RADIOPROTECTORS & RADIOSENSITIZERS

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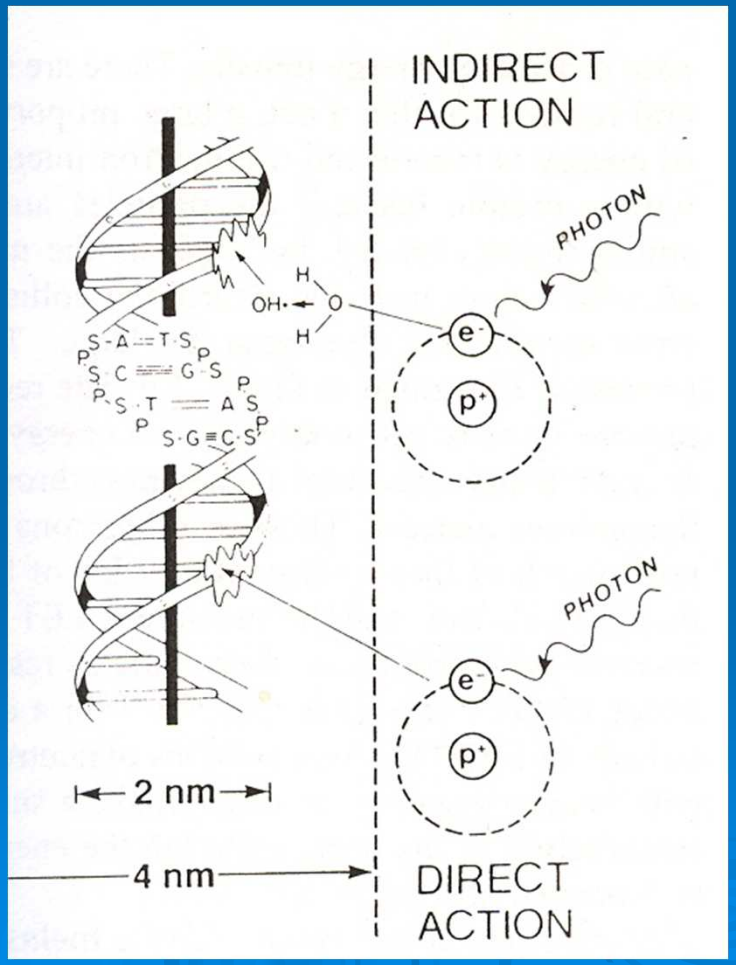
- Free and Ion radicals
- Free radicals can diffuse to DNA from within cylinder with diameter twice that of the DNA double helix

Interaction of Radiation with water

$$H_2O \longrightarrow H_2O^+ + e^-$$

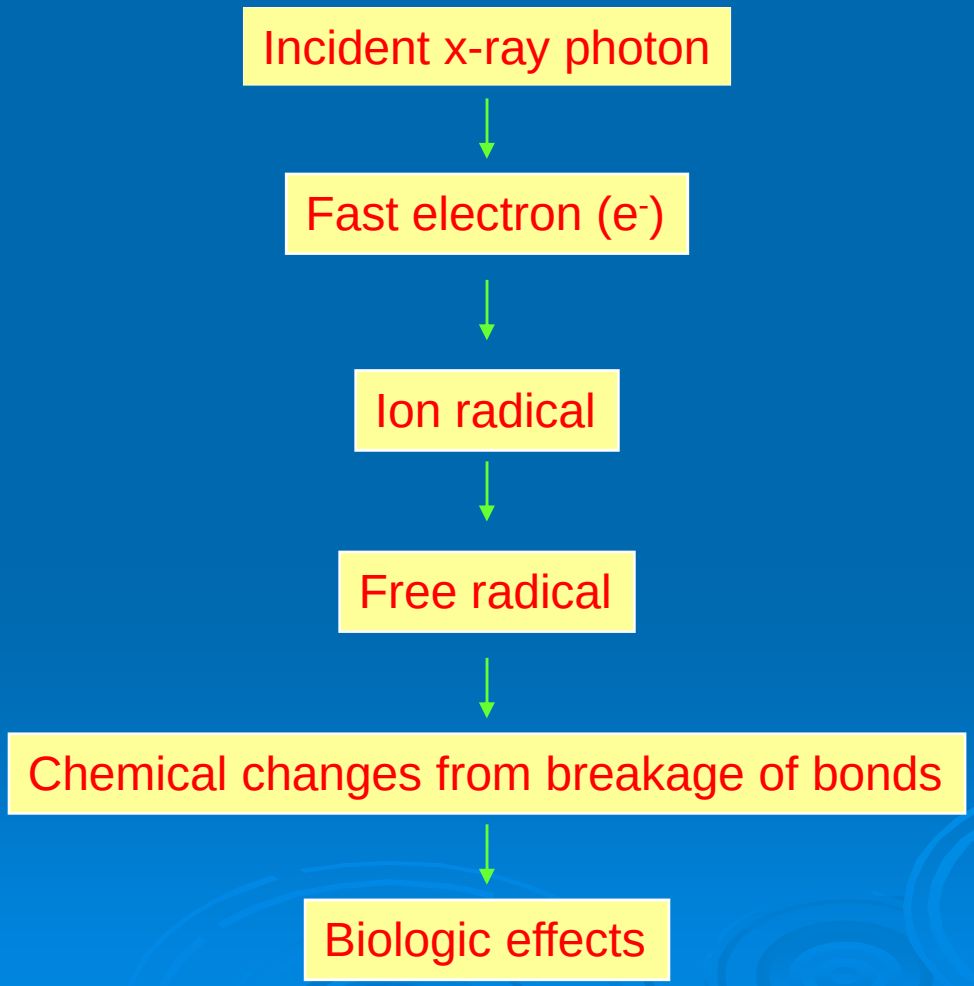
$$H_2O^+ + H_2O \longrightarrow H_3O^+ + OH^\cdot$$

