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Delivery of nanoparticulate drug delivery systems via the intravenous route for cancer gene therapy

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While the systemic route of administration enables therapeutic genes to spread through the bloodstream and access target cells, it is a challenge to achieve this. Several studies demonstrate that systemic administration of therapeutic genes or other nucleic acid-based constructs such as siRNA to solid tumors as well as cancer metastases are better with nanoparticulate systems compared to administration of free (uncomplexed) nucleic acids. Nanoparticle-based nucleic acid delivery systems might be more pertinent, due to the several privileges in terms of enhanced tissue penetrability, improved cellular uptake and to a lesser extent, targeted gene delivery to the cells of interest provided targeting ligands are used. Systemic delivery of nanoplexes has already been reported with different nanoparticles containing DNA via various routes of administration. The goal of the present article is to review the current state of intravenous delivery of nanoparticles for gene therapy of cancer.

1. Introduction

Gene therapy is the transfer of genetic material into diseased cells in an attempt to revert the cell to its normal state or to facilitate its ablation from the organism. Cancer gene therapy is an umbrella term encompassing the stimulation of protective immune response against a tumour, substitution of mutated tumour suppressor genes, inactivation of oncogenes, suicide gene therapy, or multidrug resistance genes in bone marrow or peripheral blood stem cells (Habib 2000). At present, there are more than 1500 gene therapy clinical trials worldwide, and approximately 1000 of these are for cancer (http://www.wiley.co.uk/genetherapy/clinical/, accessed 4th June 2010). However, there is a large dip in the number of gene therapy trials being introduced, and one major reason for this is the shortcoming of present delivery vectors.

Apart from viral vectors for cancer gene therapy, non-viral vectors for gene delivery also exist. These consist of three types, liposomal delivery systems (lipoplexes), polymeric delivery systems (polyplexes), and the solid nanoparticles (NPs) which bind the therapeutic payload within its dense and compact structure. NPs used in gene therapy consist of polymeric NPs, liposomes, gold NPs and magnetic NPs. Commonly, nanoparticulates used in gene delivery include nanospheres, nanocapsules, nanotubes and nanogels.

One way NPs gain entry into cells may be via endocytosis/phagocytosis (Brigger et al. 2002). It is claimed that NPs offer enhanced cellular uptake and deeper tissue penetration, are capable of crossing the blood-brain barrier, and of targeting particular cell types, though for the latter, targeting moieties are required. Moreover, some NPs are capable of interacting with and crossing mucosal surfaces, escaping endolysosomal compartments and sustaining the release of the nucleic acid payload within the cell (Alonso 2004; Basarkar and Singh 2007).

Appropriate selection of the administration route is highly important in gene delivery, due to the short degradation time of nucleic acid constructs in cells and in blood (Kawabata et al. 1995; Dass et al. 2002). Selection of the administration route could influence the ultimate therapeutic effect of the delivered nucleic acids. In some cases, use of some administration routes may not be possible due to various physiologic and safety concerns, for example in cases where the drugs are large molecules and movement through the subcutaneous route will be either slow or impossible.

Overall, systemic delivery of NPs with the purpose of gene expression has already been achieved with diverse NPs containing DNA via different routes of administration including subcutaneous (Thakor et al. 2007), intradermal (Mumper and Cui 2003; Minigo et al. 2007), intranasal (Csaba et al. 2006; Lee et al. 2007; Glud et al. 2009), intraperitoneal (Jiang et al. 2007; Intra and Salem 2008), and oral (Dass and Choong 2008) routes of delivery. This review addresses the recent state of systemic delivery of NPs focusing on cancer gene therapy via the intravenous route, focusing on *in vivo* studies.

