Modification of Radiation Response

Biological Modification
- **repair of radiation damage**
- **dose rate effects**
- **fractionation**

Repair of DNA damage

Three types of damage

- Lethal damage- irrepairable, leads to cell death
- Potentially lethal damage
- Sublethal damage

Potentially lethal damage (PLD): component of radiation damage that can be modified by post-irradiation conditions.

PLD repair has been demonstrated *in vitro.*

Survival increases when:

- Cells are prevented from dividing for $\geq$ 6 hrs.

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- Cells are kept in saline or plateau (G_0) phase for $\geq$ 6 hrs.

PLD repair has been demonstrated *in vivo*: **relevant to the clinical situation.**

- Survival increases when a delay is introduced after irradiation and before the excision assay.

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- *in vivo/in vitro* assay
- smaller tumors well oxygenated

PLD repair is significant for X rays
PLD repair does not occur with neutrons (or other high-LET radiation).

Sublethal Damage Repair: increase in survival observed if a given radiation dose is split into two fractions separated by a time interval.

Split dose experiments

24° C incubation allows repair but prevents cells from progressing through the cell cycle.

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Changing the time interval between doses illustrates several important radiobiological principles.

Split dose experiment at 37^oC Chinese hamster cells

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- Initial repair

- Partially synchronized survivors (S phase)

- 6 hours later population is in a radiosensitive phase of the cell cycle (G_2/M)

Three processes are operative: *The three "R"s of radiobiology*
- **Repair** of sublethal damage
- **Reassortment** by progression of survivors through the cell cycle
- **Repopulation** if interval between fractions > cell cycle time

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Split Dose Summary
- Split doses increase survival, the shoulder of the curve is repeated with each dose.
- Extent of SLD repair correlates with the size of the shoulder.
- β component causes survival curve to bend and results in the sparing effect.
- Large shoulders indicate a small α/β ratio.
- The dip between fractions (reassortment) is only observed with fast-cycling cells.

High-LET radiation and SLD repair

- Little or no shoulder
- Little or no repair
- Little or no SLD repair when split doses are given

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Clinical Implications of SLD repair:

If:
Early responding tissues: SLD ~ 20% of total damage
Late responding tissues: SLD ~ 50% of total damage

Repair of SLD: $t_{1/2}$ ~ 0.5 – 1.5 hrs

"fading time" time for damage to be ~ 95% repaired

3-4 hrs in early responding tissues: SLD ~ 20% of total damage
5-6 hrs in late responding tissues: SLD ~ 50% of total damage

Fraction spacing should be > 6 hours to allow normal tissues to fully repair SLD.

The Dose Rate Effect

- For low-LET radiation, dose rate is one of the principle factors that determine the biologic response.

- Low dose rate sparing results from repair of SLD during the protracted exposure.

Idealized fractionation experiment

Multiple small fractions approximate a continuous low dose rate exposure.

Continuous low dose rate composite (dashed line) *has no shoulder.*

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Chinese hamster cells

- ^{60}Co exposure at various dose rates
- Most dramatic dose rate effect is observed between 0.01 and 1 Gy/min.
- Dose rate sparing varies with cell type.

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Dose rate effect *in vivo*

- Intestinal epithelium crypt cells in the mouse.
- Cell proliferation begins to dominate at the lowest dose rate (0.57 rad/min).

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Dose rate effect summary

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- Acute doses: shoulder significant.
- Low dose rate: D_0 increases, shoulder decreases.
- Redistribution: in some cell lines, a G_2 block results in increased sensitivity " inverse dose rate effect".
- Below critical dose rate: G_2 block can be overcome, proliferation increases.

Modification of Radiation Response

Chemical Modification: radiosensitizers and radioprotectors

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Oxygen – best known and most general radiosensitizer

- The slopes of survival curves for cells exposed to sparsely ionizing radiation in hypoxia and in well oxygenated environments differ by about a factor of 3 – the oxygen effect.

- **Hypoxia** means low oxygen
- **Anoxia** means no oxygen.