Direct & Indirect Action of Radiation



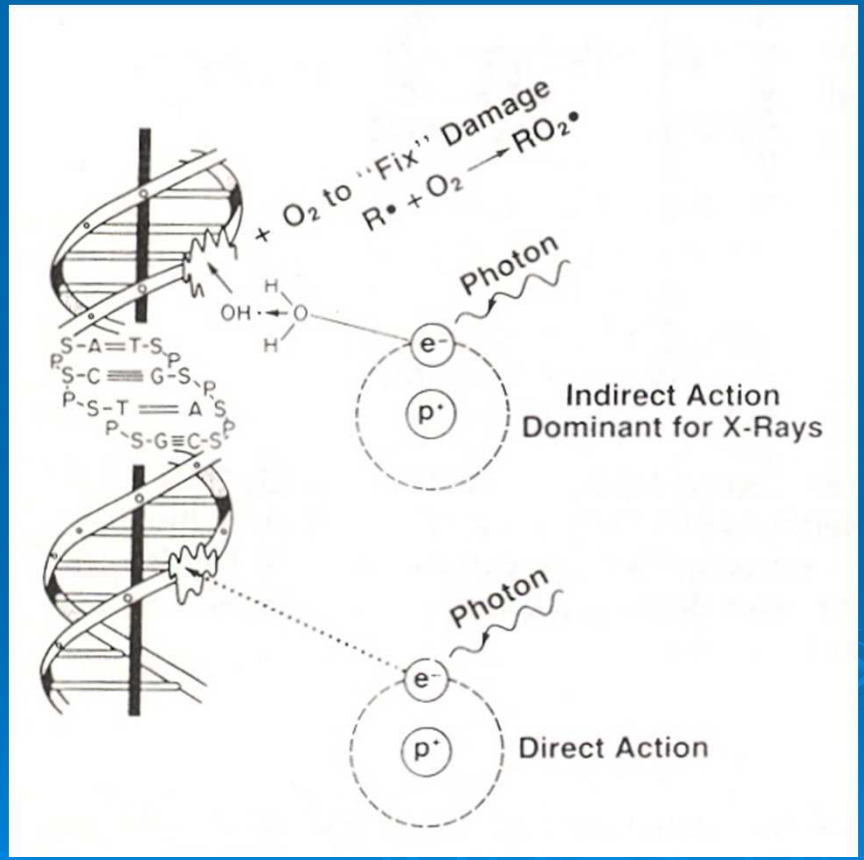
- 2/3 of DNA damage in mammalian cells caused by $OH^{\cdot}$ radical
- Indirect action of radiation can be modified by chemical means
- Direct action can not be modified

Indirect action of Radiation



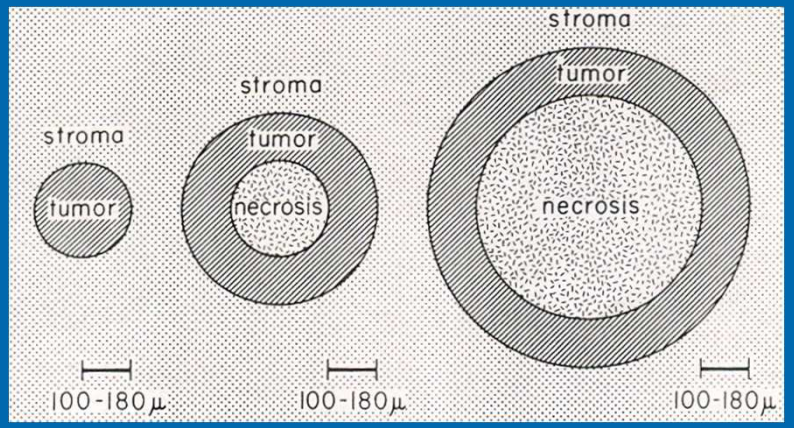
Mechansim of Oxygen Effect

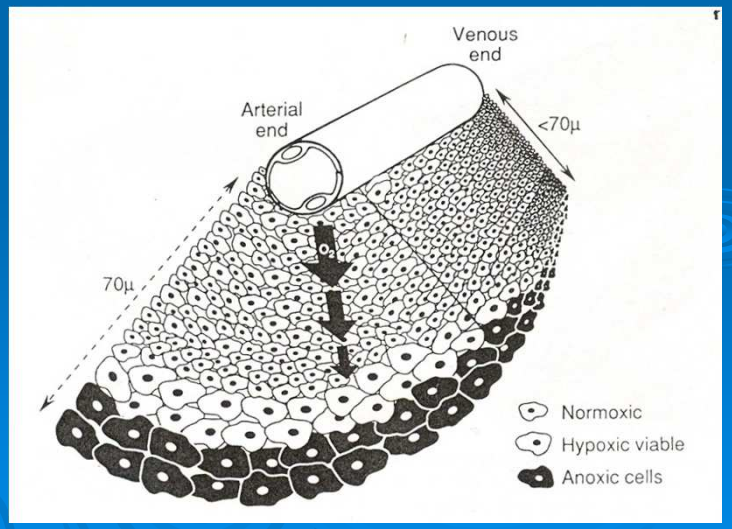
- For oxygen effect- O_2 to be present during radiation exposure- *during or within microseconds after radiation exposure*.
- Free radicals break chemical bonds, produce chemical changes & initiate chain of events- final expression of biological damage.
- If oxygen present it reacts with the free radicals- produces RO_2, an organic peroxide-a nonrestorable form of target material.
- Oxygen fixes the radiation lesion.



Chronic Hypoxia

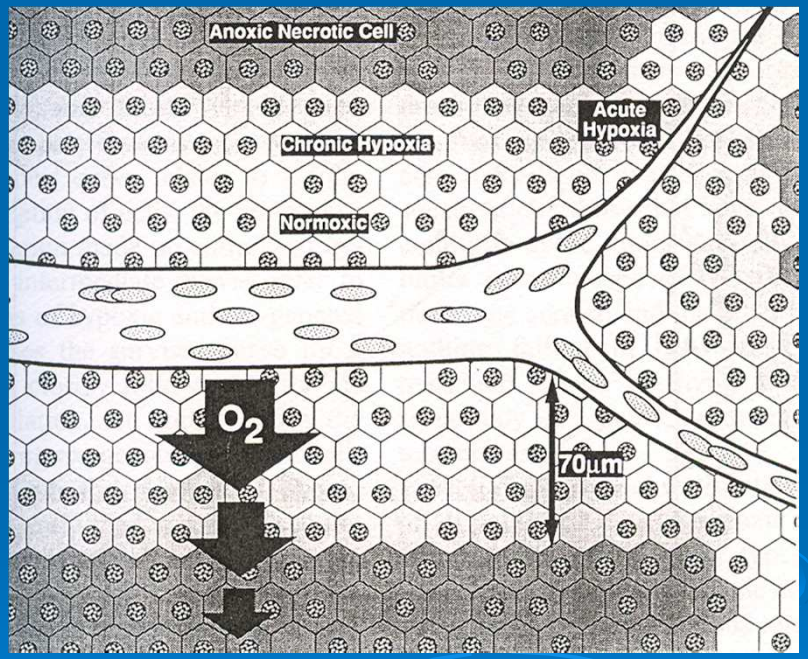
- No necrosis in small tumour cords with radius <160 μm.
- No tumour cord with radius >200 μm without a necrotic centre.
- As diameter of necrotic area increased, thickness of sheath of viable tumour – 100-180 μm.
- O_2 diffusion distance-limited by rapid rate of metabolism by respiring tumour cells.
- Hypoxic cells layer- 1-2 cells thick. O_2 concentration high for cells to be viable but low enough to be protected from radiations.
- O_2 diffusion distance- 70 μm at arterial end & < 70 μm at venous end.





