IS ONCOLYTIC VIROThERAPy A VIABLE PLATFORM FOR GLIOMA TREATMENT?

Fahad Mohammad

ABSTRACT

Gliomas are devastating cancers of the nervous system with poor prognosis. Their aggressiveness produces a mortality rate rarely seen with other malignant tumours and the lack of effective treatment has left very few options. Oncolytic viruses, with their long history of experimentation, have been deemed to be a key player in the future treatment of gliomas. This review will focus on the two main contenders, adenovirus and herpes simplex virus, for glioma treatment and discuss how far the field has come since its conception. The concept of each vector and the rationales behind their use will be contrasted before discussing the future of the field. Data was located by accessing the MEDLINE database using the PubMed search system. Data was selected on the basis of the insight its information provided as well as on the dependability of the experimental method used.

Key Words: Glioma, Glioblastoma, Oncolytic virus, Herpes simplex virus, Adenovirus

INTRODUCTION & DISCUSSION

A glioma is any tumour that has its origins from a glial cell. These are supporting cells that nourish the neurons within the nervous system. Patients experience a decline in cognitive function and score lower on quality of life scale than any other cancer. Gliomas are also accountable for the greatest number of life years lost (an average of eight years) for any given cancer patient. Furthermore, the 5-year survival rate is dismal with less than 10% surviving in patients with glioblastoma multiforme – the highest grade of glioma (Fig.1).

Gliomas account for almost 90% of brain tumours, with the highest incidence in the Southeast of England. Current treatments involve radiotherapy followed by Temozolomide chemotherapy and surgical resection. However, efficacy is limited by the fact that alternative oncogenic pathways can mutate, offering resistance. Compounding this is the problem of targeting tumour elements within the central nervous system which requires drugs to pass the blood-brain-barrier as well as the resulting haematological toxicity in patients. Financially, this therapy proves to be expensive especially as an adjuvant. Looking at all these issues, the justification for novel approaches to glioma treatment becomes apparent.

The use of oncolytic viruses to treat cancer dates back as far as the 19th century although unbeknownst to doctors at the time. Multiple observations of remission in cancer patients with concurrent viral infections paved the way for research into the field. We now know that the oncolytic effect of viruses is due to replication-mediated cell lysis.

This review will explore the current state of oncolytic virotherapy in treating glioma. It will focus on adenovirus and herpes simplex virus due to their effectiveness in clinical trials. First, the rationales behind using each virus will be discussed before comparing the various mechanisms employed. Finally, the future of the field will be questioned to see if there is potential in oncolytic virotherapy.

Adenovirus (Ad) – Concept & Rationale

Adenoviruses are a good choice for gene therapy as a result of the ease with which their genome can be hijacked to carry transgenes. They also have the ability to grow exponentially in a short space of time thus boosting their oncolytic potential.

The replication cycle follows the mechanism of many viruses in that it begins with the infection of a cell and ends with the release of many virus particles. First, adenovirus interacts with the coxsackie adenovirus receptor (CAR) expressed by host cell membranes. Internalisation is then promoted through an adenoviral penton base which recognises integrins on the cell surface and results in the formation of endosomes. This allows adenoviral particles to relocate to the nuclear membrane where viral DNA can enter the nucleus. Lastly, genomic transcription of viral DNA results in the organised production of early, immediate and late genes which trans-
locate to the cytoplasm to produce new viral particles. These are eventually released by cell lysis and are thus free to infect more cells\cite{13}.

**Adenovirus – Rb Pathway**

Retinoblastoma protein (pRb) is implicated in a variety of cancers including almost a third of malignant gliomas\cite{14}. This protein works as a tumour suppressor gene where it binds to E2F transcription factors thereby preventing the cell from moving into S-phase. A mutation in pRb thus leads to unregulated cell proliferation\cite{17}. One of the earlier experiments was to use Ad5CMV-Rb, which carried the Rb gene, in an effort to replace the aberrant protein expressed in cancer cells. The virus was made replication-deficient in order to limit gene expression in healthy tissue but this also reduced the capacity for gene transfer to a small number of cells\cite{18}. As a result, it was observed that there was growth arrest in gliomas, however there was no actual remission i.e. the virus caused a cytostatic effect but was not cytopathic\cite{16}.

