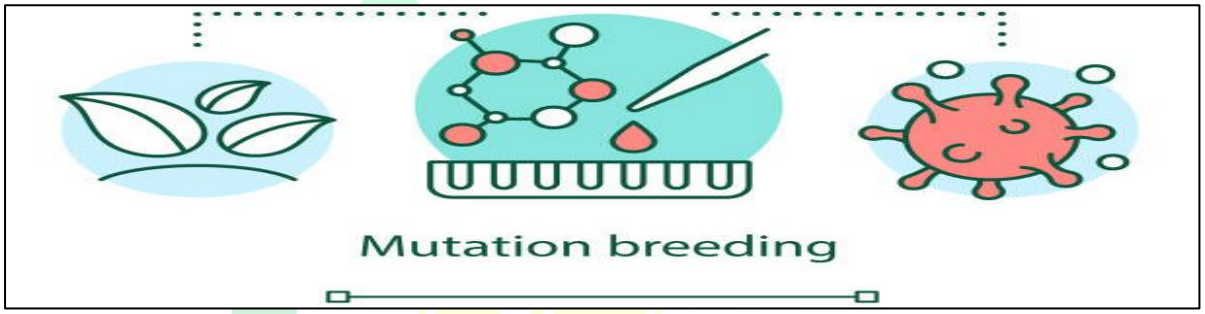
JUST AGRICULTURE
multidisciplinary e-Newsletter

Alkylating Agent Used in Mutation Breeding

Rajneesh Kumar[1] and Aman Tutlani[2*]
[1]Department of Genetics and Plant Breeding, School of Agriculture
Lovely Professional University, Phagwara-144411, Punjab, India.
[2]Department of Genetics and Plant Breeding, Sher-e-Kashmir University of Agricultural Sciences and Technology, Srinagar -190052, Jammu & Kashmir, India.

ARTICLE ID: 65



Alkylating Agent in Mutation Breeding

Chemical structure of alkylating agents Biological alkylating agents are chemicals that transfer alkyl groups to biologically important macromolecules under physiological conditions. Ross (1) has reviewed their general chemistry; Lawley (2) their reactions with nucleic acids. Table 1 lists the main classes of alkylating agent; each class is represented by one or several well-known mutagens. Cutting across this classification by structural group are differences in two properties whose role in mutagenicity has been much discussed. One is the type of alkyl group transferred, whether it is, e.g., a methyl or ethyl group or a more complex one like - CH_2 $COCH_3$. The other is the number of alkyl groups that a single molecule can donate. This property is called the 'functionality' of the compound. Thus, among the nitrogen mustards, H2 N (CH_2CH_2 Cl) is monofunctional, HN (CH_2 CH_2 Cl) $_2$ is bifunctional, and N (CH_2 CH_2 Cl) is trifunctional. The term polyfunctional may be used for all compounds whose functionality is greater than one. It should be noted that the degree of functionality cannot be inferred simply from the number of alkyl groups carried by a compound. The alkyl alkane sulphonates, for example, are mono-functional, EMS donating only its ethyl group and MMS only one of its two methyl groups. Even when two alkyl groups are bound in the same way, only one of them may be available for alkylation; this applies to the dialkyl sulphates, which act as

JUST AGRICULTURE
multidisciplinary e-Newsletter

monofunctional agents. Nitroso compounds require chemical activation, which for some compounds can take place *in vitro* or *in vivo.*

Alkylating agents are strong mutagenic, carcinogenic and cytotoxic compounds Paradoxically, the cytotoxic properties of some of the compounds are largely exploited in cancer therapy. Alkylating agents can be found among a large panoply of classes of compounds, including sulphur mustards, nitrogen mustards, epoxides, ethyleneimines and *ethyleneimides,* alkyl *methanesulphonates, alkylnitrosoureas, alkylnitrosoamines, alkylnitrosoamides, alkyl halides, alkyl sulphates, alkyl phosphates, chloroethyl sulphides, chloroethylamines, diazoalkanes*, etc. Although most are synthetically produced, a few alkylating agents are of biological origin, e.g., the strong mutagenic glucosamine-nitrosourea (Streptozotocin) is produced by *Streptomyces achromogenes.* There is currently an enormous number of known chemical compounds able to induce mutations in prokaryotic and/or eukaryotic cells and this continues to increase. The continuous search and the synthesis of new mutagenic compounds is driven, not by the needs of experimental mutagenesis, but by the paradoxal fact that several mutagenic compounds, although carcinogenic, possess simultaneously anti-neoplastic properties and find application in anti-tumour therapy. Despite the large number of mutagenic compounds, only a small number has been tested in plants. Among them, only a very restricted group of alkylating agents has found large application in plant experimental mutagenesis and plant mutation breeding. Some alkylating agents such as the methyl-donor S-adenosylmethionine (SAM), which in spite of being involved in about 40 metabolic reactions in mammalians is a weak methylating agent able to form adducts to DNA, are formed endogenously as natural products of organisms.