Acute Hypoxia

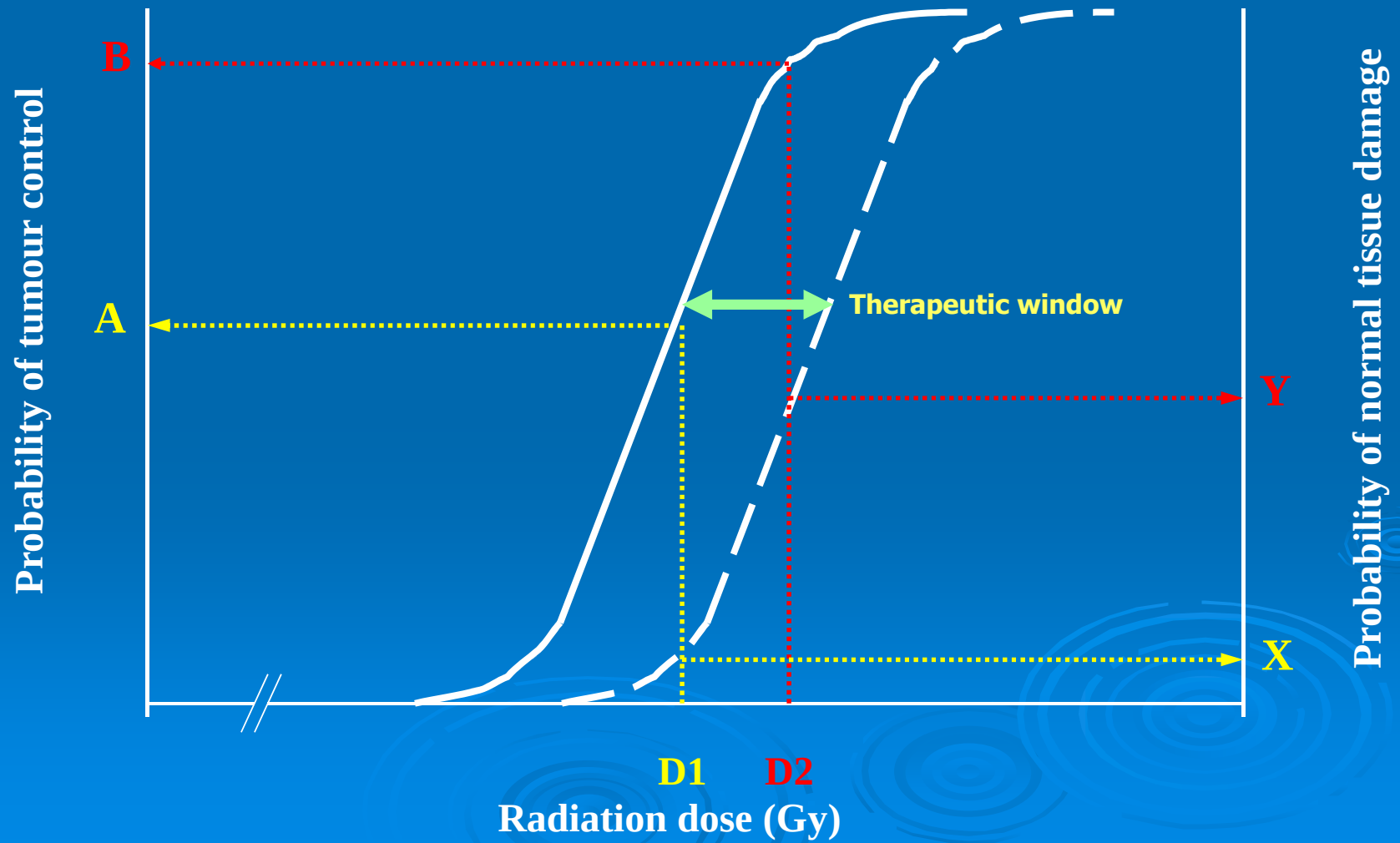
- Postulated in early 1980s by Brown. Later demonstrated by Chaplin *et al*.
- Regions of acute hypoxia-a result of temporary closing or blockage of a particular blood vessel.
- Tumour blood vessels open and close in random fashion-intermittent hypoxia.
- Acute hypoxia from blood flow instability and not a result of total stasis.
- Chronic hypoxia- limited diffusion distance of O_2.
- Acute hypoxia-from temporary closure of tumour blood vessels.



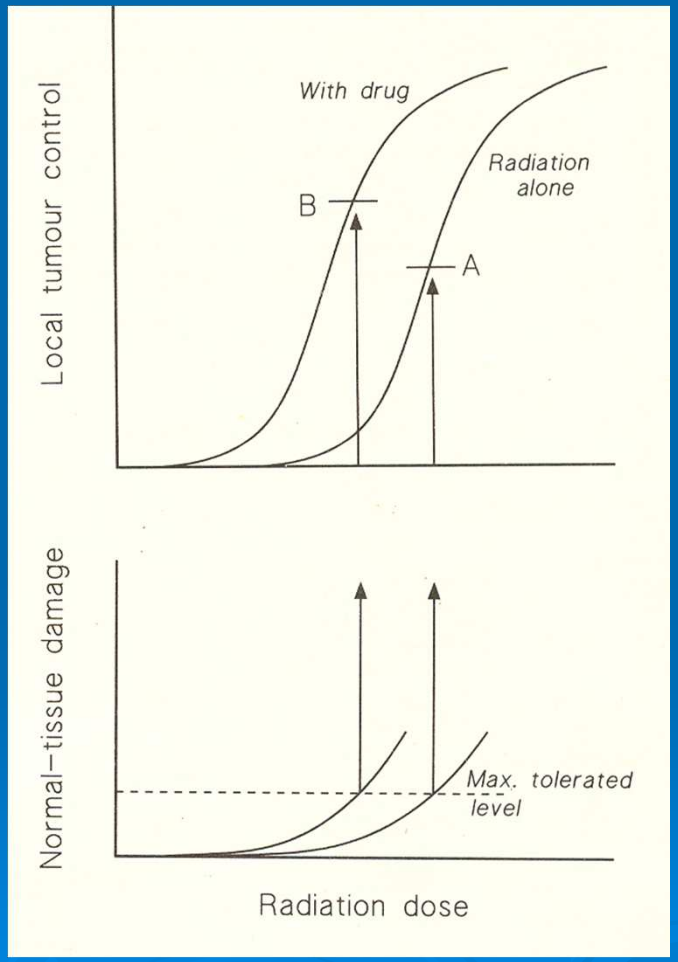
Therapeutic Index or Therapeutic Ratio

Tumour response for a fixed level of normal tissue damage

Radiotherapy dose-response effect



Improvement in Therapeutic Index



Radioprotectors

Radioprotector: A chemical compound that reduces the biologic consequences of radiation

