Assessment *In Vitro* of a Novel Therapeutic Strategy for Glioma, Combining Herpes Simplex Virus HSV1716-mediated Oncolysis with Gene Transfer and Targeted Radiotherapy

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Abstract: Genetically engineered herpes simplex virus ICP34.5 null mutants replicate only in dividing cells and have shown potential for the treatment of malignant disease, including glioma. Phase I trials have demonstrated the safety of these viruses in various clinical settings but it is envisaged that for full efficacy they will be used in combination with other therapeutic modalities. To enhance virus-induced tumour cytotoxicity, we have engineered an ICP34.5 null mutant (HSV1716) of HSV1 which expresses the noradrenaline transporter gene (NAT). This virus is designated HSV1716/NAT. We have shown previously that introduction of the NAT gene into a range of tumour cells, *via* plasmid-mediated transfection, conferred the capacity for active uptake of the radiopharmaceutical [^{131}I]MIBG and resulted in dose-dependent toxicity. In this study, combination therapy utilising HSV1716/NAT and [^{131}I]MIBG was assessed *in vitro* by the MTT assay. We demonstrate that the NAT gene, introduced by HSV1716/NAT into cultured glioma cells, was expressed 1 h after viral infection, enabling active uptake of [^{131}I]MIBG. The combination of viral oncolysis and induced radiopharmaceutical uptake resulted in significantly enhanced cytotoxicity compared to either agent alone and the response was dose- and time-dependent. These studies show that the combination of oncolytic HSV therapy with targeted radiotherapy has the potential for effective tumour cell kill and warrants further investigation as a treatment for malignant glioma.

Key Words: HSV1716, glioma, gene therapy, targeted radiotherapy, oncolytic viral therapy.

INTRODUCTION

Malignant gliomas are universally fatal even after aggressive conventional treatments such as tumour resection, chemotherapy and radiotherapy. The median patient survival time is one year. Therefore new treatment modalities are urgently required. Genetically engineered herpes simplex virus type 1 (HSV-1), which fails to express the virulence factor ICP34.5, has been proposed as a therapeutic agent for malignant glioma [1]. The strategy depends on the ability of ICP34.5 null HSV (HSV1716) to selectively replicate in and lyse rapidly dividing tumour cells but not growth-arrested or terminally differentiated cells [2, 3]. HSV1716 has a proven safety and efficacy profile in isogenic and xenograft tumour models [4, 5, 6] and its safety in humans has been demonstrated in three Phase 1 clinical trials in glioma patients [7, 8] and one trial in melanoma patients [9]. It is also currently undergoing evaluation in patients with squamous cell carcinoma of the head and neck.

Targeted radiotherapy is the selective irradiation of tumour cells by radionuclides conjugated to tumour seeking molecules. One of the most promising, non-immunogenic, targeting molecules is radiolabelled meta-iodobenzyl-guanidine (MIBG) - an analogue of the adrenergic neurone blockers guanethidine and bretylium [10]. Because it has high affinity for the noradrenaline transporter (NAT) [11], [^{131}I]MIBG is used in the imaging and treatment of tumours derived from the neural crest, such as neuroblastoma and phaeochromocytoma. Previously, we demonstrated that plasmid-mediated transfer of the NAT gene into glioma cells, which do not express NAT, endowed these cells with the capacity for active [^{131}I]MIBG, leading to their demise in a dose-dependent manner [12, 13]. Therefore this gene transfer strategy expands the use of [^{131}I]MIBG for the treatment of a wider range of tumour types.

Here we describe an investigation of tumour cell kill *in vitro,* using the genetically engineered herpes simplex virus HSV1716/NAT containing the NAT gene. Our strategy combines NAT transgene delivery and [^{131}I]MIBG treatment [12, 13, 14] with the proliferation-specific, lytic capacity of HSV1716.

We demonstrate that HSV1716/NAT is a suitable vector for delivery and expression of the NAT transgene *in vitro* and that the level of tumour cell kill, following the administration of [^{131}I]MIBG to cells infected with HSV1716/NAT, was significantly greater than that achieved by either treatment alone. These results suggest that this

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novel combination therapy is worthy of further investigation for the treatment of glioma and other malignancies.