Studies like these led to the development of conditionally replicative adenoviruses (CRAds), which are unable to replicate in normal host cells but selectively target tumour cells\cite{19}. In order to protect against infection, host cells will undergo cell cycle regulation and apoptosis\cite{20}. To counter this, the adenovirus E1A gene codes for a protein which displaces E2F from pRb thus allowing cell transition into S-phase which favours viral DNA synthesis\cite{21-23}.

Ad5-Delta24 is a genetically modified adenovirus which has a 24-bp deletion in the region of its genome coding for E1A\cite{24}. As a result, it can no longer prevent the pRb checkpoint in healthy cells and thus cannot divide. However, as pRb is already aberrant in tumour cells\cite{25}, there is nothing to stop progression into S-phase. This mechanism allows adenovirus to be conditionally-replicative in tumour cells whilst retaining its oncolytic potential. In vitro studies have shown Ad5-Delta24 to be a potent oncolytic virus in glioma cell cultures. By transferring pRb to pRb-null cells i.e. cancer cells, it was confirmed that the conditionally-replicative mechanism was indeed dependent on retinoblastoma protein. It was important to confirm this finding in order to be certain that there would be no bystander damage to brain tissue when running trials on humans. Multiplicity of infection ratios (MOI), the ratio between virus particles and tumour cells, as low as five caused noticeable cytopathic effects and within seven days some cell lines were in complete remission. With MOI ratios of ten, cell lines showed complete cytolysis within 14 days, however it must be noted that results differed between the cell lines used. In vivo studies were less marked with around 40% of live subjects showing tumour regression but multiple injections had to be used. Moreover, despite many different animals being used, to date there have been no clinical trials to authenticate the efficacy of the virus in humans\cite{24}.

Although the results of Ad5-Delta24 seemed promising, there was a stark difference between cell lines in terms of cytolytic effects. Bergelson et al. found that, despite adenovirus anchorage to tumour cells being related to CAR, internalisation of the virus relied on a secondary mechanism that was integrin dependent\cite{26,28,30}. Also, Asaoka et al. found that the expression of CAR on glioma cells was quite variable and thus not a stable cellular identifier (Fig.2)\cite{30-31}. Conclusively, Ad5-Delta24 affected cell lines differently due to their variability in CAR expression. Ad5-Delta24RGD was produced to include an RGD motif (arginine-glycine-aspartic acid), which binds strongly to integrins. Such a modification meant the virus would not have to rely on CAR but was instead integrin-dependent, resulting in a higher infectivity\cite{22,23}. To simulate clinical conditions, cells were grown in spheroids which replicated a tumour mass that may actually be encountered instead of monolayers of glioma cells. Whilst replicative-deficient viruses barely broke the border of the mass, with Ad5-Delta24RGD the viability of cells was significantly reduced. Concordantly, when tested in nude mice with xenografts of low CAR-expressing human glioma, it was found that there was complete remission in 90% of mice and they survived free of any cancer for four months\cite{31}.

Unlike the variants previously mentioned, a phase I/II trial has recently been completed to assess the safety profile of Ad5-Delta24RGD. For the first time, it has been shown that this virus can cause complete remission in patients with no evidence of relapse more than three years after the disease. However, one must note that complete oncolysis was only seen in three patients and as the trial has been published quite recently, there has been little time to evidence any side effects or relapses\cite{34}.

**Adenovirus – P53 pathway**

P53 is a tumour suppressor gene that acts to inhibit cell cycle progression and cause apoptosis in order to prevent tumour formation\cite{35}. Not only is p53 mutated in a vast array of cancers but also in more than a third of astrocytomas\cite{36,37}. By upregulating p21, p53 inhibits cyclin–dependent kinases to retard progression of the cell cycle. A secondary effect includes the transcriptional activation of Bax which leads to apoptosis\cite{38}. Thus, it makes sense that a transfer of p53 to p53-null cells results in apoptosis and indeed this has been shown\cite{39}.

