Solution
Exam TFY 4315 Stålingsbiofysikk/ Biophysics of ionizing radiation
May 22, 2014

Exercise 1. Survival curves. Oxygen effect (Credit 2)

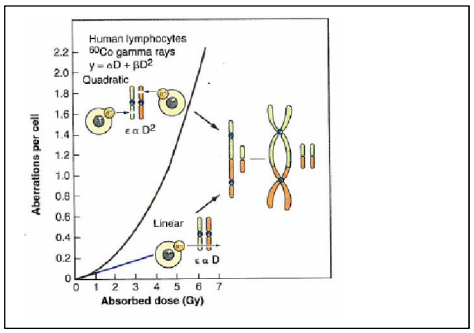
a) The figure shows typical survival curves for cells growing in culture and irradiated with x-ray. How can such survival curves be described mathematically using the linear-quadratic model? Explain the model based on chromosomal aberrations.

Cell survival can generally be described by:

$$S = e^{(-\alpha D - \beta D^2)}$$

S = survival fraction of cells
D = delivered dose
α and β are constants

α proportional to dose at lower doses and represents two double strand breaks causing lethal aberrations caused by one single electron
β proportional to the square of the dose at higher doses and represents chromosome aberrations that are the result of two separate double strand DNA breaks on two different chromatids caused by two separate electrons. The probability of an interaction between the two breaks is then proportional to the square of the doses. See figure.



b) The figures below show survival curves for cells irradiated with x-rays (left) or α-particles (right) in the presence and absence of oxygen. The change in radiation sensitivity caused by oxygen can be given by the oxygen enhancement ratio (OER). Define OER.
Why are cells more sensitive x-rays in the presence of oxygen?
Why is OER=1 for cells irradiated with α-particles?

OER = $D_{hypoxic}$/ $D_{aerated}$
$D_{hypoxic}$ and $D_{aerated}$ are the doses needed in hypoxic (extremely little oxygen) and aerated (presence of oxygen) conditions to achieve the same biological effect.

The mechanism behind the oxygen effect is called the fixation hypothesis. When cells are killed by indirect action, the electrons form free radicals, OH· and H·. These radicals are very reactive. In the presence of O_2, the DNA reacts with the radicals (R·) forming RO_2·This radical result in a chemical modification of the DNA that can not be restored. Thus the damage is the presence of oxygen increases.

α-particles damage DNA by direct action, i.e. the electrons set in motion by the α-particles damage DNA directly without the formation of radicals. Thus oxygen can not cause chemical modifications of the DNA.

c) Cells having very little access to oxygen become hypoxic. Explain how cells can become chronic and acute hypoxic

Chronic hypoxia
Areas far from blood vessels obtain low concentrations of oxygen because oxygen does not diffuse so far. The distance to which oxygen can diffuse is largely limited by the rapid rate at which it is metabolized by respiring tumor cells. The distance from a capillary to hypoxic cell is approximately 70 μm. Chronic hypoxic cells form a few layer of viable, non proliferating cells between oxygenated and necrotic cells.
Also called diffuse limited

Acute hypoxia
Temporary closing or blockage of a tumor blood vessel owing to the malformed vasculature of the tumor. Tumour blood vessels open and close randomly causing fluctuating blood flow, so that different part of the tumour becomes acute hypoxic
Also called perfusion limited.

Exercise 2. Fractionated radiotherapy. The 4 R's. (Credit 2)

a) Radiotherapy is given in fractions in order to kill as many cancer cells as possible and reduce the damage to normal tissue. Explain based on the 4 R's how this is achieved. Explain the consequences of the 4 R's for the effect on early and late responding normal tissue.

The 4 R:

- **Repair** of subletal damage between the fractions. The aim is that normal tissues are repaired to a larger extent than cancer cells.
  The time between the fractions should be so long that repair can take place.
  This occurs for early responding tissue but not to the same extent for late responding tissue.

- **Reassortment,** Progression of cells through cell cycle causes redistribution. Cells that survive a first dose of radiation are in a resistant phase of the cell cycle and within a few hr they progress to the more sensitive phase G2, M. Thus rapidly

dividing cancer cells will reach the sensitive phase, whereas slow proliferating normal cells will not. Early responding tissue consists of rapidly growing cells and reassortment might be an issue. It is not important for late responding tissue.

- **Repopulation** due to cell division increases the number of cells if the time between the doses is sufficient long. This is important for sparing early responding tissue, but repopulation will not occur for late responding tissue. However also tumour cells might be repopulated.

