Dose-Response Relationships for Model Normal Tissues

Chapter 19
Eric J. Hall, Amato Giaccia, Radiobiology for the Radiologist

Introduction

- Dose-response relationships are important for prescribing a proper therapy course
- Response is quantified as either increase of radiation effects in severity, or frequency (% incidence), or both
- In vitro vs. in vivo experiments
- Different cells have different response based on their reproduction rate (acute vs. late effects)

Cell survival curve parameters

- Multi-target model
  \[ S = 1 - (1 - e^{-\frac{D}{D_0}})^n \]
- \( D_1 \) – 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 \( n \))

Survival curves and LQ model

- Linear-quadratic model assumes there are two components to cell killing, only two parameters
- An adequate representation of the data up to doses used as daily fractions in clinical radiotherapy

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

\( \alpha/\beta \) 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 \( \alpha/\beta \) ratios can be inferred from multi-fraction experiments, assuming:
  - each dose in fractionated regime produces the same effect
  - there is full repair of sub-lethal damage between fractions
  - there is no cell proliferation between fractions

\( \alpha/\beta \) ratios

- For the total dose \( D \) divided in \( n \) equal fractions of dose \( d \)
  \[ S = (e^{-\alpha d - \beta d^2})^n \Rightarrow \]
  \[ -\ln S \Gamma nd = \alpha + \beta d \]
- If the reciprocal dose \( 1/\Gamma d \) is plotted against the dose per fraction \( d \), the ratio of the intercept/slope gives \( \alpha/\beta \)
\( \alpha/\beta \) ratios

- LQ approach is used for iso-effect calculations (equivalent fractionation schemes)
- Has its limitations, for example, when continuous radiation is used (>5cGy/h dose rate), not allowing for complete repair of SLD

\[ \alpha/\beta \]

Acute, mouse skin
Late, mouse kidney

Slopes are different

\( \alpha/\beta \) ratios

- Acute, mouse skin
- Late, mouse kidney

\( \alpha/\beta \) ratios

- Tumors should follow \( \alpha/\beta \) for early-responding tissues …

\( \alpha/\beta \) ratios

- Dose-response relationships
  - Curves are typically sigmoid (S)-shaped for both tumor and normal cells (y-axis is “flipped” compared to cell survival curves)
  - Therapeutic ratio (index)
  - TR: 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 radiosensitizer may improve the TR
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
  - Cell shrinks, chromatin condenses, cell breaks into fragments, no inflammation

Mechanisms of cell death after irradiation

- Additional mechanisms under investigation:
  - Autophagic: cell degradation of unnecessary or dysfunctional cellular components through lysosomes
  - Necrotic: cell swells, leakage of membrane, inflammation
  - Entosis: cell death by invasion
- Bystander (abscopal) effect – cells directly affected by radiation release cytotoxic molecules inducing death in neighboring cells

Assays for dose-response relationships

- Clonogenic end points
  - Depend directly on reproductive integrity of individual cells (cell survival)
  - Cell re-growth in situ and by transplantation into another site
- Functional end points
  - Reflect the minimum number of functional cells remaining in a tissue or organ
  - Dose-response can be inferred from multifraction experiments
  - More pertinent to radiation therapy

Early and late responding tissues

- Observation: cells of different tissues demonstrate different response rates to the same radiation dose
- 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

Clonogenic end points: Skin clones

- Survival curve for mouse skin cells
- Central area is given a test dose and monitored for cell re-growth

Clonogenic end points: Skin clones

- There are practical limits to the range of doses
- Determine $D_0 = 1.35$ Gy from the single dose curve
- Determine $D_q = 3.4$ Gy from the curve separation
- Values for $D_0$ and $D_q$ are similar to those obtained in vitro
Clonogenic end points: Crypt cells of the mouse jejunum

- Intestinal epithelium is a classic example of a self-renewing system
- Crypt cells divide rapidly and replenish cells on the top of villi
- Mice are given total body irradiation and are sacrificed after 3.5 days
- Radiation effect is assessed based on the number of regenerating crypts per circumference of the sectioned jejunum

