Review Article

Dendrimers in combination therapy of cancers

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Abstract

Objective: In the last 3 decades the nanotechnology has impacted researchers with promising results and newer hope in the treatment of complex diseases. Apart from other nanocarriers dendrimers have shown to be one of the most successful nanocarrier as per as the drug delivery is concerned. The present review article is an attempt to comprehensively compile the work in the dual or cocktail delivery using different dendrimers and specially PAMAM. **Methods:** Bibliographic information were collected and analysed from text books and peer reviewed journals, consulting worldwide accepted scientific databases from the libraries and internet sources. Dendrimers are highly branched macromolecular carriers with the size below 100 nm. The nanometric size and tailor made functionality provides another opportunity for the drug delivery. In the recent past, the dual or cocktail delivery of drugs and genes has opened a new avenue in the treatment of diseases with multiple drug therapy. This is a step ahead possibility shown by the dendrimers which is purely due to its unique features and properties. **Conclusion:** The applications of dendrimers extends even beyond drug delivery i.e. in diagnostics, non pharmaceutical applications etc. The combined delivery of siRNA and drugs has better potential in overcoming the drug resistance in the diseases such as breast cancer others.

Keywords: PAMAM, siRNA, Combined delivery, Drug Delivery

Introduction

Cancer is a most frightened and dangerous disease, according to WHO 14.1 million peoples conceived cancer and 8.1 million were died in 2012 and 32.5 million peoples diagnosed within five years 2010-2015. According to estimation with this increasing rate burden of cancer will increase to 23.6 million by 2030. (WHO report, 2012) It becomes 'disease of civilization' and the main reasons are change in the life style and eating habits of the peoples (Chen et al, 2015). Several treatment strategies of cancer were explored and till date are several more are being explored and in pipeline too. Very tellingly, despite drastic improvement in medical care the mortality rate is still around 60% of the overall 5 years (Chen et al, 2015). Treatment of cancer is still burdensome in both terms patient compliance as well as in pharmacological context. Most of the anticancer agents highly cytotoxic and causes severe side effects to the patient and single drug medication is not effective because cancer cells often show (Duesberg et al, 2007; Wang et al, 2009) drug resistance against anti cancer agents which is a long standing challenge, it overcomes the activity of the drugs/agents against tumor cells, one of the possible reason of resistance is single drug therapy often activates the alternative paths at the cellular level, and it leads to chemoresistance and tumor may get relapsed (Hu CMJ et al, 2012). There are several vital mechanisms responsible for chemoresistance in malignant cells were investigated, 1) the increased expression of ATP-binding cassette (ABC) efflux transporters, 2) changes in the protein levels, 3) enhanced DNA repair, 4) overexpression of antiapoptotic proteins or down regulation of proapoptotic proteins, 5) detoxification by phase II conjugating enzymes, such as glutathione S-transferases and UDP-glucuronosyltransferases, 6) multidrug resistance associated protein and breast cancer resistant protein, 7) alteration in lipid metabolism, 8) drug test sequestration inside lysosome, 9) Altered drug targets such as topoisomerase II, 10) hypoxia up-regulated

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expression of MDR-linked genes such as ABC transporters, Bcl-2 family genes, glutathione, metallothionein, etc. through activation of transcription factor HIF1, 11) chromosomal abnormalities in cancer cells lead to over-expression of anti-apoptotic genes (m) altered signal transduction pathways in cancer cells governed via integrin receptors, growth factor receptors etc (Gatti et al, 2005; Abdullah et al, 2013; Mistry et al, 2014; Broxtterman et al, 2003). Plethora of literature documented for the combination therapy of cancer treatment and it has been established as an effective treatment to overcome or to delay the chemoresistance. In combination therapy more than one drugs applied to a single or multiple target due to functional synergy leads to higher therapeutic efficacy to the target. Despite the merely combining two or more drugs also had some hurdles like: varying pharmacokinetics it makes the dose and schedule optimization difficult and also causes severe side effects (Chen et al, 2015).

