Introduction to Radiation Biology

Survey of Clinical Radiation Oncology

Lecture 2

Outline

- Ionizing radiation
- Development of radiobiological damage
- Cell cycle
- Cell survival curves
- Tissue response and fractionation

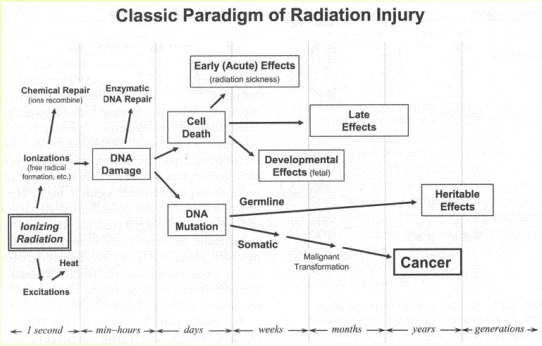
Radiation biology

- **Radiation biology** is the study of the action of ionizing radiation on living organisms
- The action is very complex, involving physics, chemistry, and biology
  - Different types of ionizing radiation
  - Energy absorption at the atomic and molecular level leads to biological damage
  - Repair of damage in living organisms
- Basic principles are used in radiation therapy with the objective to treat cancer with minimal damage to the normal tissues

Indications for radiation therapy

- Radiation therapy may be used to treat almost every type of solid tumor, including cancers of the brain, breast, cervix, larynx, lung, pancreas, prostate, skin, spine, stomach, uterus, or soft tissue sarcomas
- Radiation can also be used to treat leukemia and lymphoma (cancers of the blood-forming cells and lymphatic system, respectively)
- Radiation dose to each site depends on a number of factors: the type of cancer and whether there are tissues and organs nearby that may be damaged by radiation
- Palliative radiation therapy also can be given to help reduce symptoms such as pain from cancer that has spread to the bones or other parts of the body

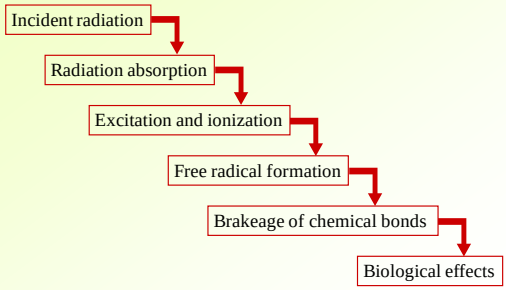
Development of radiobiological damage



Characteristic time scales

- The physical event of absorption occurs over about 10^{-15} seconds
- The biologic lifetime of the free radical is on the order of 10^{-10} - 10^{-9} seconds (10^{-5} seconds in the presence of air)
- The expression of cell death may take up to days to months
- The expression of carcinogenesis may take years or generations

Development of radiobiological damage



Absorption of radiation

- Biological systems are very sensitive to radiation
- Absorption of 4 Gy in water produces the rise in temperature ~10^{-3} ^{o}C (~67 cal in 70-kg person)
- Whole body dose of 4 Gy given to human is lethal in 50% of cases (LD50)
- The potency of radiation is in its concentration and the damage done to the genetic material of each cell

Types of ionizing radiations

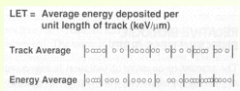
- Electromagnetic radiations
  - X-rays and Gamma-rays
- Particulate radiations
  - Electrons, protons, α-particles, heavy charged particles
  - Neutrons
- All charged particles: directly ionizing radiation
- X and γ-rays, as well as neutrons – indirectly ionizing radiation

Types of ionizing radiations

- If radiation is absorbed in biologic material, ionizations and excitations occur in a pattern that depends on the type of radiation involved
- Depending on how far the primary ionization events are separated in space, radiation is characterized as *sparsely ionizing* (x-rays) or *densely ionizing* (α-particles)
- Heavier particles with larger charge produce higher ionization density
- For a given particle type, the density of ionization decreases as the energy (and velocity) goes up