Have to use high minimal dose of 10 Gy to produce enough damage

Clonogenic end points: Crypt cells of the mouse jejunum

To obtain the data for doses below 10 Gy - use multiple fractions of smaller dose

The data are fitted equally by multi-target and LQ models

Clonogenic end points: Kidney tubules

- Late responding tissue
- One kidney per mouse is irradiated and examined 60 weeks later
- Summary of response rates as time required for depletion of the epithelium after a single dose of 14 Gy:
  - 3 days in jejunum
  - 12 to 24 days in the skin
  - 30 days in the seminiferous tubules of the testes
  - 300 days in the kidney tubules

Clonogenic end points: donor-recipient approach

- Systems in which cell survival is assessed by transplantation into another site: bone-marrow stem cells, thyroid and mammary gland cells
- Un-irradiated cells are transplanted into recipient animals irradiated supralethally
- Irradiated cells are injected into white fat pads of healthy recipient animals to produce a growing unit
Normal tissue clonogenic assays summary

- Radiosensitivity of a cell line is determined by the curve shoulder ($D_0$ parameter)
- Significant variability observed

Dose-response curves for functional end points

- Can be obtained on pig and rodent skin by assessing skin reaction
- For mouse lung system based on breathing rate, assess early and late response
- Spinal cords of rats by observing myelopathy after local irradiation
  - complex system
  - various syndromes are similar to those described in humans

Myelopathy - functional disturbance or pathological change in the spinal cord

Spinal cord system

- Assess late damage caused by local irradiation of the spinal cords of rats
- First symptoms develop after 4 to 12 months
- Delayed injuries peak at 1 to 2 years postirradiation
- The regimen of dose delivery has a strong effect on the resultant effect such as the extent of necrosis, loss of functionality, etc.
- Obtain the information on the tolerance to radiation

Spinal cord system

- Dose-response relationship is steep
- Fractionation demonstrates dramatic sparing (lower graph)
- Latency decreases with increase in dose
- Variation with the region of cord irradiated

Spinal cord system

- Total dose vs. dose per fraction
- Data fitted with LQ model for $\alpha/\beta=1.5$ Gy
- LQ model overestimates the tolerance for small doses per fraction
  - Could be the result of too short repair time between the fractions

Spinal cord system

- The total volume of irradiated tissue has to have an effect on the resultant injury
- Functional subunits (FSUs) in a spinal cord are arranged in linear fashion therefore above a certain length of ~1cm the dependence is very weak
Clinical Response of Normal Tissues

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Normal tissues in radiation therapy

- The target volume in radiotherapy necessarily includes normal tissues
  - Malignant cells infiltrate into normal structures, which must be included as a tumor margin
  - Normal tissues within the tumor (soft tissue and blood vessels) are exposed to the full tumor dose
  - Normal structures in the entrance and exit areas of the radiation beam may be exposed to clinically relevant doses

Tissue response to radiation damage

- Cells of normal tissues are not independent
- For a 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
  - The kinetics of the tissue
  - The way cells are organized in that tissue

Effects beyond cell killing

Mediated by inflammatory cytokines

- Nausea or vomiting that may occur a few hours after irradiation of the abdomen
- Fatigue felt by patients receiving irradiation to a large volume, especially within the abdomen
- Somnolence that may develop several hours after cranial irradiation
- Acute edema or erythema that results from radiation-induced acute inflammation and associated vascular leakage

Response to radiation damage

- In tissues with a rapid turnover rate, damage becomes evident quickly
- In tissues in which cells divide rarely, radiation damage to cells may remain latent for a long period of time and be expressed very slowly
- Radiation damage to cells that are already on the path to differentiation (and would not have divided many times anyway) is of little consequence - they appear more radioresistant
- Stem cells appear more radiosensitive since loss of their reproductive integrity results in loss of their potential descendants
- At a cell level survival curves may be identical, but tissue radioresponse may be very different

α/β ratios

- The value of the α/β ratio tends to be
  - larger (~10 Gy) for early-responding tissues and tumors
  - lower (~2 Gy) for late-responding tissues
- There are exceptions
  - prostate cancer ~3 (or less)
  - breast cancer ~4
Early and late effects

