Synthesis of Cyclophosphamide with Deuterium substituent

Mena Hanna

Alkylating Agents and DNA

- Strategy where DNA function is disrupted

1) Function by different means to disrupt DNA duplication

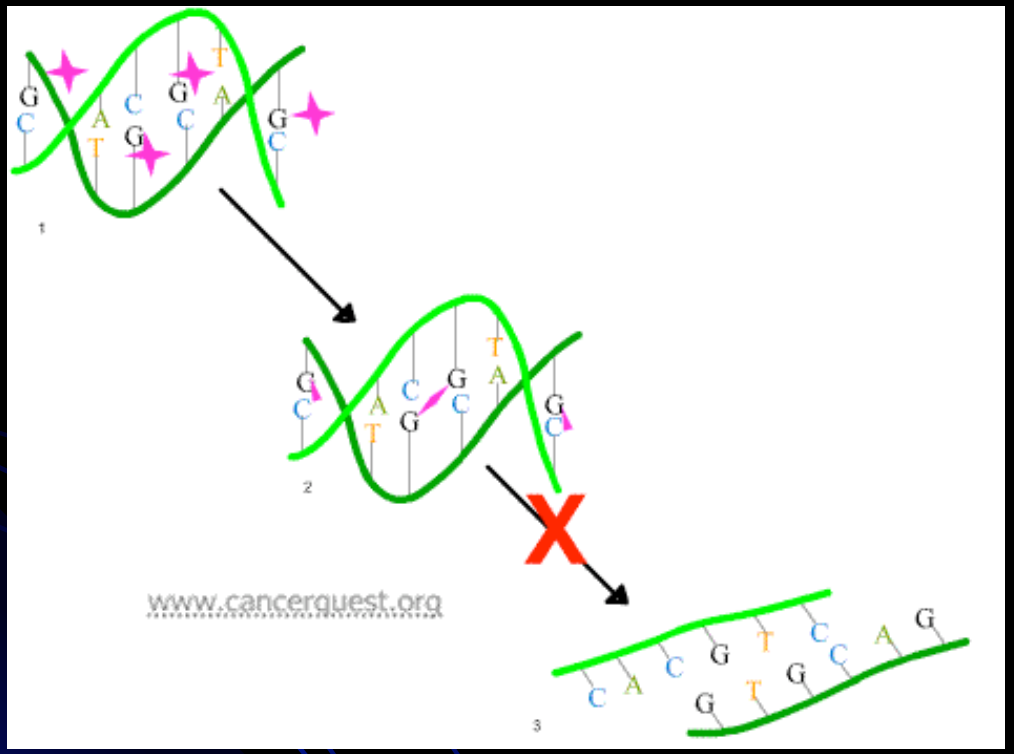
2) Proves successful since cancer cells generally multiply faster and with less error-correcting than healthy cells.

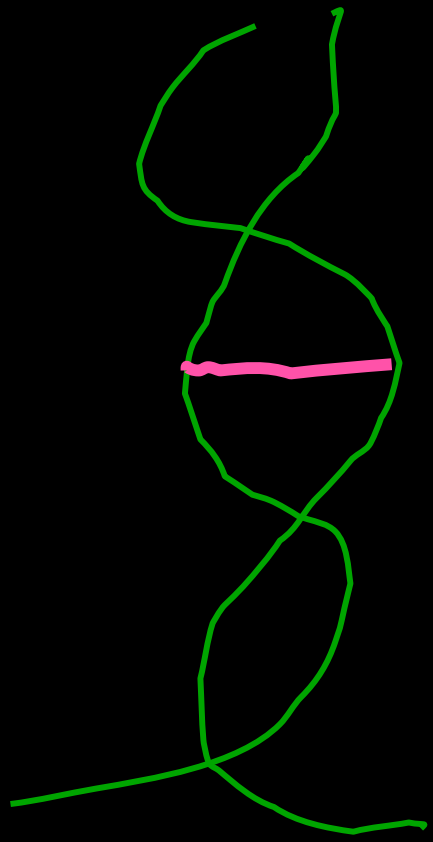
3) Example mechanism: Alkylating agents cause DNA damage through the formation of cross-bridges between two molecules of DNA.

4) Cross-linking prevents DNA from being separated for synthesis or transcription.

5) Results in cell death.