RESULTS

Replication of HSV1716 Variants in Cell Lines

BHK21/C13 cells are routinely used for propagation of HSV-1. HSV wild type strain 17+ and the ICP34.5 null mutant HSV1716, have been shown to have similar growth kinetics in this cell line [2]. The genetically engineered variant, HSV1716/NAT, whose structure is shown in Fig. (**1**), also grew with similar kinetics in BHK21/C13 cells (Fig. (**2A**)). Growth arrested mouse embryo fibroblast cells (3T6) have previously been shown to be permissive for growth of the wild type virus 17+ but not for HSV1716 [2]. HSV1716/NAT was also replication-deficient in this cell line, (Fig. (**2B**)). In the glioma cell line MOG, the growth patterns of HSV1716 and HSV1716/NAT were indistinguishable from those of wild type virus (Fig. (**2C**)). The growth kinetics of HSV1716/NAT and HSV1716 were similar in BHK21/C13, 3T6 and MOG cells suggesting that insertion of the NAT gene within the RL1 locus of HSV-1 did not adversely effect its proliferation *in vitro*.

Transgene Expression Following Viral Transduction

Untreated MOG cells exhibited negligible expression of the bovine NAT gene by TaqMan RT-PCR analysis whereas HSV1716/NAT-infected MOG cells generated amounts of gene product which reflected the capacity of these cells for active uptake of [^{131}I]MIBG (Table **1**). Uninfected PN3 cells showed substantial expression of the bovine NAT gene. However, treatment with HSV1716/NAT increased the number of transcripts of the transgene and this corresponded to greater capacity for active accumulation of the radiopharmaceutical.

In both MOG and PN3 cells, transgene expression was related to multiplicity of infection – expressed in plaque forming units (pfu) (Table **1**).

Cellular Uptake of [^{131}I]MIBG Following Viral Transduction

MOG cells do not endogenously express NAT. Therefore they have no capacity for active uptake of [^{131}I]MIBG. At 24 h after HSV1716/NAT infection at 0.1 pfu/cell, MOG cells exhibited a 5-fold increase ($P < 0.001$) in uptake of [^{131}I] MIBG (Fig. (**3A**)). Compared with the [^{131}I]MIBG concentration capacity of untreated cells, a 16-fold enhancement of uptake resulted from infection of MOG cells at 1 pfu/cell. This level of active accumulation of radiopharmaceutical was significantly greater than that achieved by infection at 0.1 pfu/cell ($P < 0.001$).

PN3 cells, which are stably transfected with the NAT transgene [12], also took up significantly more [^{131}I]MIBG ($P < 0.001$) following infection with HSV1716/NAT at 0.1pfu/cell (Fig. (**3A**). Again, significantly greater uptake, than that obtained by viral treatment at 0.1pfu/cell, was achieved by infection at 1 pfu/cell ($P < 0.001$). This suggests that cells which already express NAT may be induced to synthesise more NAT following HSV1716 infection.

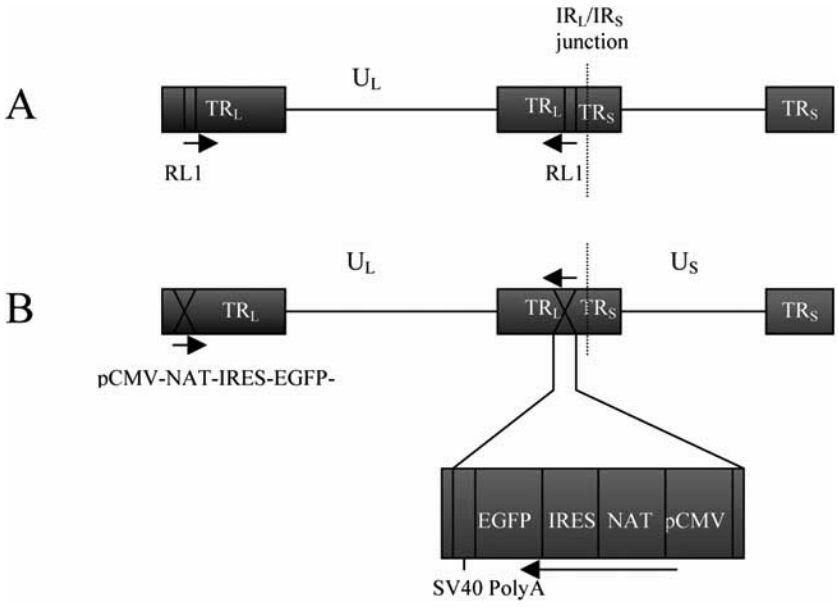

Fig. (1). Schematic representation of the HSV-1 genome (A) and the HSV1716/NAT genome (B).

