

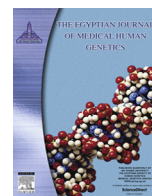
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Review

Oncolytic virotherapy – A novel strategy for cancer therapy

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ABSTRACT

Oncolytic virotherapy is a new modality of cancer treatment which uses competent replicating viruses to destroy cancer cells. This field progressed from earlier observations of accidental viral infections causing remission in many malignancies to virus drugs targeting and killing cancer cells. More competent and specific viruses which attack tumor cells but not healthy cells could be made with advancements in the field of genetic engineering. Studying virus as a drug has benefits of secure handling of all aspects related to this advancing field. In many ways virus given for treatment is comparable to a drug. The virus lies in the grey area of life and death and thus outside the body it is same as an unopened drug. Once inside a biological system, it starts acting targetting specific systems sine qua non as a drug. This review compares virus to a drug and deals with its pharmacokinetics, pharmacodynamics, virus drug interactions and combination virotherapy of this new treatment modality.

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1. Introduction

Cancer treatment is ever-changing, and researchers are in search of newer modalities to fight cancer. It was a known fact that certain viruses have oncolytic or cancer-killing properties. There were reports of chickenpox infection improving the WBC (White Blood cells) count and lymph node status in patients with lympho-

cytic leukaemia [1]. Measles caused an improvement in the case of leukemia, Hodgkin's, and Burkitt's lymphoma [2]. A lot of research is going on in this field utilizing this property of virus to make new treatment options for cancer. Using genetic engineering better virus are created which have more specificity for its action. Chinese state FDA (Food and Drug Administration) approved the first oncolytic virus-drug 'ONCORINE' an Adenovirus type 5 injection in 2005 for head and neck malignancy [3]. Many new viral drugs are on trial for metastatic malignancies like malignant melanoma. In October 2015 the US FDA approved a genetically engineered herpes virus called talimogene laherparepvec (T-VEC) to treat advanced melanoma [4]. T-VEC is genetically modified so that it

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will not cause herpes but attacks specifically tumor cells and destroys it. More candidate viruses are under trial for its oncolytic potential. The viruses kill neoplastic cells as well as trigger immune response against the tumor. Virotherapy along with chemotherapy, i.e., combination virotherapy, may fill the lacunae of current treatment options by reducing adverse events as it has specificity for cancer cells.

2. Virus kinetics

2.1. Dosage and administration

To begin with, we need to have an idea of the dosage form of virus drug. The dosage of the virus is measured using several methods, e.g., TCID₅₀ (50% Tissue Culture Infective Dose) a measure of the infective virus titer, plaque forming units (pfu), Focus forming assay, Haemagglutinin assay, electron microscopic methods, TRPS (Tunable Resistive Pulse Sensing), etc. Adequate dosage is required for the significant action of the virus at the tumor site. Studies in measles virus (Moraten strain) showed that for efficient antitumor activity, Moraten dose more than 10⁸ TCID₅₀ is needed, which was assessed by the prolonged survival after administration of the strain in the murine intraperitoneal model of human ovarian cancer. Therapeutic range studies of the virus can be done to determine the dose which matches the efficacy and safety of virus drug. In mice, doses up to 1 × 10⁷ plaque forming units (pfu) of the second generation HSV inoculated into brain resulted in no adverse effects [5,6]. Measles virus given as a single intravenous dose of 4 × 10⁶ TCID₅₀ per kilogram caused tumor regression and prolongation of survival in KAS-6/1 myeloma model [2]. Unlike drugs which can be readily prepared in any concentrations, there is difficulty in getting a maximum concentration of virus drug to mark the actual start of toxicity. It is practically possible only in few cases. In the virus, JX-594 maximum tolerated dose was 1 × 10⁹ pfu [7,8]. Drugs are given at lower doses and given more frequently considering the half-life so that adequate concentration is reached without causing a much toxic effect. Safer viral drugs are also available which can be provided in more massive doses. ONYX 015, even when administered up to 10¹¹ pfu did not cause any dose-limiting toxicity [9]. Virus infection in cancer cells and its destruction induces an immune response against cancer cells as many tumor antigens get exposed to the immune system. There is a dose-dependent increase in levels of the inflammatory cytokines like IL-6 and IL-8 released by malignant melanoma cells upon infection with measles virus. This increase shows the need of optimal dosage for the bystander response also [9]. Threshold dose to ensure that the virus acts on tumors can be calculated by conducting dose range studies and doing tumor biopsies to recover the virus. JX594, an oncolytic vaccinia virus is under trial for non-resectable hepatocellular carcinoma appeared in tissue biopsies only when the threshold dose was more than 10⁹ infectious units [10].