Some may protect whole animals as they cause vasoconstrictions/upset metabolism to $<O_2$ concentration. e.g. NaCn, CO, epinephrine, serotonin, histamine

- Most remarkable group of true protectors- SH compounds
- 1948-Harvey Patt et al- cysteine afforded mice protection from death by WBI

- Bacq et al-cysteamine also a protector

$SH\text{-}CH_2\text{-}CH_2\text{-}NH_2$

- Following years-many similar compounds discovered
- Same general structure:
  i. A free SH or a potential SH group at one end
  ii. Strong basic function, i.e. an amine or guanidine at other
  iii. A straight chain of 2 or 3 carbon atoms
- Problem was their toxicity- cysteine- nausea and vomiting
- After WW-II greater interest

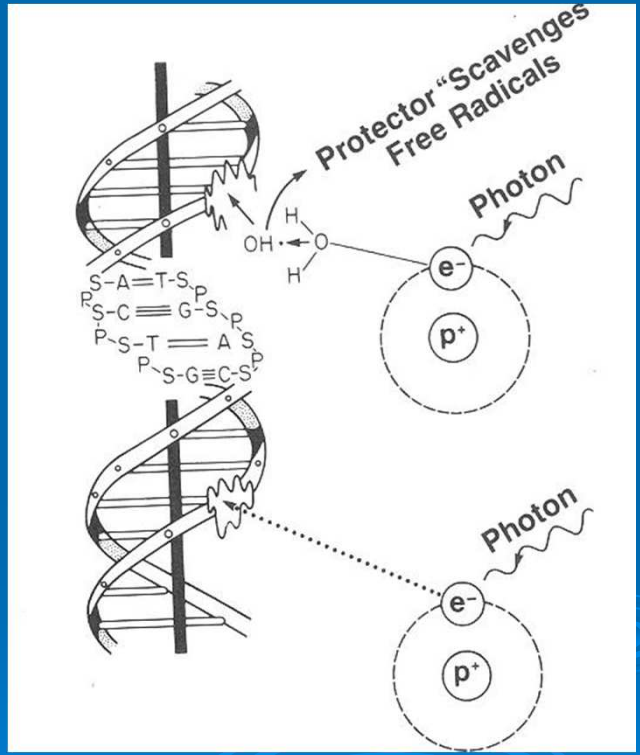
- 1959- Development programme at Walter Reed Army Hospital-over 4000 compounds similar to cysteine and cysteamine synthesized
- Aim- reduce toxicity
- First breakthrough- covering the SH group with phosphate- dose causing a given level of toxicity doubled, i.e. the toxicity of the compound decreased

Radioprotectors-Mechanism of Action

- SH compounds effective against sparsely ionizing radiation.
- Mechanism:

  i. Free radical scavenging- O_2 based free radical generation or chemotherapy agents

  ii. H_2 atom donation to facilitate direct chemical repair at sites of DNA damage
- Protective effect parallel O_2 effect- maximal for sparsely ionizing and minimal for densely ionizing radiation



Radioprotectors-Mechanism of Action

- Dose reduction factor:

$$DRF = \frac{\text{Dose of radiation in presence of drug}}{\text{Dose of radiation in absence of drug}}$$

- The largest possible value of DRF for sparsely ionizing radiation would equal OER with value 2.5-3
- SH radioprotectors, in reality, have more effect with densely ionizing radiations than would be expected based on theoretical mechanism of action. May be other factors involved.

Effect of adding a Phosphate-covering function on the free SH of Cysteamine

Drug	Formula	Mean 50% lethal dose (Range) in mice	Dose reduction factor
MEA	NH_2-CH_2-CH_2-SH	343 (323-364)	1.6 at 200mg/kg
MEA-PO3	NH_2-CH_2-CH-SH_2PO_3	777(700-864)	2.1 at 500mg/kg

Two Radioprotectors in Practical Use

Compound	Dose (mg/kg)	Dose reduction factor		Use
		7 days (GI)	30 days (Haematopoetic)	
WR-638 Cystaphos	500	1.6	2.1	Carried in field pack by Russian army
WR-2721 Amifostine	900	1.8	2.7	Protector in radiotherapy and carried by US astronauts on lunar trips

Amifostine(WR-2721)

Radioprotective drug approved by FDA for use in RT-prevention of xerostomia in patients treated for H&N cancers.

Phosphorothioate prodrug-nonreactive, does not readily permeate cells.

Alkaline phosphatase (expressed on plasma membrane cells of endothelial cells lining blood vessels & surface of proximal renal tubular cells)

Dephosphorylated & converted to active free thiol metabolite WR1065

Enters normal cells by facilitated diffusion

Free thiol acts as a potent scavenger of oxygen free radicals and superoxide anions

Taken up much more slowly by tumour tissues due to inferior vascularization or difference in membrane structure of tumours that impedes entry of this relatively hydrophilic compound

Absorption- Not orally bioavailable. Appr. 50% of drug bioavailable after sc. Inj.

Distribution- confined primarily to intravascular compartment. Rapidly cleared from Plasma- distribution half life <1 min and <10% drug remains in plasma 6 min after admin. Active metabolite widely distributed in body tissues. Does not cross blood brain barrier or distribute into skeletal muscle.

Metabolism-Activation to greater extent in normal cells when compared to tumour cells because of higher level of expression of Alk.Phosphatase, higher pH & increased vascularity in normal cells. Elimination half time 8 min & <5% of drug excreted in urine either as parent form or as Amifostine metabolites

Indications:

- Reduce incidence of nephrotoxicity in patients receiving cisplatin based chemotherapy
- Reduce incidence of mod. to severe xerostomia where radiation port includes significant portion of parotid gland.

Dose range:

1. For reduction of cumulative renal toxicity-910 mg/m^2 IV, o.d. 30 min before cisplatin chemo.
2. For reduction of xerostomia-200 mg/m^2 o.d. 15-30 min before RT.
3. Alt. regimen for H&N cancer-500mg sc o.d. 20 min before RT.

Special consideration:

1. Antiemetics
2. Well hydrated and in supine position
3. Antihypertensives stopped 24hrs before starting therapy
4. BP and vitals monitored every 5 mins during and 10 mins after infusion.
5. Infuse over period of 15 min when used for CT and over 3 min when used for RT. Longer infusion- greater risk of side effects.
6. SC admin. Less nausea/vomiting & hypotension
7. Pregnancy Category C drug. Breast feeding avoided.