2. Intravenous cancer gene therapy

Intravenous administration offers at least initial complete bioavailability. Thus, it is the best choice in emergencies when there is an urgent need for rapid drug delivery. Besides it is the best alternative route when there are problems with oral absorption or stability in the gastrointestinal tract. However, there are some problems with intravenous injection, for

Table: Summary of *in vivo* studies examining intravenous delivery of nanoparticles for cancer gene therapy

Polymer	Gene/construct	Cancer type	Major findings	Ref.
Poly-L-lysine	Green fluorescent protein (GFP)	Not determined	Selective delivery to lung and brain	Xiang et al. (2003)
Polyethylenimine	VEGFR2 siRNA	N2A (neuroblastoma) xenograft	Selective delivery to tumour due to RGD ligand and PEG	Schiffelers et al. (2004)
Polyethylenimine	GFP	Not determined	PEG reduces haemolysis and aggregation in blood	Brownlie et al. (2004)
Atelocollagen	Luciferase siRNA	PC3 (prostate cancer) xenograft	Reduced luciferase expression in tumours by 90%	Hanai et al. (2006)
Atelocollagen	Enhancer of zeste homolog 2 siRNA	PC3 (prostate cancer) xenograft	Reduction of metastases	Hanai et al. (2006)
Cationic albumin	Apo2L/TRAIL	Glioma	Induced tumour cell apoptosis, reduced tumour growth	Lu et al. (2006)
Gelatin	Soluble Flt1	MDA-MB435 (breast cancer) grown orthotopically	Reduced angiogenesis and tumour growth, PEG prolonged circulation	Komareddy and Amiji (2007)
HBsAg L	GFP	Hepatic (orthotopic)	HBsAg allowed selective delivery to tumours	Iwasaki et al. (2007)
HBsAg L	Herpes Simplex Virus – thymidine kinase	Hepatic (orthotopic)	Suppressed tumour growth	Iwasaki et al. (2007)
Cyclodextrin-containing polycations	Ribonucleotide reductase	Neuro2A xenograft (subcutaneous)	Inhibited tumour growth	Bartlett and Davis (2008)
Calf thymus DNA+polycation peptide+cationic liposome	siRNAs to MDM2, c-myc and VEGF	Melanoma (B16F10) in lungs (metastasis model)	Reduction in tumour growth and metastasis	Li et al. (2008)
Reximmune C	Granulocyte macrophage colony stimulating factor	MiaPaca2 pancreatic cancer (subcutaneous)	High level expression of transgene	Gordon et al. (2008)
Poly-L-lysine	NM23-H1	B16F10 melanoma cell pulmonary metastasis	Suppression of metastasis	Li et al. (2009)

example, a certain degree of haemolysis is possible in the intravenous administration of therapeutic genes (Brownlie et al. 2004). On the other hand, interactions with plasma proteins and uptake by the macrophages of the monocyte phagocytic system (MPS) should be avoided. This probably cause the formation of aggregates which are either entrapped in the lung endothelial capillary bed or taken up by the MPS. Moreover, biocompatibility problems are a main problem associated with intravenous injection. size is also important, as just small particles have the ability to cross a permeable endothelium such as in neovascularized tumours or inflammation through the fenestrated barriers (Fattal and Bochot 2008). All this is discussed to update the reader on the current state of intravenous delivery of NPs for cancer gene therapy.

In 2003, Xiang et al. developed a non-viral vector which is formed by modifying poly-L-lysine to iron oxide NPs (IONP-PLL) (Xiang et al. 2003). They investigated the transfection efficiency of IONP-PLL-plasmid DNA *in vitro* as well as *in vivo* following intravenous injection of DNA complexes into adult BALB/C mice. Profile of cell uptake and the tissue distribution of IONP-PLL/DNA were investigated by transmission electron microscopy and iron stain in many organs subsequent to the intravenous injection. The results showed that IONP-PLL NPs incorporating the EGFP (encoding green fluorescent protein) was efficiently delivered to lung, brain, spleen and kidney, whereas liver, heart and stomach did not show noticeable gene expression. Furthermore, transfection efficiency of IONP-PLL-DNA was much higher in the lung than in other organs. Besides, IONP-PLL had the ability to distribute in the glial and neuron cells of brain after penetrating the blood-brain barrier; most probably as a consequence of small size, enzymatic stability and hydrogen bonding potential. In addition, since iron oxide NPs can accumulate in tumor cells and tumor-associated macrophages and IONP-PLL had the capability to transport the desired genes to lung and brain intravenously, IONP-PLL offers a promising gene delivery system for gene therapy.

