

## UNIT IV: Consequences and Targets of Alkylating Agents

### Assigned Reading:

Hsu et al. (1991) Mutational hotspots in the P53 gene in human hepatocellular carcinomas. *Nature*, 350:427

Bressac et al. (1991) Selective G to T mutations of P53 gene in hepatocellular carcinoma from southern Africa. *Nature* 350:429-431.

### Targets and consequences of alkylating agents (electrophiles).

- 1) A large number of chemicals act through electrophilic intermediates that form adducts on cellular nucleophiles
- 2) The cellular targets of electrophiles include N, S and O atoms on protein, DNA, RNA, lipids and carbohydrate. GSH is a major scavenger of electrophiles that can be overwhelmed.
- 3) The consequences of DNA adducts are heritable changes resulting from point mutations, frame shift mutations, deletions and strand breaks.

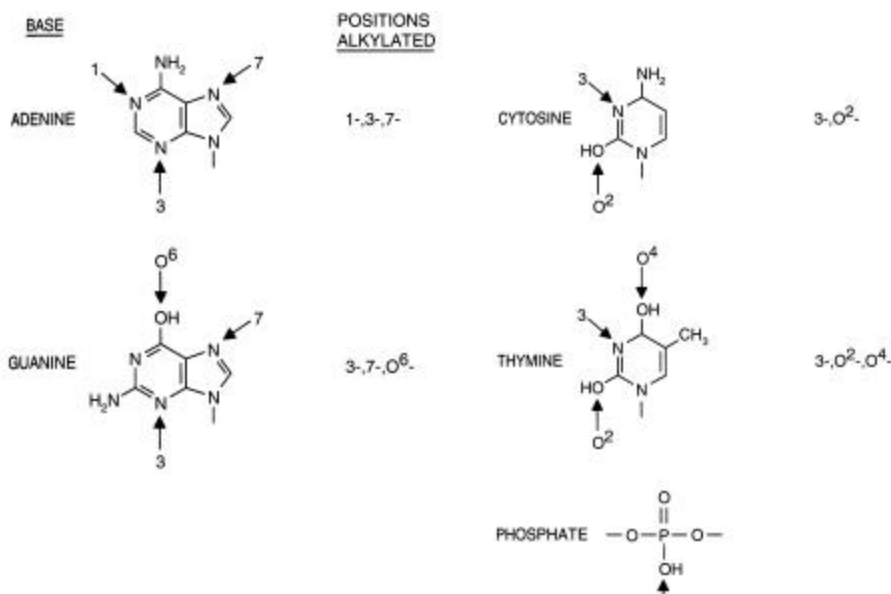
### Spectrum of Damage by Alkylating Electrophiles:

In the preceding lectures, we have been considering those chemicals that initiate the carcinogenic process via their capacity to alkylate cellular nucleophiles. In this session, we will begin to consider the consequences of those interactions. In the first part of our discussion we will describe the different types of chemical damage that can result from the production of intracellular electrophiles. It is important to understand the spectrum of chemical damage that they can create so that you can understand the biological consequences.

Electrophiles are compounds that have an electron deficient atom. Because it is electron deficient, it will attack atoms that are electron rich, called nucleophiles. Another way to think of this is “electrophiles are electron loving”. They interact with nucleophiles that are electron rich and “nucleus loving.” “Opposites attract”. The consequence of this interaction is often formation of a covalent bond and the generation of a novel product. For this course, it will not be necessary for you to delve into electron orbital theory to understand these concepts. Although the chemistry can be quite complicated, the principle is quite simple. Metabolism of certain carcinogens generates a chemically reactive species called an electrophile. This species can then alkylate cellular macromolecules, inhibiting their function or fidelity. As mentioned previously nucleophilic sites are easy to spot. In general, S, O: and N: atoms in macromolecules are nucleophilic targets of many electrophiles. They have unpaired electrons that are the targets of attack.

### Heritable changes resulting from covalent modification of electrophile DNA interactions:

**DNA.** Arguably, the most important cellular nucleophiles is DNA. The double helix has a number of N and O atoms that are targets of electrophiles. These atoms exist not only in the base, but also in the phosphodiester backbone. Although all of these sites are observed, the most common sites of alkylation are; 1) N7 of Guanine, 2) N3 of adenine, 3) N1 of adenine, N3 of Guanine, and O6 of guanine. Alkylations of phosphate are also quite frequent. The remaining sites are fairly rare and may be a “footprint” of a specific carcinogen.