Toxicity:

- Nausea, vomiting & other GI effects
- Transient hypotension- in 60%. Mean time of onset is 14 mins into infusion. BP reverts in 5-15 min.
- Infusion related such as flushing and feeling of warmth, Chills, Dizziness, somnolence, hiccups & sneezing
- Allergic reactions rare and include anaphylaxis
- Hypocalcemia in <1%- clinically asymptomatic
- Seizure

- Extent to which Amifostine protects normal tissues from radiation effects varies considerably among tissues
- Haematopoietic, gut lining and salivary glands well protected
- No protection to brain and low level of protection of lung
- For maximum radioprotective effect- admin. at maximum tolerable concentration immediately before RT

- Use has been slow in coming
- Toxicity have tended to limit amount of drug given to less than the dose needed to achieve maximum protection
- Study from mainland China (Kligerman et al, 1992)- 100 patients with inoperable, unresectable or recurrent adenoca. of rectum.

  Amifostine group- protection of skin, mucous membranes, bladder, and pelvic structures against moderate and severe reactions- none of the 34 pts had these reactions

  RT only group- 5 of 37 evaluable had reactions- significant difference
- Use not fail safe

- More modest but achieveable goal is to use them to reduce side effects
- RTOG phase III RC trial (Brizel et al.1998): Amifostine reduced xerostomia without affecting early tumour control in H&N cancers. 15 min before radiation, 4 days each week, for 5 weeks. Three months after treatment, incidence of xerostomia significantly reduced.
- MDACC trial (Komaki et al. 2004): whether Amifostine reduced acute toxicity associated with concurrent chemoradiation. Amifostine twice a week at 20-30 min before doses of IV cisplatin, oral etoposide or irradiation. Did reduce incidence and severity of esophageal, pulmonary and hematologic toxicity. Did not affect survival

Radioprotector & Chemothearpy

- Also protect against the cytotoxicity effects of a number of CT agents.
- Significant protection against:
  1. nephrotoxicity, ototoxicity and neuropathy from cisplatin
  2. haematologic toxicity of cyclophosphamide
- No obvious antitumour activity

Other uses of Amifostine

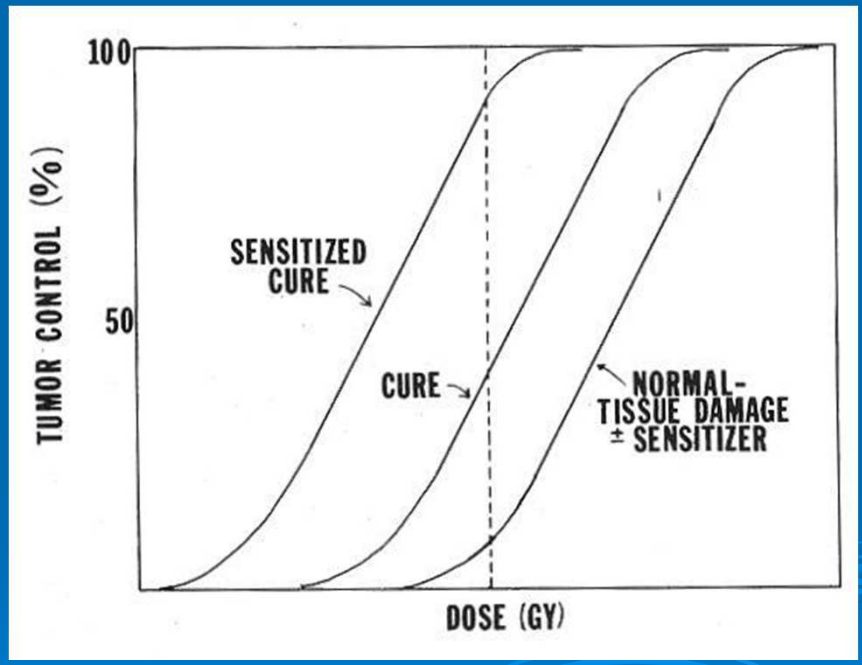
- Dose of 400mg/kg for optimal cytoprotection, but its antimutagenic effect persists at a low nontoxic dose of 25mg/kg
- Antimutagenic property not explained by antioxidant property-occurs if drug added 3 hrs following irradiation-due to its polyamine like properties which stabilize DNA damage site and promote error free repair

Radiosensitizers

Chemical or pharmacologic agents that increase the lethal effects of radiation if administered in conjunction with it.

Basic Strategy of Radiosensitizers

- Aim: Move TCP curve to lower doses by sensitizing tumour cells but not affecting NTCP curve or not altering it as much
- Outcome-Increase TCP for a given level of normal tissue complications



Mechanisms of Radiosensitization by Chemotherapy

CT can act as radiosensitizing agent by various mechanisms:

1. Direct enhancement of initial radiation damage by incorporating drugs into DNA
2. Inhibiting cellular repair mechanisms
3. Accumulating cells in radiosensitive phase or eliminating radioresistant phase
4. Eliminating hypoxic cells
5. Inhibiting accelerated repopulation of tumour cells

Mechanisms of Radiosensitization by Chemotherapy

Reduction in hypoxic fraction:

- Causes reduction in number of viable cells leading to potential decrease in number of hypoxic cells by mechanism analogous to that seen with tumour reoxygenation after RT.
- Improved oxygenation leads to improved response to ionizing radiation when combined with CT
- At same time reduce repair in normal tissue- increase normal tissue toxicity.
- Final net clinical effect will depend on balance of toxicity to benefit.

Mechanisms of Radiosensitization by Chemotherapy

Inhibition of repair of sublethal damage by radiation:

- RT causes sublethal and potentially lethal damage
- Fractionated RT allows these damages to be repaired
- Combining RT and certain pharmacological substances that prevent repair of this damage could enhance tumour response
- Likely to benefit only if differential effect on normal tissue repair
- Ideal radiosensitizer would have synergistic or additive effect on tumour but a sub-additive effect on normal tissues
- Halogenated purines or pyrimidines- BUdR, IUdR, and especially 5FU