The HSV-1 genome consists of two regions of unique sequences designated unique long (U_L) and unique short (U_S), flanked by a set of repeat sequences designated terminal repeat long (TR_L) and terminal repeat short (TR_S), inverted long (IR_L) and inverted short (IR_S). Within the repeat sequences flanking the unique long segment (TR_L and IR_L) are two copies of the RL1 gene which codes for the neurovirulence factor ICP34.5. In HSV1716, the majority of both copies of this gene has been removed and replaced with an expression cassette consisting of CMV IE promoter (pCMV), upstream of the bovine noradrenaline transporter cDNA (NAT), the encephalomyocarditis virus internal ribosome entry site (IRES), the enhanced green fluorescent protein gene (EGFP) and the SV40 polyadenylation sequences (SV40 PolyA). HSV1716/NAT expresses NAT and EGFP but does not express ICP34.5. The structure of the construct was verified by restriction enzyme digestion and expression of GFP *via* fluorescent microscopy and NAT by [^{131}I] MIBG uptake.

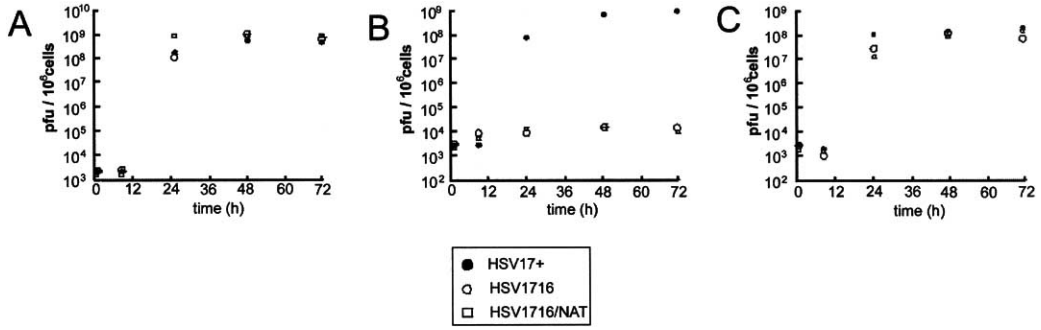

Fig. (2). Growth kinetics of HSV-1 variants 17+, 1716, and 1716/NAT in (A) BHK21/C13, (B) 3T6 and (C) MOG cells.
Cells were infected with 0.1 pfu per cell. At various times after infection, cells were harvested and cell-associated virus particles were released by sonication before titration on BHK21/C13, cells.

[^{131}I]MIBG uptake in MOG cells increased with increasing time up to 24 h following infection (Fig. (**3B**). However, no significant further increase in uptake capacity was observed at time points after 24 h.

Cytotoxicity of Combined Virus and [^{131}I]MIBG Treatment

Tumour cell kill by HSV1716/NAT oncolytic activity, [^{131}I]MIBG and the combination of both treatments, was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Fig. (**4**)). After incubation of PN3 cells (which expressed the NAT prior to viral infection) with HSV1716/NAT, followed by treatment with [^{131}I]MIBG for 24 h, we observed significant increases in cell kill 48 h, 72 h and 144 h later (Fig. (**4A**)) compared to treatment with HSV1716/NAT alone. Furthermore, combination treatment was also significantly more toxic than [^{131}I]MIBG alone at the time points 48 h (0.1 pfu/cell: $P < 0.001$; 1 pfu/cell: $P < 0.001$), 72 h (0.1 pfu/cell: $P < 0.05$; 1 pfu/cell: $P < 0.01$) and 144 h (0.1 pfu/cell: $P < 0.001$; 1 pfu/cell: $P < 0.001$). At 144 h after combination therapy, 1 pfu/cell viral infection was more effective than 0.1 pfu/cell ($P < 0.01$).