Different electrophilic carcinogens will often display different preferences for nucleophilic sites in DNA and different spectra of damage. A comparison of dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) makes the point. P450 oxidation of DMN generates a methyl carbonium ion ( $\text{CH}_3^+$ ) and P450 oxidation of DEN generates an ethyl carbonium ion ( $\text{CH}_3\text{CH}_2^+$ ). Despite the structural similarities of the ultimate electrophiles, they display significant differences in alkylation profiles. (data taken from reference 1) **Given that DEN and DMN yield different spectra of alkylation, why is it not surprising that structurally more divergent compounds like the direct acting carcinogens N-methyl-N-nitrosourea or 1,2-dimethyl hydrazine yield the exact same spectra as DMN? (Put another way, why do DEN and the direct acting carcinogen N-ethyl-N-nitrosourea yield the same alkylation patterns?)**

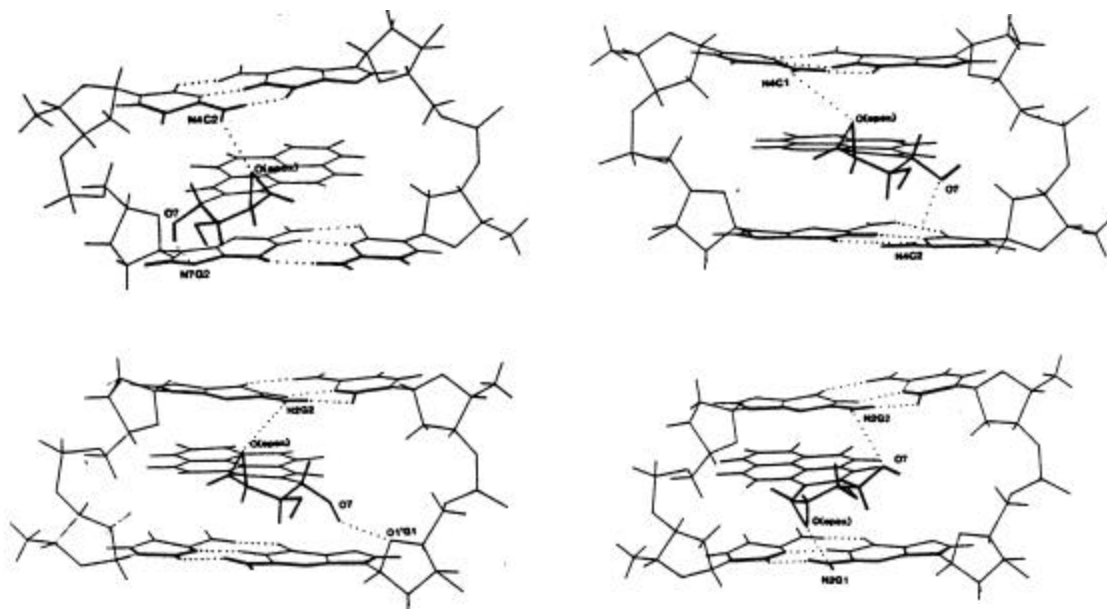
|                               | PERCENTAGE OF<br>TOTAL ALKYLATION BY   |   |
|-------------------------------|--|---|
|                               | DIMETHYL-<br>NITROSAMINE,<br>N-METHYL-N<br>NITROSOUREA,<br>or 1,2-DIMETHYL-<br>HYDRAZINE | DIETHYL-<br>NITROSAMINE<br>or N-ETHYL-N-<br>NITROSOUREA |
| 1-Alkyladenine                | 0.7  | 0.3   |
| 3-Alkyladenine                | 8  | 4   |
| 7-Alkyladenine                | 1.5  | 0.4   |
| 3-Alkylguanine                | 0.8  | 0.6   |
| 7-Alkylguanine                | 68   | 12  |
| O <sup>6</sup> -Alkylguanine  | 7.5  | 8   |
| 3-Alkylcytosine               | 0.5  | 0.2   |
| O <sup>2</sup> -Alkylcytosine | 0.1  | 3   |
| 3-Alkylthymine                | 0.3  | 0.8   |
| O <sup>2</sup> -Alkylthymine  | 0.1  | 7   |
| O <sup>4</sup> -Alkylthymine  | 0.1-0.7  | 1-4   |
| Alkylphosphates               | 12   | 53  |

SOURCE: Adapted from Pegg (1984).

Even if you were an experienced organic chemist you would have difficulties making predictions regarding the spectra of damage induced by any given electrophile. In reality, an understanding of these spectra often comes in retrospect (i.e., knowing the adduct identity and working backwards to the pathways). However, it is useful to be able to recognize a few potentially damaging electrophilic moieties and their nucleophilic targets (see below). You will find that if you recognize a few prototypes, you will understand the mechanism by which the majority of alkylating carcinogens act.

#### A number of factors influence what adducts are formed.

- 1) In general, the stronger electrophiles display a greater range of nucleophilic targets (i.e., they can attack weak and strong nucleophiles). Weaker electrophiles will only be able to alkylate strong nucleophiles (like S: in amino acids).
- 2) Biological macromolecules (DNA proteins etc.) don't behave like organic molecules in simple systems. For example, DNA is in an alpha helix, each helix is wound around histones, each strand is involved in hydrogen bonding with the other strand etc. etc. All of these interactions affect how atoms interact, where electron densities exist etc. Thus, any conclusions you might draw from an understanding of chemistry in vitro, will only be an approximation (and may often lead you astray).
- 3) The 3D structure of the DNA ladder in vivo imposes certain limits on how bulky electrophiles can attack. Benzo(a)pyrene is unique in that it attacks the N2 of guanine. To explain this, computer modeling studies of benzo(a)pyrene were performed. We have talked previously about the diol-epoxide as being the ultimate carcinogen. If you think about it, you will realize that there are four isomers of the 7,8 diol, 9-10 epoxide. The two most important are called (+)anti and (-)anti. Modeling studies demonstrate how these different isomers might interact with nucleophilic sites in DNA. Left side are the (+)anti isomer intercalating via the major groove (upper) and minor groove (lower). Right side are the same predictions for the (-)anti isomer. Although it is subtle, the point to remember is that the positioning of the epoxide near the N2 of guanine is best accomplished when one particular isomer enters the base stack via a given pathways (in this case, (+)anti entering through the minor groove).



