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GENOTOXICITY: MALIGNANCY, DAMAGE TO DNA; A VOLUMINOUS REVIEW

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ABSTRACT

A chemical agent that causes DNA damage is genotoxin. Germline mutation, somatic mutation lead to malignant transformation. The in-vivo, in-vitro test is performed for genotoxicity. The various assay is helped to detect DNA damage and is also used to evaluate the safety of environmental chemicals and to know the mechanism of action of suspected carcinogens. Many mutagens undergo metabolic activation for binding with DNA and DNA adduct in a reactive species can be detected in cells and human tissues by using various sensitive techniques. The characterization and the detection of DNA adducts provide causes of human cancer.

KEYWORDS: Carcinogens, mutagenicity, DNA adduct, immunoassay, genotoxin.

INTRODUCTION

Genotoxicity is a Latin term used for a chemical or biological agent having the ability to damage the DNA or genetic code of a cell, resulting in genetic mutation or carcinogenicity. A destructive effect on chromosomal material affects the integrity of the cell. Genotoxicity and mutagenicity both are different terms as all mutagens are genotoxic, but not all mutagenic compounds are genotoxic. Chemical substances and radiations are genotoxins. Indirect or

direct effects on DNA are observed where the induction of the mutation, DNA damage, and mistimed event activation occurs. In organisms, somatic cells or germ cells are affected by permanent and heritable changes and it leads to cause defects in a future generation. Apoptosis is used to prevent genotoxic mutation, not always damage will be repaired. Interaction between DNA sequence and genotoxic substances causes damage to genetic material.

Genotoxins are classified into three types

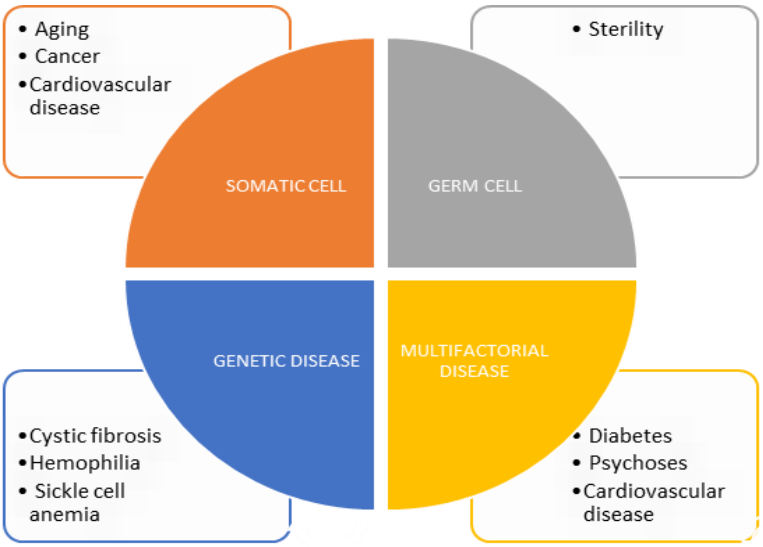
Carcinogens

Mutagens

Teratogens

Malignancy is caused due to damage to damage genetic material of somatic cells of eukaryotic organisms. While birth defects are caused due to heritable changes or damage to the genetic material of germ cells. Duplication, insertion, or deletion of genetic information are the forms of mutation. These mutations cause various types of cancer and chronic disease in the host. Genotoxic incidents can seem at both the DNA during mitosis or whole-genome level.

GENOTOXIC RISK



SHORT-TERM TEST

The short-term test is performed for genotoxicity and mutagenicity. The tests are used to detect genetic alterations. The genotoxic agent inserts the gene amplifications, deletions, point mutations, and chromosomal rearrangement. The genetic alterations may be of one or

multiple types. Genetic alterations or such biological properties may affect DNA directly or indirectly. Neither the other test nor any assays can detect all genotoxic chemical entities. That's why several tests are conducted to evaluate whether the chemical is genotoxic or not. Some guidelines recommended - test battery for genotoxicity must include-1) gene mutation test for bacteria. 2) An in-vitro test for the cytogenetic evaluation of chromosomal damage in the mammalian cell. 3) An in-vivo test for chromosomal damage rodent hematopoietic cells.

BACTERIAL MUTAGENICITY TESTING

The chemically induced gene mutations are detected by a bacterial mutagenicity test. This is the reverse mutations assay in which salmonella Typhimurium strains contain defined mutations. Bacteria are incubated with the range of concentration of the test compound. The second mutation-induced which directly suppresses the functional mutation, which restores biological function to the non-functional histidine gene. The endogenous metabolic 6 pathways required for bio-activation of the test chemical are lacking by bacteria, extract of the mammalian liver is incorporated as an exogenous activation system. Using genetically-engineered S. Typhimurium strains blocks enzymes required for the bioactivation of different carcinogens which provides major information on their metabolism. While 'humanized' S. Typhimurium with human enzymes are developed to identify the human enzymes involved in bioactivation and to improve the quality of Ames Salmonella assay to detect human hazardous agents.

