

Environmental mutagens and teratogens

Mutagens

Physical mutagens

Ionizing radiation represents a radiation with shorter wavelength and larger energy in comparison with other type of radiation (X-ray, gamma-ray, cosmic rays).

Above radiation exhibits high energy and high penetration through the tissues. Ionizing radiation by passing through the tissue collides with atoms with subsequent release of the electrons; along the trace of the radiation free radicals and ions, capable of reacting with biological macromolecules, including DNA, arise (H^+ , OH^-). Ionizing radiation may directly attack DNA itself. This kind of radiation induces oxidation of DNA bases and disrupts pentose-phosphate bond in DNA helix. Mutagenic effect depends on the amount of arising ions. Absorbed dose of ionizing radiation is being expressed in Grays [$Gy = J/kg$].

Mutagenic effect depends on:

1. The dose
2. The duration of exposure
3. The phase of the cell cycle in the target cell
4. The capacity of the DNA repair system

Ionizing radiation induces gene mutations, chromosomal aberrations and, ultimately, chromosomal translocations. There is no threshold for ionizing radiation, even small quantities may induce mutations. The quantity of radiation, which may duplicate the amount of mutations in humans, is important in genetics for prediction of the risk (especially in the etiology of neoplasia).

Ultraviolet radiation

Damage of DNA molecule induced by UV radiation UV radiation exhibits lower energy than ionizing radiation, but even UV is capable to cause electron excitation. UV radiation is absorbed by several organic molecules, namely by pyrimidines and purines. UV acts as potent mutagen in unicellular organisms, in more complex organisms it alters cells on the surface. In humans UV induces or contributes to the induction of skin neoplasia (carcinoma, melanoma). The risk of exposure to UV radiation increases with decreasing ozone content in the atmosphere. UV radiation induces mutations mainly due to generation of hydrated purines and pyrimidine dimers. Thymine alterations are mutagenic due to: a) distortion of DNA double-helical structure hinders the procedure of DNA polymerase along the template with subsequent block of DNA replication; b) in the course of the repair of altered thymines a base mispairing often occurs. The repeated interruption of DNA replication due to thymidine dimers and their incomplete repair cause gaps in newly synthesized DNA chain with subsequent chromosomal breaks. Thymidine dimers may give rise base substitutions and/or deletions.

Chemical mutagens

Chemical mutagens are chemicals exerting mutagenic effects. They comprise: a) food stains based on acridine; b) combustion products in cigarette smoke (more than 400 carcinogens and mutagens); c) chemicals in car exhausts; d) monomers in plastics industry (polychlorinated biphenyls, styrene, butadiene, vinyl chloride etc.).

Mode of action of chemical mutagens

1. Compounds that are mutagenic only during replication (base analogues and acridine stains).
2. Compounds that are mutagenic by attacking DNA unless this is replicated.
3. Compounds causing alkylation, deamination and hydroxylation of bases.

Base analogues

These compounds are structurally related to nucleotides and are therefore mis- incorporated into DNA during replication. Their differences in comparison with physiological nucleotides cause base mispairing and mutations. These compounds are employed in investigation of mutagenic processes and as anticancer drugs (2-aminouracil, 5-bromouracil, 5-fluorouracil). 5-bromouracil is analogous to thymine. Br atom replaces methyl group on C5 of pyrimidine and increases a chance of tautomeric shift. If in enol form, 5-BU pairs with guanine. If 5-BU in enol form is incorporated into a new strand, during the following replication 5-BU in keto form pairs with adenine and GC:AT transition arises. Acridine stains (such as proflavin and acridine blue) induce a shift in the reading frame. Molecules of bases are incorporated in between base pairs and the double-helix conformation of DNA is altered during the replication. During the replication insertion or deletion of one or more bases occurs with all associated phenotype consequences.

Alkylation compounds

Many chemicals may be donors of alkyl groups. Yperit (or its nitroso derivative) was the first reported mutagen. Nitroso guanidine, on the other hand, belongs among the most potent mutagens. Alkylating agents cause mispairing by attaching the functional group (methyl-, ethyl- etc.) to the nucleophilic centers of purines and pyrimidines. Alkylating agents may induce all kinds of mutations and result ultimately in chromosomal aberrations and translocations.

Deaminating compounds

They act via (oxidative) deamination of aminogroup in adenine, guanine and cytosine (as an example: nitrates and nitric acid). Amino- group is converted into keto- group. Deamination alters the ability of bases to form hydrogen bonds. In general, hypoxanthin (deaminated adenine) pairs with cytosine, while uracil pairs with adenine. Deamination results usually in transitions (CG:AT as well as AT:CG). Nitric oxides are generated by combustion of fossil sources and by car exhausts. Nitrates, which are used in canning of smoked meat, are particularly dangerous towards gastrointestinal cells.