- 4) Histones can influence the nature of the adducts in two ways. DNA winding around histones can protect sites from DNA attack. DNA bends that are exposed may be sites for attack. Regions of DNA that are not associated with histones (more actively transcribed regions) may also be more susceptible to electrophilic attack.
- 5) Finally and maybe most important, the metabolic profile of a given cell will dictate which electrophiles are generated.

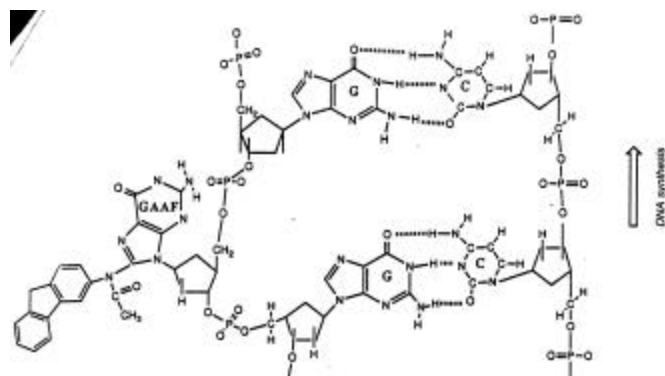
### Consequences of electrophilic attack on DNA

Modification of DNA by electrophilic carcinogens can lead to a number of products. These events are dependent upon when in the cell cycle the adduct is formed, where the adduct is formed and the type of repair process (repair is initiated at all) used in response to the damage.

**Transitions and transversions. Transitions:** substitution of one pyrimidine by the other or one purine by the other (changes within the class). **Transversions:** a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine (changes across class). Chemicals can cause transitions and transversions in a number of ways. 1) When adducts (or resultant apurinic or apyrimidinic sites) are encountered by the replicative machinery, they may be misread. Preferentially (but not exclusively), the polymerase will insert an Adenine (A) in response to a noninformative site. Thus, the daughter strand of an A, C, G or T adduct may contain an A. This mutation is now fixed and resistant to subsequent repair leading to a change in the genetic code (mutation).

**Under what conditions of carcinogen exposure and cell cycle might such a situation occur?** Another example is when a modified nucleotide can pair counter to standard Watson and Crick rules. O6 MeG and O4 MeT are examples of such lesions (causing G to A and T to C transitions, respectively). **What carcinogens that we have talked about might induce these effects?**

**Frame shifts.** A shift in the reading frame can also result from a carcinogen-DNA adduct. In model systems studying AAF adducts it was postulated that strand slippage was a mechanism that allowed a bulky adduct to be bypassed. This model predicts that most frame shift mutations will be deletions and that they may occur more frequently when the adduct is formed on a nucleotide that is part of a repetitive sequence or a polynucleotide stretch. **Why would this mechanism be more likely to yield more loss of function mutations than the models described above?**



**DNA strand breaks.** DNA strand breaks can also result from DNA adducts. These can arise either through 1) direct alkylation of the phosphodiester backbone leading to chemical cleavage of the backbone or 2) result from excision repair mechanisms that are incomplete during DNA replication. Strand scission can lead to double strand breaks, recombination, loss of heterozygosity etc, etc.

### Non heritable changes

It is important to keep in mind that electrophiles are modifying a vast array of macromolecules in the cell. Although we often focus on DNA because of its role in heritability of cancer phenotypes, other targets are important. We will come back to this point later, but the carcinogenic properties of many chemicals may also be a function of their ability to disrupt cellular function. In particular their ability to kill cells. Electrophiles can kill cells by altering protein function (again covalent modification of thiols and oxygen and nitrogen containing proteins), chemical modification of gens that blocks their transcription etc. Cell death can lead to cell division in certain tissues (regeneration). Cell division can have a marked effect on the rates of mutation by a chemical.

**Can you explain why this might be the case?**

### Additional Reading:

- Chemical Carcinogenesis, in "Casarette and Doull's Toxicology, The basic Science of Poisons. Pages 201-267 (CD Klaassen ed.) 5th Edition, McGraw Hill, San Francisco, CA.)
- Strauss, BS. (1991). The A rule of mutagen specificity: A consequence of DNA polymerase bypass of nonfunctional lesions? Bioessays 13: 79
- Subbiah, et al. Molecular modelling studies on the non-covalent intercalative interactions between DNA and the enantiomers of anti-benzo[a]pyrene 7,8-diol-9,10-epoxide. (1983). Carcinogenesis 4:211.
- Scholl et al. (1995). Molecular biomarkers for aflatoxins and their application to human liver cancer. Pharmacogenetics 5:S171-S176.