- **Reoxygenation,** x-rays kills aerated cells more efficiently than hypoxic cells. Thus after the first dose the fraction of hyoxic cells increases. Part of the hypoxic cells becomes reoxygenated and sensitive to the next dose of x-rays.
  Reoxygenation is not important for normal tissue which will be well oxygenated.

b) The α/β-ratio is an important parameter in radiotherapy. Explain how you based on the numbers in the table below can determine the α/β-ratio. You should not calculate the exact value, just indicate how you will determine the ratio.
The survival curve giving n fraction of dose d is.

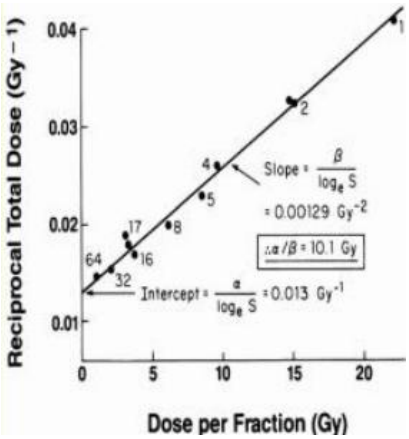
$$S = (e^{-\alpha d - \beta d^2})^n$$

$$-\ln s / nd = \alpha + \beta d$$

The α/β-ratio can be determined from the graph plotting reciprocal dose (1/Gy) as a function of dose per fraction (Gy). Thus from the table the total dose has to be calculated D=Number of fraction (n) x dose per fraction (d)
1/D is plotted as a function of d.

This will give a straight line with a slope β/lnS
The intersection with y-axis =α/lnS

$$\frac{\frac{\alpha}{\ln S}}{\frac{\beta}{\ln s}} = \frac{\alpha}{\beta}$$



c) Explain the difference between hyperfractionation and accelerated treatment, and why such treatment regimens are used. Is there any constrain on the time between two successive fractions?

Hyperfractioning:
Same total dose as in conventional treatment regimen in the same overall time. Each dose per fraction is reduced and the number of fraction increased. For instance deliver twice as many fraction giving two fraction per day.

The aim of hyperfractioning is to reduce late effects without increasing significantly the early effects and maintain or improve local tumor control.

Accelerated treatment
Same total dose delivered in shorter time. Increasing the number of doses per day, not changing the number of fractions compared to conventional treatment. For instance the overall treatment time is half of what is used in conventional treatment and two fractions are given per day.
This treatment is given to reduce repopulation and is given for rapidly growing cancers.

The time between each fraction should be long enough for repair of sublethal damage to take place

Exercise 3. (Credit 2)

a) Ionizing radiation can induce deterministic and stochastic effects. Explain the difference between the two effects. Give examples of tissue where the two effects occur.

Deterministic effect:

- Severity increase with dose
- Threshold in dose
- Probability of occurrence increase with dose
- Example: cataract

Stochastic effect:

- Severity independent of dose
- No dose threshold
- Probability of occurrence increase with dose
- Example: cancer

b) Explain how radiant energy is deposited in tissue and how this deposition can explain that the relative biological effect (RBE) has a maximum when plotted as a function of the linear energy transfer (LET) as shown in the figure below.

The energy is localized along tracks of individual charged particles depending on the type of radiation. The spatial distribution of the ionizing events produced by different particles varies enormously. The energy density is given by the linear energy transfer (LET) defined as the energy transferred pr. unit length of the track
The LET (L) of a charged particle in medium is the quotient: L = *dE/dl*

(The relative biological effectiveness RBE is the ratio between a test radiation and a reference radiation defined by:

$$RBE = \frac{Dose\ of\ reference\ radiation}{Dose\ of\ test\ radiation} = \frac{Dref}{Dtest}$$

Where D_{ref} and D_{test} are the doses of reference and test radiation required for equal biological effect.)

The optimal RBE occurs at coincidence between the diameter of the DNA helix and the average separation of ionizing events, i.e. about 2 nm. LET of approx100 kev/μm gives an average separation between ionizing event of approx. 2 nm which is equal to the diameter of the DNA helix. Radiation of this density of ionizations has the highest probability of causing double-strand breaks

c) Hyperthermia can increase the radiosensitivity. Explain various ways this can be achieved

Hyperthermia and radiation have different target, protein versus DNA. Thus protein damage represents an important mechanistic difference to ionization radiation and can induce additive or synergistic effect on survival. This leads to:

- Protein damage can damage DNA repair proteins preventing sublethal damage repair
- Damage of proteins cause apoptosis or necrosis depending on the temperature, thus the inactivation mechanisms is different from radiation which causes mitotic death.
- Hyperthermia can kill differentiated and non-proliferating cells that radiations can not kill

Radiation and hyperthermia affects different cells in the cell cycle. G2/M cells are most sensitive to radiation, whereas S-phase cells are most sensitive to hyperthermia.

