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Genotoxicity: A deep insight into drug induces genetic toxicology

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ABSTRACT

Genotoxins are chemicals which have the ability to cause damage to genetic materials within the cell. There are three major effects that genotoxins can have on organisms: carcinogens, mutagens, and teratogens. By identifying and understanding genotoxins properties of the compounds, we can get early alert about safety of the drugs and prevent the potential risk of the drugs by avoiding those Active pharmaceutical ingredients (API) in the drug formulation. In this review, we provide deep insight about the drug induced genotoxicity which creates potential damage to the genetic material, its mechanism, and the necessity of the genotoxicity testing. We also discussed various types of in-vivo and in-vitro assays to confirm genotoxicity, also in-silico methods to predict the genotoxicity.

INTRODUCTION

The Drug markets and the drugs are expanding globally as never before. With this diversifying, various chemicals are being synthesized. These chemicals have shown damage to genetic material and vital parts. However, damage of genetic material is more of a concern. Among these chemicals, genotoxin plays a major role of damaging genetic materials (materials (DNA, RNA) [1]. Genotoxins are chemicals which possess the chemical properties of the ability to cause damage to genetic materials within the cell which terms as “drug induced genotoxicity”. The drug induced genotoxicity creates several alterations on genetic materials, such as the mutation induction, chromosomal aberrations, mistimed event activation, and breaking of the DNA double strands and also severe potential mutations. Single and double strand DNA breaks, structural and numerical chromosomal aberrations, point mutations, and the loss of excision repair are common damage caused by genotoxins. Permanent, genetic changes can influence either the organism's somatic cells or the transfer of germ cells to future generations. Whereas this will usually be mitigated by the organism by the repairing of DNA or the apoptosis mechanism (controlled cell death), the damage that contributes to mutagenesis cannot always be prevented or fixed [1].

Among the several genotoxicity effects caused by the chemical drugs, the most severe effects are carcinogenic and mutagenic effects. These genotoxins can act as potential carcinogens, mutagenic agents which cause mutations, or teratogens which cause birth defects [2]. Genotoxicity outcomes can affect different cells

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and damage the genetic materials. This impacts normal functioning of the cells and leads to mutations in different cells. These mutations are responsible for several diseases that are known as "Genotoxic disease syndrome (GDS)" which are responsible for impaired enzymatic functions, cytotoxicity in the cells, growth inhibition and degenerative processes. It is important to understand that all mutagenic substances are genotoxic, whereas not all genotoxic substances are mutagenic [1].

Genotoxicity Risks and how it affects DNA-

In many aspects, genotoxic agents are present, such as in drugs, pesticides, air, water, soil. Humans are exposed to these genotoxic agents and this increases risk of various diseases. A broader spectrum of endpoints is covered by genotoxicity. For example, breakage of DNA strands, exchange of sister chromatin, unscheduled DNA synthesis [8]. Data has shown that genotoxic agents may be responsible for various types of cancer and other major problems such as reproductive problems [6]. Genotoxicity can damage the DNA and chromosomes of the cells. Genetic damage to somatic cells in eukaryotic organisms can lead to Tumor. It may adversely affect reproduction or induce inheritable mutations in germ cells [7]. Noncovalent chemical/DNA interactions such as groove binding and intercalation also can play an important role in genome integrity there may be genotoxic consequences of that binding [10].

Through interactions with the DNA sequence and structure, genotoxic substances cause damage to the genetic material in the cells. For example, in its high-valent oxidation state, the transition metal chromium interacts with DNA with exchange of the electrons and generation of the free radicals in order to induce DNA lesions that lead to carcinogenesis [4]. Therefore, early detection of these genetic damages and knowledge about genotoxic chemicals is very important for taking necessary measures to reduce it.

Mechanism of Genotoxicity –

Gene mutations are one of the major endpoints of genotoxicity. Gene mutation is caused mainly by mutagenic chemicals, which are generally not lethal, but can cause considerable damage to chromosome integrity and cell viability. The damage to genetic material is caused by interactions of DNA structure by binding on the DNA double helix, intercalation or groove binding on the DNA or breaking the DNA and its sequence with genotoxins. These genotoxins interact at a specific location or the base sequence of the DNA**.**

This results in DNA damage and mutation due to lesions, breakage, fusion, deletion, and mis-segregation.
For example, the transition metal chromium in its high-valent oxidation state interacts with the DNA by creation of the free radicals and by causing DNA lesions that lead to carcinogenesis. According to researchers, the mechanism of DNA damage as well as base oxidation products for the interaction between DNA and high-valent chromium are important to in-vivo DNA damage formation, which leads to cancer in chromate-exposed humans. [5]. In the experiment it was concluded that chromium was specific to the guanine nucleotide.