Mechanism
- Mechanism(s) of the oxygen effect really not known, although, clearly, O_2 acts at the free radical level.
- The reactions involved may be:

$$O_2 + e^-_{aq} \rightarrow O_2\bullet- \quad \text{or} \quad O_2 + R\bullet \rightarrow R\,O_2\bullet$$

The later reaction is sometimes called "fixation" of damage in the lethal form and occurs in competition with chemical repair of damage, perhaps by H atom donation from thiols.

Oxygen Enhancement Ratio (OER)

- OER = dose(hypoxia)/dose(oxygenated) for ***the same biological effect***.

- OER is usually about 3 at high radiation doses, but often has a lower value of about 2 at low doses (at or below 2 Gy).

- OER decreases as LET increases: i.e., fraction of radiation damage from direct action increases.

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How much O₂ is required?

Survival curves show that relatively little O_2 is needed, e.g., as little as 100 ppm O_2 (0.075 mm Hg) causes significant sensitization compared to the response in anoxia.

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- "K-curve" – plot of relative radiosensitivity vs. oxygen concentration.
- Half-maximal effect of oxygen at about 0.5% (3 mm Hg) O_2.
- For comparison, venous pO_2 is about 50 mm Hg and arterial is about 100 mm Hg; air is 155 mm Hg.

Importance of the oxygen effect

- Thomlinson and Gray (1955) studied sections of bronchial carcinoma
- Small tumors (<160 μm) – no necrosis
- Tumors over 200 μm - necrotic centers surrounded by sheath of healthy cells
- Sheath of growing cells always 100-180 μm
- They also calculated O_2 diffusion in tissues and found that all O_2 should be metabolized at a distance of 150-200 μ from a capillary, in good agreement with the observations of necrosis.
- Actually, there will be an O_2 concentration gradient through the tumor, so some tumor cells will have enough O_2 to grow but will be radiobiologically hypoxic (and therefore radioresistant). These cells may limit the effectiveness of radiation therapy of tumors.

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This model is really a gross oversimplification of tumor oxygenation, but emphasizes the importance of oxygen in radiation therapy and explains why a great deal of research has been conducted into ways to overcome hypoxic cells.

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Hypoxic cells may be of two types:

- *Diffusion-limited* – as described by Thomlinson and Gray (chronic hypoxia)
- *Perfusion-limited* – cells intermittently hypoxic only when the blood flow transiently stops on their vessel (acute hypoxia).

Do hypoxic cells really exist in tumors?

The first demonstration of hypoxic cells in an experimental animal tumor was by Powers and Tolmach using the dilution assay technique.

A two-component survival curve was observed:
- Low doses: D_0 = 1.1 Gy (normal)
- High doses: D_0 = 2.6 Gy

- The ratio of about 2.5 between the two D_0 values suggested the OER: the low dose component was from oxygenated cells and the high dose component from hypoxic cells.

- Back-extrapolation of the high dose component to the y-axis gives the % hypoxic cells in the tumor.

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Paired survival curves

- Steepest: fully oxygenated
- Shallowest: fully hypoxic
- Intermediate curve reflects the actual mixture and is biphasic.

- Asphyxiate animal with N_2 several minutes before irradiation and subsequent in vitro assay.
- Air breathing

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Evidence for the presence of hypoxic cells in human tumors:
- Tumor histology
- Oxygen electrode measurements
- Clinical gains with hyperbaric oxygen
- Studies showing anemia is poor prognostic factor also associated with local failure, but pre-transfusion help.

Reoxygenation

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First indications came after observations of 15% hypoxic cells in experimental tumors after a variety of different radiation doses and fractionation schemes.

Implication was that the tumor cell population was dynamic and able to readjust: reoxygenate.

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A single 10 Gy dose kills virtually all oxic cells.

Time course of reoxygenation varies among different tumors.

Mechanism:
Vessel re-opening at short times.
Neovascularization at long times.

Radiation Sensitizers

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Radiosensitizers
- Agents which enhance the response of cells to radiation.

- Ideally, radiosensitizers would selectively sensitize tumor cells while having no effect on normal tissues.