2.2. Distribution of virus

Viral titer, which is similar to drug concentration measures the in vivo distribution of the viral drug. As the virus causes cytolysis, it releases some detectable molecules which can be used to assess the activity indirectly. Adeno virus used in the treatment of prostatic adenocarcinoma if infects the tumor cells significantly, cause PSA (Prostate-specific Antigen) to rise by many times, which confirm its effect [11]. The virus levels may spike several times over a period due to replication [12]. Due to this; the total viral load will increase that which was introduced. There are other ways to study the in vivo monitoring of the spread of the oncolytic virus. Visual

analysis of distribution can be done using fluorescent labelling of the vector with isothiocyanate [11]. By genetic engineering of the viral genome, transcription units coding for soluble marker peptides is inserted. When the virus infects the cells, this soluble substance is secreted from the cell to detect it quickly from the body fluids. In case of measles virus, β-hCG (β sub-unit of human chorionic gonadotropin) and soluble extracellular domain of CEA (human Carcino Embryonic Antigen) is used for this purpose [13]. So once a virus infects the cells, this soluble marker starts appearing in the blood and urine and thus confirms the infection and not mere excretion of the agent or destruction by the immune system.

In the case of measles virus, a recombinant version that codes human thyroidal sodium iodide symporter (NIS) is added [14]. So the infected cells, with the help of this symporter concentrates iodine in the cells. Radioactive iodine given through the intravenous route gets concentrated only in the virus-infected cells by the uptake through NIS. Radioactivity measured by serial SPECT (Single Photon Emission Computed Tomography), serial gamma camera imaging of iodine-123 or PET (Positron Emission Tomography) at the site of tumor confirms the action of a virus on the tumor. A Vaccinia virus GLV-1h153 engineered to carry the human sodium iodide symporter (hNIS), facilitated detection by PET with both intratumoral and systemic administration. Dana et al. studied the tissue distribution, spread of GLV-ih153 and timing dynamics between viral infection, uptake of radioiodine and oncolysis in human pancreatic carcinoma cells [15]. A benefit of virus drug is that its action can be confirmed by immunohistochemistry method. G207, another second generation oncolytic HSV virus is a multmutated virus with both copies of γ34.5 gene deleted and *E. coli* LacZ gene inserted. Histochemical detection of β-galactosidase helps to confirm and monitor the preferential affinity of the virus to glioma cells instead of healthy neural tissue [16]. Distribution in tissues depends on the chemical properties of the drug. More lipophilic molecules will spread easily through the body and will have a higher volume of distribution. Entry of drugs into the central nervous system is limited due to tight junctions which form the blood-brain barrier. This limitation can be circumvented by using new drug delivery methods like CED (Convection Enhanced Devices). CNS tumors can also be targeted by using a virus like Parvo virus which, unlike other oncolytic viruses crosses the blood brain barrier and attack the tumor cells [17].

2.3. Metabolism and excretion

The main route of elimination of virus drug is by the immunological mechanism. But studies have not shown much correlation between viral titers and increasing anti-viral antibody titer. Antitumor action persisted in spite of high antibody response against them [18]. O'Riordan and colleagues described PEGylation of virus that is adding polyethylene glycol covalently to the protein covering of Adenovirus. It prevents the immunogenicity of the virus, prevents antibody binding, and increases the solubility and thus the serum half-life [19]. The Virus will concentrate in the reticuloendothelial system, and from there it is slowly removed by immune-mediated mechanisms. Preconditioning the MPS scavenger receptors or by destroying the macrophage or endothelial cells can reduce this loss. In animal studies, it is seen that polyinosinic acid, which will bind to the scavenger receptors will reduce the sequestration of adenovirus [15]. Clodronate-loaded liposomes can destroy the liver Kupffer cells and splenic macrophages in mice [20]. Some cells can function as vehicles for the therapeutic virus. The cell carriers can be solid tumor cells, Hematological malignant cells, Xenogeneic/allogeneic cells, T cells, CIKs, mesenchymal stem cells, neural stem cells, etc. An efficient carrier should have a natural habit of being at the tumor site. This vehicle should pass

through areas in the tumors, which are hostile due to hypoxia and other deregulated homeostasis. Once in tumor environment, they should act like a source for virus production. Stem cells are attracted towards certain substances called chemo attractant molecules and also some receptors in the tumor cells, like SDF1/CXCR4, VEGF/VGFR, growth factors, and chemokines, etc., which are expressed more in cancer cells during uncontrolled growth and thus are tried for this.

