environmental factors. Recent studies have revealed that different alleles in human populations can influence whether a substance is harmless or dangerous to the fetus. For example, if the fetus of a heavy smoker mother has a particular allele (A 2) of the gene for growth factor TGF –Beta, then the tobacco smoke absorbed through the placenta can raise the risk of abnormalities such as cleft lip and palate ten folds. This clearly indicates that teratogenic influence depends upon the genetic constitution of the individual.

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