Halogenated pyrimidines, BUdR and IudR

- Are incorporated into DNA in place of thymine. Therefore, the tumor cells must be cycling faster than the nearby dose limiting normal tissues.
- IUdR and BUdR have similar sensitization with X-rays, but IUdR is preferable clinically because it sensitizes cells much less to fluorescent light, so less harmful side effects.
- Sensitize both hypoxic and oxygenated cells.
- The degree of sensitization depends on the amount of halogenated pyrimidine incorporated into a cell.
- Clinical trials with IUdR are underway with gliomas and sarcomas with encouraging early results. The agent is being delivered with brachytherapy as well as external beam therapy.

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Hypoxic cell radiosensitizers

Electron-affinic compounds that selectively sensitize hypoxic cells while having no effect on oxygenated cells.

Ideal properties of sensitizer:
- Selectively sensitize hypoxic cells
- Chemically stable and slowly metabolized
- Highly soluble in water and lipids so can diffuse to hypoxic tumor cells
- Effective throughout cell cycle
- Effective at low daily doses of radiation

Sensitization enhancement ratio (SER) = D_0(without sensitizer)/D_0(with sensitizer)

Nitroimidazole class of compounds most studied.

- Metronidazole (flagyl – a 5-nitroimidazole) gave *in vitro* SER = 1.7 and *in vivo* SER = 1.3.
- Misonidazole (Ro-07-0582 – 2 – nitroimidazole) better sensitizer, with *in vitro* SER = 2.5 for hypoxic cells (no effect on oxygenated cells) and tumor SER up to 1.8.

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Hypoxic cell cytotoxins; Bioreductive agents

Drugs that are preferentially toxic to hypoxic cells.

SR4233 (tirapazamine)
<u>Hypoxic Cell Cytotoxicity Ratio</u>** Drug dose required to kill given proportion of aerobic cells divided by that needed to kill same fraction of hypoxic cells

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Killing hypoxic cells may have greater therapeutic advantage than radiosensitizing them because:
- hypoxic cytotoxins kill cells resistant to radiation and most chemotherapy, producing complementary cytotoxicity.
- random fluctuations in acute hypoxia could create a situation where hypoxia could be used to advantage.
- Modeling studies show that if a hypoxic cytotoxin is given with every dose fraction, the overall kill in a hypoxic tumor can be greater than if the tumor is fully oxygenated. This occurs when the drug kills at least 50% of the hypoxic cells each time it is given.

Results of clinical trials have been disappointing.

Table 1. Results of randomized controlled trials of radiosensitizing methods

	Hyperbaric Oxygen	Metronidazole	Misonidazole
Therapeutic Benefit	3	0	2
Significantly Improved Results	0	2	4
Margin in favor (not significant)	6	0	2
No Difference	6	4	30
Margin Against (not significant)	0	0	1
Adverse Response	0	0	0
	15	6	39

Possible reasons for failure of misonidazole in clinical trials include:

- Relatively small sample size and heterogeneous population may have precluded observation of a small effect.

- SER may be lower at clinically relevant doses.

- Misonidazole has cumulative neuropathy, which limited the dose that could be given and the number of fractions with which it could be given. (Total of 12 g/m^2, so with single dose of 2 g/m^2, which might be expected to give ER in hypoxic cells of about 1.3-1.4, could only give misonidazole with 6 fractions.)

Radioprotectors

Agents which decrease the response of cells to radiation.

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Best radioprotectors are thiols

Dose Reduction Factor (DFR) = Protection Factor (PF) =

$$\frac{\text{dose of radiation in the presence of the drug}}{\text{dose of radiation in the absence of the drug}}$$

to produce a given level of effect.

Proposed mechanisms for radioprotection by thiols:

$TH + \bullet OH \rightarrow T\bullet + H_2O$ (TH = target)

$RSH + \bullet OH \rightarrow RS\bullet + H_2O_2$ chemical protection
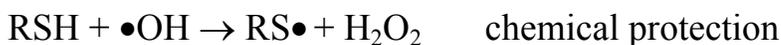
The rate of the scavenging reaction is independent of the presence or absence of oxygen, so scavenging will not explain the differential protection by thiols in hypoxia and air.