It clearly shows how high-valent chromium could also act as a carcinogen by forming xenobiotics with modified 8-oxo-G nucleotide base [1].

Necessity of genotoxicity tests –

In most of the countries, genotoxicity testing is a critical aspect of regulatory toxicity evaluation. The purpose of genotoxicity is to examine the

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safety and effectiveness of new chemical entities prior to their market release, to recognize the responsibility for heritable effect on germ cells which will impose risk on future generations. It also helps in providing a quantitative estimation of the contribution of chemical agents to the occurrence of genetic diseases and risk characterization of cancer [4]. Genetic modification, such as the repair of DNA damage by gene mutation or large-scale chromosomal damage or recombination or numerical chromosomal changes, plays a role in the complex process of hereditary effects and malignancy, and genotoxicity assays allow us to identify them [5].

Genotoxicity assays allow early detection of the genotoxicity potential of a drug in drug development. These assays are designed to be more vulnerable to hazard identification [4]. As part of the safety assessment process, all regulatory guidelines strongly imposed to obtain genotoxicity data for each medicine and validation by specific methods such as micronucleus and Ames assays. During the pre-clinical phase of drug screening, several toxicological end points need to be accessed for the New chemical entities (NCE) and provide safety assessment data to the regulatory authorities during approval of the drugs. Therefore, Assays of genotoxicity have become an important part of regulatory requirements [1].

Tests to confirm genotoxicity –

The purpose of genotoxicity testing is to determine whether genetic material will be affected by a substrate or whether it can cause growth. These tests may be performed in various cell types (i.e., mammalian, bacterial, yeast cells). With the data from these tests, the early advancement of defenceless life forms of genotoxic substances can be monitored. Genotoxicity tests can be characterized in two types of tests, i.e., in-vivo and in-vitro tests. These tests detect compounds that cause genetic damage directly or indirectly through various mechanisms[2].

IN VITRO TESTING:

In vitro genotoxicity assays are a rapid and inexpensive way to test for specific toxicity and molecular mechanisms. In-vitro genotoxicity testing can be done in a variety of ways.

1. Bacterial reverse mutation test or Ames test –

This test is performed to determine the mutagenic potential of given chemical compounds. The test was developed by Bruce Ames, thus the name Ames test [2]. It involves application of using amino acid-requiring strains of Salmonella typhimurium and Escherichia coli to detect mutation points that may involve substitution, deletion, or addition of one or more base pairs of DNA. The basic principle of the Ames test involves first to detect the mutation, then reverts it back and restores the cell's functional capability to synthesize Histidine [5]. Salmonella typhimurium bacteria is used with mutations in genes which involve Histidine biosynthesis. As a result, bacterial cells that require histidine for growth are referred to as histidine auxotrophs. Mutagenic agents can cause reverse mutations. This results in bacteria to grow on histidine-deficient media. The number of bacteria that form colonies is then used to determine a compound's mutagenic potential [2]. As it's rapid, inexpensive, and convenient to perform, the bacterial reverse mutation test is commonly used as an initial screening test for genotoxicity or mutagenicity.

2. Mammalian chromosome aberration test –

The mammalian chromosome aberration test reveals information about those agents that can

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induce structural mutations in chromosomes or chromatids.[5]. This test is performed in-vitro in cultural mammalian cells. Microscopic analysis of chromosomes in mitotic metaphase cells scores structural and numerical damage [13]. Other types of chromosomal changes, such as polyploidy and duplication, can be detected with this examination. A positive test result indicates that the agent may be mutagenic or carcinogenic, although there is no perfect relation [2].