- Early (acute) effects result from death of a large number of 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 onset 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 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 late reactions are very sensitive to dose fractionation
- Late damage may improve but is never completely repaired (could have a vascular component and loss of parenchymal cells)

Consequential late effect - a late effect consequent to, or evolving out of, a persistent severe early effect; an early reaction in a rapidly proliferating tissue may persist as a chronic injury
- Occurs upon depletion of the stem-cell population below levels needed for tissue restoration
- The earlier damage is most often attributable to an overlying acutely responding epithelial surface. Example: fibrosis or necrosis of skin consequent to desquamation (skin shedding) and acute ulceration

The survival of structurally defined FSUs depend on the survival of one or more clonogenic cells within them, which are easily depleted by low doses
- Surviving clonogens cannot migrate from one unit to another
- Tissue survival in turn depends on the number and radiosensitivity of these clonogens
- Examples: the lung, liver, and exocrine organs (salivary glands, sweat glands, etc.)

In structurally undefined FSUs the clonogenic cells that can re-populate after the depletion by radiation are not confined to one particular FSU
- Clonogenic cells can migrate from one FSU to another and allow repopulation of a depleted FSU
- Examples: reepithelialization of a denuded area of skin can occur either from surviving clonogens within the denuded area or by migration from adjacent areas.
Tissue rescue unit

- To link the survival of clonogenic cells and functional survival, introduce a concept of the **tissue rescue unit**: the minimum number of FSUs required to maintain tissue function. Model assumptions:
  - The number of tissue rescue units in a tissue is proportional to the number of clonogenic cells
  - FSUs contain a constant number of clonogens
  - FSUs can be repopulated from a single surviving clonogen
- Not all tissue fit the classification by this model

The volume effect in radiotherapy

- Generally, the total dose that can be tolerated depends on the volume of irradiated tissue
- However, the spatial arrangement of FSUs in the tissue is critical
  - FSUs are arranged in a series. Elimination of any unit is critical to the organ function
  - FSUs are arranged in parallel. Elimination of a single unit is not critical to the organ function

Casarett’s classification of tissue radiosensitivity

- Based on histological observations of early cell death
- All parenchymal cells are divided into four major categories I (most sensitive) through IV; supporting structure cells are placed between groups II and III
- The general trend: sensitivity decreases for highly differentiated cells, that do not divide regularly, and have a longer life span
- Exception: small lymphocytes – do not divide, but are very radiosensitive

Michalowski’s classification

- Tissues are following either “hierarchical” or “flexible” model, many tissues are hybrids of these two extremes
- Hierarchical model tissue consists of cells of three distinct categories (bone marrow, intestinal epithelium, epidermis)
  - Stem cells, capable of unlimited proliferation
  - Functional cells: fully differentiated, incapable of divisions, die after a finite lifespan
  - Maturing partially differentiated cells: descendants of stem cells, still multiplying
- Flexible model tissue consists of cells that rarely divide under normal conditions, no strict hierarchy (liver, thyroid, dermis)
Growth factors

- Growth factors are proteins that bind to receptors on the cell surface, with the primary result ofactivating cellular proliferation and/or differentiation. Many growth factors are quite versatile, acting on numerous different cell types, while others are specific to a particular cell type.
- The response of a tissue to radiation is influenced greatly by a host of growth factors:
  - Interleukin-1 acts as radioprotectant of hematopoietic cells
  - Basic fibroblast growth factor induces endothelial cell growth, inhibits radiation-induced apoptosis, and therefore protects against microvascular damage
  - Platelet-derived growth factor β increases damage to vascular tissue
  - Transforming growth factor β (TGF-β) induces a strong inflammatory response
  - Tumor necrosis factor (TNF) induces proliferation of inflammatory cells, and endothelial cells as well, is associated with complications. TNF protects hematopoietic cells and sensitizes tumor cells to radiation.