The cytotoxic effect of the various treatments was also examined in MOG cells which had no endogenous capacity for [^{131}I]MIBG uptake and therefore did not succumb to [^{131}I]MIBG therapy. The combination of HSV1716/NAT infection and [^{131}I]MIBG was statistically significantly more toxic than treatment with virus alone at all time points examined (Fig. (**4B**)). Administration of virus and radiopharmaceutical was also significantly more toxic than single [^{131}I]MIBG treatment at 24 h (1 pfu/cell: $P < 0.01$), 48 h (0.1 pfu/cell: $P < 0.01$; 1 pfu/cell: $P < 0.001$), 72 h (0.1 pfu/cell: $P < 0.001$; 1 pfu/cell: $P < 0.001$) and 144 h (0.1 pfu/cell: $P < 0.001$; 1 pfu/cell: $P < 0.001$). At all time points, combination treatments which incorporated viral infection at 1 pfu/cell were more effective than those involving infection at 0.1 pfu/cell ($P < 0.001$).

DISCUSSION

Due to its ability to preferentially replicate in cycling glioma cells but not in quiescent neurons and supporting cells of the normal brain, selectively replication competent HSV has shown promise in the treatment of malignant glioma [7, 8, 15, 16, 17]. These HSV variants fail to express the neurovirulence factor ICP34.5 and, although capable of infecting cells at any stage in the cell cycle, discriminate between fully differentiated non-dividing cells and cycling malignant cells in their ability to replicate and produce an oncolytic response. The ICP34.5 null mutant, HSV1716, lacks both copies of the RL1 gene which encodes the protein

Table 1. Bovine NAT Gene Expression in MOG and PN3 Cells – Uninfected or Treated with HSV1716/NAT at 0.1 or 1 pfu/cell

Cells	multiplicity of infection (pfu / cell)	Copies of NAT per 10^3 copies of GAPDH
MOG	0	0.21 ± 0.05
	0.1	93.63 ± 9.82
	1.0	257.77 ± 35.70
PN3	0	288.39 ± 42.11
	0.1	341.18 ± 39.04
	1.0	528.44 ± 66.87

Bovine NAT and GAPDH expression were assessed by reverse transcription and real-time PCR amplification of RNA derived from the cells. Results are means ± s.d. of three determinations in triplicate.

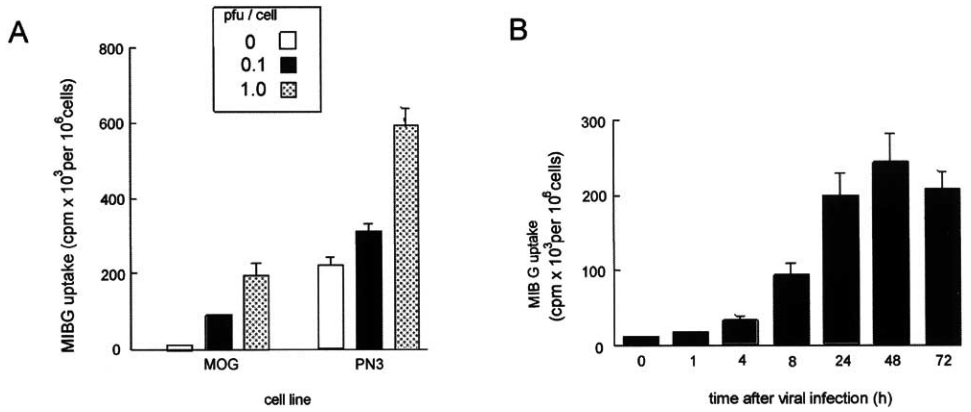

Fig. (3). Active accumulation of MIBG by MOG cells following HSV1716/NAT infection.

(A) MOG cells were infected with 0.1 or 1 pfu per cell. Controls were not exposed to virus but were treated with the same activity concentration of [^{131}I]MIBG as the virally infected cells. (B) The time-dependence of enhancement of [^{131}I]MIBG uptake was measured at various times after treatment of MOG cells with HSV1716/NAT virus at 1 pfu per cell.

Active uptake was determined by subtracting, from total uptake, passive uptake in the presence of desmethylimipramine - a specific inhibitor of the NAT. Data are means and standard deviations of five experiments.