3. Mammalian cell gene mutation test or the mouse lymphoma test –

This test is used to determine if chemical compounds have triggered gene mutations. The most commonly used mammalian gene mutation assay is the mouse lymphoma assay (MLA) [14]. L5178Y mouse lymphoma cells, CHO, CHO-AS52, and V79 Chinese hamster cell lines, and TK6 human lymphoblastic cells are among the most widely used cell lines [5]. The L5178Y system is the most recommended in-vitro mammalian cell mutation assay because it can detect a wide range of genetic alterations, including both mutations and chromosomal damage. It can also identify end points such as thymidine kinase (TK) and Hypoxanthine-guanine-Phosphoribosyltransferase (HPRT), as well as a mutation in the Xanthineguanine phosphoribosyltransferase (XPRT) transgene [15].

4. The micronucleus assay –

Another type of genotoxicity assay is the micronucleus assay. This assay helps to identify genotoxicity in micronucleus. Chromosomal fragments or complete chromosomes can often be located outside the nucleus in one of the daughter cells as a result of DNA damage or errors in chromosome separation during the cell cycle. After the division of the nucleus these DNA fragments will disintegrate and form a so-called micronucleus. These micronuclei can be seen and measured under the microscope using DNA staining techniques. Genotoxicity is determined by the number of these micronuclei per 1,000 (bi-nucleated) cells. This assay can be performed in-vitro on cell lines like CHO-k1 [2].

IN VIVO TESTING:

The in vivo testing for genotoxicity of the compounds is to determine the potential of DNA damage that can alter chromosomal structure or disturb the mitotic apparatus, which changes chromosome number. It can also detect genotoxic agents that have been missed by in vitro tests. Some of the in-vivo testing methods are given below [1].

1. **In-vivo comet assay –**

The in vivo comet assay has several advantages: It has broad applicability to identified DNA damage in the different tissues, and/or special cell types, higher degree of sensitivity which can detect very low levels of DNA damage caused by the compounds, the requirement for a small cell count per sample, and the general ease with which test results can be obtained, and the short time required to complete the test and its relatively low cost. The aim is to identify how to determine the risk of heritable mutations (germ cells) and cancer progression (somatic cells). The results of the in vivo comet assay help with hazard identification, dose–response evaluation, and mechanistic identifying of the mode of action of a substance. It's especially useful for evaluating local genotoxicity, especially for organs/cell types that are difficult to assess with other standard tests [16].

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2. In-vivo micronuclei test/In-vivo chromosome aberration test –

This test used to identify the extent of chromosome or spindle damage. When a cell is exposed to a mutagen, it may be damaged, and when it divides, it may form smaller micronuclei in addition to the main nucleus [5]. When the in-vivo micronucleus assay also came up negative, the compound is likely to have no genotoxic potential and may move forward in growth. further research is required in a rare situation, where after getting positive results from in-vitro, the in-vivo shows negative results [2]. This can be performed on red blood cells and bone marrow in-vivo. It is possible to determine whether micronuclei contain complete chromosomes or chromosome fragments using centromeric probes. These findings can then be used to assess whether a compound's mode of action is clastogenic or aneugenic

Table 1: In-vivo and In-vitro assays for Genotoxicity testing

Name of the Test	Type of Assay	Primary Principle	Cell/ organism used
Bacterial reverse mutation test or Ames test	In-vitro	The main principle of this test is that after detecting the mutation, it reverts it back and restore the cell's functional capability to synthesise Histidine	Amino acid-requiring strains of Salmonella typhimurium and Escherichia coli
Mammalian chromosome aberration test	In-vitro	The primary purpose of the mammalian chromosome aberration test is to find agents that can induce structural mutations in chromosomes or chromatids, with chromatid mutation being the most common	Cultural mammalian cells
Mammalian cell gene mutation test or the mouse lymphoma test	In-vitro	The purpose of this test is to detect a wide range of genetic alterations, including both mutations and chromosomal damage. It can also identify end points such as thymidine kinase (TK) and	L5178Y mouse lymphoma cells, and V79 Chinese hamster cell lines, CHO, CHO-AS52, and TK6 human lymphoblastic cells
In-vitro micronucleus assay	In-vitro	The purpose of this assay is to help to identify genotoxicity in micronucleus	CHO-k1 cell line
In-vivo comet assay	In-vivo	The primary purpose is to evaluate local genotoxicity, especially for organs/cell types that are difficult to assess with other standard tests	Any cell line or tissue

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In-vivo micronuclei test/In-vivo chromosome aberration test	In-vivo	The purpose of this test is to determine the extent of chromosome or spindle damage and identify genotoxicity in micronucleus	Red blood cells and bone marrow