Linear Energy Transfer

- **Linear energy transfer** (LET) is the energy transferred per unit length of the track
- The special unit usually used for this quantity is keV/μm of unit density material
- It is an average quantity, typically *track* averaged



$$LET = dE / dl$$

Linear Energy Transfer

Typical Linear Energy Transfer Values

Radiation	Linear Energy Transfer, keV/μm	
Cobalt-60 γ-rays	0.2	
250-kV x-rays	2.0	
10-MeV protons	4.7	
150-MeV proton	0.5	
	Track Avg.	Energy Avg.
14-MeV neutrons	12	100
2.5-MeV α-particles	166	
2-GeV Fe ions (space radiation)	1,000	

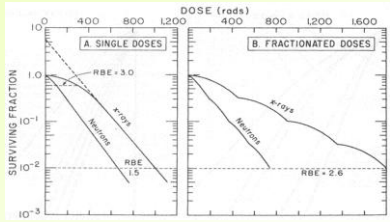
- The method of averaging makes little difference for x-rays or for mono-energetic charged particles, but the track average and energy average are different for neutrons

Relative Biological Effectiveness

- The amount or quantity of radiation is expressed in terms of the *absorbed dose*
- Equal doses of different types of radiation do not, however, produce equal biologic effects: 1 Gy of neutrons produces a greater biologic effect than 1 Gy of x-rays due to the difference in the pattern of energy deposition at the microscopic level
- **The relative biologic effectiveness (RBE)** of some test radiation (r) compared with 250 kV x-rays is defined by the ratio D_{250}/D_r where D_{250} and D_r are, respectively, the doses of x-rays and the test radiation required for equal biological effect

Relative Biological Effectiveness

The RBE increases as the dose is decreased



- Because the x-ray and neutron survival curves have different shapes the resultant RBE depends on the level of biologic damage chosen
- The RBE for a fractionated regimen with neutrons is greater than for a single exposure (because the RBE is larger for smaller doses)

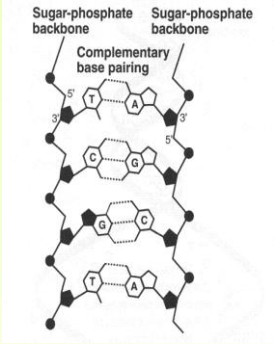
Biological effect

- The biological effect is expressed in cell killing, or cell transformation (carcinogenesis and mutations)
- The primary target of radiation is DNA molecule, suffering breaks in chemical bonds
- Depending on the extent of the damage, it can be repaired through several repair mechanisms in place in a living organism

Classification of radiation damage

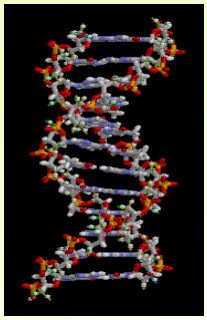
- Radiation damage to mammalian cells can operationally be divided into three categories:
  - **Lethal damage**, which is irreversible and irreparable and, by definition, leads irrevocably to cell death;
  - **Potentially lethal damage** (PLD), the component of radiation damage that can be modified by post-irradiation environmental conditions; and
  - **Sublethal damage** (SLD), which under normal circumstances can be repaired in hours unless additional sublethal damage is added (e.g., from a second dose of radiation)

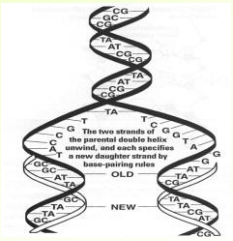
The structure of DNA



- DNA molecule has many deoxyribo-nucleotides (bases) linked in a chain-like arrangement
- Bases are held by hydrogen bonds and are paired complimentary:
  - adenine with thymine
  - cytosine with guanine
- Each half constitutes a template for reconstruction of the other half

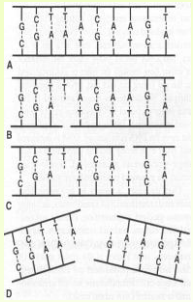
The structure of DNA





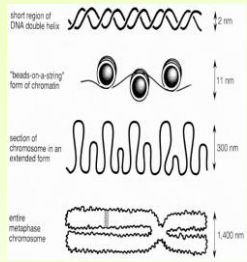
- The molecule is composed of two anti-parallel helices, twisted like a ladder
- During cell division each strand is self-replicated resulting in identical molecules

