Bystander Effects elicited by targeted radionuclide therapy

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Targeted Radiotherapy

$^{131}$I-conjugate
**Neuroblastoma**

- 2nd commonest solid tumour in children
- peak age < 2 years
- mostly disseminated at 1st presentation: poor prognosis
- rapid growth
- chemo/radiosensitive
Catecholamines, adrenergic neurone blockers and MIBG

Adrenaline

Noradrenaline

Guanethidine

Meta-iodobenzylguanidine (MIBG)

Noradrenaline transporter
Preoperative $^{131}$I-MIBG treatment

3 year old neuroblastoma stage IV patient
(PA Voute, 2001)

- 3 consecutive treatments; interval = 4 weeks
- 1st scan: bone and bone marrow invasion + large retroperitoneal tumour
- 2nd scan: decreased uptake in bone, bone marrow and primary tumour
- 3rd scan: bone and bone marrow cleared; primary tumour resectable
Transfer of the noradrenaline transporter (NAT) gene for $[^{131}\text{I}]$MIBG targeted radiotherapy of
- glioma
- urological tumours
- neuroblastoma
Introduction of NAT gene into human UVW glioma cells induces active uptake of $[^{131}\text{I}]\text{MIBG}$
Toxicity of $[^{131}\text{I}]$MIBG to NAT transfectants

Clonogenic assay

Growth Delay

\[ \text{T.I.} = 1000 \]
Expression of the NAT transgene in malignant tissue using tumour-specific elements

- telomerase promoters
- radiation-inducible WAF1 promoter
Viral delivery

Replication selective Herpes Virus (HSV 1716)
[Prof Moira Brown, Institute of Neurological Sciences, Glasgow]

- HSV1716 deleted in RL1 gene - replicates and lyses only dividing tumour cells
- avirulent in normal brain
- viral replication and oncolysis enhanced by radiation?
- MRC trial:
  Patients with relapsed high grade glioma
  No toxicity
  Some long term survivors
DOCS INJECT ROB’S BRAIN WITH COLD SORE VIRUS TO KILL TUMOUR

Site where doctors injected herpes virus, inset, into brain tumour.

ALIVE AND KICKING: Robert, above right, expects four extra years thanks to the injection.
Viral therapy for glioma

HSV1716-NAT

GLIOMA CELL

[131I]MIBG

NAT

Cell kill by lysis +
MIBG targeted radiotherapy
Images of mouse tumours 1 and 7 days after intravenous injection of HSV1716

Uninfected tumour cells stain blue. HSV-infected cells stain brown.

X 5 magnification
Areas of virus concentration are indicated by arrows

Day 1

Area A at x 20 magnification
These cells show classic signs of HSV infection; multi-nucleated and necrotic.

Day 7

Area E at x 20 magnification
Classic signs of HSV infection: multi-nucleation; giant cells; holes due to cell death.

X 5 magnification
There are more and larger areas of positive staining compared with Day 1.

Classic signs of HSV infection: multi-nucleation; giant cells; holes due to cell death.

Highly necrotic; most cells have lysed due to HSV infection.
The effect of scheduling of viral delivery on tumour uptake of $^{[131\text{I}]}$MIBG
Effect on tumor growth of HSV1716/NAT + [¹³¹I]MIBG delivered simultaneously or sequentially

N=12, mean and sd
Transfection of multicellular tumour spheroids with the gene encoding the jellyfish green fluorescent protein (GFP)
UVW glioma cells infected with HSV1716 / lac-Z
GENETICALLY ENHANCED TARGETED RADIOTHERAPY FOR GLIOMA

Herpes virus for tumour cell lysis + transfer of NAT gene, controlled by WAF1 or telomerase promoter

Targeted radiotherapy

[\textsuperscript{123}I]MIBG
[\textsuperscript{131}I]MIBG
[\textsuperscript{211}At]MABG
# 3 types of therapeutic decay particle

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>decay particle</th>
<th>range</th>
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<tbody>
<tr>
<td>$^{123}$I</td>
<td>Auger electrons</td>
<td>10 nm</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>beta particles</td>
<td>800 m</td>
</tr>
<tr>
<td>$^{211}$At</td>
<td>alpha particles</td>
<td>60 m</td>
</tr>
</tbody>
</table>
Incorporation of the Auger electron emitter $^{123}$I into DNA using the thymidine analogue $[^{123}$I]I UdR
$^{131}\text{I}$ particle bombardment of DNA using the noradrenaline analogue $[^{131}\text{I}]\text{MIBG}$
$^{211}$At -particle bombardment of DNA using the noradrenaline analogue [$^{211}$At]MABG
Bystander Effects

- Killing/damage of un-irradiated cells due to irradiation of adjacent cells

- **Physical**: radiation cross-fire, i.e., direct traversal

- **Biological**: direct traversal not required
Manifestation of radiation-induced biological bystander effects (RIBBE)

- Transfer of medium
- Transfer of serum
- Unirradiated cells
- Unirradiated cells
- Cell death
- Chromosomal aberrations
- Mutations
- Genomic instability

Esp: low dose & low dose rate
Are RIBBEs significant in targeted radiotherapy?

How do we investigate RIBBE in our gene therapy/targeted radiotherapy scheme?

