Are low doses of radiation in the environment PROTECTING you from cancer?

Pam Sykes

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Flinders University and Medical Centre
Outline

• Put low doses of radiation into perspective

• Discuss current risk assessment paradigm

• Present data which contradicts the paradigm
  – low dose radiation induced protection from cancer

• Attempts to study mechanism of protection
  – *In vivo*
  – At relevant doses
Radiation is everywhere

We live in a sea of radiation…

1-2 mSv/year

0.7 mSv
Inhaled Radon

0.3 mSv
Bodies

0.3 mSv
Radioactive Elements

0.6 mSv
Rocks

0.3 mSv
Plants

0.3 mSv
Cosmic

Background Range 1-100 mSv
Yearly Estimate of man-made exposure over and above 2 mSv background (minus medical diagnostic exposure)

<table>
<thead>
<tr>
<th>Man-made exposure</th>
<th>0.07 mSv</th>
</tr>
</thead>
<tbody>
<tr>
<td>(some paints, smoke detectors etc)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nuclear industry</th>
<th>&lt;0.001 mSv</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Weapons test fall-out</th>
<th>&lt;0.001 mSv</th>
</tr>
</thead>
</table>

This is the radiation that people are worried about!!
Pre-existing dogma and public perception: High dose radiation is bad.

Nuclear tests

Chernobyl, 1986
Radiation is not the poison - the dose is!
Why are people so scared of the R word?

• Indoctrination
  – There is no dose of radiation that is safe
  – We live in a litigious society and so authorities try to protect us from even tiny doses of radiation (so it must be dangerous)
  – The NEWSPAPERS say so!
What is a low dose of ionising radiation?

- US DOE Low Dose Radiation Research Program
  - < 100 mGy low LET radiation

- For X-rays
  
  \[ 1 \text{ mGy} = 1 \text{ mSv} \]
Typical doses
Dose-rate, radiation quality and extent of exposure vary

<table>
<thead>
<tr>
<th>Exposure</th>
<th>~Dose (mSv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiotherapy to tumour</td>
<td>70000</td>
</tr>
<tr>
<td>Lethal Dose 50</td>
<td>4,200</td>
</tr>
<tr>
<td>Typical Space Station mission</td>
<td>1,000</td>
</tr>
<tr>
<td>Spiral CT scan –full body</td>
<td>15-100</td>
</tr>
<tr>
<td>Mammogram</td>
<td>2.5</td>
</tr>
<tr>
<td>Dental X-ray</td>
<td>0.6</td>
</tr>
<tr>
<td>Chest X-ray</td>
<td>0.1</td>
</tr>
<tr>
<td>Adelaide to Sydney flight</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Occupational exposure limit 20 mSv
Population exposure limit 1 mSv
Why study low dose ionising radiation?

• Increased use of diagnostic radiology
• Increased use of radiation for security screening
• Accidents involving radiation
• Space exploration
• Potential need for increased nuclear power energy generation
• Potential low dose radiation therapy
  – Many diseases
Current radiation risk regulation uses the Linear No-threshold Model

LNT
The LNT model of risk assessment states that even the tiniest dose of radiation is harmful and that risk increases linearly with dose.

\[ \text{Radiation dose} \]

- Cancer & DNA damage
- Data
- < 100 mSv
- Data???
- high exposure
- Death Cancer

LNT = Linear no-threshold
LNT model of risk assessment

- Risk is directly proportional to dose

- Even the tiniest dose of radiation is harmful
  - ↑cancer

- Everyone must be protected from tiny doses
  - Billions of $’s
Low dose of ionising radiation can protect from mutation and cancer.
Hormesis

– occurs when a system overcompensates for a disruption in homeostasis, effectively overshooting homeostatic feedback controls and that the modest overcompensation re-establishes homeodynamic balance.
Should we use LNT for **LOW DOSES**?

- **Pro-LNT vs Anti-LNT Groups**
  - Use the same data, different interpretations
    - Gives a hint about the clarity of the data

- **Pro-LNT**
  - Concentrate on Epidemiology data
    - Confounding effects at low doses?

- **Anti-LNT**
  - Biological data
    - Relevance to humans?
Predictions

• Pro-LNT
  – Predict that large numbers of cancers will result from increased use of CT scanning
      – 0.4-2% of all cancers in US

• Anti-LNT
  – Predict that such low doses are below a threshold and may even be protecting from cancer
Long-term effect
Evacuated population 336,000
average 2X previous background

4000 cancer deaths predicted by LNT

- 2006 UN Chernobyl Forum
  - Increase in thyroid cancer (4X)
    - 15 deaths estimated
  - No increase in leukaemia
  - No increase in solid tumours
  - No increase in incidence of hereditary disorders
Post-Evacuation in Belarus, Russia and Ukraine

- Mass psychosomatic disturbances
- Great economic loss
- Traumatic social consequences
Japanese Earthquake and Tsunami
March 2011