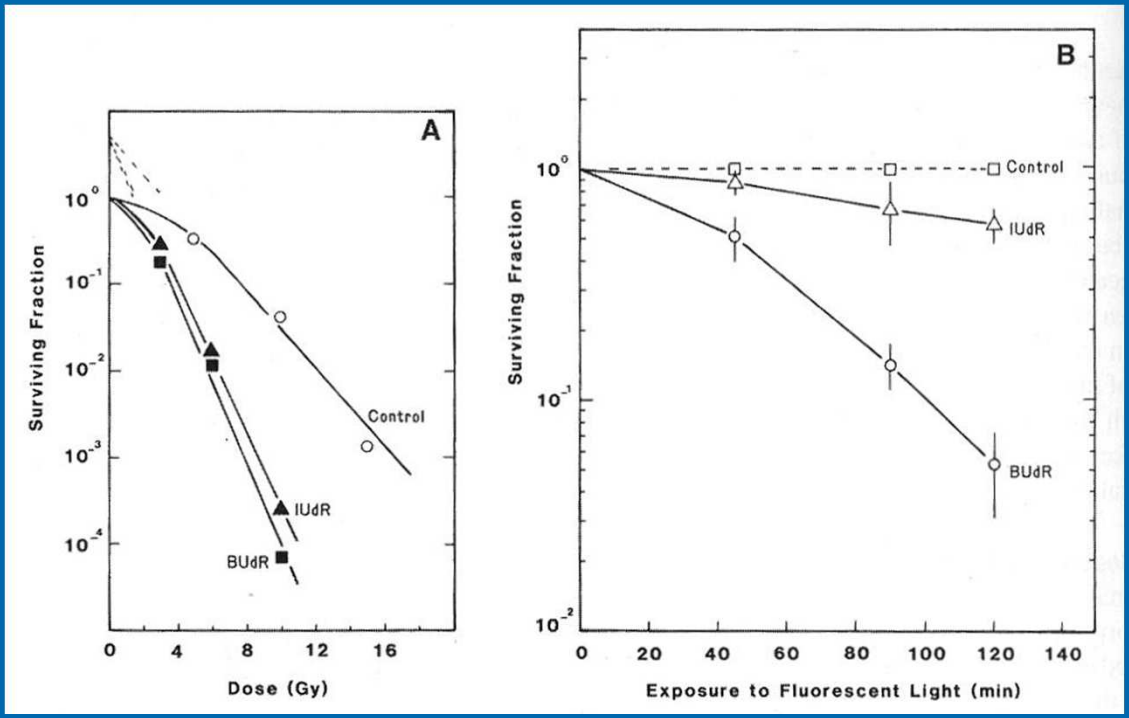
Mechanisms of Radiosensitization by Chemotherapy

Gemcitabine-deoxycytidine acitve against pancreas, lung, breast, bladder and head and neck.

- Exact mechanism of radiosensitization still unclear and various mechanisms suggested.
- One suggested mechanism is early ‘S’ phase block by gemcitabine, making tumour cells to synchronously progress into ‘S’ phase leading to radiosensitization by subsequent RT
- Other suggesed mechanism is induction of apoptosis
- In addition to known cytotoxicity effect it has been considered to have potent radiosensitizing effect

Halogenated Pyrimidines

- IUdR & BUdR very similar to thymidine- a halogen in place of CH3
- Incorporated into DNA in place of thymidine
- ‘Substitution’ weakens DNA chain, making cells more susceptible to damage by γ rays or uv rays
- Effective as sensitizers only if made available to cells for several generations
- As % of thymidine bases replaced increases, so does extent of radiosensitization
- Effectiveness first shown in bacteria



- BUdR much more efficient sensitizer for fluorescent light
- Rash- unpleasant side effect of BUdR in some patients, casused by phototoxicity
- Much less with IUdR

- Use of halogenated pyrimidines as adjunct to RT began in 1970s
- Rationale- tumours cycling more rapidly than normal tissues- more drug incorporated in tumour cell DNA
- H&N tumours were among those treated at Stanford University
- Tumor response good but normal tissue damage unacceptable
- Not used for many years
- Subsequently evaluated in other tumours- high grade glioblastomas & large unresectable sarcomas

Radiosensitizing Hypoxic Cells

- In transplanted tumours in animals-tumour control in animals frequently limited by foci of hypoxic cells

- Methods to overcome this problem:
  1. T/t in hyperbaric O_2 chambers
  2. High LET radiations - neutrons, heavy ions etc.
  3. Chemical sensitizers

Hyperbaric Oxygen Treatment

- First use- Churchill Davidson of St. Thomas Hospital
- Trials involved small number of patients
- Unconventional fractionation schemes
- Time consuming
- Patient compliance was poor-claustrophobia
- Serious risk of fire
- Fallen into disuse: partly because cumbersome & partly because drugs thought to achieve same end by simpler means

Hyperbaric Oxygen Treatment

- MRC- largest multicentre trial of Hyperbaric O_2
- Significant benefit both in LC and survival for CaCx & advanced H&N but not Ca Urinary bladder
- 6.6% improvement in LC, with suggestion of increase of late normal tissue damage.

Carbogen

Blood Transfusion & perfluorocarbons

Quit smoking

Hypoxic Cell Radiosensitizers

- Search underway in early 1960s for compounds that mimic oxygen in sensitizing biologic material to radiation
- O_2 substitutes that diffuse into poorly vascularized areas of tumours
- Vital difference- not rapidly metabolized by cells in tumour through which they diffuse
- Sensitizing efficiency related directly to electron affinity of compounds

Hypoxic Cell Radiosensitizers

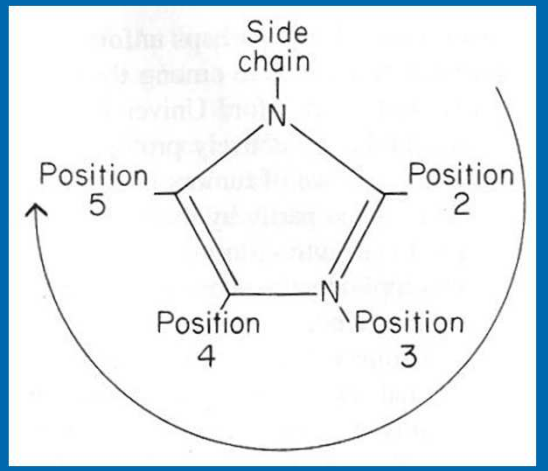
Properties essential for clinically useful hypoxic cell sensitizer (Adams et al.) :

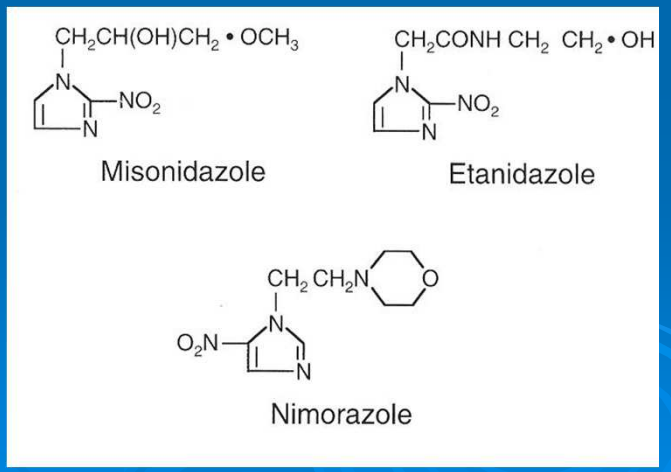
- Has to selectively sensitize hypoxic cells at conc. resulting in acceptable normal tissue toxicity.
- Chemically stable and not subject to rapid metabolic breakdown
- Highly soluble on water or lipids & capable of diffusing a considerable distance
- Should be effective at relatively low daily doses of a few gray used in conventional RT.