Alkylating Agents and DNA



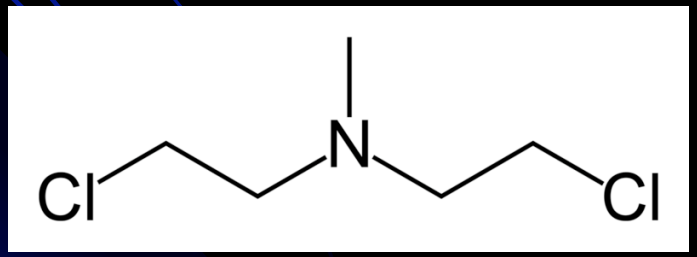


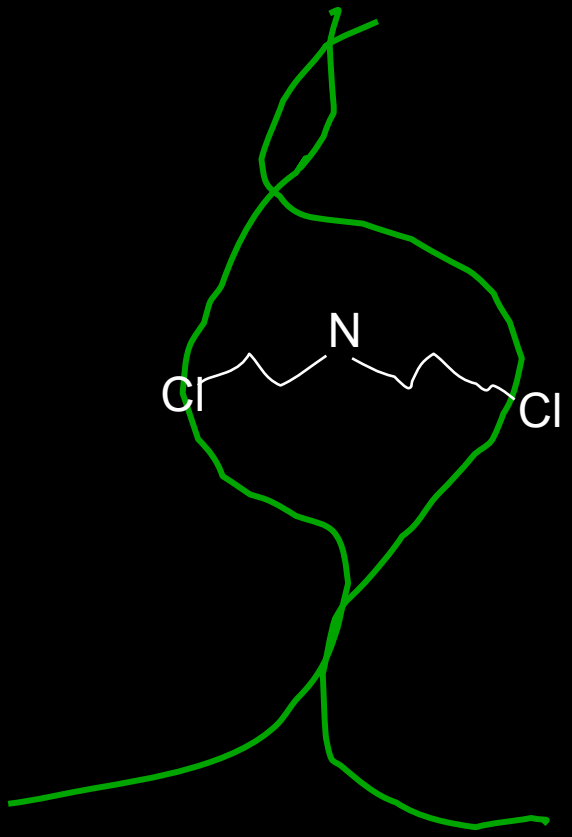
Nitrogen Mustards

- Oldest and most widely used alkylators

1) Based on the "mustard gas" used in chemical warfare during WWI
   a. Lower White Blood cells level and attack rapidly dividing cells
   b. Thought to be useful against leukemia

2) Active nitrogen mustards contain two chloroethylamine chains

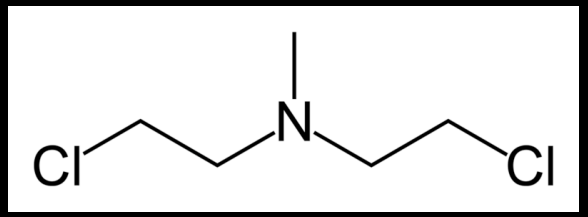


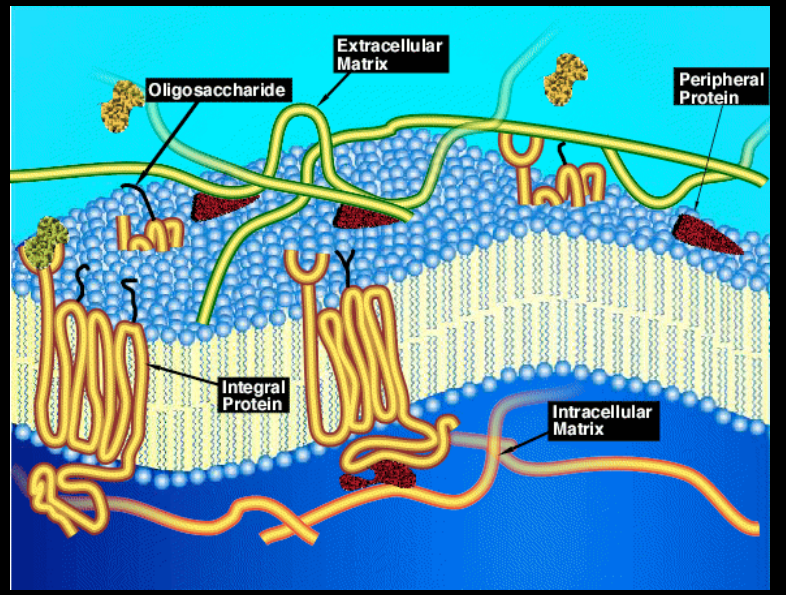


CycloPhosphamide

- Prodrug

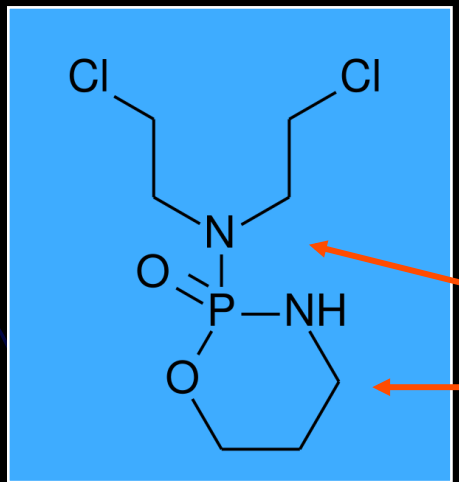
  1) Cell membrane is made of lipids: hydrophobic (non polar)





2) Membrane permeability calls for a hydrophobic segment that can force the drug into the cell