DNA strand breaks



- **Single-strand breaks** are of little biologic consequence because they are repaired readily using the opposite strand as a template
- **Double-strand breaks** are believed to be the most important lesions produced in chromosomes by radiation; the interaction of two double-strand breaks may result in cell killing, carcinogenesis, or mutation

Chromosomes

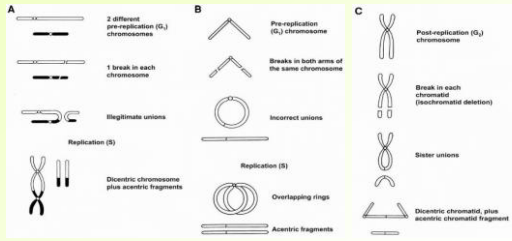


- DNA molecules carry the genetic information
- Chromosome is an organized structure of DNA and DNA-bound proteins (serve to package the DNA and control its functions)
- Chromosomes are located mostly in cell nucleus (some amount is in mitochondria)

Chromosome aberrations

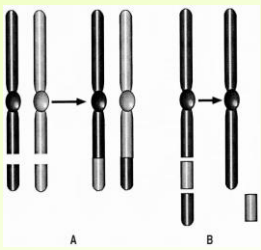
- Damage to DNA may result in lethal damage to the cell
- The cell will attempt to repair the damage
- Efforts to repair the damage may result in chromosome aberrations
- Depending on the types of aberration they can be lethal or non-lethal, but result in **mutations** which can be perpetuated in subsequent cellular divisions

Radiation-induced aberrations



Lethal aberrations include dicentrics (A), rings (B), and anaphase bridges (C)

Radiation-induced aberrations



Symmetric translocations and small deletions are nonlethal

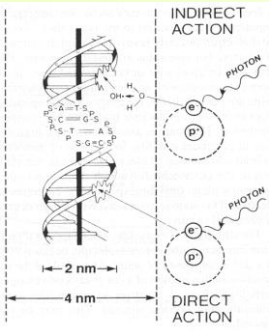
A: Symmetric translocation: radiation produces breaks in two different pre-replication chromosomes. The broken pieces are exchanged between the two chromosomes, and the "sticky" ends rejoin.

B: Deletion: radiation produces two breaks in the same arm of the same chromosome

Mutations

- If occur in the germ cells (sperm and ova) they can be passed on as genetic abnormalities in offspring
- If they occur in the somatic cells (the cells that make up an organism) they can lead to the development of diseases including cancer - this is called **carcinogenesis**
- There are genes called *oncogenes* that affect cancer incidence
- If an inhibitory oncogene is lost due to a deletion the patient is at higher risk for cancer formation

Direct and indirect actions



- In direct action, a secondary electron resulting from absorption of an x-ray photon interacts with the DNA to produce an effect
- In indirect action, the secondary electron interacts with, for example, a water molecule to produce a hydroxyl radical (OH-), which in turn produces the damage to the DNA
- The DNA helix has a diameter of ~ 2 nm; free radicals produced in a cylinder with a diameter ~ 4 nm can affect the DNA
- Indirect action is dominant for sparsely ionizing radiation (x-rays)

Free radicals

- **A free radical** is an atom or molecule carrying an unpaired orbital electron in the outer shell. This state is associated with a high degree of chemical reactivity
- Since 80% of a cell is composed of water, as a result of the interaction with a photon or a charged particle, the water molecule may become ionized:

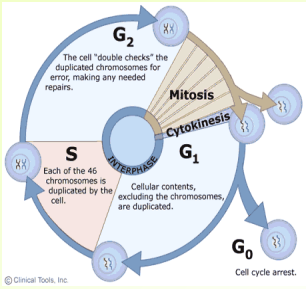
$$H_2O \rightarrow H_2O^{+\bullet} + e^-$$

- $H_2O^{+\bullet}$ is an ion radical with a lifetime of ~10^{-10} s; it decays to form highly reactive hydroxyl free radical *OH•*

$$H_2O^{+\bullet} + H_2O \rightarrow H_3O^+ + OH\bullet$$

- About 2/3 of the x-ray damage to DNA in mammalian cells is caused by the hydroxyl radical (lifetime of ~10^{-3} s)