Schiffelers et al. (2004) utilized siRNA for inhibiting vascular endothelial growth factor receptor-2 (VEGF R2) expression which results in tumor angiogenesis using ligand-targeted sterically stabilized NPs. For this purpose, they developed NPs with PEGylated polyethyleneimine (PEI) with an Arg-Gly-Asp (RGD) peptide ligand attached at the distal end of the polyethylene glycol, for targeting tumor neovasculature expressing integrins, to deliver the siRNA inhibiting VEGF R2 expression. They prepared three forms of nanoplexes: one with a branched polyethyleneimine (PEI) (p), the other PEI with a PEG having an RGD peptide at its distal end: RGD-PEG-PEI (RPP) and the last PEI with a PEG missing the peptide: PEG-PEI (PP). Administration of free siRNA intravenously did not produce considerable FITC-siRNA fluorescence in the tumor, and very little FITC fluorescence was observed in the liver and lung. The authors attributed this to a rapid clearance of the FITC-siRNA into the urine, poor tissue accumulation except liver. Metabolic instability may also cause rapid excretion or liver metabolism of the FITC. In contrast, FITC-siRNA incorporated in P-nanoplexes produced appreciable FITC-siRNA fluorescence in liver and

lung with a punctate profile, whereas RPP-nanoplexes produced considerable FITC-siRNA fluorescence in the tumor, but reduced liver and lung accumulation as well as a reduced punctate fluorescence pattern. This is probably due to reduction in non-specific tissue interactions of the RPP nanoplex resulting in accumulation in tumor by ligand binding, and reduction in uptake by liver and lung. Additionally, siRNA in the RPP-nanoplex were more stable than aqueous siRNA. Intravenous administration of RPP-nanoplexes incorporating siRNA facilitated sequence-specific inhibition of tumor growth, suggesting that the RPP siRNA nanoplex acts through an endothelial cell uptake mechanism. Thus, RPP-nanoplex is capable of delivery of siRNA to tumor tissue via intravenous administration and has the ability to inhibit gene expression sequence-specifically in tumor.

Another research group synthesized and tested PEI derivatives *in vitro* and *in vivo* (Brownlie et al. 2004). They aimed to combine complementary properties of cationic lipids and polymers into a hybrid material. For this purpose, palmitoylated (PA) derivatives with PEI and with quaternary ammonium PEI (QPEI) were synthesized. The synthesized PEI derivatives include: PEI–PA, PEG–PEI–PA, QPEI–PA, PEG–PEI–PA/cholesterol, and QPEI–PA/cholesterol. Since haemolysis is a possible side-effect of the intravenous administration of synthetic gene delivery systems, they evaluated haemolytic activity of PEI derived polymer systems following the intravenous administration to the lateral tail vein of a mouse model. The results revealed that both water-soluble and particle/vesicle forming derivatives cause less than 10% haemolysis, whereas only PA-PEI exhibits a dose-dependent haemolysis, significantly lower than that of the parent polymer which showed a dose-dependent tendency to cause haemolysis (27% at 1 mg/ml). Furthermore, *in vivo* transfection efficacy was studied by assessing GFP transgene expression in the liver which was carried out 24 h after intravenous injection of 'naked' DNA and different DNA-polymer complexes in mice. The rank order of histochemical staining of liver sections was found to be: PEI<PEG–PEI–PA<PEI–PA<QPEI<QPEI–PA. Histochemical staining was observed at the centre of the liver lobules or in its periphery which might be due to the effect of blood flow in the portal triads. Moreover, the modified PEI carriers have a reduced tendency to induce aggregation in serum, plasma and erythrocytes. In addition, cytotoxicity was considerably reduced; however transfection efficiency did not increase *in vitro*.