>30,000 deaths, >590,000 homeless

Fukushima Nuclear Power plant
0 deaths

All doses calculated in the prefectures affected were much lower than the 1 mSv/yr dose limit for the public.
According to LNT, doses must be directly additive
Adaptive response
Low dose protects from a high dose
Low dose protects from endogenous damage

![Graph showing adaptive response](image-url)

- **Expected**
- **Challenge dose**
- **Observed**

<table>
<thead>
<tr>
<th>Radiation Dose</th>
<th>Effect frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>conditioning dose</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

- Low dose protects from a high dose
- Low dose protects from endogenous damage
Low dose radiation increases latency of radiation-induced lymphoma in mice

# Dose-rate

The effectiveness of the dose is dependent on the dose-rate.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Dose -Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 bottle of Aspirin</td>
<td>Death</td>
</tr>
<tr>
<td>or</td>
<td>Minimal health risk</td>
</tr>
<tr>
<td>25 Sv of Radiation</td>
<td>Death</td>
</tr>
<tr>
<td></td>
<td>Minimal health risk</td>
</tr>
</tbody>
</table>

- Over 50 seconds??
- Or over 50 years??
Low doses of radiation protect from diabetes

Total dose ~ 10 Gy

Low doses of radiation stimulate the immune system

Sakai K, IHS 2006
Importance of dose threshold


0.33 mGy/day for:
- 30 weeks (48 mGy)
- 60 weeks (97 mGy)
- 90 weeks (146 mGy)

B and T lymphomas

Sarcomas
Dose range showing protection

• <50 mSv (dose-rate?)

• Most evidence - low LET
  – (X-rays, γ-rays, β-particles)

• Total body irradiation

• Protection not restricted to cancer
  – life span, diabetes, cardiac disease

• If the dose is too low then protection may not occur
Radiation Biology
Undisputed Fact

The biological responses to LOW dose radiation are DIFFERENT from the biological responses to HIGH dose radiation.
Our approach to study biological effects of very low dose radiation

- In vivo – mouse
- Low doses relevant to OH&S and population exposure
  - 1 µGy – 10 mGy; 250 mGy
- With and without a high challenge dose
- End-points
  - DNA damage, cell fate
- In situ analysis
- Temporal studies
pKZ1 assay for studying chromosomal inversions

Chromosomal inversions are common in cancer cells
Detection of chromosomal inversions *in situ* in pKZ1 spleen and prostate after low dose radiation

X-gal staining of mouse tissues for chromosomal inversions
In vivo inversion frequency for pKZ1 spleen and prostate tissue 3 days after single whole body X-irradiation

Inversion frequency +/- SE (% of sham-treated)

Zeng et al, 2006

*, p < 0.05

Linear?

Low end of radiotherapy

Adelaide – Sydney return flight

CT scan

X-Radiation (mGy) = mSv
In vivo inversion frequency for pKZ1 spleen and prostate tissue 3 days after single acute whole body X-irradiation.
Is this complex in vivo dose-response a permanent effect?
The temporal response for inversions in spleen is different for different doses.

Inversion frequency (% of sham-irradiated)

Treatment (mGy)

- 7 hours
- Day 1
- Day 3
- Day 7

n ≥ 5 transgenic mice per

Alex Staudacher
How can low dose radiation remove inversions? increase tumour latency?
Low doses of radiation can up-regulate removal of pre-tumour cells *in vitro*.

Portess *et al*, 2007 (Cancer Res. 67:1246)
Where have our inversions gone?
Removed by apoptosis?

At such low doses, expected increase in apoptosis by direct effects would be very small – so need a precise method
Development of sensitive detection of apoptosis

- *TUNEL in situ*

- Minimum of 110,000 cells studied in spleen/mouse

- Precision of 0.001%

- The range of mean sham-treated apoptosis frequencies in 14 separate experiments represented a maximal difference of 1.2 apoptotic cell /1000 cells
Temporal study of apoptosis frequency in spleen after low dose X-radiation  

N ≥ 10, Experiments performed twice
Total of 14 experiments

274 mice
You need a very large number of mice to detect apoptotic changes based on LNT at these low doses (Staudacher et al, Radiat. Res., 2010)

<table>
<thead>
<tr>
<th>Dose (mGy)</th>
<th>Apoptosis Induction</th>
<th>Required Sample Size $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>1.4% $^b$</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>0.14%</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>0.014%</td>
<td>124</td>
</tr>
<tr>
<td>1.0</td>
<td>0.0014%</td>
<td>12,325</td>
</tr>
<tr>
<td>0.1</td>
<td>0.00014%</td>
<td>1,232,500</td>
</tr>
<tr>
<td>0.01</td>
<td>0.000014%</td>
<td>123,249,910</td>
</tr>
</tbody>
</table>

$^a$ Sample size required per group for 80% power at $\alpha$ level = 0.05, Compared to the sham-irradiated apoptosis frequency of 0.293 ± 0.0442% (± SD). Assumes equal variance.