Misonidazole-a 2-nitroimidazole- first candidate fulfilling the criteria

Nitroimidazole

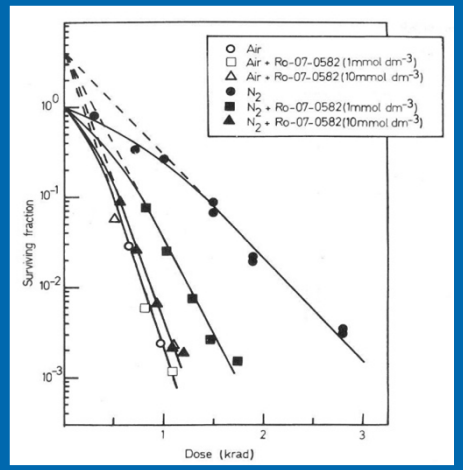
- Side chain determines position 1
- Position of nitro(NO2) group leads to classification of drugs
- 2-nitroimidazole have higher electron affinity than 5-nitroimidazole

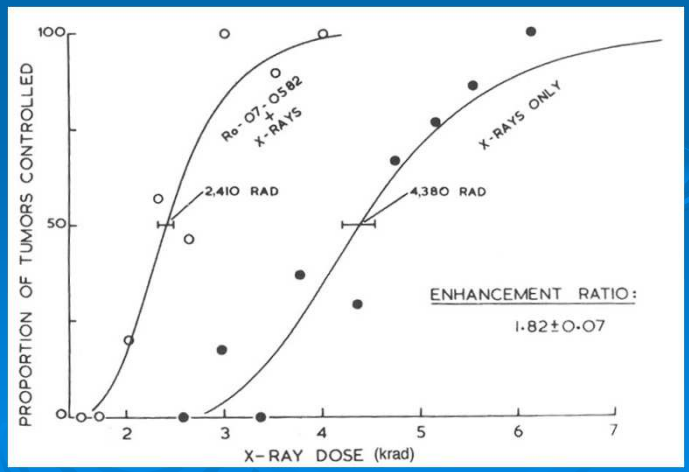




Misonidazole

- Produces appreciable sensitization with cells in culture
- Hypoxic cells in presence of 10mM concent. have RTsensitivity approaching that of aerated cells
- Dramatic effect on tumours in experimental animals
- Enhancement ratio of 1.8 for single fraction treatment
- In multifraction treatment-reoxygenation-enhancement ratio much less than for single fraction





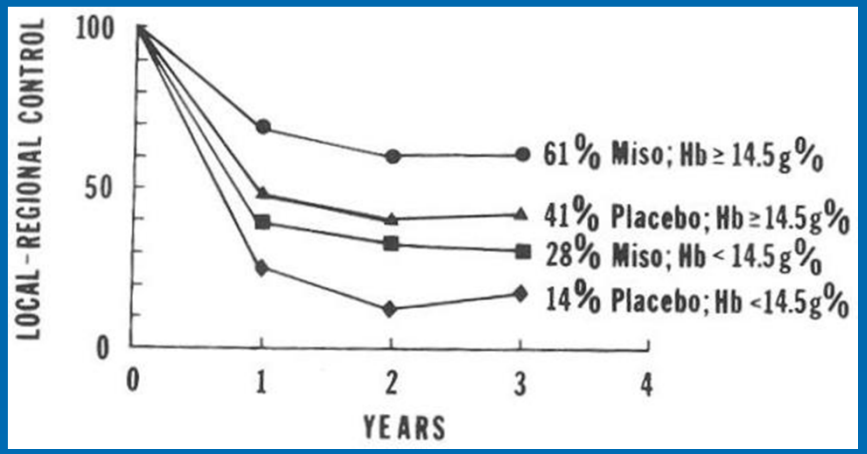
Misonidazole

- Encouraging results in lab.- introduced into many clinical trials involving many types of human tumours
- Results disappointing
- RTOG- 20 or more prospective RCCT-none gave significant advantage, some showed slight benefit
- Danish H&N trial- largest single trial with the drug. Only trial showing advantage
- In Clinic- dose limiting toxicity- peripheral neuropathy progressing to CNS toxicity-preventing use at adequate dose
- Poor results in clinic attributed to inadequate dose due to toxicity

Misonidazole

Danish H&N trial

- In all patients- Miso+RT no benefit
- Subgroup analysis- males with high Hb & Ca. pharynx showed benefit. TC at 3yrs- double in Miso.+RT group
- Ca. Larynx-no benefit



Etanidazole & Nimorazole

Following Misonidazole efforts made to find better drugs

Etanidazole-

- 2-nitroimidazole.
- Equals misonidazole as sensitizer but less toxic- dose increased by factor of 3
- Lower toxicity- shorter half life in vivo + lower partition coefficient
- CCT by RTOG & European consortium- no benefit when added to RT

Etanidazole & Nimorazole

Nimorazole-

- 5-nitroimidazole.
- Less effective than Misonidazole or Etanidazole as sensitizer but much less toxic- large doses can be given
- Danish H&N cancer trial- significant improvement in both locoregional control and survival compared with RT alone in patients with supraglottic larynx
- Has not been used elsewhere

Nicotinamide & Carbogen Breathing

Hypoxic cell sensitizers- nitroimidazoles- designed to deal with chronic hypoxia.

Nicotinamide- Vit B_3 analogue- prevents transient fluctuations in blood flow, atleast in mouse tumours

ARCON trial- in European centres-

- Nicotinamide- to overcome acute hypoxia
- Carbogen breathing- to overcome chronic hypoxia
- Accelerated RT- to overcome proliferation
- Hyperfractionated RT- spare late responding normal tissue