The cell cycle



- **M** - mitosis, identifiable by light microscopy and the most constant time (~ 1 hr)
- **S** - DNA synthesis phase
- **G_1** - the first gap in activity, between mitosis and the S phase (most variable length)
- **G_2** - the second gap in activity, between S phase and the next mitosis
- If the cells stop progressing through the cycle (if they are arrested) they are in **G_0**

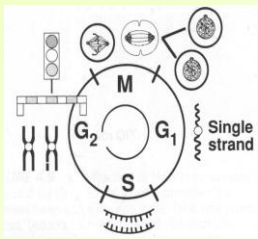
Variation of radiosensitivity with cell age in the mitotic cycle

- Cells are *most sensitive* at or close to M (mitosis)
- G2 phase is usually as sensitive as M phase
- *Resistance is usually greatest* in the latter part of S phase due to repairs that are more likely to occur after the DNA has replicated
- If G1 phase has an appreciable length, a resistant period is evident early in G1, followed by a sensitive period toward the end of G1

Cell cycle times

- Fast-growing cells in culture and some cells in self-renewing tissues have total cell-cycle time length of 10 hours
- Stem cells in resting normal tissue, such as skin, have a cell-cycle time of 10 days
- It usually is found that the malignant cells have the shorter cycle time than the normal-tissue cells

Molecular checkpoint genes



- Cell-cycle progression is controlled by a family of *molecular checkpoint genes*
- Their function is to ensure the correct order of cell-cycle events
- The genes involved in radiation effects halt cells in G2, so that an inventory of chromosome damage can be taken, and repair initiated and completed, before the mitosis is attempted
- Cells that lack checkpoint genes are sensitive to radiation-induced cell killing, and carcinogenesis

Molecular checkpoint genes

Table 2.2 Radiation-induced cell-cycle checkpoints and their characteristics

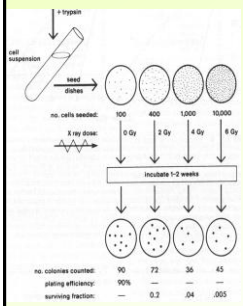
Position	Primary signalling proteins	Applies to cells irradiated in	Features
G1	ATM, p53, p21	G1	Prevents entry into S
S	ATM, Chk1/Chk12, CDC25A/ CDC25C, BRCA1, BRCA2	S	Slows progression through S
G2-early	ATM, Chk1/Chk12, CDC25A/ CDC25C, BRCA1, BRCA2	G2	Prevents entry into mitosis
G2-late	ATR, Chk1, CDC25A/CDC25C	All phases	Accumulation of cells in G2

- DNA damage repair involves activation of a group of highly interrelated signaling pathways; two major groups: sensors and effectors
- As many as ~700 proteins may be involved

Mechanisms of cell death after irradiation

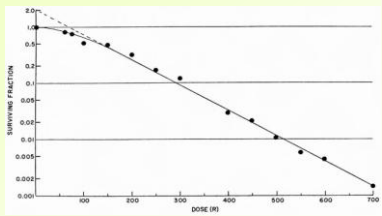
- The main target of radiation is cell's DNA; single breaks are often reparable, double breaks lethal
- Mitotic death – cells die attempting to divide, primarily due to asymmetric chromosome aberrations; most common mechanism
- Apoptosis – programmed cell death; characterized by a predefined sequence of events resulting in cell separation in apoptotic bodies
- Bystander effect – cells directly affected by radiation release cytotoxic molecules inducing death in neighboring cells

Cell survival curves



- A **cell survival curve** describes the relationship between the radiation dose and the proportion of cells that survive
- They usually are presented in the form with dose plotted on a linear scale and surviving fraction on a logarithmic scale
- Straight-line dependence means that the surviving fraction is an exponential function of dose
- At higher doses the curve bends

Cell survival curves



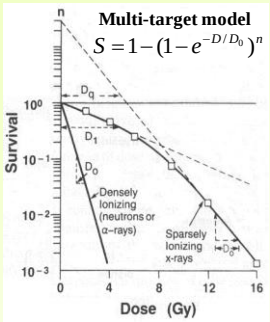
• Survival curve for HeLa cells in culture exposed to x-rays
• All mammalian cells, normal or malignant, regardless of their species of origin, exhibit similar x-ray survival curves