ICP34.5 [1, 18]. HSV1716 and other ICP34.5 null mutants have demonstrated anti-tumour efficacy in a range of isogenic and xenograft models [4, 5, 6]. Moreover, an encouraging lack of toxicity has been demonstrated in Phase 1 clinical trials [7, 8, 9]. Phase II efficacy trials using mutant HSV are being conducted in patients with glioma and other malignancies.

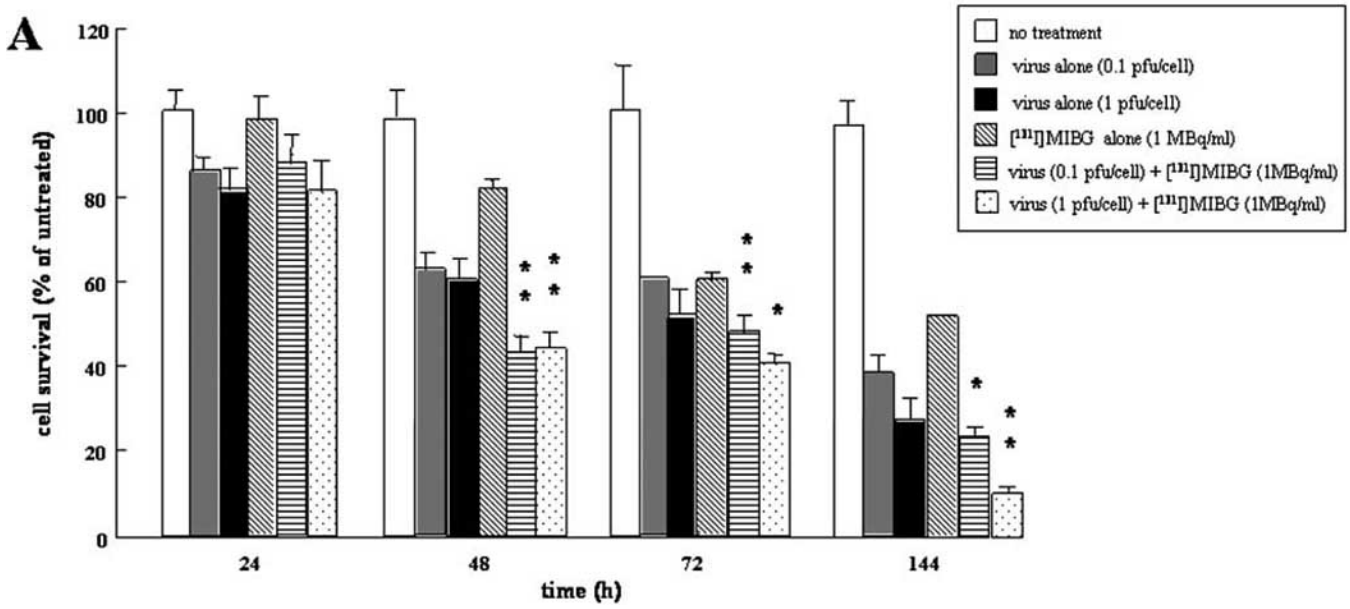

Fig. (4). Survival of PN3 cells (A) and MOG cells (B) following treatment with HSV1716/NAT alone, [^{131}I]MIBG alone or a combination of virus and [^{131}I]MIBG.

Cells were infected at 0.1 or 1 pfu per cell. One hour after infection, medium was removed and the cells were incubated with 1 MBq/ml [^{131}I]MIBG for 24 h. At various times thereafter, cell survival was determined by MTT assay. Data are means and standard deviations of three experiments performed in triplicate. At the various time points, the efficacy of combined [^{131}I]MIBG and virus treatment was compared with that of virus alone, delivered at the corresponding multiplicity of infection. The significance of differences are indicated by one, two or three asterisks, corresponding to $P < 0.05$, $P < 0.01$ or $P < 0.001$ respectively (one-way ANOVA with Bonferroni post-hoc test adjusted for five simultaneous comparisons).