Adenoviruses express the E1B–55K protein which binds to p53 in an attempt to stop apoptosis and allow the production of viral progeny\cite{40}. Also they produce the E1B–19K protein to regulate free Bak and Bax proteins in a further attempt to reduce mitochondrial-dependent apoptosis\cite{41}.
ONXY-015 is the earliest example of a genetically engineered adenovirus and has a deletion in the E1B-55K gene. As a result, in normal cells ONXY-015 is unable to replicate but in cancerous cells where p53 is already mutated, the adenovirus is able to produce progeny. In this way, ONXY-015 is a conditionally replicative adenovirus. However, this has been contested with recent studies that suggest ONXY-015 works by deflecting the export of mRNA from the nucleus. Phase I trials have shown that this virus has a good safety profile when injected into resected tumours. A team in China have also completed a phase III trial of the virus with enough success to warrant FDA approval for its use in patients with head and neck cancer albeit in combination with chemotherapy. Despite this, there has been much criticism of ONXY-015. Studies have found that many gliomas express functional p53 and may contain a small population of p21-expressing tumour cells rendering the virus ineffective. Moreover, as E1B-55K is also involved in the translocation of nuclear viral mRNA to ribosomes and ONXY-015 is E1B-55K-null, the replicative potential is attenuated and indeed viral transduction in glioma models has not shown any substantial amount of oncolytic activity. Likewise, progression was shown no more than two months after treatment on average in addition to the fact that only a third of the patients treated with the maximum dose were alive after 19 months. Thus, although ONXY-015 is safe, its therapeutic efficacy is questionable. As such, one of the biggest hurdles in virotherapy is maintaining a fine balance between oncolytic potential and tumour selectivity.

Herpes Simplex Virus (HSV-1) – Concept & Rationale

HSV-1 is a neurotropic virus i.e. it preferentially infects the nervous system hence why it is favoured by current research for glioma (Fig.3). It has a relatively large genome which does not integrate with that of the hosts. As a result, it can be modified to carry a large number of transgenes and exhibit latency without causing any insertional mutagenesis that may affect the cell unpredictably. Likewise, the genes associated with its neurovirulence are nonessential and can thus be modified without affecting the virus’ survival. The virus is inherently cytolytic and in case therapy goes awry, antivirptic drugs such as gancyclovir can be used as a failsafe. One caveat is that in the general population, a high rate of immunity already exists which may cause difficulties with regards to viral proliferation, although its habitation of a nuclear episomal state may avoid provoking an immune response altogether.

The first modified replicative-competent herpes virus was a mutant with a deletion in the tk gene and was called dlspkt. Briefly, for herpes virus to replicate, it requires the presence of thymidine kinase. This allows the phosphorylation of deoxythymidine – a precursor for deoxythymidine triphosphate which is used in DNA synthesis. In normal cells there are two types, TK1 and TK2, with the former only being present during cellular division (as more substrate is needed). HSV-1 codes for its own thymidine kinase allowing it to replicate in both dividing and non-dividing cells, however dlspkt is tk-null. As a result, it must rely on the cells inherent thymidine kinase activity, and as only replicating cells have both TK1 and TK2, only replicating cells are sufficiently suitable for HSV-1 replication. As a result, HSV-1 cells only replicate in dividing cells such as tumour cells in the nervous system. In vivo studies proved that dlspkt was a potent oncolytic vector, however the deletion in the tk gene also meant the mutant was resistant to antiviral treatment. This, in combination with noxious effects at high doses led to the dismissal of dlspkt. Nevertheless, this example proved as a proof of concept that HSV-1 could indeed affect gliomas vigorously.