In last two decades nanocarriers are gaining attraction form the researchers in the drug delivery in various diseases like cardiac disease (Wang et al, 2001), topical, brain tumor (Katare et al, 2015). Various nanocarriers are nowadays are being used in drug delivery liposomes, nanoparticles, quantum dots, polymeric micelles etc. In cancer it is more relevant due to targeting of the drug at the targeted site active as well as passive targeting. The phenomenon called as enhanced permeability and retention is the drive force for the passive targeting of drugs. Dendrimers are mono dispersed, three dimentional, hyperbranched macro molecules generating from a central molecule and branching molecules by iterative steps of reactions and structure depends on the generation, lower generations are flexible while higher globular and denser (Gupta et al, 2010; Singh et al, 2008; Gothwal et al, 2015). Dendrimers are well established for enhancing solubility of insoluble drugs PTX, Doxorubicin, Docetaxel, adriamycin, MTX etc and targeted delivery active (Ligand mediated) and passive as well.

In the past few years several studies have been documented to make the chemotherapy effective by suppressing or blocking the drug efflux process by co-delivery of siRNA and chemotherapeutic agent or two chemotherapeutic agents. In this review we focus on the combinational drug/ bioactive delivery in cancer using dendrimer as a carrier. This field is not so much explored but many researchers tried to deliver multiple drugs with dendrimers.

Drug-siRNA combined delivery using dendrimers in cancer

As the conventional chemotherapy have high cyto-toxicity and less effectively because the exposure of the anticancer agents to the normal cells is as same as to carcinoma cells and in combined administration of two anticancer drugs the risk increases several folds. Hence, dendrimers are the effective approaches for combined drug delivery. Very tellingly, plethora of literature is documented for most of anticancer drugs like doxorubicine, paclitaxel, deocetaxel, 5- FU, adriamycine etc were delivered using different dendrimers but very less literature is available for the combined delivery of drug and siRNA *via* dendrimers (Figure 1).

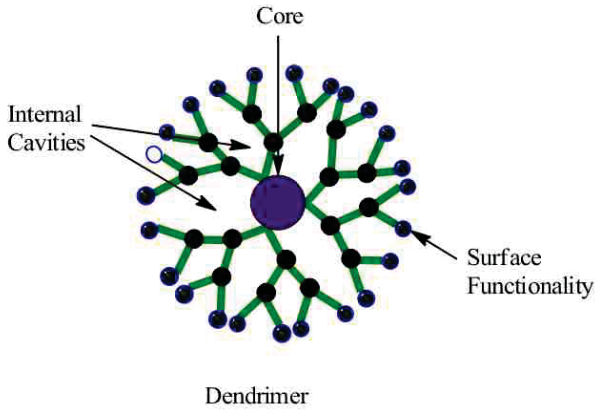

Figure 1. Structure of a Dendrimer

Very first, in 2009 Zhao et al documented the host-guest chemistry of dendrimer-drug complex for competitive binding multiple drugs for combination therapy. The authors analyzed the interaction between multiple drugs sulfamethoxazole, phenylbutazone, mycophenolic acid, methotrexate, phenobarbitol and benzoic acid and G3 PAMAM dendrimer by H^1 NMR and 2D-NOESY (two-dimensional nuclear Overhauser enhancement spectroscopy) and DOSY (diffusion-ordered spectroscopy). In NMR spectra they observed that when drugs such as phenylbutazone, mycophenolic acid, and sulfamethoxazole forms ion-pairs with dendrimer and it leads to downfield shift of the associated protons and protons takes place in H-bonding shown upfield shift, furthermore, in NOESY and DOSY the broadening of line was observed due to reduced degree of freedom and behalf of NMR spectra six types of protons were observed during the interaction. In different competitive combinations of dendrimer-drugs like G3/phenylbutazone/ mycophenolic acid the numbers of molecules attached with dendrimer were 20 and 41 respectively due to higher stability of NH_3^+ /COO^- than NH_3^+/CO^- because carboxylate is more negative and in G3/ sulfamethoxazole/phenylbutazone competitive binding system 27-28 molecules were loaded of both drugs, sulfamethoxazole has higher pK_a value but phenylbutazone is much more hydrophobic which split the difference. In the same time period, Kang et al employed PAMAM dendrimer as a carrier for anticancer agent paclitaxel and antisense micro RNA-21 gene for treating glioma and breast cancer,

antisense micro RNA-21 was used for the suppression of overexpressed micro-RNA-21 gene in breast cancer and observed that the nanosystem has the higher efficiency of inhibiting cell migration and cell apoptosis as well. Similarly, Mei et al, 2009 conjugated 5-flluorouracil with PAMAM dendrimer with combination of antisense micro-RNA-21 gene.