Cell survival curve: multi-target model

- Multi-target single hit model: assume the cell has *n* targets to be 'hit' for the cell to not survive
- Probability of each 'hit' not being successful is $e^{-D/D0}$
- Probability of each 'hit' being successful is $1-e^{-D/D0}$
- Probability of all n targets within a cell to be 'hit' is $(1-e^{-D/D_0})^n$
- The probability of survival of cell containing n targets:

$$S=\frac{N}{N_0}=1-(1-e^{-D/D_0})^n$$

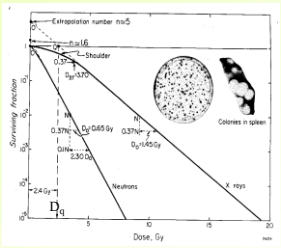
Cell survival curve parameters



- D_1 – initial slope (the dose required to reduce the fraction of surviving cells to 37% of its previous value); D_0 – final slope
- D_q – quasi-threshold, the dose at which the straight portion of the survival curve, extrapolated backward, cuts the dose axis drawn through a survival fraction of unity
- n – extrapolation number
- Radiosensitive cells are characterized by curves with steep slope D_0 and/or small shoulder (low n)

Cell survival curve parameters

Multi-target model



$$S = 1-(1-e^{-D/D_0})^n$$

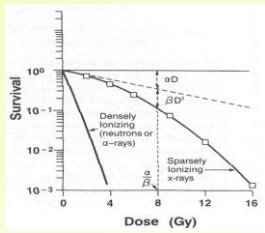
for $D >> D_0$

$$S = ne^{-D/D_0}$$

- Setting D=0, find n – the number of targets
- To find D_q, set S=1
- Relationship between n and D_q:

$$1 = ne^{-D_q/D_0},\ D_q = D_0 \ln n$$

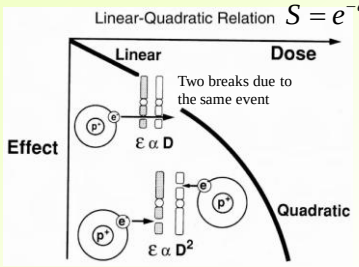
Survival curves and LQ model



- **Linear-quadratic (LQ)** model assumes there are two components to cell killing, only two adjustable parameters
- No final straight portion that is observed experimentally
- An adequate representation of the data up to doses used as daily fractions in clinical radiotherapy

$$S = \frac{N}{N_0} = e^{-\alpha D-\beta D^2}$$

Survival curves and LQ model

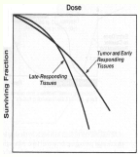


α/β ratios

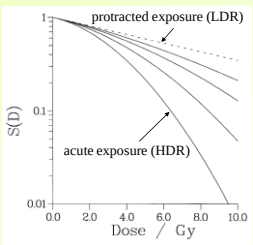
- If the dose-response relationship is adequately represented by LQ-model:

$$S \sim e^{-\alpha D-\beta D^2}$$

- The dose at which $\alpha D=\beta D^2$, or $D= \alpha/\beta$
- The α/β ratios can be inferred from multi-fraction experiments
- The value of the ratio tends to be
  - larger (~10 Gy) for tumors and early-responding tissues
  - lower (~2 Gy) for late-responding tissues



Repair of sub-lethal damage



- In the presence of repair mechanisms sublesions may be eliminated *before* the next hit arrives - dose rate becomes relevant
- As the dose rate decreases the quadratic term (βD^2) becomes smaller
- At very low dose rates only the linear term, αD, remains

Fractionation

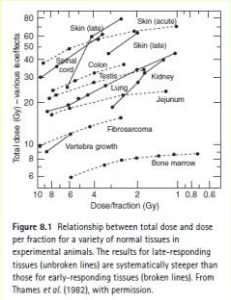


Figure 8.1 Relationship between total dose and dose per fraction for a variety of normal tissues in experimental animals. The results for late-responding tissues (unbroken lines) are systematically steeper than those for early-responding tissues (broken lines). From Thames *et al.* (1982), with permission.