Herpes Simplex Virus 1 – PKR Pathway

Unlike adenovirus, a single gene deletion in HSV-1 does not render the virus innocuous to non-dividing cells. Thus, to prevent herpes relapsing to its wild-type form, HSV-1 is often modified to have a double knockout. Viruses with such mutations are often referred to as 2nd generation oncolytic viruses with the first variant termed G207. G207 has paired deletions in both of its 34.5 genes which regulate the neurovirulence of HSV-1 and overcome host cell defence. Usually when HSV-1 infects a cell, RNA-dependent protein kinase (PKR) leads to an antiviral response that induces protein synthesis shutoff. This is carried out by the phosphorylation of eukaryotic initiating factor 2-alpha (eIF-2α) by PKR and culminates in the cessation of virus replication. The 34.5 genes code for infected cell protein 34.5 (ICP34.5) which blocks PKR mediated protein synthesis shutoff and therefore allows HSV-1 to continue replicating. Genetic engineering of HSV-1 with its 34.5 gene deletions means that the virus can no longer overcome host defences in normal cells. However, in cancer cells with an oncogenic Ras system, PKR is already in a repressed state and so G207 no longer has to rely on its 34.5 genes. Moreover, the U39 region of the HSV-1 genome codes for a subunit of ribonucleotide reductase called ICP6. This enzyme is needed for the synthesis of nucleotides post-infection and just as with tk deletions, can be provided by cells undergoing active cell division but not quiescent cells. G207 has a LacZ reporter gene (from bacteria) inserted into the U39 region to act as a disruption and allow detection by histochemical methods. Overall, these two modifications result in a doubly attenuated virus to decrease the chance of wild-type reversion whilst also increasing its sensitivity to acyclovir thus ensuring a high safety margin.
Preclinical studies in glioma cell lines showed that an MOI as low as 0.1 managed to completely lyse the entire cell population within two days60.

However, as HSV-1 is one of the commonest causes of viral encephalitis, it is important to test the safety of the virus. When the highest dose possible was given to mice either intracerebrally or intraventricularly, there were no symptoms for over five months. Even with the most susceptible mouse strain, there was only slight non-fatal symptoms in a quarter of the population72. Additionally, mice that survived a previous infection with wild-type HSV were given a G207 inoculation. Despite both infections localising to the same area, there was no reactivation of latent HSV-173. To further commend the safety of G207, trials were carried out in New World owl monkeys, primates with a propensity for HSV infection, but they were also asymptomatic74. Clinical trials in human patients did not elicit any adverse reactions nor could a maximum tolerated dose be identified. 20% of patients had reduced tumour volume and eight patients survived more than nine months with one example remaining alive even after five years. In a trial of 21 patients, only three patients suffered from side effects such as seizures or brain oedema75. Although the safety of G207 is almost unquestionable, almost all test subjects showed progression after ten months and even with immunosuppressive steroid therapy, most had seroconverted against the virus. One point to note is that G207 seems to hinder growth not only by replicative oncolysis, but also through an inflation of cytotoxic T-cell activity76. One could reason that immunosuppressive drugs to prevent seroconversion against HSV-1 could also decrease antitumor efficacy via the T-cell mechanism.

**CONCLUSION**

With the current state of treatment offering little improvement in prognosis, oncolytic virotherapy has shown promise as a main player in future glioma therapy. Not only have there been effective responses in preclinical studies but virotherapy has also proven to be extremely safe in humans despite using some of the most lethal viruses in existence.

In spite of this, more work still needs to be done to advance the field. Initial hypotheses of oncolytic mechanisms have had to be rethought several times illustrating just how complex the biology is. One area that still lacks significant knowledge is how the immune system interacts with both viral and tumour mechanisms, further highlighting that more research needs to be done to truly see how all innate processes interplay with each other.

Future approaches may seek to combine virotherapy with other novel approaches currently being developed such as virus-directed enzyme prodrug therapy (VDEPT) as well as cancer immunology. These collaborated efforts could potentially allow a deeper understanding of the field whilst also providing a substantial therapy for glioma.
IS ONCOLYTIC VIROTHERAPY A VIABLE PLATFORM FOR GLIOMA TREATMENT?

Figure 2: Correlation between different CAR expression on different glioma cell lines and viability after infection with different adenovirus mutants

Figure 3: Mechanism of replicative-competent HSV-1

REFERENCES


340:1255-1268.


67. He B, Gross M, Roizman B. The gamma(1) 34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1 alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. Proc Natl Acad Sci USA 1997; 94:843-8.


70. Goldstein DJ, Weller SK. Factor (s) present in herpes simplex virus type 1-infected cells can compensate for the loss of the large subunit of the viral ribonucleotide reductase: characterization of an ICP6 deletion mutant. Virology 1988; 166:41-51.