Table 1. Combined delivery of bioactives through PAMAM dendrimers

Dendrimers	Bioactives		Cancer types	Outcomes	References
	RNA	Drug			
PAMAM	as-miRNA-21	PTX	Glioma and breast	Increased efficiency, apoptosis, reduced mitigation	Kang et al
PAMAM	as-miRNA-21	5-FU	Breast cancer	Increased apoptosis, reduced mitigation	Mei et al
PAMAM	As-miRNA-21	Taxol	Breast cancer	Transfection of cells decreased, apoptosis increased	Mei et al
PAMAM	pORF-hTRAIL	Dox	Liver cancer	Higher cellular uptake & gene expression	Han et al
PAMAM	siRNA	Metformin & Doxorubicin	Prostate cancer	Enhances the anti-tumoral activity of metformin and doxorubicin	Monteagudo et al
PAMAM	miR-2Li	Temozola-mide	glioma cells	Synergistic anti proliferative and proapoptotic activity	Qian et al
PAMAM dendrimer	Akt siRNA	Paclitaxel	ovarian cancer	Effective for gene silencing *in vitro* and *in vivo*	Kala et al
PAMAM	poly (I:C)	Doxorubicin	Breast cancer	Most effective against dox resistant MCF-7 cells	Khodadust et al
PAMAM	mdr1 siRNA	Cisplatin	Lung cancer	Accumulation of bioactives was increased and P-gp efflux and MRP expression both were decreased in A549 cells	Zheng et al
PAMAM	siRNA	Cisplatin	hepatic cancer	PLK-1 mRNA level was reduced and enhancing expression of pro-apoptotic molecules and down-regulation of anti-apoptotic proteins	Sherwani et al

Results shown efficacy of 5-FU was increased by as-miRNA. The author's found that as-miRNA-21 not only apoptosis was increased but it could also reduce the MCF-7 cell migrations ability. In another work Mei et al, 2010 evaluated involvement of non-coding RNAs in cancer development and cancer resistance as well. The authors combined anticancer taxol with mRNA-21 inhibitor using PAMAM dendrimer as a carrier. Authors observed IC_{50} of taxol was reduced significantly with miR-21 inhibitor than free taxol, transfection efficiency of cancer cells was inhibited and percentage apoptosis was increased. Behalf of results they concluded that miR-21 is an important factor in developing resistance of blastoma in breast cancer and indicated that taxol with miR-21 inhibitor may be a newer therapeutic approach in treatment of breast cancer.

In 2011, Han et al reported a combination therapy of therapeutic gene encoding human tumor necrosis factor-related apoptosis-inducing ligand (pORF-hTRAIL) and doxorubicin (DOX) against liver cancer using a tumor-targeting carrier, peptide HAIYPRH (T7)-conjugated polyethylene glycol-modified polyamidoamine dendrimer (PAMAM-PEG-T7). They constructed the nanosystem by conjugating Dox with one pORF-hTRAIL and then electrostatically attached with dendrimer. T7 modified dendrimer mediated co-delivery shown higher cellular uptake and gene expression than unmodified co-delivery system in bel-7402 cell line and accumulation in mice bearing bel-7402 xenografts. Interestingly, in 2012 Monteagudo et al, reported a new concept of removing p42 MAPK protein in prostate cancer by blocking MAPK pathway using dendrimer/siRNA complex and potentiate anti-tumoral activity of an ant-diabetic agent metformin and docetaxel. Authors found that the siRNA/G1 polyamidoamine dendrimer complex was effective in reducing level of p42 MAPK mRNA and protein by 80% and also enhances the anti-tumoral activity of metformin and doxorubicin in prostate cancer. Bottom line of the study was concluded that the therapy may be useful for cancer treatment. Interestingly Qian et al, 2012 explored the most effective schedule of combined therapy of miR-21 inhibitor (miR-2Li) and temozolamide (TMZ) using PAMAM dendrimer as a carrier against three different glioma cells U251 phasphatase and tension homologue (PTEN) mutant, LN229 (wild-type) and U87 (PTEN loss function), they scheduled a predose of miR-2Li for 4/8 h and followed by TMZ treatment and further a treatment of TMZ for 4/8 h and then a subsequent miR-Li treatment or a attended treatment *in vitro*. The authors observed synergistic anti proliferative and proapoptotic activity when a predose was administered for 4 h before any other agent was administered and the best result was observed when concomitant treatment was followed in LN229 cells. Behalf of results the authors concluded that time of administration plays a crucial role in glioma combination therapy. In 2013 Acton et al, synthesized non-toxic, non-hemolytic novel janus dendrimers for combination therapy in cancer and evaluated with two model drugs benzyl alcohol (BA) and 3-phenylpropionic acid (PPA) by conjugating via linkers carbonate and ester respectively and observed BA was released faster than PPA. Authors suggested that the janus dendrimer has great potential for combination therapy.