IN SILICO METHOD:

Computational approaches for predicting genotoxicity based on chemical structures and properties are accepted as an alternative due to the high cost and laboriousness of experimental tests. In-silico method such as Quantitative structure-activity relationships (QSAR) is one of the best alternative method to assess safety assessment of the compounds chemical risk assessment, genotoxicity and carcinogenicity, with the goal of providing quicker, more cost-effective, and animal-free tools to predict toxicity [17]. Quantitative Structure-Activity Relationship (QSAR) is a computational modelling technique for determining relationships between biological activities and structural properties of chemical compounds. It is used to predict the activity of new chemicals. There are many softwares by which genotoxicity can be predicted.

1. OECD QSAR Application Toolbox -

This toolbox is a stand-alone software application for determining chemical hazards with data gaps. The data gaps are filled using read-across or local QSARs. The integrated method in the toolbox such as "profilers" can be used to find the similar chemicals from the database to find common mechanisms or modes of action. The Toolbox contains more than > 5000 compounds with data obtained from experimental studies to support read-across and trend analysis [18].

2. Leadscope -

The Leadscope software is based on the QSAR approach to predict rodent foetal developmental toxicity and developmental toxicity (foetal growth retardation and weight loss) [1].

3. TopkatPredictor -

This QSAR-based model was developed to predict a number of toxicological endpoints, such as developmental toxicity. The TOPKAT software's Developmental Toxicity Potential (DTP) module was created using experimental studies selected after a study of literature citations on rat oral data. TOPKAT consists of three QSAR models, each of which is applicable to a specific chemical class. The result is the probability that a chemical structure submitted is a developmental toxicant in rats. In order to assess the certainty of predictions, the TOPKAT model automatically decides if the submitted structure belongs to the model's Optimum Prediction Space (OPS) [1].

4. T.E.S.T.Predictor -

The T.E.S.T. predictor is a QSAR based Toxicity Estimation Software tool created by US EPA to estimate the toxicity of a compound [1].

Table 2: In-silico QSAR based software used for prediction of Genotoxicity.

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Name of Software	Method used	Prediction	Applicability	Availability
OECD QSAR Application Toolbox	(Q)SAR Toolbox	The Toolbox also includes a number of profilers to quickly assess chemicals for common mechanisms or modes of action	It includes several databases, including reprotoxicity data, despite being primarily a tool for chemical categories and read-acros, such as 166,072 experimental ER binding affinity values from the OASIS commercial database, as well as 166,072 ER binding data from the Danish EPA (pre-generated forecasts, not experimental values).	Freely Available
Leadscope	QSAR methodologies	The Leadscope software includes a module with QSAR models for predicting rodent foetal developmental toxicity, such as dysmorphogenesis (structural and visceral birth defects), foetal survival (foetal death, post-implantation loss, and preimplantation loss) and developmental toxicity (foetal growth retardation and weight loss)	Classification models for developmental toxicity in the rodent fetus dysmorphogenesis (structural and visceral birth defects), developmental toxicity (fetal growth retardation and weight decrease), and fetal survival (fetal death, post-implantation loss, Preimplantation loss).	Commercial
TopkatPredictor	QSAR statistical methods	This QSAR-based model predicts a number of toxicological endpoints, such as developmental toxicity.	Optimum predictive space	Not freely Available

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T.E.S.T.Predictor	QSAR methodologie, Method of consensus: average of predicted toxicity values from three models: Food and Drug Administration, neural network, and hierarchical clustering.			Freely Available

Conclusion:

Our review paper provides a brief overview of Drug induced genotoxicity, and what are the mechanism of the genotoxicity. We describe information about how different in vitro methods have been used to find the genotoxins agents that bind with DNA, causing mutations and structural damage that can lead to cancer. Tests such as mammalian chromosome abbreviation, Comet assay and micronucleus assay are recommended by the OECD and different regulatory authorities for providing safety of the compounds and to determine genotoxicity potential of the compound during pre-clinical screening stage of the drug development. However, these tests are very expensive to carry out also need lots of resources and time. Therefore, different in silico methods have been developed specially QSAR based method to fasten the process of toxicity prediction. Identification of genotoxic agents allows us to better understand the mechanism of mutation and genotoxicity, paving the way for us to reduce the occurrence of such mutations and genotoxicity.

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