Definition

Alkylating agents are a family of anticancer drugs that interfere with cell's DNA and inhibit cancer cell growth. They are so named because of their ability to add alkyl groups to negatively charged groups on biological molecules such as DNA and proteins. Alkylating agents are among the first group of chemicals determined to be useful in cancer treatment or chemotherapy. They remain to be the most important components of modern chemotherapeutic protocols (individually or in combination with other drugs) because of their proved and significant clinical anticancer activities.

JUST AGRICULTURE
multidisciplinary e-Newsletter

Mechanism of Action

Alkylating agents are a diverse group of chemical compounds with a common characteristic of formingpositively charged (electrophilic – electron poor) alkyl groups in aqueous solutions under physiologicalconditions. The positively charged alkyl groups are capable of reacting with basic/negatively charged (nucleophilic – electron rich) groups present in DNA and proteins/peptides. Such reactions lead to adding alkyl groups at oxygen, nitrogen, phosphorous, or sulfur atoms (nucleophilic centers), thus altering thebiological function of DNA and proteins. The most important reaction of alkylating agents with regard to their antitumor activity is their reactions with DNA nucleobases. Other nucleobases alkylated and the atomic positions at which alkylation occurs in order of preference includeN1 and O6 positions of guanine; N_1, N_3 and N_7 positions on adenine; N_3 position on cytosine; and O4position of thymidine.

Cause of Alkylating Agents

Alkylating agents may cause critical vomiting and nausea and decreases the number of white blood cells and red blood cells as well. The decrease in the white blood cell count results in susceptibility to the infection. Alkylating agents have been found to be used in the treatment of leukaemia, lymphoma, melanoma, testicular cancer, breast cancer, and brain cancer. Often, they are the most used ones in combination with other anticancer drugs.

Characteristics

Discovery of alkylating agents as anticancer drugs has its origin in the use of sulfur mustard gas for warfare during World War I. Sulfur mustard gas was not only fatal but it also showed myelosup- pression/immunosuppression in its victims as well as in animal models. The latter observation led to the development of less volatile mustargen (mechlorethamine) with strong antitumor activity against lym- phomas and other cancers. Eventually mustargen (nitrogen mustard) was developed for clinical use to treat Hodgkin disease. Following the discovery of mustargen, less toxic and more clinically effective nitrogen mustard derivatives, e.g., cyclophosphamide, and other alkylating agents in clinical use today were developed. Cyclophosphamide is a bifunctional nitrogen mustard that is a most commonly used drug in combination chemotherapy and is a DNA alkylating agent that is used as an immunosup- pressive drug. It acts by killing rapidly dividing cells.

Alkylating agents, as suggested by their names contain reactive alkyl groups. An alkyl is an univalentreactive group containing only carbon and hydrogen atoms arranged in a chain with a general formula ofC_n H_{2n+1}, e.g., *methyl*, CH_3 (derived from *methane*) and *butyl* C_4H_9 (derived from *butane*). Alkylating agents used as anticancer drugs are cable of reacting with biological molecules such as DNA and proteins, and disrupt cellular function by either killing the

JUST AGRICULTURE
multidisciplinary e-Newsletter

cell or by preventing its growth. The most common biological functional moiety alkylated by these compounds is guanine, a nucleobase. The anticancer activities of alkylating agents are caused in two ways: (i) through cross-linking two different DNA strands via the reaction with guanine nucleobases present on the opposing strands of DNA and (ii) preventing/affecting the activities of critical DNA processing enzymes and thereby stimulating apoptosis via the reaction with guanine nucleobases on a single DNA strand. The cross-linking of DNA makes it impossible to uncoil DNA during cell division thus preventing its growth. Based on the reactivity, alkylating agents are of two types:

(i) Monofunctional (monoalkylating – alkylate nucleobases on one DNA strand)

(ii) Bifunctional (dialkylating – alkylate nucleobases on both DNA strands and cross-link them).