Donation of H atoms to organic radicals, in competition with damage "fixation" of those radicals by oxygen

$T\bullet + RSH \rightarrow TH + RS\bullet$ chemical repair
$T\bullet + O_2 \rightarrow TO_2\bullet$ damage fixation

WR2721 (amifostine) and related compounds

"Covering" the SH with a phosphate group decreased the toxicity

TABLE 9.1. *Effect of adding a Phosphate-Covering Function on the Free Sulfhydryl of β-Mercaptoethylamine (MEA)*

Drug	Formula	Mean 50% Lethal Dose (Range) in Mice	Dose-reduction Factor
MEA	$NH_2-CH-CH_2-SH$	343 (323–364)	1.6 at 200 mg/kg
MEA-PO$_3$	$NH_2-CH_2-CH-SH_2PO_3$	777 (700–864)	2.1 at 500 mg/kg

In 1969, Yuhas and colleagues reported that WR2721 could protect normal tissues with less protection of tumors.

TABLE 9.3. Summary of Normal-Tissue Responsiveness to Protection by WR-2721

Tissues Protected[a]	Tissues not Protected
Bone marrow (2.4–3)	Brain
Immune system (1.8–3.4)	Spinal cord
Skin (2–2.4)	
Small Intestine (1.8–2)	
Colon (1.8)	
Lung (1.2–1.8)	
Esophagus (1.4)	
Kidney (1.5)	
Liver (2.7)	
Salivary gland (2.0)	
Oral mucosa (>1)	
Testes (2.1)	

[a]Numbers in parentheses are the dose *reduction* factors or factor increases in resistance associated with WR-2721 injection.

Note that the degree of protection depends on the normal tissue type.

TABLE 9.2. *Three Protectors in Practical Use*

Compound	Structure	Use
WR-638	$NH_2CH_2CH_2SPO_3HNa$	Carried in field pack by Russian army (cystaphos)
WR-2721	$NH_2(CH_2)_3NHCH_2CH_2SPO_3H_2$	Protector in radiotherapy and carried by US astronauts on lunar trips (amifostine)
WR-1607	$CH_3(CH_2)_9NHCH_2CH_2SSO_3H$	Marketed as rat poison (d-CON)

Comparison of Hematopoietic and Gastrointestinal Dose Reduction Factors in Mice for the Three Compounds Listed Above

Compound	Drug Dose, mg/kg	Dose-reduction Factor	
		7 Days	30 Days
WR-638	500	1.6	2.1
WR-2721	900	1.8	2.7
WR-1607	10	—	2.1

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However, radioprotection by WR2721 is not restricted to normal tissues; tumors can be protected.

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Some factors which affect the degree of radioprotection of normal tissue or tumor by WR2721 include:
- Rate and extent of uptake of WR2721, which depends on:
- Concentration of alkaline phosphatase in plasma membrane
- pH
- Oxygen concentration
- Endogenous thiol level
- Fractionation pattern (single *versus* multiple doses)

Clinical trials have shown side effects such as hypotension, nausea, vomiting, and hypocalcemia. Hypotension has been the dose limiting toxicity.

- Only a few clinical trials with radiation have been undertaken. Although acute reactions to radiation appear to be protected, the effects on late radiation reactions remain to be evaluated in long term studies. Acute reactions do not always predict late reactions, so dose escalation must proceed cautiously.
- Clinical trials suggest amifostine may be useful to protect against nephro-neuro- and oto-toxicity from *cis*-platin treatment and cyclophosphamide-induced granulocytopenia.
- More recently, interest in WR2721 and its derivatives has centered on observations that these drugs effectively protect against radiation-induced and some drug-induced mutations and neoplastic transformation.

Protector	Treatment	Endpoint	PF
WR-1065	Gamma rays	HGPRT mutations	5.1
WR-1065	Neutrons	HGPRT mutations	3.3
WR-1065	*cis*-PT	HGPRT mutations	7.1
WR-1065	HN_2	HGPRT mutations	3.4
WR-1065	BLM	HGPRT mutations	2.8
WR-1065	gamma rays	Transformation	6.0
WR-2721	gamma rays	Preneoplastic lesions	9.7
WR-2721	gamma rays	Tumor induction	3.1
Mixture	X-rays	Tumor induction	1.4