While inhibition of the growth of human tumour xenografts and improved survival of experimental animal hosts have been observed following the administration of oncolytic HSV [reviewed by 19], only a small fraction of such animals have been cured by this means. It is probable that the optimal application of selectively replication competent HSV in cancer therapy, will be in combination with standard treatment modalities including surgery, radiotherapy and chemotherapy. Indeed, several studies have highlighted the enhanced toxicity of HSV oncolytic therapy in combination with cytotoxic drugs [20, 21] or with ionising radiation [22, 23, 24]. Other investigators have used modified HSV as gene delivery vectors, combining the oncolytic activity of the virus with its capacity to deliver genes expressing prodrug-activating enzymes and immunostimulatory antigens [reviewed by 19].

Our purpose was to determine *in vitro* the potential of HSV1716/NAT to express the NAT transgene and to examine whether the combination of viral oncolytic activity and induced susceptibility to [^{131}I]MIBG-mediated cell kill, would recommend this therapeutic scheme for further pre-clinical development. Introduction of the NAT cDNA into the RL1 locus of HSV strain 17+ resulted in a virus with growth characteristics indistinguishable from those of HSV1716. Furthermore, the expression of the NAT gene from the HSV1716 genome did not alter the selective replication competence of the virus in a range of cell lines.

We have previously demonstrated that plasmid mediated introduction of NAT into glioma cells, conferred upon them the capacity for active uptake of [^{131}I]MIBG [12]. Similarly, treatment of MOG cells with HSV1716/NAT, resulted in expression of a functional transporter allowing the cells to actively concentrate [^{131}I]MIBG. Enhanced radiopharmaceutical uptake was apparent one hour after viral infection, suggesting rapid induction of transgene expression. The induced accumulation of radiopharmaceutical increased with time after infection and with increasing dose of HSV1716/NAT, indicating the effectiveness of HSV1716 as a transgene delivery vehicle.

We examined cell kill by viral oncolysis and [^{131}I]MIBG treatment independently or in combination in PN3 cells. These were derived from MOG cells by introduction of the NAT gene *via* plasmid-mediated transfection. The combination of HSV1716/NAT and [^{131}I]MIBG resulted in increased cell kill 48 hours or more after treatment, compared to that induced by virus or [^{131}I]MIBG treatment alone. When HSV1716/NAT and [^{131}I]MIBG were applied to MOG cells, which have no inherent capacity for uptake of the radiopharmaceutical, the combination therapy similarly induced greater cell kill than either single agent treatment. The MOG cell line, which is incapable of active uptake of [^{131}I]MIBG due to its lack of endogenous expression of NAT, provides a more representative model than PN3 cells of glioma in patients. Our results indicate that the combination of HSV 1716/NAT and [^{131}I]MIBG could be an effective tumour cell killing strategy.

It is expected that the effectiveness of HSV1716-induced lysis of glioma cells in patients will be restricted to cycling cells. This limitation to treatment, imposed by proliferative heterogeneity, may be overcome by our proposed combination strategy. Even in quiescent virally infected tumour cells where viral replication is inhibited, NAT will still be expressed, allowing accumulation of [^{131}I]MIBG and targeted radiation cell kill. Furthermore, as we have previously demonstrated in plasmid-transfected three dimensional spheroid models, tumour cells which fail to express NAT (therefore have no capacity for accumulation of [^{131}I]MIBG) can nonetheless be subjected to cross-fire irradiation and radiation-mediated bystander effects from targeted cells [25].

The acute hypoxia of gliomas probably accounts in part for their resistance to treatment with radiation and anticancer drugs [26]. In recent decades, many efforts have been made to overcome hypoxia-induced resistance by increasing oxygenation, by the use of radiosensitizers or by the administration of agents which are especially toxic to hypoxic cells. Clinical studies have indicated that enhanced therapeutic benefit can be obtained by such schemes, but none has yet produced a significant, reproducible increase in the therapeutic ratio [27].

An alternative, potentially beneficial strategy is the tumour-targeted delivery of α-emitting radionuclides such as [^{211}At]astatine. The α-decay particles from this radiohalogen cause localised damage (having a mean range of only six cell diameters) and their high linear energy transfer quality ensures toxicity which is not compromised by low intracellular oxygen tension [28]. Furthermore, the short, 7.2 h, half-life of [^{211}At]astatine suggests that it may be particularly appropriate for glioma therapy following its intracerebral administration because most of the radionuclide will have decayed before gaining access to the systemic circulation. Recently, the first clinical study of the therapeutic use of ^{211}At commenced, in Duke Medical Centre, North Carolina. This phase I trial involves the intra-cavitary injection of ^{211}At-labelled anti-tenascin antibody [29] for the treatment of brain tumours.