Hypoxic cells being resistant to radiation can be killed by hyperthermia. Hypoxic cells might be more sensitive to hyperthermia than well oxygenated cells, especially chronic hypoxic cells due to hypoxic cells having no access to blood have an acidic environment, and cells at acidic pH and deficient in nutrients are more sensitive to killing by heat.

Moderate hyperthermia 41.41,5 C is found to promote tumor reoxygenation, due to increased blood flow and reduced oxygen consumption. Increased reoxygenation will improve the response to radiotherapy.

Exercise 4. Biologically effective dose (Vekttall 2)

a) The biologically effective dose (BED) for fractionated radiotherapy can be written as:

$$\mathbf{BED = D\ (1+\ d/(\alpha/\beta))}$$

Define the parameters in the equation, and show how this equation is related to the linear quadratic model.

D = total dose
d = fractionation dose
α/β = the ratio of the parameters α and β in the linear quadratic model, (used to quantify the fractionation sensitivity of the tissue)

Linear quadratic model:

$$S = e^{-(\alpha d + \beta d2)}$$

For a single acute dose d the biological effect E is given by:

$$-\ln S = E = (\alpha d + \beta d^2)$$

For n well separated fractions of dose d, the biological effect is given by;

$$E = n\ (\alpha d + \beta d^2)$$

Rewritten:

$$E = nd\ (\alpha + \beta d) = \alpha(nd)(1+d/(\alpha/\beta))$$

nd = D

$$E = \alpha D(1+d/(\alpha/\beta))$$

Where $(1+d/(\alpha/\beta))$is the relative effectiveness

$$E/\alpha = D(1+d/(\alpha/\beta)) = \text{BED Biologically effective dose}$$

b) How can EQD_2 (equivalent total dose in 2 Gy fractions) be expressed by using the parameters in the equation for BED.

$$BED = D(1+\ d/\ (\alpha/\beta)) = EQD_2\ (1+\ 2/(\alpha/\beta))$$

Rewritten:

$$EQD_2\ = D(d+\ (\alpha/\beta))\ /\ (2+\ (\alpha/\beta))$$

For a change in a fractionation regimen (from $D_1$and d_1 to D_2 and d_2) absolute values of change in BED and EQD_2 are not equal. Show how the relative change in BED and EQD_2 are related for the given change in the fractionation regimen.

Regimen 1:	Total dose: D_1, fraction dose: d_1	BED_1 $(EQD_2)_1$
Regimen 2:	Total dose: D_2, fraction dose: d_2	BED_2 $(EQD_2)_2$

The differences in BED and EQD_2 when changing from regimen 1 to regimen 2 are:

$$\Delta BED = BED_2 - BED_1 = D_2 (1+ d_2/ (\alpha/\beta)) - D_1 (1+ d_1/ (\alpha/\beta))$$

$$\Delta EQD_2 = (EQD_2)_2 - (EQD_2)_1 = D_2(d_2+ (\alpha/\beta)) / (2+ (\alpha/\beta)) - D_1(d_1+ (\alpha/\beta)) / (2+ (\alpha/\beta))$$

The absolute values of ΔBED and ΔEQD_2 are not equal.

The relative change in BED and EQD_2 are:

$$BED_2 / BED_1 = D_2 (1+ d_2/ (\alpha/\beta)) / D_1 (1+ d_1/ (\alpha/\beta))$$
$$= D_2 (d_2+ (\alpha/\beta)) / D_1 (d_1+ (\alpha/\beta))$$

$$(EQD_2)_2 / (EQD_2)_1 = (D_2(d_2+ (\alpha/\beta)) / (2+ (\alpha/\beta))) / (D_1(d_1+ (\alpha/\beta)) / (2+ (\alpha/\beta)))$$
$$= D_2 (d_2+ (\alpha/\beta)) / D_1 (d_1+ (\alpha/\beta))$$

The <u>relative change in BED and EQD_2 are equal</u>

c) A tumour with $\alpha/\beta = 10$ is irradiated with a conventional fractionation regimen of 2 Gy x 25 = 50 Gy. If the fraction dose is increased to 3 Gy, how many fractions must be delivered to give the same tumour effect?

The calculations can be done either with EQD_2 or BED, (as the relative change is equal demonstrated above).