Hanai et al. (2006) prepared on atelocollagen-mediated oligonucleotide delivery system applied to systemic siRNA and antisense oligonucleotide treatments in animal disease models. They investigated the efficiency of the prepared siRNA/atelocollagen complexes for luciferase gene silencing via intravenous administration. A tumor metastatic model was developed by injection of PC-3M-luc-C6 tumor cells (bioluminescent human prostate carcinoma cells) into the bloodstream of mice. The results of bioluminescent imaging indicated around 90% Inhibition of luciferase production by metastatic tumor in mice treated with the siRNA/atelocollagen complex which was noticeably more than that of mice treated with just atelocollagen or luciferase siRNA alone. Furthermore, suppression of tumor growth metastasized into bone tissue by siRNA/atelocollagen complexes were studied. The bioluminescence on day 28 in mice treated with just atelocollagen or siRNA alone was about 25-fold of that on the initial day whereas bioluminescence in mice treated with the siRNA/atelocollagen complexes did not show an increase. The researchers proposed that atelocollagen-mediated oligonucleotide delivery system could be efficacious in systemic treatments *in vivo*.

Lu et al. (2006) studied cationic albumin–conjugated pegylated NPs (CBSA-NP) for glioma gene therapy with the plasmid pORF-hTRAIL (pDNA). They selected the proapoptotic Apo2 ligand/tumor necrosis factor–related apoptosis inducing ligand (Apo2L/TRAIL) for gene therapy as Apo2L/TRAIL is thought to selectively kill tumor cells. The results revealed that the CBSA-NP-hTRAIL can cross the BBB and accumulated in intercranial glioma xenografts by absorptive-mediated transcytosis (AMT) following intravenous administration to BALB/c mice bearing the C6 gliomas. At 48 hours subsequent to administration of CBSA-NP-hTRAIL, hTRAIL protein was identified in normal brain and tumors. In addition, repeated injections of CBSA-NP-hTRAIL induced apoptosis of glioma cells but not of normal of cells *in vivo* and notably delayed tumor growth. Also, CBSA-NP-hTRAIL was found to have a moderate effect on median survival of tumor-bearing mice (41 versus 22 days for control animals). Finally, as retroviral vectors need intratumoral delivery and possess safety risks, repeated intravenous administration of nontoxic CBSA-NP-hTRAIL prove to be a better choice for noninvasive gene therapy of malignant glioma.

The feasibility of gelatin-NPs for intravenous delivery of plasmid DNA encoding vascular endothelial growth factor receptor-1 or soluble fms-like tyrosine kinase-1 (VEGF-R1 or sFlt-1) was examined by Kommareddy and Amiji (2007). VEGF is one of the growth factor proteins known to have a main role in vasculogenesis and angiogenesis. Generally, VEGF, over-expressed by most types of cancers, leads to angiogenesis, and is one of the most potent pro-angiogenic factors known. For entrapment of VEGF produced by tumor cells, they used plasmid DNA which encodes the extracellular domain of Flt-1 VEGF receptor excluding the transmembrane and cytoplasmic domains. The plasmid DNA was encapsulated with gelatin (Gel), thiolated gelatin (SHGel), polyethylene glycol-modified gelatin (PEG-Gel) as well as polyethylene glycol-modified thiolated gelatin (PEG-SHGel). According to the results obtained, both the tumor growth suppression and anti-angiogenic effects of expressed sFlt-1 were significantly superior with PEG-SHGel than that with PEG-Gel NPs. Also, liver transfection with plasmid in PEG-SHGel was lower than that with plasmid-PEG-Gel NPs; however in the skeletal muscle there was no transfection of sFlt-1 subsequent to the intravenous administration of all types of formulations studied. Also, the PEG chains enhanced the *in vivo* circulation time upon intravenous administration. On the whole, PEG-Gel NPs possibly will offer a safe and efficient approach for intravenous administration of plasmids to solid tumors.