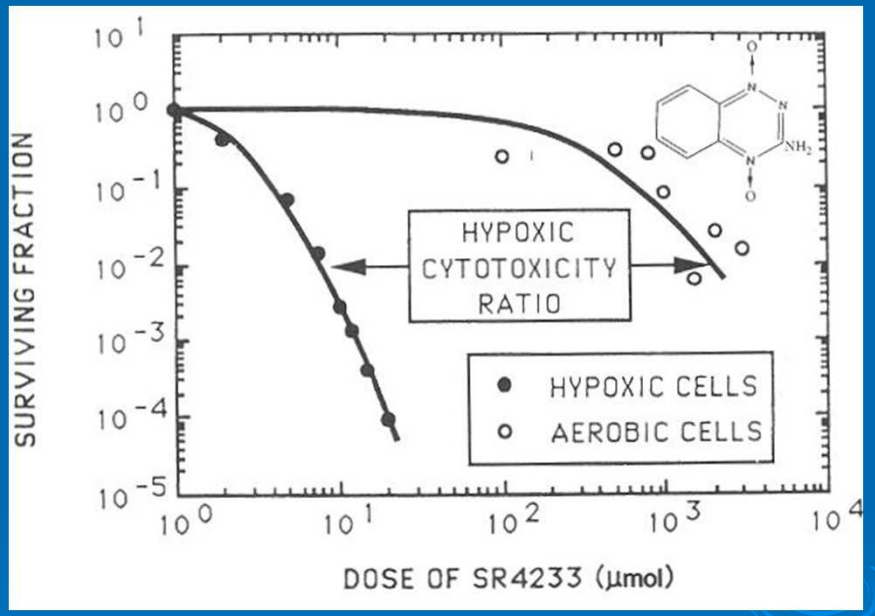
Hypoxic Cytotoxins

Alternative to drugs that preferentially radiosensitize hypoixc cells , is develop drugs that selectively kill hypoxic cells

- Quinone antibiotics- Mitomycin C- aerated - hypoxic differential is relatively small
- Nitroaromatic compounds- normal tissue toxicity high
- Benzotriazine di-N-oxides- Tirapazamine- high selective toxicity towards hypoxic cells *in-vitro* and *in-vivo*

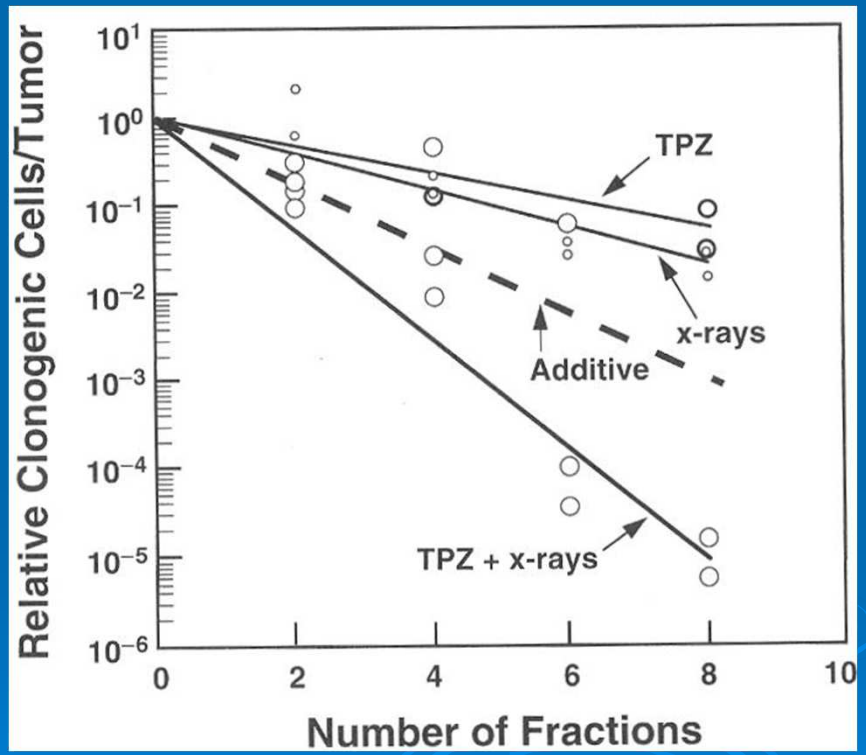
Tirapazamine

- Organic nitroxide synthesized by Stanford Research
- Cells deficient in O_2 are killed preferentially
- Hypoxic cytotoxicity ratio variable- for Chinese Hamster cells 100, for humans 20



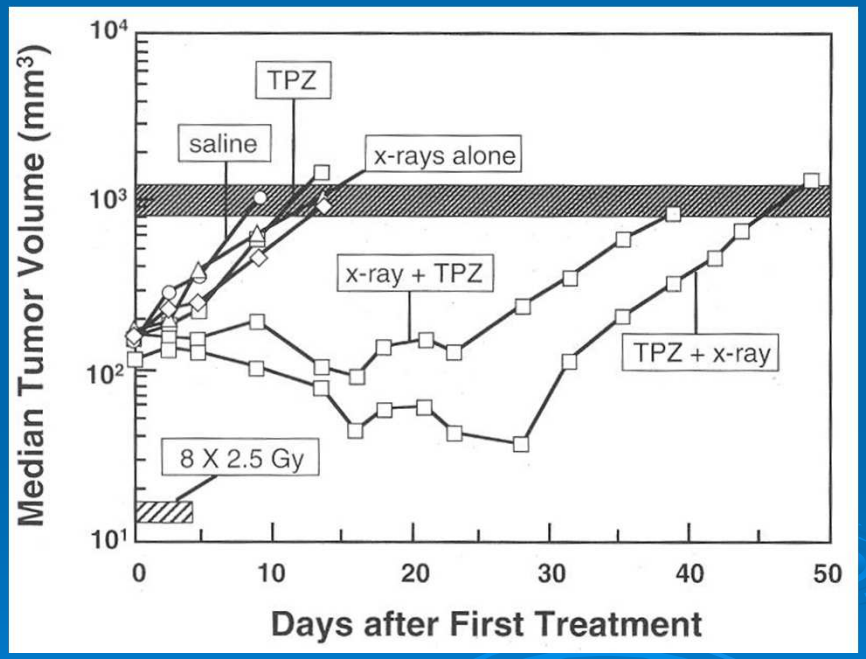
Tirapazamine

- Transplanted mouse carcinoma treated with x-rays alone, TPZ alone or x-rays+TPZ
- TPZ injected 30 min before each irradiation
- 2.5 Gy fractions twice daily
- RT or drug treatment given every 12 hrs for 8 fractions
- Effect of x-rays+TPZ much greater than additive



Tirapazamine

- Study *in-vivo*, scoring regrowth delay
- x-rays alone, TPZ alone or x-rays+TPZ
- 8X2.5 Gy fractions
- X-rays+TPZ caused significant growth delay
- X-rays following TPZ caused greater effect
- In parallel exp. no radiosensitization or additive cytotoxicity on skin reactions



Clinical Trials with Tirapazamine

- Clinical trials have shown only modest success
- Phase III trial (von Pawel J et al, 2000) comparing cisplatin or cisplatin +TPZ for Stage IIIB or Iv NSCLC, pts. Given combination had twice the response rate and longer survival. Systemic cisplatin toxicity not increased. TPZ associated nausea and muscle cramping reported.
- Rischin et al (2001,2005) TPZ+ RT trial from Australia. TPZ+Cisplatin & RT superior to FU+Cisplatin & RT for patients with locally advanced H&N cancers