2 Gy x 25 and $\alpha/\beta = 10$:

$$EQD_2 = D(d+ (\alpha/\beta)) / (2+ (\alpha/\beta)) = 50Gy (2 + 10)/(2 + 10) = 50 Gy$$

3 Gy fractions with equal EQD_2:

$$EQD_2 = 50 Gy = n3Gy(3+10)/ (2+10)$$

<u>n = 15.4</u>

$$BED = 50Gy(1+\frac{2}{10}) = 60Gy$$

$$BED = 60Gy = n3Gy(1+\frac{3}{10})$$

$$n = 15,4$$

A fraction regime of 3 Gy x 15 approximately equals the 2 Gy x 25 regimen when concerning tumour control

An organ at risk (OAR) with α/β = 3 is located nearby the tumour and will get the same radiation dose as the tumour. How will the change in fractionation affect the relative effect of the OAR?

2 Gy x 25 and α/β= 3

$EQD_2 = D(d+ (\alpha/\beta)) / (2+ (\alpha/\beta)) = 50Gy (2 + 3)/(2 + 3) = 50$ Gy

3 Gy x 15 and α/β= 3:

$EQD_2 = D(d+ (\alpha/\beta)) / (2+ (\alpha/\beta)) = 45Gy (3 + 3)/(2 + 3) = 54$ Gy

$$BED = 50Gy(1+\frac{2}{3}) = 83,3\,\text{Gy}$$

$$\text{BED} = 45Gy(1+\frac{3}{3}) = 90Gy$$

The change in fractionation will give a the relative increase in effect on OAR of 54Gy/50Gy = 1.08 (or 90Gy/83,3Gy), which represent 8 % increase in effect on the OAR.

What is the highest number of 3 Gy fractions possible if no increase in biological effect to the OAR is allowed, and how will this fractionation affect the relative tumour effect?

$EQD_2 = D(d+ (\alpha/\beta)) / (2+ (\alpha/\beta)) = 3n (3 + 3)/(2 + 3) < 50$ Gy

$n<13.9$

$$BED = n \cdot 3Gy(1+\frac{3}{3}) < 83,3\,\text{Gy}$$

$$n < 13,9$$

The highest number of 3 Gy fractions possible if no increase in biological effect is allowed is 13. (n=14 will give a to high dose)
This will give the following tumour effect:
3 Gy x 13 and α/β= 10:

$EQD_2 = D(d+ (\alpha/\beta)) / (2+ (\alpha/\beta)) = 39Gy (3 + 10)/(2 + 10) = 42.25Gy$

BED=39Gy(1+3/10)=50,7Gy

This gives a relative decrease in tumour effect of 42.25Gy / 50Gy = 0.85 or 50,7Gy/60 Gy representing a 15 % reduction of tumour effect

Exercise 5 Multiple choice (Credit 1)

You have 3 possible answers. Mark the correct answer.

a) The number of DNA double strand breaks in a cell irradiated with 2 Gy is approximately:
   - ***40***
   - 100
   - 400

b) Radiation induced chromosomal aberrations can be induced pre-or post replication. Which of these aberrations is induced post replication:
   - Dicentric
   - Ring
   - ***Anaphase bridge***

c) What is the most common mechanisms for cell death after ionizing radiation:
   - ***Mitotic death***
   - Apoptosis
   - Necrotic death

d) What is the most important factor which determine the late effects in late responding tissue after ionization radiation:
   - ***Dose per fraction***
   - Number of fractions
   - Total dose

e) A reduction in radiation rate and increased overall radiation time lead to:
   - Reduced survival
   - ***Increased survival***
   - No change in survival when the total dose is not changed

f) Which diagnostic method will give highest dose of radiation per examination:
   - ***CT***
   - Nuclear medicine and the use of radioactive isotopes
   - X-ray imaging

g) Equivalent dose is the product of absorbed dose and a radiation weighting factor. Which value does the radiation weighting factor have for photons:
   - ***1***
   - 5
   - 10

h) Which of these tissues respond early to ionizing radiation:
   - Lung
   - Kidney
   - ***Skin***

i) Which of these cell types are most radiosensitive:
   - Neurons
   - ***Stem cells***
   - Muscle cells

j) What is the unit for effective dose:
   - Gy
   - ***Sivert***
   - Bequerel

k) IMRT is used in dose planning in radiotherapy. One of the following statements is not correct:
   - ***The gantry rotate continuously around the patient while the dose is given towards the target***
   - The gantry is positioned in several defined positions while the dose is given towards the target
   - The intensity of the ionizing radiation varies from different gantry angles

l) Which statement is not valid for proton therapy:
   - The damage to surrounding normal tissue is reduced
   - Limited repair damage
   - ***Hypoxic cells are resistant to proton therapy***

m) Which method is **not** used to spread the proton beam laterally:
- ***Using a rotating wheel with varying thickness***
- Placing a scattering material (foil) in front of the proton source
- Scanning the proton beam across the tumour volume