Radiosensitivity of specific tissues and organs

- Compilation of data (1968) in Table 20.2, pp.334-5
- Tolerance for each organ and for a partial organ irradiation (volume fraction)
  - TD5/5, Gy: dose for complication probability 5% in 5 years
  - TD50/5 dose for complication probability of 50% in 5 years
- Organs are classified as:
  - Class I - fatal or severe morbidity
  - Class II - moderate to mild morbidity
  - Class III - low morbidity

Radiosensitivity of specific tissues and organs

- Our clinic uses compilation of “Timmerman’s” dose constraints (hypofractionation, 2012)

Hematopoietic system

- Tissues are located primarily in the bone marrow
- In the normal healthy adult, the liver and spleen have no hematopoietic activity, but they can become active after partial-body irradiation
- The hematopoietic system is very sensitive to radiation, especially the stem cells
- There is little sparing from either fractioning the dose or lowering the dose rate

Skin

- The skin from the perspective of a radiation biologist, the skin has a thickness of about 100 cm; it is a matter of fact that cutaneous (SK) cells in contact with IR in 2-5% of body weight, the skin is a primary organ of radioprotection
- The skin is a primary organ of radioprotection

Hematopoietic system

- The complex changes seen in peripheral blood count after irradiation reflect differences in transit time from stem cell to functioning cell for the various circulatory blood elements
Lymphoid tissue and the immune system

- The lymphoid tissues (e.g., nodes, spleen) are very radiosensitive and get depleted by small radiation doses.
- The effect of irradiation on the immune function is complex, depending on the volume irradiated and the number of surviving cells.
- A total-body dose of 3.5 to 4.5 Gy inhibits the immune response against a new antigen.
- Partial-body irradiation, characteristic of ordinary radiation therapy, has only a limited effect on the immune response, and whether it influences metastatic dissemination is controversial.

Other organs

- The lung is an intermediate- to late-responding tissue. Two waves of damage can be identified, an acute pneumonitis and a later fibrosis. The lung is among the most sensitive late-responding organs.
- Together with the lung, the kidney is among the more radiosensitive late-responding critical organs. Dose of 30 Gy in 2-Gy fractions to both kidneys results in nephropathy.
- In terms of radiosensitivity, the liver ranks immediately below kidney and lung. FSUs are in parallel, so that much larger doses are tolerated if only part of the organ is exposed. Fatal hepatitis may result from 35 Gy (conventional fractionation) to the whole organ.
- The nervous system is less sensitive to radiation than other late-responding organs.

Radiosensitivity of tissues and organs

<table>
<thead>
<tr>
<th>High Radiosensitivity</th>
<th>Lymphoid organs, bone marrow, blood, testes, ovaries, intestines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fairly High Radiosensitivity</td>
<td>Skin and other organs with epithelial cell lining (cornea, oral cavity, esophagus, rectum, bladder, vagina, uterine cervix, ureters)</td>
</tr>
<tr>
<td>Moderate Radiosensitivity</td>
<td>Optic lens, stomach, growing cartilage, fine vasculature, growing bone</td>
</tr>
<tr>
<td>Fairly Low Radiosensitivity</td>
<td>Mature cartilage or bones, salivary glands, respiratory organs, kidneys, liver, pancreas, thyroid, adrenal and pituitary glands</td>
</tr>
<tr>
<td>Low Radiosensitivity</td>
<td>Muscle, brain, spinal cord</td>
</tr>
</tbody>
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Scoring systems for tissue injury: LENT and SOMA

- The European Organization for Research and Treatment of Cancer (EORTC) and the Radiation Therapy Oncology Group (RTOG) formed working groups to produce systems for assessing the late effects of treatment on normal tissues.
- Two acronyms introduce the new scoring system for late effects toxicity and the key elements forming the scales:
  - LENT = Late Effects Normal Tissues (grades 1–minor through 4–irreversible functional damage)
  - SOMA = Subjective, Objective, Management, and Analytic (descriptors of toxicity)

LENT and SOMA example

- There is a number of anatomical sites for which LENT and SOMA are scored.

Summary

- Dose-response relationships based on cell assays
  - Clonogenic end points
  - Functional end points
- Clinical response of normal tissues
  - Functional subunits
  - Other complicating factors
  - Tissue tolerance