- The main objective of fractionation is sparing of the normal tissues
- Using LQ model can potentially find an equivalent treatment

Equivalent treatment

- To find biologically equivalent treatments, one would use the following expression:

$$\alpha D + \beta q D^2 - \frac{t}{T_{pot}} = \alpha_1 D_1 + \beta_1 q_1 D_1^2 - \frac{t_1}{T_{pot,1}}$$

- The dose-rate function quantifying sublesion damage repair that occurs between events $q(t)$
- T_{pot} – potential doubling time characterizes cell kinetics
- This equation must be applied separately to early- (e.g., tumor) and late-responding tissues

The four Rs of radiobiology

- Fractionation of the radiation dose typically produces better tumor control for a given level of normal-tissue toxicity than a single large dose
- Radiobiological basis for fractionations (4 Rs):
  - *Repair* of sublethal damage in normal tissues
  - *Reassortment* of cells within the cell cycle move tumor cells to more sensitive phase
  - *Repopulation* of normal tissue cells; however too long treatment time can lead to cancer cell proliferation
  - *Reoxygenation* of tumor cells as tumor shrinks
- Prolongation of treatment spares early reactions

Early and late responding tissues

- Rapidly dividing self-renewing tissues respond early to the effects of radiation; examples: skin, intestinal epithelium, bone-marrow
- Late-responding tissues: spinal cord, lung, kidney
- Early or late radiation response reflects different cell turnover rates

Early effects

- *Early* (*acute*) effects result from death of a large number cells and occur within a few days or weeks of irradiation in tissues with a rapid rate of turnover.
- Examples: the epidermal layer of the skin, gastrointestinal epithelium, and hematopoietic system
- The time of on-set of early reactions correlates with the relatively short life span of the mature functional cells
- Acute damage is repaired rapidly and may be completely reversible

Late effects

- *Late* effects occur predominantly in slow-proliferating tissues, and appear after a delay of months or years from irradiation
- Examples: tissues of the lung, kidney, heart, liver, and central nervous system
- The time of on-set of late reactions correlates with the relatively long life span of the mature functional cells
- Late damage may improve but is never completely repaired

Dose rate effect

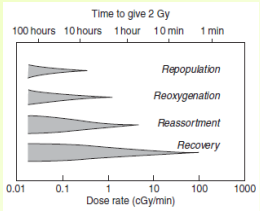


Figure 12.1 The range of dose rates over which repair, reassortment and repopulation modify radiosensitivity depends upon the speed of these processes. From Steel *et al.* (1986), with permission.

Kogel, et al., Basic clinical radiobiology

- LDR – 0.4-2 Gy/h
- HDR – over 12 Gy/h (20 cGy/min)
- EBRT – 100-500 cGy/min
- Dose rate effect results primary from repair of sublethal damage; repopulation may play a role for treatment times >1-2 days
- Most normal tissues show considerable sparing as the dose rate reduces

The Law of Bergonie and Tribondeau

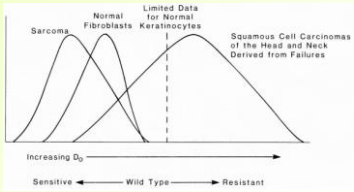
- Formulated in 1906: "X-rays are more effective on cells which have a greater reproductive activity"
- Now refined to the more rapidly responding a population of cells the more radioresponsive they are

Radiosensitivity of cancer cells

- Highly *radiosensitive* cancer cells are rapidly killed by modest doses of radiation. These include leukemia, most lymphomas, some sarcomas (connective tissue cancers), and germ cell tumors
- The majority of epithelial cancers (carcinomas) have only moderate radiosensitivity
- Some types of cancer, such as renal cell cancer and melanoma, are notably radioresistant, with much higher doses required to produce a radical cure than may be safe in clinical practice

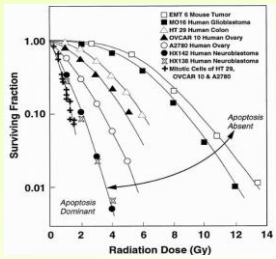
Radiosensitivity of cancer cells

Summary of D_0 values for cells of human origin (in vitro studies)



- Cells from human tumors have a wide range of radiation sensitivities
- In general, squamous cell carcinoma cells are more resistant than sarcoma cells

