Radiation Induced Biological Bystander Effect Elicited in vitro by Targeted Radiopharmaceuticals Labeled with $\alpha$-, $\beta$- and Auger Electron Emitting Radiohalogens

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ABSTRACT
Recent studies have shown that indirect effects of ionizing radiation may contribute significantly to the effectiveness of radiotherapy by sterilizing malignant cells that are not directly hit by the radiation. However, there have been few investigations of the importance of indirect effects in targeted radionuclide treatment. Our purpose was to compare the induction of bystander effects by external beam $\gamma$-radiation with those resultant from exposure to three radiohaloanalogues of meta-iodobenzylguanidine (MIBG): $[^{131}\text{I}]$MIBG (low linear energy transfer (LET) $\beta$-emitter), $[^{123}\text{I}]$MIBG (potentially high LET Auger electron emitter), and meta-$[^{211}\text{At}]$astatobenzylguanidine ($[^{211}\text{At}]$MABG) (high LET $\alpha$-emitter). Methods: Two human tumor cell lines - UVW (glioma) and EJ138 (transitional cell carcinoma of bladder) – were transfected with the noradrenaline transporter (NAT) gene to enable active uptake of MIBG. Medium from cells that accumulated the radiopharmaceuticals or were treated with external beam radiation was transferred to cells which had not been exposed to radioactivity and clonogenic survival was determined in donor and recipient cultures. Results: Over the dose range 0 to 9 Gy of external beam radiation of donor cells, 2 Gy caused 30 to 40% clonogenic cell kill in recipient cultures. This potency was maintained but not increased by higher dosage. In contrast, no corresponding saturation of bystander cell kill was observed after treatment with a range of activity concentrations of $[^{131}\text{I}]$MIBG, which resulted in up to 97% death of donor cells. Cellular uptake of $[^{123}\text{I}]$MIBG and $[^{211}\text{At}]$MABG induced increasing recipient cell kill up to levels that resulted in direct kill of 35 to 70% of clonogens. Thereafter, the administration of higher activity concentrations of these high-LET emitters was inversely related to the kill of recipient cells. Over the range of activity concentrations examined, neither direct nor indirect kill was observed in cultures of cells not expressing the noradrenaline transporter and thus incapable of active uptake of MIBG. Conclusion: Potent toxins are generated specifically by cells which concentrate radiohalogenated MIBG. These may be LET-dependent and distinct from those elicited by conventional radiotherapy.
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 $[^{131}I]\text{MIBG}$ 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 now resectable

For mets but...
Transfer of the noradrenaline transporter (NAT) gene for $[^{131}\text{I}]$MIBG targeted radiotherapy of
- glioma
- urological tumours
- neuroblastoma
Dose Heterogeneity is the Bane of Targeted Radiotherapy

• Even with long range radionuclides, because of their low LET, sub-populations of tumor cells will be underdosed
• Current gene delivery vehicles for cancer gene therapy cannot access every tumour cell – at best, get only about 30% transgene introduced into a tumour
Biological effects of ionising radiation: the traditional perspective
Incorporation of the Auger electron emitter $^{123}\text{I}$ into DNA using the thymidine analogue $[^{123}\text{I}]\text{IUdR}$
$^{131}$I $\beta$-particle bombardment of DNA using the noradrenaline analogue $[^{131}$I]MIBG
$^{211}\text{At}$ α-particle bombardment of DNA using the noradrenaline analogue [${}^{211}\text{At}$]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
Transfer of medium

Transfer of serum

unirradiated cells

manifestation of radiation-induced biological bystander effects (RIBBE)

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).
$^{211}$At]astatine

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

$^{[211\text{At}]}$MABG  
meta-astatobenzylguanidine
Survival of NAT gene-transfected UVW spheroids after treatment with $^{[211}\text{At}]$MABG

Greater TMS killing than attributable to physical cross fire, implicating RIBBE
Transfer of medium

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]

DONOR

Recipient

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

ACTIVITY CONTROL

Clonogenic Assay
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 $\beta$-emitter $[^{131}\text{I}]\text{MIBG}$ of UVW/NAT cells

- 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 \(\alpha\)-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
- Cell line shows RIBBE in response to external beam irradiation
- **Recipient cells** - dose response at low doses then plateau
RIBBE following exposure of NAT transfected-EJ138 cells to β-emitter $[^{131}I]MIBG$

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 NAT transfected-EJ138 cells to Auger electron emitter ([\(^{123}\text{I}\)]MIBG) and \(\alpha\)-emitter ([\(^{211}\text{At}\)]MABG) - high LET

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

RIBBE from targeted radionuclides appear distinct from external beam irradiation:

- follow a dose response
- LET dependent
- Higher LET (auger & alphas) see 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]UdR effect similar to [\textsuperscript{131}I]MIBG

![Graph showing the effect of [\textsuperscript{131}I]UdR and [\textsuperscript{131}I]MIBG on surviving fraction. The x-axis represents the activity concentration (MBq/ml) of [\textsuperscript{131}I]UdR, while the y-axis represents the surviving fraction. The graph compares the effects of direct and indirect pathways, as well as the donor and recipient groups.]
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 α-beam irradiation

- In our system:
  - media transfer expts - using cells stably transfected with the SUPEROXIDE DISMUTASE gene
RIBBE after external $\gamma$-irradiation of untransfected cells and SOD-transfectants (recipients)

SOD transfection abolishes bystander effect
RIBBE after $[^{131}I]$IUdR treatment of untransfected cells and SOD-transfectants (recipients)

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

$[^{123}\text{I}]\text{I UdR}$ - SOD abolishes bystander effect only at low activity conc
Are macromolecules involved in RIBBE elicited by targeted radionuclides?

- Boil medium before transfer to recipient cultures
Effect on RIBBE of boiling the medium from external beam $\gamma$-irradiated cells before transfer to recipients

![Graph showing the effect of boiling the medium on recipient cell surviving fraction. The x-axis represents different treatment groups, and the y-axis shows the surviving fraction. The bars are color-coded to indicate different conditions: boil -> room temp, boil -> 4°C. The graph includes controls and treatment groups.](image-url)
Effect on RIBBE of boiling the medium from $[^{131}I]\text{MIBG}$-treated cells before transfer to recipients

- **Controls**
- **Treatment groups**
- **Medium only controls**

- 0 MBq no treatment
- 4 MBq no treatment
- 0 MBq boil -> RT
- 0 MBq boil -> 4°C
- 4 MBq boil -> RT
- 4 MBq boil -> 4°C

Recipient cells' surviving fraction

- boil -> room temp
- boil -> 4°C
Bystander signals

- Subcellular localisation of radionuclide is not critical
- Reactive oxygen species are implicated
- One component is a macromolecule(s) whose 3-D structure is necessary for its cytotoxicity
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