Hydroxylating agents

May convert cytosine into hydroxy aminocytosine, which pairs with adenine forming CG:AT transition.

Biological mutagens

Viruses

In the course of lysogenic cycle viruses may become incorporated into the DNA of the host. Incorporation of virus into the sequence of the gene affects substantially its function, the gene loses its function with subsequent consequences, such as chromosomal breaks, tumors.

Transposons

Represent elements capable to transpose from one site of the genome to the other. In human genome there are two classes of transposable elements: LINE (long interspersed nuclear element) and SINE (short interspersed nuclear element). Their shifts within a genome may have mutagenic effects.

Testing of mutagens

Most mutations negatively affect human health and, additionally, mutagenic compounds are often teratogenic and carcinogenic. Testing of the new compounds for their tentative mutagenic effect is a standard procedure within obligatory tests prior to the compounds is released on market.

Ames test

Enables to disclose mutagenic activity, type of the mutations induced and test potential mutagens. For that purpose a special auxotrophic strain *Salmonella typhimurium*, capable to grow on medium containing histidine, was created. Compounds to be tested are added into the medium. By the Ames test we evaluate the emergence of mutations following the transfer on the minimal medium. By adding histidine into the minimal medium the limited amount of cell divisions is allowed and the mutagenic compounds are tested only during the replication. By adding enzymes from liver extract we may stimulate biotransformation of xenobiotics and therefore follow mutagenicity arising after the metabolic activation.

Currently available scope of methods can test both the mutagenicity and genotoxicity.

Complex screening for genotoxicity may be carried out on three levels:

1. monitoring of environmental pollution;
2. monitoring of biological effects (the response of the organism towards genotoxic compounds, exposure levels, effectiveness of the preventive measures);
3. genetic monitoring (epidemiological studies regarding spontaneous abortions, incidence of congenital malformations in relation to the genotoxic compounds).

Teratogens

Teratogens are external factors capable to cause (or substantially increase a risk of) congenital malformations. Alike mutagens, teratogens may arbitrarily be classified into three major groups: biological teratogens, chemical teratogens and physical teratogens.

Classes of teratogens

Biological teratogens

Several pathogenic viruses are members of this class. Proven teratogens are following viruses: Rubivirus (rubella), Cytomegalovirus, Herpesvirus, Parvovirus B-19, influenza virus, HIV and others, but also bacteria *Treponema pallidum* (syphilis) and protozoon *Toxoplasma gondii* (toxoplasmosis). Teratogenic risk may also be elevated by

serious diseases of the mother, such as diabetes mellitus, phenylketonuria, myasthenia gravis and others.

Chemical teratogens

This class of teratogens comprises several industrially and agriculturally employed chemicals (organic solvents, polychlorinated biphenyls, heavy metals etc.). Particularly important group of chemical teratogens is constituted by drugs and medicaments, where prominent teratogens are cytostatics, several antibiotics (namely tetracyclins), antiepileptics (fenytoin, valproate), lithium, warfarin, thalidomide, ACE-inhibitors, steroids, retinoids etc. Teratogenic effects have been spotted in the case of ethyl alcohol (its abuse in gravidity causes foetal alcohol syndrome) and drugs such as pervitin.

Physical teratogens

This class of teratogens involves various kinds of radiation (X-rays, gamma-radiation), high temperature and mechanical teratogens.

Mode of action

Teratogenic effect has a complex character and a simplification mutagen = teratogen is not applicable. By assessing teratogenic effect we have to take into consideration:

Factor of the dose

The dose of the teratogenic agent has a decisive role for its effect. Lower doses may not induce any malformation, or may lead to a moderate or differently located damage.

Factor of the time

The sensitivity towards various teratogens is not constant during the pregnancy. In general, the exposure to teratogens during the first trimester of gravidity has the worst prognosis, however, adverse effect exhibits the exposure during the second and third trimester as well. For each teratogen there is so called critical period during which is the fetus most sensitive towards the particular teratogen or during which the target organ/system is most vulnerable. It is not surprising that the effects of the same dose of the same teratogen may be different depending on the phase of gravidity. Embryo in its early stage of development (embryogenesis) reacts towards teratogens by means All or Nothing. It means that no malformations are fixed within this period; embryo either compensates and restores all the damage or it perishes. In the later period (period of organogenesis) exposure to teratogens induces malformations.

Genetic and interspecies differences

Genetic background of individuals significantly predisposes the susceptibility towards teratogens. Although intraspecies variability may not be clearly pronounced, certainly significant is than interspecies variability. This assumption is of importance, particularly with respect to the testing of teratogenic effects. While the same dose of the same teratogen is very effective in humans, it may not be effective at all in rodents (for instance resistance of mice towards teratogenic effects of thalidomide).