Classification

Alkylating agents currently used as anticancer drugs are divided into *fi*ve major classes. The examples ofthe clinically used agents (most common) under each of these classes and their clinical utility are shown in Table

Class	Clinically used agents	Cancer/other disease treated
Nitrogen mustards	Cyclophosphamide	Breast cancers, most lymphomas, and childhood cancers
	Ifosfamide	
	4-Hydroxycyclophosphamide	High dose therapies in conjunction with bone marrow transplantation
	Mafosfamide	
	Melphalan	Multiple myeloma, melanoma, and sarcomas
	Chlorambucil	B-cell chronic lymphocytic leukemia and immunosuppressive therapy for autoimmune diseases
Aziridines and epoxides	Thiotepa	Breast, ovarian, and bladder cancers
	Mitomycin C	Esophageal, breast, and bladder cancers
	Dianhydrogalactitol	Breast, cervical, and brain cancers
Alkyl sulfonates	Busulfan	Bone marrow transplantation for chronic myelogenous leukemia
Nitrosoureas	BCNU [*N*,N^0-bis(2-chloroethyl)- *N*-nitrosourea]	

JUST AGRICULTURE
multidisciplinary e-Newsletter

	CCNU [*N*-(2-chloroethyl)-N^0-cyclohexyl-*N*-nitrosourea]	Brain tumors (glioma, glioblastoma, medulloblastoma, andastrocytoma), multiple myeloma, and lymphoma
	MeCCNU [*N*-(2-chloroethyl)-N^0 (4-methylcyclohexyl)-*N*-nitrosourea]	
Hydrazine and triazine derivative	Procarbazine	Hodgkin lymphoma and certain brain cancers such as glioblastoma multiforme astrocytoma, and melanoma
	Dacarbazine	
	Temozolomide	

Examples of Alkylating Agents

A few examples of alkylating agents can be given as:

- cisplatin, nitrogen mustards (cyclophosphamide and chlorambucil),
- alkyl sulfonates (busulfan),
- nitrosoureas (lomustine, semustine, and carmustine),
- triazines (dacarbazine), and
- ethyleneimines (thiotepa).

Types of Molecular Changes

The types of molecular changes that are induced by the alkylating agents can be given as cross-linking between the DNA strands and the loss of a basic component (which is purine) from or the nucleic acid breaking. The result is, nucleic acid will not be replicated. Either the altered DNA will be not able to carry out the cell functions, resulting in cell death (which is called cytotoxicity), or the altered DNA will change the characteristics of the cell, resulting in an altered cell (which is called mutagenic change). This change can result either in the ability or tendency to produce cancerous cells (which is called carcinogenicity). Normal cells can also be affected and become cancer cells.

Alkylating agents Drugs

Alkylating agents were one first class of drugs to be used against cancer. There exist five traditional categories of alkylating agents, which are given as follows:

- Nitrogen mustards (for example, chlorambucil, bendamustine, ifosfamide, cyclophosphamide, melphalan, and mechlorethamine)
- Alkyl sulfonates (for example, busulfan)
- Nitrosoureas (for example, lomustine, carmustine, and streptozocin)

JUST AGRICULTURE
multidisciplinary e-Newsletter

- Ethylenimines (for example, thiotepa and altretamine)
- Triazines (for example, temozolomide and dacarbazine).

Some types of Alkylating Agents

Non-specifically Acting Agents

A few of the alkylating agents are active under the conditions present in cells, and the similar mechanism that makes them toxic allows them to be used as anti-cancer drugs. They stop the tumour growth by cross-linking guanine nucleobases in the double-helix strands of DNA, directly attacking DNA. This process makes the strands unable to separate and uncoil. As this is quite necessary for DNA replication, the cells may no longer divide. These particular drugs act non-specifically.

Agents Requiring Activation

A few of the substances that require conversion into the active substances in vivo (for example, cyclophosphamide). Cyclophosphamide is the most potent immunosuppressive substance. In small doses, it is much efficient in the therapy of autoimmune hemolytic anaemia, systemic lupus erythematosus, granulomatosis with polyangiitis, including the other autoimmune diseases. High dosages will cause pancytopenia and hemorrhagic cystitis.

Dialkylating Agents

Dialkylating agents may react with two various 7-N-guanine residues, and if these are in varied DNA strands, the result can be cross-linkage of the DNA strands that prevents the DNA double helix from uncoiling.

Monoalkylating Agents

Monoalkylating agents can only react with one 7-N of guanine.

Limpet Attachment

Monoalkylation and limpet attachment do not prevent the two DNA strands' separation of the double helix but do prevent the vital DNA-processing enzymes from DNA accessing. The final result is given as the inhibition of cell growth or stimulation cell suicide, apoptosis.