In 2007, Iwasaki et al. described a gene delivery system based on hepatotropic NPs to be tested against human liver tumors. The NPs contained the hepatitis B virus surface L antigen (HBsAg) on the surface to proffer hepatic specificity, but did not contain the viral genome. Generally, viral vectors can cause inadvertent transgene expression in non-target cells, causing somewhat unpredicted side-effects, such as bone marrow suppression. Iwasaki et al. used green fluorescent protein (GFP) expression plasmid as reporter gene as well as Herpes simplex virus thymidine kinase (HSV-tk) gene with ganciclovir (GCV) as suicide gene/prodrug combinations. GFP expression after injection of GFP-L antigen NPs to the tail vein of rats bearing human hepatic (NuE) and non-hepatic tumors was detected merely in NuE-derived tumors but not in the non-hepatic tumor. Furthermore, intravenous administration of L antigen-NPs incorporating the HSV-tk gene, in combination with ganciclovir led to growth suppression of NuE-derived tumors in rats, but not of the non-hepatic tumor control. In addition, the GFP biodistribution profile in hepatic and non-hepatic tumors and in various rat tissues showed that GFP expression is restricted to the transplanted liver tumors (NuE). Thus the authors proposed that the L antigen NPs are appropriate for targeted gene delivery and expression in human liver tumors.

Bartlett and Davis (2008) investigated the effect of transferrin-targeted and non-targeted siRNA-containing NPs formed with cyclodextrin-containing polycations in A/J mice bearing subcutaneous Neuro2A tumors after intravenous injection. siRNAs were designed to target ribonucleotide reductase, the main enzyme for nucleotide preparation for DNA replication (Cerqueira et al. 2005). Three consecutive daily doses of transferrin-targeted NPs of two different siRNA sequences targeting ribonucleotide reductase subunit M2 (RRM2) contributed to tumor growth inhibition, while non-targeted NPs showed less inhibition of tumor growth at the same dose. On the other hand, administration of the three doses on consecutive days or every 3 days caused no statistically significant differences in tumor growth delay. The results proved the importance of tumor-specific targeting as well as dose and dose frequency for siRNA NP delivery.

In 2008, Li et al. presented a NP formulation composed of siRNA, a carrier DNA (calf thymus DNA), a polycationic peptide, and cationic liposomes. They encapsulated a combination of three different siRNA sequences in order to improve the antitumor/antimetastasis effect by attacking multiple oncogene pathways. To this end, MDM2 (inactivator of p53), c-myc (an activated transcription factor that promotes cell proliferation) and VEGF were combined. In addition, there was a ligand on the NPs targeting sigma receptor–expressing murine melanoma cells, B16F10. Gene silencing was studied after two repeated intravenous injections to the lung metastases-bearing mice. According to the results obtained, the siRNA-targeted NP led to simultaneously silencing of MDM2, c-myc, and VEGF in the lung metastases. A considerable reduction in the tumor load compared to the untreated control was seen with the NPs ($P < 0.01$). Also, metastasis nodules were appreciably decreased in the mice lung after two consecutive injections of siRNA-targeted NP. The mean animal survival times after two consecutive intravenous injections of siRNA-targeted NP notably decreased the lung metastasis (~70–80%), compared to free siRNA and the non targeted NP. Moreover, siRNA by the targeted NP revealed little local and systemic immunotoxicity and did not reduce the body weight or damage the major organs. Hence, the authors claimed that siRNA formulated in the targeted NP could be a useful tool in cancer therapy.