3) Cyclophosphamide



Nitrogen mustard

Hydrocarbons

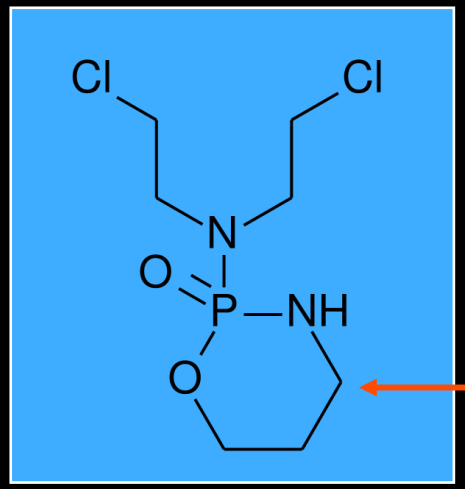
Metabolism of Cyclophosphamide

- Experimenting was not successful in cell culture, but only in living organism
- Oxidation through hepatic P450 enzyme

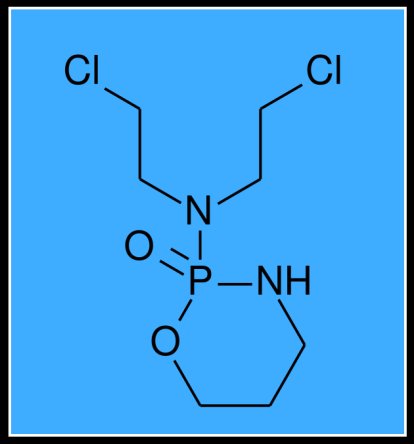
  1) occurs on the 4th carbon in the chain

  2) Once oxidation occurs, the drug is separated into its active form as it enters the cell and cross-link with DNA

- Oxidation triggers a cascade or series of metabolites that contribute to its toxicity and selectivity



Drug Metabolism



Prodrug: converted to active form through P450 and mixed function oxidase enzymes

4-hydroxycyclophosphamide

aldophosphamide

Aldehyde dehydrogenase (ALDH)

Acrolein (toxic)

Phosphoramide Mustard

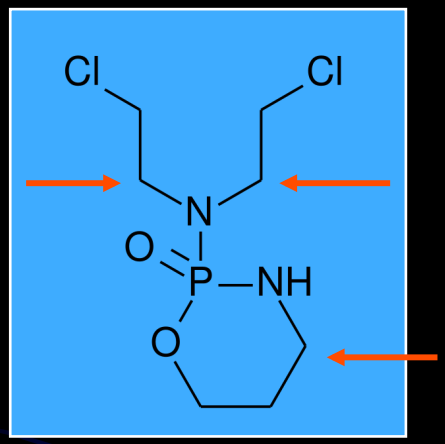
Forms DNA Cross links (Cell Death)

Carboxyphosphamide

Waste

Acrolein (toxic)

- Oxidation on the wrong carbon

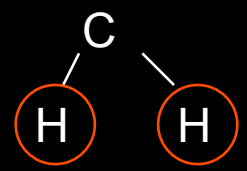


1) P450 can not differentiate between similar carbons

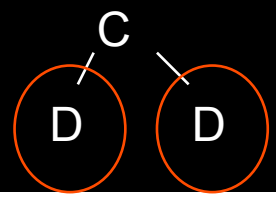
2) Mechanism is unclear. Possibly enzyme works on all three carbons: mustard forms if the right carbon (4C) is oxidized first, acrolein forms if the wrong carbon is oxidized.

3) Avoid acrolein by favoring the enzyme for the 4th carbon while disfavoring the other two similar carbons

4) Each carbon contains 2 hydrogen bonds



5) Substitute each hydrogen in the mustard with its isotope (deuterium)



Hypothesis

- Oxidation will continue to work on all three carbons however with the addition of deuterium, the C4 will be favored.
- Once oxidation occurs on the C4, the metabolism will follow the phosphoramide mustard pathway.

Materials

1. Diethyl iminodiacetate
2. $LiALD_4$
3. Thionyl Chloride

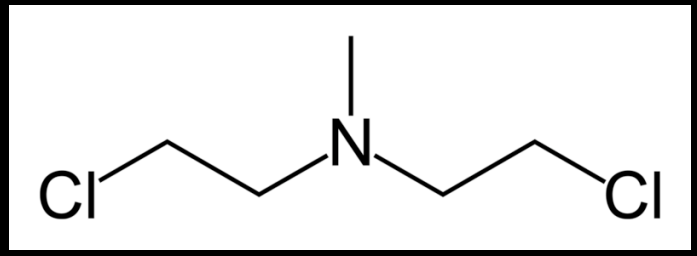
Reagents

4. Soxhlet
5. Roter Vap
6. Pump

Extract compound from solvents

7. TLC
8. NMR

Analyze compounds



Conclusion

- By modifying cyclophosphamide in changing the mustard hydrogen to its deuterium isotope, we believe the oxidation will occur on the 4^{th} carbon, thus avoiding the acrolein side effect of the drug.
- Drug will effect only cancer cells due to the prescence of ALDH in normal ones.
- A break through in cancer research if proved effective.

Future work

- Continue working on synthesizing the Prodrug form, then sending it out for further testings in cell cultures, mice, and mammals.

References

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know cancer