- transfectant mosaic spheroids

- media transfer experiments
Non-mosaic multicellular spheroids

100% GFP-expressing cells 100% GFP-non-expressing cells
Transfected mosaic spheroids derived from the human glioma cell line UVW

The spheroids, ranging in size from 100 to 500 μm diameter, are composed of mixtures of cells transfected with the GFP gene (green) and cells transfected with the NAT gene (red).
[\textsuperscript{211}At]astatine

\[\text{\textsuperscript{131}I}MIBG\]
\text{meta-iodobenzylguanidine}

\[\text{\textsuperscript{211}At}\text{MABG}\]
\text{meta-astatobenzylguanidine}
Media transfer protocol

After 1hr incubation, gamma count the transferred medium and add activity, equivalent to that of radiopharmaceutical which leaked from cells, to a third flask - [= Activity control]

Remove targeted radionuclide Non-irradiated cells
Replace medium and Incubate for 1hr

24h

Clonogenic Assay
Groups evaluated

**Direct + Indirect:** incubate with MIBG 2h; wash; incubate to allow generation of “bystander poisons” (direct + bystander effect)

**Donor:** as Direct + Indirect but remove medium then replace with fresh medium (~ direct effect only)

**Recipient:** receive medium from Donor (~ bystander effect only)

**Activity control:** to correct for small amount of [*I]MIBG leaked from Donor cells and transferred to Recipients

**Untransfected:** wild-type UVW cells (do not express NAT) recipients of medium from MIBG-treated wild-type UVW donors
This cell line demonstrates RIBBE after external beam irradiation.

- **Direct irradiation** is more cytotoxic than RIBBE at high dose.

- RIBBE are most significant at low doses.
RIBBE after treatment with -emitter $[^{131}\text{I}]\text{MIBG}$

- medium from irradiated cells is very cytotoxic - dose response
- significant effect at high doses; RIBBE cell kill almost as potent as direct kill in cells treated with radiopharmaceutical
RIBBE elicited by treatment with Auger electron emitter ([^{123}I]MIBG) and -emitter ([^{211}At]MABG) - high LET

- U-shaped survival curves for RIBBE-kill - i.e. dose-related cytotoxicity at low activity concentration and diminishing bystander kill at higher activity concentration

- RIBBE elicited by high LET targeted radionuclides result in cell kill of magnitude similar to that caused by direct irradiation - hence potentially powerful therapeutic effect
RIBBE in a second cell line - EJ138 bladder carcinoma

- Cell line shows RIBBE in response to external beam irradiation
- **Recipient cells** - dose response at low doses then plateau
RIBBE following exposure of EJ138 cells to \(^{131}\text{I}\)MIBG

Effect similar to that observed in UVW cell line:
- no U-shaped survival curve
- RIBBE cell kill less than in donor cells
RIBBE following exposure of EJ138 cells to Auger electron emitter ([\(^{123}\)I]MIBG) and -emitter ([\(^{211}\)At]MABG) - high LET

Similar to effect observed in UVW cell line:
- U-shaped survival curves for RIBBE-kill
Conclusion

Radiation-induced biological bystander effects may contribute significantly to the efficacy of targeted radionuclide therapy
Conclusions

RIBBE from targeted radionuclides appear distinct from external beam irradiation:
- dose response
- LET dependent
- U-shaped survival curves

RIBBE from high LET radionuclides at low doses are more cytotoxic than direct irradiation

Only cells which take up radiopharmaceutical produce RIBBE
Is subcellular localisation of radiopharmaceutical important?
[\textsuperscript{131}I]IUdR effect similar to [\textsuperscript{131}I]MIBG
What are the bystander toxins?
**Do Reactive Oxygen Species play a role in RIBBE elicited by targeted radionuclides?**

- low mol. wt. chemical scavengers
  perturbed bystander effects following X-irradiation
  or -beam irradiation

- In our system:
  - media transfer expts - using cells stably transfected with the SUPEROXIDE DISMUTASE gene
RIBBE after γ-irradiation of untransfected cells and SOD-transfectants

SOD transfection abolishes bystander effect
RIBBE after $[^{131}\text{I}]\text{I UdR}$ treatment of untransfected cells and SOD-transfectants

SOD transfection abolishes bystander effect
RIBBE after $^{[123]}$IUdR treatment of untransfected cells and SOD-transfectants

$^{[123]}$IUdR - SOD abolishes bystander effect only at low activity conc
Is a protein involved in RIBBE elicited by targeted radionuclides?

- Boil medium before transfer to recipient cultures
Effect on RIBBE of boiling the medium from -irradiated cells before transfer to recipients
Effect on RIBBE of boiling the medium from $[^{131}\text{I}]$MIBG-treated cells before transfer to recipients

Controls

Treatment groups

Medium only controls

boil -> room temp

boil -> $4^\circ$C
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**Sources of funding**
- Cancer Research Campaign
- British Urological Foundation
- Urology Dept., Glasgow
- European Community
- Neuroblastoma Society
- Scottish Office
- Department of Health
- Dr Hadwen Trust
- Scottish Hospitals Endowment
- Research Trust
- North Glasgow NHS Trust
- Clerk Maxwell Foundation
- Ian Sunter Trust