MAMMALIAN MUTAGENICITY TESTING

The most widely used mammalian gene mutation assay in mouse lymphoma assay (MLA). In the mouse lymphoma cells, the mutation in the thymidine kinase (TK) gene in L5178Y/TK+/-3.6.2C is detected by mammalian mutagenicity testing. On the seven mouse chromosome no.11, the gene coding is performed for thymidine kinase, which allows reuse of the salvage nucleotide from the culture medium in the metabolism. Trifluorothymidine which is a thymidine analog is used to detect the mutants. The L5178Y system is used in in-vitro mammalian cell mutation assay, as it detects genetic alteration (mutation and chromosomal damage).

CHROMOSOME DAMAGE TEST

Chromosome aberrations (CA), micronuclei (MN), and sister chromatid exchanges (SCE) are detected structural chromosome changes through in-vitro cytogenetics. Structural chromosome aberrations are observed in normal chromosomes or numbers expressed over the

chemical exposure in the cell. Direct DNA breakage, replication on a damaged DNA template, inhibition topoisomerase, inhibition of DNA synthesis, and other mechanism shows the result for chromosome damage test. Human primary cells (peripheral lymphocytes) are generally used in in-vitro studies because these cells have a low spontaneous rate of chromosomal damage and can be easily cultured with a stable karyotype (2n=46). The structural CA observed over 30 years in human peripheral blood lymphocytes used in occupational and environmental settings shows the risk for cancer.

COMET ASSAY

Single-cell gel electrophoresis assay is also known as comet assay. It's a simple method for measuring alkali labile sites and DNA strand breaks in the DNA of mammalian cells. The assay includes the cells suspended in an agar medium which contains alkaline conditions, forwarded to gel electrophoresis then neutralized and stained with the propidium iodide DNA dye. The DNA damaged cells show the migration of chromosomal DNA from a nucleus in the shape of a 'comet'. The percentage of migrated DNA is analyzed by using image analysis software parameters. The tail length, the tail moment are measured as DNA damage. As in in-vivo genotoxicity assay, it is applied to a single-cell suspension of material from any animal tissue. While non-carcinogens may show the test positive as mutagens, the activity is carried due to a metabolic pathway that is not expressed in vivo or due to the absence of competing detoxification 12 pathway or lack of DNA repair.

DNA ADDUCTS FORMED BY CHEMICAL CARCINOGENS

Genotoxic chemical carcinogens are mostly DNA reactive. Not all chemical carcinogens are chemically reactive but undergo metabolic activation in mammalian cells to reactive intermediate which reacts with DNA. Carcinogens in DNA damage can result in breaks in the sugar-phosphate backbone of the molecule. In one or two strands of the double helix which forms single-strand breaks, or in both causes double-strand breaks. "Covalent binding of the carcinogens results in the formation of a chemically altered base or phosphate group in DNA is known as an Adduct." These can originate from endogenous processes, metabolism, oxidative stress, and chronic inflammation.

METHODS FOR ADDUCT DETECTION

Various methods are developed for the detection of DNA adducts. Methods which apply for assay to human exposure should-1) sensitive to low-level adduct 2) requires the minute quantity of DNA 3) gives result simultaneous with human exposure 4) unknown adducts

developed from complex mixture 5) can resolve, quantitate and identify adduct. Radiolabelled compounds on the molecule are required for continuing isotope during metabolic activation and binding to DNA for identifying adducts.

BIOLOGICAL SIGNIFICANCE OF DNA ADDUCTS

Initiating stages of the carcinogenesis the DNA damage and binding by carcinogens are observed earlier while increasing damage to DNA is also a feature of the next stages of the multistage process as is known as mutation of a gene in some type of tumor. The formation of extra DNA adducts in carcinogenesis is frequently detected in target and non-target tissue. It is shown that inhibition of the formation of DNA adduct decreases the tumor formation, and increasing adduct levels gives the higher tumor yield. DNA adduct formation in the initial stages of tumor it is demonstrated, XPA knockout mice, that are deficient in nucleotide excision repair that is highly sensitive from carcinogens to tumor induction, which forms stable adducts would be removed by this repairing mechanism.

ADDUCTS AS BIOMARKERS OF OCCUPATIONAL AND ENVIRONMENTAL EXPOSURE TO CARCINOGENS

Not all detection methods require radiolabeled carcinogens in some methods the DNA may be isolated from human tissue. Blood, sputum, buccal mucosa, cervical mucosa, sperm, and bladder, placenta, and hair roots such tissues provides the DNA for studies. From all of this most commonly used source is blood cells, as these are not target cells for malignancy. The risk of lung cancer and many others have been observed due to widely spreading industries including steel production, aluminum production, graphite electrode, and coke ovens manufacture. The formation of DNA adduct in workers of industries is detected through many studies. Environmental exposure to genotoxic carcinogens can be detected by DNA adduct.

CONCLUSION

DNA adduct formation, the causes of malignancy or DNA damage these are properties of genotoxic agents. Various tests are used to determine the carcinogenic potential of chemicals as short-term-test, comet assay, based on the DNA damage or its biological consequences. Most human carcinogens are genotoxic. Most of the methods required the radiolabeled compounds while some alternative method with a high degree of sensitivity has been developed. Reduced DNA adduct formation reduces carcinogenicity, as increased DNA

adduct formation gives an opposite effect. Continued research on DNA adduct characterization in human tissues will detect the causative agents of malignancy.

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