Phasphatedylinositol 3-kinase/Akt is a pathway, has been explored as a reason for ovarian cancer development. In 2014 Kala et al, targeted Akt pathway for the combination therapy of PAMAM G6 dendrimer mediated Akt siRNA and paclitaxel in ovarian cancer. The authors observed that dendrimer/Akt siRNA complex is effective for gene silencing *in vitro* and *in vivo* as well. More

interestingly the complex increases the potency of anticancer agent paclitaxel. Interestingly Khodadust et al, 2014 claimed for the first reported combined delivery of synthetic analogue of double stranded RNA which directly triggers apoptosis in many types of human malignant cells, poly (I:C) (polyinosinic:polycytidylic acid) binding on doxorubicin loaded DcMNP (Dendrimer-coated magnetic nanoparticles). The authors observed that the doxorubicin enhanced the binding efficiency of poly (I:C). Authors observed that ploy (I:C) on the surface of dendrimer dicreases the cytotoxicity of the PAMAM dendrimer, nanocomposite was most effective against dox resistant MCF-7 cells additionally also cause apoptosis into the tumor cells. Combined delivery of drug and gene/siRNA is still the field of interest and gaining attention of the researchers, evidences for this are here that researchers still trying to explore new combination paradigms. In the current year Zheng et al, 2015 synthesized G5 PAMAM dendrimer modified selenium nanoparticles and used as a carrier for combined deliver mdr1 siRNA and cisplatin (cis-diamminedichloroplatinum-(II), DDP). The authors observed DDP enhances the RNA loading, release and gene silencing. Accumulation of bioactives was increased and P-gp efflux and MRP expression both were decreased in A549 cells. Interestingly they also observed down regulation and expression of c-myc, cyclin D1, P-AKT and P-ERK, and upregulated the expression of caspase-3, behalf of the results they suggested that the complex could overcome drug resistance by arresting A549/ DDP cells at G1 phase and inducing apoptosis via the MAPKs and PI3K/AKT signaling pathways in drug resistance A549/DDP cells. In the same time somewhere else Sherwani et al, 2015 demonstrated a new poly functional delivery system for siRNA and cisplatin for hepatic carcinoma to deliver the bioactive agents in a targeted manner. They constructed the nanosystem by encapsulating siRNA in PAMAM dendrimer and antibody was conjugated at the surface of dendrimer and on another hand cisplatin was loaded in PLGA nano-particles and then NPs were used to provide scaffold to the dendrimer. Authors claimed for the first example of *in vivo* and simultaneous delivery of siRNA and cisplatin by dendrimer-PLGA based immuno-nanocomposites demonstrating synergistic anti-tumor therapy in hepatic cancer, additionally also claimed that the nanocomposite system is non-immunogenic, non-toxic and bears minimum adverse effects. Authors observed that PLK-1 mRNA level was reduced and enhancing expression of pro-apoptotic molecules and down-regulation of anti-apoptotic proteins. Authors suggested it is a promising nano carrier system for combined delivery in cancer.

Conclusion and future perspective

Cancer therapy is always a typical challenge for the researchers due to associated cytotoxicity of the drugs. As we have discussed above that dendrimer is a most effective approach for the combined delivery of bioactives in the early stages of cancer and can also be effective in management of resistant cancer. Hence it is essential to develop such strategies for efficient delivery of drugs and minimize the health risk of patients as well. Therefore we suggest to more and more explore the dendrimer based combinational therapy in cancer.

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