$^b$ Measured in this study
It is very difficult to detect any changes in conventional biological end-points after very low doses alone because the changes that occur are likely to fall within the normal homeostatic range for the end-point.
Why is the pKZ1 assay so sensitive?
Responses observed in pKZ1 assay

Mechanism?
Increased age
Over expression c-myc

High doses
-etoposide
-X-rays
-mitomycin C
-cyclophosphamide
-methylene chloride

Very low doses of
- X-rays

Balance?

Inversions

Mechanism?
Loss of msh-2
Expression of SV40 T antigen
Loss of TP53

Low doses
-etoposide
-X-rays
-mitomycin C
Mechanism of pKZ1 assay

- Chromosomal inversion breakpoints identified at DNA level
- Inversions are transcribed into RNA
- Transcripts off a cryptic pKZ1 promoter identified
- pKZ1 gene expression affected by demethylating agent and histone deacetylase inhibitor
We are working in unchartered territory
10^{-7} \quad 10^{-6} \quad 10^{-5} \quad 10^{-4} \quad 10^{-3} \quad 10^{-2} \quad 10^{-1} \quad 10^{0} \quad 10^{1} \quad 10^{2}

Dose Rate (Gy/hr)

10^{-6} \quad 10^{-5} \quad 10^{-4} \quad 10^{-3} \quad 10^{-2} \quad 10^{-1} \quad 10^{0} \quad 10^{1} \quad 10^{2}

Dose (Gy)

---

Natural Background Radiation

50yr

1yr

Damage

Damage Reduction

No Effect

Conventional Expts.

A-bomb

Mitchel

Sakai

Azzam

Sykes

Redpath
Gran Sasso Laboratories (LNGS)

- Largest underground laboratories in the world
- 1,400 m below the top of the Gran Sasso massif
- 70X lower background dose-rate (γ)
- 25X lower radon
Would adaptive responses occur if we were not “conditioned” by background radiation doses on earth?

_In vitro/in vivo_ MUrine coSmiC siLEnce experiment (MUSCLE)

Collaboration with:
G. Simone, MA Tabocchini, G. Esposita, Istituto Superiore di Sanità, Rome
R. Amendola, M Balduzzi, ENEA Casaccia, Roma
E. Alesse, F Zazzeroni, L Fuci, Universita dell’Aquila
M. Balata, L. Ionnucci, Laboratori Nazionali del Gran Sasso,
Dr Luigi Satta, INFN – Laboratori Nazionali di Frascati
Background radiation is required for an efficient adaptive response

- Yeast (Satta et al, 1995)

- V79 Chinese hamster cells (Satta et al, 2002)

- TK cells human lymphoblastoid cells (Carbone et al, 2009)

Extremely important experiments as they allow us to effectively study homeostasis
We need some radiation for normal homeostasis
Experiments at Gran Sasso using the pKZ1 assay
Will these adaptive responses still occur?

Day et al, 2006; 2007

*, p < 0.05

Inversion frequency +/- SE (% of sham-treated)

X-Radiation (mGy)

Spleen
Prostate

Linear?

Protects from high doses
Summary – *In vivo*

- Studying very low doses of whole body X-rays *in vivo*

- pKZ1 assay is 3 orders of magnitude more sensitive than other assays

- Observed adaptive responses for chromosomal changes between 0.001 and 50 mGy

- Temporal studies suggest that changes at the pKZ1 transgene at very low doses may be short-lived, whereas the high dose induced changes may be more permanent.

- Intercellular induced apoptosis is a candidate mechanism for low dose radiation induced protection from cancer – but no evidence *in vivo* to date

- There is evidence that below a certain dose-rate adaptive responses are less effective.

- Will the pKZ1 adaptive response occur in reduced background radiation?
Take-home messages

- Life on earth is dependent on radiation

- Radiation is not the poison, the dose is.

- Background radiation is likely required for maintenance of normal homeostasis

- Low doses of radiation may be protecting you from cancer and other diseases

- If we can understand the mechanism then we should be able to harness low dose radiation to prevent cancer and other diseases
Implications of low dose radiation protection for radiation regulation

• Identify safe doses of radiation
  – prevent costly (billions of dollars) clean-up of low level radiation

• Protect groups that are at high OH&S risk of radiation damage
  – radiation clean-up emergency workers, astronauts

• Better informed response to radiation disasters
  – Do more good than harm
Applications for low dose X-radiation in cancer prevention

- Destroy early stage cancer cells

- Protect from secondary tumours after radiotherapy and chemotherapy

- Stimulate the immune system

- Use the key molecules as biomarkers of radiation damage/protection
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Mark Lawrence
Michelle Newman
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