Cell radiosensitivity

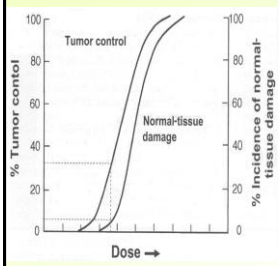


- There is a number of factors that influence cell radiation sensitivity even in vitro (position in cell cycle, genetic abnormalities, environment)
- The mechanism of cell death can be dominated by apoptosis or mitosis; most cells are in-between
- Cells in mitosis show the same sensitivity

Genetic control of radiosensitivity

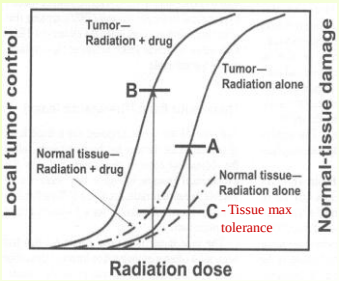
- A number of genes is involved in determining radiosensitivity of mammalian cells
- In many cases this sensitivity has been related greatly reduced ability to repair double-strand DNA breaks
- Some of the inherited human syndromes are associated with high radiosensitivity
  - Ataxia telangiectasia (AT), Down's syndrome, etc.

Dose-response relationships



- Curves are typically sigmoid (S) -shaped for both tumor and normal cells
- Therapeutic ratio (index): tumor response for a fixed level of a normal tissue damage

Therapeutic ratio



- The time factor is often employed to manipulate the TR (hyperfractionation for sparing of late-responding normal tissues)
- Addition of a drug, a chemotherapy agent, or a radio-sensitizer may improve the TR

Chemical factors influencing cell response

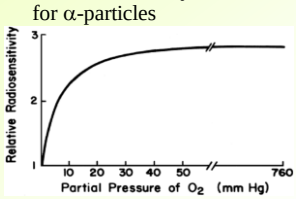
- **Radioprotectors** – chemicals that reduce the biological effects of radiation
- Mechanisms of action:
  - Scavenging of free radicals
  - Facilitation of direct chemical repair at sites of DNA damage
- Amifostine (trade name Ethyol) is the only FDA approved drug for use in radiation therapy, e.g. head and neck cancer treatments

Chemical factors influencing cell response

- **Radiosensitizers** are employed to increase the lethal effects of radiation in tumor cells
- Have to show the differential affect between tumor and normal cells
- Two types have found practical use in radiotherapy:
  - Halogenated pyrimidines: more drug is incorporated in fast-proliferating tumor cells
  - Hypoxic cell sensitizers: hypoxic cells occur only in tumors

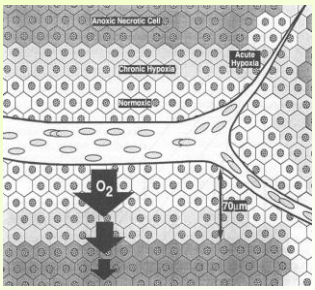
Oxygen effect

- Oxygen makes the damage produced by free radicals permanent; the damage can be repaired in the absence of oxygen
- Oxygen enhancement ratio OER=3 can be achieved for x-rays; OER=1.6 for neutrons; only 1 for α-particles



Only 3 mm Hg, or about 0.5% of oxygen is required to achieve a relative radiosensitivity halfway between anoxia and full oxygenation

Tumor oxygenation



- Oxygen can diffuse at only about 70 µm from the blood vessel
- Solid tumors often outgrow their blood supply and become hypoxic
- Cells not receiving oxygen and nutrients become necrotic

Tissue response to radiation damage

- Cells of normal tissues are not independent
- For an tissue to function properly its organization and the number of cells have to be at a certain level
- Typically there is no effect after small doses
- The response to radiation damage is governed by:
  - The inherent cellular radiosensitivity and position in the cell cycle at the time of radiation
  - The kinetics of the tissue
  - The way cells are organized in that tissue

The volume effect in radiotherapy

- Generally, the total dose that can be tolerated depends on the volume of irradiated tissue
- It also depend on the structural organization of the tissue in terms of the spatial arrangement of functional sub-units, FSU's (the nephron in the kidney, the lobule in the liver):
  - If FSUs are arranged in a series, elimination of any unit is critical to the organ function (example: spinal cord)
  - If FSUs are arranged in parallel, elimination of a single unit is not critical to the organ function (kidney and lung)

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