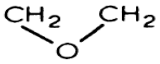
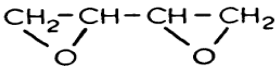
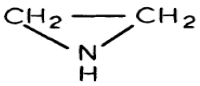
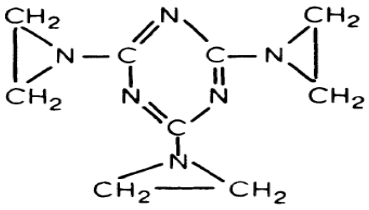
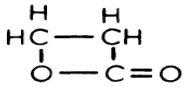
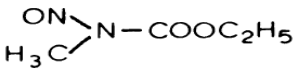
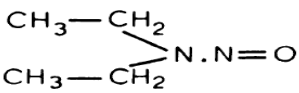
Nitrogen Mustard

JUST AGRICULTURE
multidisciplinary e-Newsletter

Nitrogen mustards are the cytotoxic organic compounds having the functional group - chloroethylamine ($Cl(CH_2)_2NR_2$). Although originally it is produced as chemical warfare agents, they were the first chemotherapeutic agents for cancer treatment. Nitrogen mustards are said to be the nonspecific DNA alkylating agents.

Table 1

Main classes of alkylating agents, each represented by one or more well-known mutagens.

I	Sulphur mustards	$S(CH_2CH_2Cl)_2$	mustard gas
II	Nitrogen mustards	$HN(CH_2CH_2Cl)_2$	nitrogen mustard (HN_2)
III	Epoxides		ethylene oxide (EO)
			diepoxybutane (DEB)
IV	Ethylene imines		ethyleneimine (EI)
			triethylenemelamine (TEM)
V	Alkyl alkanesulphonates	$C_2H_5OSO_2CH_3$	ethyl methane-sulphonate (EMS)
		$CH_3OSO_2CH_3$	methyl methane-sulphonate (MMS)
VI	Dialkyl sulphates	$SO_2(OC_2H_5)_2$	diethylsulphate (DES)
VII	β-lactones		β-propiolactone
VIII	Diazo compounds	$CH_3N=N$	diazomethane
IX	Nitroso compounds		*N*-nitroso-*N*-methyl urethane (NMU)
			diethylnitrosamine (DEN)
		$CH_3.N(NO).C(NH).NH.NO_2$	*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG or NTG or MNNG)

JUST AGRICULTURE
multidisciplinary e-Newsletter

Limitations

Alkylating antineoplastic agents have some limitations. Alkylating antineoplastic agent's functionality has been found to be limited in the presence of the DNA-repair enzyme, which is O-6-methylguanine-DNA methyltransferase (MGMT). The cross-linking of double-stranded DNA by the alkylating agents can be inhibited by a mechanism of cellular DNA repair, MGMT. If the MGMT promoter region gets methylated, the cells will no longer produce the MGMT, and they are thus more responsive to the alkylating agents. In gliomas, methylation of the MGMT promoter is a valuable indicator of tumour responsiveness to alkylating agents.

References

Ferguson, L.R. and Denny, W.A. 1995. Anticancer drugs: an underestimated risk or an underutilized resource in mutagenesis? *Mutat Res-Fund Mol M.* 331: 1–26.

Ferguson, L.R. and Denny, W.A. 2007. Genotoxicity of non-covalent interactions: DNA intercalators. *Mutat Res*. 623: 14–23.

Georgieva, M. and Stoilov, L. 2008. Assessment of DNA strand breaks induced by bleomycin in barley by the comet assay. *Environmental and Molecular Mutagenesis*. 49: 381–387.

Gichner, T., Ptáek, O., Stavreva, D.A. *et al.* 1999. Comparison of DNA damage in plants as measured by single cell gel electrophoresis and somatic leaf mutations induced by monofunctional alkylating agents. *Environmental and Molecular Mutagenesis*. 33: 279–286.

Gichner, T. 2003. Differential genotoxicity of ethyl methanesulphonate, N-ethyl-N-nitrosourea and maleic hydrazide in tobacco seedlings based on data of the Comet assay and two recombination assays. *Mutat Res.* 538(1-2): 171–179.

Gichner, T., Patková, Z. and Kim, J.K. 2003. DNA damage measured by the comet assay in eight agronomic plants. *Biologia Plantarum*. 47 (2): 185–188.

Grant, W.F. and Owens, E.T. 2001. Chromosome aberration assays in Pisum for the study of environmental mutagens. *Mutat Res*. 488: 93–118.

Kumari, S., Kumar, R., Chouhan, S., & Chaudhary, P. L. 2023. Influence of Various Organic Amendments on Growth and Yield Attributes of Mung Bean (*Vigna radiata* L.). *International Journal of Plant & Soil Science*, *35*(12), 124-130.