Radiation Biochemistry

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Objectives

- Radiation chemistry and chemical effects
- DNA/Chromosome damage and repair mechanisms
- mechanisms of cell death and cell death
Overview

- **Fundamentals of Radiobiology**
- DNA Strand Breaks
- Radiosensitivity
- The Four Rs of Radiobiology
- Summary
What is Radiobiology?

- The study of the effects of ionizing radiations on living things.
- Examines the biological absorption of energy from radiation.
Absorption of Energy

- May lead to *excitation* or *ionization*.
  - *Excitation* – an electron in an atom or molecule is elevated to a higher energy level without actual ejection
  - *Ionization* – one or more orbital electrons are ejected from the atom or molecule
Ionizing Radiation

- *Sparsely ionizing radiation*
  - primary ionizing events are spatially well separated
  - x- and γ-rays

- *Densely ionizing radiation*
  - dense column of ionization produced
  - α particles, low-energy neutrons, and other charged particles
Energy Released

- 33 eV - energy dissipated per ionizing event
- 4.9 eV - energy associated with a C=C bond
- Each ionizing event releases enough energy to break at least one strong chemical bond.
- The most frequent energy transfer event by fast moving electrons is called SPUR and involves about 60 eV of energy transfer, on average.
Fundamentals of Radiobiology

- Two categories of biological effects
  - Stochastic effects
  - Non-stochastic, or deterministic, effects

- Two mechanisms of biological damage
  - Direct action
  - Indirect action
Direct Biological Action

- Involves the direct insult to a molecule by ionization and excitation.

- *Directly ionizing radiation* – contains sufficient kinetic energy to directly disrupt the atomic structure of the absorber and produce chemical and biological changes (electrons, protons, alpha particles, beta particles, etc.)
Indirect Biological Action

- *Indirectly ionizing radiation* – give up their energy in the absorber to produce fast-moving charged particles
- Typically involves a *free radical*.
  - A free atom or molecule carrying an unpaired orbital electron in the outer shell.
  - Associated with high degree of chemical reactivity
  - Formed from interactions with water (or other atom or molecule) and diffuses to reach and damage the critical targets (principally DNA).
Free Radical Production

- Usually involve water molecules - 80% of a cell is composed of water.
- The water molecule is ionized following interaction with a photon or a charged particle.
- $\text{H}_2\text{O}^+$ is an ion radical - electrically charged and contains an unpaired electron in the outer shell.

$$\text{H}_2\text{O} \rightarrow \text{H}_2\text{O}^+ + \text{e}^-$$
Free Radical Production (cont.)

- The ion radical reacts with another water molecule to form the highly reactive hydroxyl radical.

- The hydroxyl radical diffuses to the critical target in a cell, and is estimated to cause approximately two thirds of the x-ray damage to mammalian cell DNA.

\[
H_2O^+ + H_2O \rightarrow H_3O^+ + OH\cdot
\]
Synopsis of Indirect Action
Overview

- Fundamentals of Radiobiology
- **DNA Strand Breaks**
- Radiosensitivity
- The Four Rs of Radiobiology
- Summary - Factors Influencing Radiation Effects
Direct vs. Indirect Actions for X-rays

- **Direct action** –
  - damage to DNA results from a fast $e^-$ ejected following interaction with the photon.

- **Indirect action** –
  - fast $e^-$ interacts with a water molecule and free radicals are produced which interact with and damage DNA.
Direct vs. Indirect Effects

**Direct effect**

Cleaves (breaks) DNA

**Indirect effect**

Free radicals attach to broken ends of DNA not allowing repairs to occur!!

(Mutations might occur)

http://www.chem.fsu.edu/~alabugin/HomePage.htm
DNA Lesions

- **Single strand breaks**
- **Double strand breaks**
  - believed to be the most important lesion produced in chromosomes by radiation and may result in cell killing, mutation, or carcinogenesis
- **Interstrand cross link**
- **Base Change**
- **Dimer formations** – bond between two base pairs in same strand
Beta Radiation Damage to DNA

- Most common DNA strand break in the C=C bond
DNA Strand Breaks

- Critical target in cell is DNA

- Single strand breaks
  - Many single-strand breaks are produced in DNA by radiation
  - Usually repaired – the opposite strand is used as template
  - Misrepair may result in mutation

- Breaks involving both strands
  - If breaks are well separated, repair usually occurs because the two breaks are handled separately.
  - If breaks are opposite one another or only separated by a few base pairs, may lead to a double-strand break (i.e. piece of chromosome snaps into two pieces), resulting in DNA fragments.
DNA Fragments Actions

- Breaks may rejoin in their original configuration.
- Breaks may fail to rejoin and give rise to an aberration.
- Broken ends may reassort and rejoin other broken ends, resulting in grossly distorted chromosomes.
Chromosome Breaks
Overview