We expect that [^{211}At]astatinated benzylguanidine ([^{211}At]MABG), could also be effective in the selective eradication of glioma cells which have been transduced with the NAT gene *via* the HSV1716/NAT virus and have already demonstrated that plasmid mediated NAT gene transfer induced similar enhancement of the uptake of [^{131}I]MIBG and [^{211}At]MABG [25]. However, in terms of tumour cell kill, [^{211}At]MABG was more effective than [^{131}I]MIBG by two to three orders of magnitude [25, 30, 31]. Therefore it is expected that the administration of [^{211}At]MABG rather than [^{131}I]MIBG following HSV1716/NAT administration, would be especially efficacious for treatment of hypoxic tumour regions, thereby improving tumour treatment.

Recent studies have reported synergy between viral mediated gene delivery and ionising radiation. The conditionally replicating ONYX-15 adenovirus, which is thought to replicate selectively in p53 deficient cells, reportedly had a synergistic effect in combination with radiation *in vitro* and *in vivo* [32, 33]. However multiple injections of ONYX-15 virus were required to achieve antitumour effects in xenografts models. This suggests low replication of this adenovirus compared to the proliferation

rate of tumours and also that their capacity for efficient transgene expression is short lived [34].

Ionising radiation is a standard treatment option for many malignancies and previous studies have indicated that it increases the lytic activity of HSV1 and enhances dispersion of virus from inoculation sites, resulting in higher viral titres in recovered tumours which had been exposed radiation [22, 23, 24, 35]. Tumour xenografts treated with both radiation and recombinant HSV, also showed enhanced tumour regression compared to either modality alone [22, 23, 24]. Taken together these results suggest a role for radiotherapy in enhancing the efficacy of HSV-mediated oncolytic therapy in human tumours.

The combination of HSV1716 with a transgene that allows tumour specific uptake of radiopharmaceutical, has a distinct advantage over external beam irradiation in terms of selectivity of damage. Our present results of the effectiveness of combined viral-induced lysis and radionuclide therapy *in vitro* indicate promise for the future treatment of malignant glioma. On the basis of these encouraging *in vitro* results, studies are now underway to optimise the various aspects of this therapeutic approach in pre-clinical models.

MATERIALS AND METHODS

Reagents

Tissue culture media and supplements were purchased from Life Technologies (Paisley, UK). All other reagents were obtained from Sigma-Aldrich Co Ltd (Dorset, UK), unless otherwise stated. [^{131}I]MIBG of specific activity 45-65 MBq / mg, was obtained from Amersham, UK.

Cell Lines

Baby hamster kidney cells (BHK21/C13), human glioblastoma cells, (MOG-G-UVW - hereinafter designated as MOG) and 3T6 Swiss albino mouse fibroblast cells were obtained from the European Tissue Culture Collection. PN3 is a glioma cell line derived from MOG stably transfected with the bovine NAT gene under the control of the CMV immediate early promoter. BHK21/C13 cells were propagated in Glasgow modified Eagle's medium (GMEM) supplemented with 5% (v/v) tryptose phosphate broth. MOG cells were grown in MEM and 3T6 cells in Dulbecco's modified medium (DMEM). PN3 cells were propagated in MEM in the presence of 100mg/ml geneticin. All media were supplemented with 10% (v/v) foetal calf serum, 2mM glutamine, 5% (v/v) fungizone, 100g penicillin / streptomycin, and maintained at 37C, in 5% CO_2.