3. Virus dynamics

The infectivity of a virus can be assessed quantitatively using Multiplicity of infection (MOI) ratio. It is the ratio of infectious agents to the targets. As the MOI increases the percentage of cells infected with at least one viral particle also increases.

3.1. Receptor mediated mechanism

Viruses will have an affinity towards different receptors. It is found that CD46 (membrane co-factor protein) is overexpressed in cancer cells compared to normal cells to protect them from complement mediated destruction. This is seen in leukemias, multiple myeloma, gastrointestinal, hepatocellular, colorectal, endometrial, cervical, ovarian, breast, renal, and lung carcinomas. Measles virus has a tropism to CD46, and they use these receptors to enter into the cells [21,22]. It is known that the cytopathic effect of measles infection is syncytium formation due to cell-cell fusion. CD46 has a role in cell-cell fusion also. A particular threshold CD46 density is required for this system formation to occur following the infection so that the normal cells are more resistant to measles attack. Also, the measles used is attenuated as the wild type is not found to act via CD46 mechanism but by SLAM (Signalling Lymphocytic activation molecule) mechanism [23]. Adeno virus B1 species uses CD46, and B2 species use DSG-2 (Desmoglein-2) for cell entry [24]. There is another fiber knob: CAR-mediated pathway (Coxsackie and Adenovirus receptor) used for cell entry which is an important receptor pathway for oncolysis. Tuve et al. found that Ad11 utilizes another unidentified receptor called 'receptor X' which is significant in cancer cell entry [25]. NSLC (Non-Small Cell Lung Cancer) expresses high levels of CAR and DAF (Decay Accelerating factor). The oncolytic activity of CVB3 (Coxsackie B3 virus) is found to be proportional to the percentage of CAR expressing cells times the percentage of DAF expressing cells [26]. CD20 is a receptor seen in normal and neoplastic B cells but not in other tissues. Rituximab is a CD20 monoclonal antibody in use against NHL (Non-Hodgkin's Lymphoma). Use of a CD20-targeted recombinant measles virus can be used as a substitute to Rituximab in the FCR (Fludarabine, Cyclophosphamide, and Rituximab) regimen for lymphoma management [27]. PVS-RIPO, a novel oncolytic virus consist of the genome of Sabin strain polio virus type 1 and human rhinovirus type 2 (HRV2) internal ribosomal entry site (IRES). It has an affinity for CD155 which is up regulated in neuroectodermal malignancies like high grade gliomas and thus is under trial for such malignancies [28]. In many other virus infections, the actual receptor on which virus gets attached is unknown, or they may enter via interaction with different types of receptors. Sindbis virus is an alpha virus that can interact with heparin sulphate (HS) moiety and mediates its oncolytic activity

3.2. Aberrant signalling pathway in cancer cells

Interferons have anti-viral and anti-proliferative properties. Their antiviral mechanism involves activation of PKR pathway, 2–5 A synthases and activation of some specific proteins. PKR gets activated if comes in contact with ds RNA derived from the virus. It

phosphorylates itself and other substrates like eIF2 which is a translation initiation factor of viral translation. Phosphorylation leads to eIF2 inactivation and thus inhibition of virus and host protein translation. They act at different levels in different viral infections. E.g. they inhibit genomic transcription of Influenza virus, but interferes with protein translation of Adeno virus. It is said that the mutation lies in the heart of carcinogenesis. Mutations help in the proliferation of the cancer cells. As interferons inhibit growth, proliferation, cancer cells will switch the genes off for its better survival and proliferation thus making them susceptible to easy viral entry and spread to neighboring cells. The inherent properties of cancer cells make them more susceptible to viral invasion. Reo virus multiplies specifically in cells with activated RAS pathway, sparing normal cells. RAS inhibits PKR pathway and enhances viral infectivity thus can be used in tumors with an aberrant RAS pathway, especially solid tumors [29]. Infected cells mostly undergo apoptosis, but some tumor cells are resistant to normal apoptotic pathways which may not respond to this treatment.