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- **Radiosensitivity**
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In 1906, Bergonie and Tribondeau equated radiosensitivity to reproductive capacity.\textsuperscript{13}

All physicians have observed with the same interest mixed with surprise that x-irradiation can kill the cells of a neoplasm without impairing the neighboring healthy tissues or even the tissues invaded by the tumor. But experiments on animals have shown that x-rays have a selective action among healthy tissues. For example, in our experiments on the testicle of rat, we have been able to destroy the germinal cells whereas the interstitial tissue and Sertoli syncytium were unimpaired. As a result of these experiments, it has been possible to formulate the following law: X-rays are more effective on cells which have a greater reproductive activity; the effectiveness is greater on those cells which have a longer dividing future ahead, on those cells the morphology and the function of which are least fixed. From this law, it is easy to understand that roentgen radiation destroys tumors without destroying healthy tissues.\textsuperscript{13}
Radiosensitivity

- Directly proportional to the reproductive capacity
  - Most radiosensitive are cells that are most active in reproducing themselves

- Varies inversely with the degree of differentiation
  - Bone marrow, lymphoid tissue, reproductive organs among most radiosensitive
  - Muscle and bone cells least radiosensitive

- Also affected by the metabolic state of the cell, state of cell division, and state of nourishment
  - Most radiosensitive are cells that have a high metabolic rate and are more nourished than others
  - Cells not fully mature will be more harmed by radiation than mature cells
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3 Categories for Cell Damage

1. LD (Lethal damage) –
   - irreversible, irreparable, and leads to cell death

2. SD (Sub-lethal damage) –
   - usually repaired in hours unless additional sub-lethal damage added
   - Cell survival is increased when a given radiation dose is split into two fractions separated by a time interval.
   - repair varies with type of radiation used.

3. PLD (Potentially lethal damage) –
   - component that can be modified by post-irradiation environmental conditions
   - Experiments indicate no PLD repair following exposure to high LET radiation.
   - Repair seen when mitosis is delayed
THE FOUR Rs OF RADIOBIOLOGY

Now, more than 60 years later, we can account for the efficacy of fractionation based on more relevant radiobiological experiments. We can appeal to the four Rs of radiobiology:

Repair of sublethal damage
Reassortment of cells within the cell cycle
Repopulation
Reoxygenation

Eric J. Hall, M.D.
Synopsis of the 4 Rs

• Dividing a dose into a number of fractions *spares* normal tissue because of *repair* of SD between dose fractions and *repopulation* of cells if overall time is sufficiently long.

• At the same time dividing a dose into a number of fractions *increases* damage to the tumor because of *reoxygenation* and *reassortment* of cells into radiosensitive phases of the cycle.
Dose-Rate Effect

- Curve A – survival curve for a single acute exposure of x-rays
- Curve F – idealized curve obtained if each dose is given as a series of small fractions of size $D_1$, with an interval between fractions sufficient for repair of SD to occur
- Multiple small fractions approximate to a continuous exposure to a low dose rate
dose-rate effect: repair of SD, redistribution in the cycle, and cell proliferation
The Oxygen Effect

Indirect Action Dominant for X-Rays

Direct Action
Reoxygenation

- Figure shows reoxygenation of previously hypoxic cells following each dose of radiation, such that additional cells are killed with each subsequent dose.
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**Summary**
Factors Influencing Radiation Effects

1. Sensitivity of the individual
   - Effects may differ greatly for a select group compared to a heterogeneous group

2. Nature or type of radiation
   - Equal doses of different types of radiation do not produce equal biological effects

3. The radiation absorbed dose

4. Time distribution or fractionation
   - A lethal dose given in a short time may not be lethal if protracted over a long time

5. Dose distribution
   - Whole-body or only a specific organ involved

6. Age at irradiation
   - Response is altered during growth in some systems
Q1. Free Radical Production from water molecules involves all of the following except______.

a) The water molecule is ionized following interaction a charged particle.

b) Ion radical is formed from ionized water molecule by carrying a paired orbital electron in the outer shell.

c) The ion radical reacts with another water molecule to form the highly reactive hydroxyl radical.

d) Free radical production is estimated to cause approximately two thirds of the x-ray damage to mammalian cell DNA.
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Q2. DNA lesions are the result of

__________________.

a) Indirect beta particle interactions with a water molecule that forms free radicals that break the bond between two base pairs in same strand
b) Direct beta particle interactions resulting in single or double strand breaks
c) A and B
d) None of the above
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Q3. Dividing a dose into a number of fractions increases damage to the tumor because ______________.

a) there is **repair** of damage between dose fractions.

b) **repopulation** of tumor cells overall time is sufficiently long.

c) **Reoxygenation** results in a change in chemical composition of free radicals

d) **reassortment** of cells is into radiosensitive phases of the cycle.
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d) *reassortment of cells* is into radiosensitive phases of the cycle.