Viral Constructs

HSV1716, was derived from HSV Glasgow strain 17+ as previously described [18]. The pREP9/NAT plasmid was constructed as detailed previously [12] and the NAT gene was subcloned from this plasmid into the multiple cloning site of the pIRES2-EGFP vector (BD Biosciences Clontech, UK). The 5.4kb DNA fragment, containing the CMV IE promoter upstream of NAT/IRES/EGFP, was excised from the pIRES2-EGFP and ligated into HpaI-digested 'RL1.del' vector. RL1.del, is the pGEM 3zf(-) vector (Promega, UK), into which has been cloned the entire ORF of RL1 (and flanking sequences), followed by deletion of the majority of RL1 (deleted- 'del') and insertion of a multiple cloning site (MCS). Clones which contained the CMV/NAT/IRES/EGFP/PolyA insert were linearised using SspI and co-transfected with HSV17+ DNA on to 80% confluent BHK21/C13 cells using the $CaPO_4$ transfection method. Fluorescent, recombinant, viral plaques were purified and a stock (designated HSV1716/NAT) was grown and titrated in BHK21/C13 cells as previously described [36].

Viral Growth Kinetics

Confluent monolayers containing approximately $2x10^6$ of BHK21/C13, 3T6 or MOG cells were infected with HSV17+, HSV1716, or HSV1716/NAT at 0.1 (pfu) per cell. After incubation for 1 h at 37^0C, cells were washed, overlaid with appropriate medium and the incubation continued at 37^0C. At various times after infection, samples were harvested and progeny viruses were released by sonication and applied to BHK21/C13 cells.

Real-time RT-PCR

Total RNA was extracted from cell lines using the Qiagen RNeasy® method according to manufacturer's instructions (Qiagen Ltd. West Sussex, UK) and polymerase chain reaction (PCR)-amplified using TaqMan® RT-PCR methodology, as previously described by us [37]. Primer and probe sequences were designed from the published sequence for the bovine NAT gene (accession number U09198) using the ABI prism PrimerExpress™ v1.0 software, and were custom synthesised (MWG-Biotech, Milton Keynes, UK). The sense primer corresponded to bases 1583-1602 of the bovine NAT sequence; the antisense primer was complementary to bases 1637-1657. These primers generated a PCR product of 75 base pairs. The internal probe corresponded to bases 1612-1635 of the gene sequence. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal standard for all real-time PCR reactions. Real-time PCR was carried out using the commercially available TaqMan® Reverse Transciption reagents and TaqMan® Universal PCR Master Mix (Perkin-Elmer Applied Biosystems, Warrington, Cheshire, UK: P/N N808-0234 and 4304437 respectively) [37].

Cellular Uptake of [^{131}I]MIBG

Cells were seeded at a density of $0.5x10^6$ cells per well in 6-well tissue culture dishes and cultured for 24 h. Virus, at a dose of 0, 0.1 or 1 plaque-forming units (pfu)/cell, was added in a 100µl volume and after incubation for 1 h, the cells were washed twice with phosphate buffered saline (PBS) to remove free virus and overlaid with 2ml of appropriate medium. At various times after infection, [^{131}I]MIBG uptake capacity of host cells was determined as previously described [12]. Active uptake was determined by subtracting, from total uptake, passive uptake in the presence of desmethylimipramine - a specific inhibitor of the NAT.

Cellular Viability Assay (MTT).

Cells were seeded into 96-well microtiter plates at a density of $1x10^4$ cells per well. After culturing for 24 h, the cells were treated with virus at 0, 0.1 or 1 pfu/cell (100µl

total volume). After 1 h, the medium was removed and replaced with fresh medium containing 1 MBq/ml [^{131}I]MIBG or the equivalent volume of PBS. After incubation for 24 h, the cells were washed once in fresh medium. At various times after experimental treatment, medium was replaced with 100µl fresh medium containing 10µl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-tetrazolium bromide, (Sigma Aldrich, Dorset, UK) per well (7mg/ml). Four hours later, the formazan in the MTT was dissolved by addition of 100µl per well of 10% (w/v) SDS in 0.1M HCl. After a further 16 h incubation at 37^{0}C, viable cell numbers were determined from measurement of the absorbance at 570 nm of well contents using an ELISA plate reader (MRX II, Dynex Technologies Inc., USA).

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ABBREVIATIONS

GAPDH	=	Glyceraldehyde-3-phosphate dehydrogenase
HSV	=	Herpes simplex virus
MABG	=	Meta-astatobenzylguanidine
MIBG	=	Meta-iodobenzylguanidine
MTT	=	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NAT	=	Noradrenaline transporter
pfu	=	Plaque forming units
PBS	=	Phosphate buffered saline
PCR	=	Polymerase chain reaction

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