3.3. Augmenting immune response against cancer

Cell death or cytolysis of the cancer cells exposes cancer antigen to the circulation and launches an immune response against the same. The antigen presenting cells presents the tumor associated antigen (TAA) to the T cells, which carry out further cell killing. Augusto et al. used Adeno viral vector with HSV-TK protein which functions as a super antigen and stimulates T cells and secretion of cytokines like IL-2 [30]. The release of tumor antigens evokes a systemic response against homologous tumors metastasized. So the local intratumoral injection of viruses will have a global effect. The long-term effect of the viruses is due to this immunological activation. Working on the immunological memory may further prevent the relapse of the tumor. The immune system may not be successful against large, bulky tumors, but against residual tumors it can have a significant action. The Virus itself will increase the IFN-gamma levels in the blood, which also helps in anti-tumor action [31].

4. Combination virotherapy

Cyclophosphamide is highly toxic to the immune system, so giving it along with virus drug will suppress the anti-viral immune response. Pre-administering of cyclophosphamide prevents clearance of viral particles and increases HSV replication in glioma [32]. Many preclinical studies demonstrate that cyclophosphamide is better than dexamethasone in this aspect. Reo virus is found to be well tolerated by the patients along with cyclophosphamide. G207, NV1020, and 1716 are tested with chemotherapy with better results. It was found that G201-cisplatin combination therapy achieved 100% cure rate in case of cisplatin sensitive human head and neck squamous cell carcinoma in animal models [12]. Increased efficacy is noted in 1716 plus mitomycin in a human lung cancer model. ONYX-015 and either cisplatin or 5FU for head and neck cancer or metastatic colorectal cancer showed good results. Histone deacetylase inhibitors (HDAC) inhibited the already limited interferon response of tumor cells, thus increasing the effect of co-administered virus drug. This has been tried in pox virus (vaccinia) which showed an increased survival of mouse bearing colon tumor xenografts HCT116 [33]. Rapamycin enhances the growth of many viruses by attacking the TORC1-dependent production of interferon and disrupting the phosphatidylinositol 3-kinase AKT pathway. SVV-001 is administered with Rituximab to reduce the immune response [34]. Lytic activity of NV1020 was enhanced with radiation in HuH7 xenografts in athymic mice. G207 and radiation in cervical cancer are found to have increased

efficacy [19]. ONYX-015 produced an additive effect in the radiation sensitive p-53 mutant cell line. The potency of NIS-expressing virus can be increased by administering I-131, which delivers high-energy beta particles, into the infected tumor [14].

Combining treatments can be done using recombinant DNA technology. ONCOS-102 is a chimeric Ad5/knob3 vector added with GM-CSF production has completed phase-1 trial in advanced solid tumors [35]. GM-CSF is a potent immunostimulator which augments CD8 + T cell response. Ganesh et al. used hyaluronidase enzyme along with Ad5/35GFP fiber-chimeric adenovirus which enhanced virus transduction and spread within prostate cancer [22]. Losartan is an angiotensin receptor blocker used for control of hypertension. It enhances the intratumoral spread of oncolytic HSV as it disturbs the transforming growth factor beta1 signalling. This results in reduced collagen production and fibrotic activity helping the easy spread of the virus inside the tumor [36]. But it takes some time for its action. The vascular permeability of tumor blood vessels can be increased so that the effect of both virus, as well as drugs, can be enhanced. VEGF (Vascular Endothelial Growth Factor) plus paclitaxel or cisplatin increases it and thus improves the delivery of Sindbis virus into tumors [12]. Similarly, in the B16 melanoma model, a combination of VEGF165 and reovirus resulted in enhanced infection of the tumor [37]. Adeno virus delivered to the tumor bed following surgical resection, radiotherapy, or chemotherapy in prostatic carcinoma reduced the development of secondaries in the lungs as well as local recurrence. Combining virus with complementing virulence proteins can be done. Interferon sensitive VSV, which is an RNA virus is combined with the pox virus encoding a secreted interferon antagonist had a better oncolytic effect by VSV and better intratumoral spread due to pox.

5. Conclusion and future perspective

Oncolytic viruses initially can be tried as an add-on therapy for malignancy, then trying to replace it if found to be effective. Utilization of recombinant DNA technology to engineer the best killer virus which targets only cancer cells is the future. It should have a very high margin of safety and cost should be less for the benefit of all. Proper regulations regarding assessing the bioavailability, bioequivalence, standard of procedure for use, guidelines for health workers all need to be made available for this therapy. More and more phase 2/3 trials should be done to confirm the effectiveness of the virus drug as very few drugs has reached the phase 3 trials. Utilizing the advances in the fields of recombinant DNA technology, virology and pharmacology will help to achieve this target and help to place these virus drugs in the standard treatment of clinical oncology shortly.

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