The possibility of cytokine gene delivery to cancerous lesions via intravenously administered pathotropically targeted NPs *in vivo*, for anti-cancer vaccination, was studied by Gordon et al. (2008). One of the main privileges of exact tumor-targeted genes delivery is simple delivering of therapeutic genes to surgically inaccessible and remote sites by intravenous infusion. In general, granulocyte/macrophage colony stimulating factor (GM-CSF, Reximmune-C) recruits antigen presenting cells which leads to the activation of tumor-infiltrating B and T lymphocytes against proteins expressed by cancer cells. For this purpose, subcutaneous tumor xenografts were established in athymic nu/nu mice by subcutaneous implantation of MiaPaca2 human pancreatic cancer cells to assess the efficiency of a targeted gene delivery system. According to the results, subsequent to intravenous infusion of Reximmune-C to tumor-bearing nude mice, the vector accumulated rapidly in cancerous tissues within minutes of infusion and efficiently transduced resident tumor cells. Furthermore, immunohistochemical analysis revealed high-level expression of human GM-CSF in resident cells (~35%) of Reximmune-C vector-treated mice in comparison with <1% in the non-targeted GM-CSF vector-treated and targeted null vector-treated mice. Also, the recruitment of host mononuclear cells, including CD40+ B cells and CD86+ dendritic cells was confirmed by immunohistochemical staining. As athymic mice are lacking in T cells, recruitment of host mononuclear cells was due to the immunomodulatory action of the GM-CSF protein secreted by the cancerous cells targeted by Reximmune-C.

The ability of poly-L-lysine-modified iron oxide NPs (IONP-PLL) to deliver the NM23-H1 gene as a suppressor gene, to tumor cells *in vivo* was investigated by Li et al. (2009). The results revealed that intravenous injection of IONP-PLL incorporating NM23-H1-GFP notably extended the survival time of pulmonary metastasis in a mouse model. Furthermore, metastasis suppression was noticeably higher in the IONP-PLL/NM23-H1-GFP-treated group in comparison with a free NM23-H1-GFP plasmid treated group. Also, the number of metastatic nodules on the surface of the lungs after treatment with IONP-PLL incorporating pNM23-GFP plasmid DNA, cyclophosphamide (a chemotherapeutic agent) or with the combination of these two compounds was found to be 23.9, 62.2 and 8.9%, correspondingly. Thus, intravenous administration of IONP-PLL NPs incorporating the NM23-H1 gene may be an efficacious strategy in metastatic tumors treatment particularly in combination with chemotherapeutic agents.

3. Future directions

While systemic delivery of therapeutic nucleic acids is a challenging endeavour, it is a useful administration route in the treatment of disseminated or deep-seated tumours. The genetic material must be protected from degradation in the biological environment, must extravasate and diffuse throughout the tissues to achieve the target site, and possess an adequate circulation half-life. Furthermore, it should extravasate from the blood, and be taken up into the target tumour cells. In view of the fact that most of the present NPs systemically delivered for cancer gene therapy lack both efficiency and specificity, much work remains to be done in the future both at the discovery and developmental level, as well as in the oncological testing arena. The benefits far outweigh the effort in this case, as in the end, patient-friendly delivery systems for therapeutic genetic constructs will result.

4. Summary

Amongst other critical factors involved in successful gene delivery and subsequently therapy, a proper choice of the administration route may well be a major factor. The systemic route of administration facilitates spread of the therapeutic genetic material throughout the organism and facilitates natural access to the target cancer cell. However, under numerous conditions, one being solid tumours with aberrant patterns of growth and angiogenesis, the target cells will not be directly accessible. Hence, NP-based gene delivery systems might be more pertinent since NPs could provide enhanced tissue penetrability and improved cellular uptake, while reducing exposure of normal healthy cells to treatment. Systemic gene delivery with NPs has already been reported with various types of NPs and various routes of administration. In this discussion paper, we have reviewed those related to the current state of systemic delivery of NPs, concentrating on cancer gene therapy via the intravenous administration route.

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