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Carbosilane dendrimers as gene delivery agents for the treatment of HIV infection ☆

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ABSTRACT

Despite the use of siRNA in the downregulation of HIV-1 replication which has been reported, CD4 T lymphocytes are difficult to transfect with non-viral vectors. We determined whether second generation carbosilane dendrimers (2G-NN16 and 2G-03NN24) may be efficient transfectants in CD4 T lymphocytes. Dendrimers were also tested on macrophages to determine whether they can modify macrophage phenotype and induce an inflammatory response. The nanoconjugate formed by 2G-03NN24/siRNA-Nef presents the highest inhibition of HIV-1 replication. Dendrimers presented safety properties because they did not induce proliferation on CD4 T lymphocytes and decrease the release of TNFα and IL-12p40 by macrophages. Both dendrimers also decrease the phagocytosis activity. Additionally, 2G-03NN24 dendrimer decreases the CCL2 and CCR2 expression in macrophages. Carbosilane dendrimers 2G-NN16 and 2G-03NN24 can be used as efficient non-viral vectors for gene therapy applications, mainly in the treatment of HIV infection.

1. Introduction

Highly active antiretroviral therapy (HAART) has played an important role in controlling the spread of HIV/AIDS. However, HAART has major drawbacks, such as its limited availability, high drug cost and adverse side effects. Furthermore, it necessitates the adherence to a life-long treatment regimen, can cause the emergence of drug resistance and requires a wide array of viral isolates [1–3]. Therefore, research on HIV-1 infection needs to be continued to improve the current therapies. A promising strategy is to use gene-silencing approaches that employ antisense oligonucleotides or small interfering RNA (siRNA) [4,5]. The use of siRNA in the downregulation of HIV-1 and host mRNA expression against viral and cellular targets has been reported [5–8]. However, the most HIV-susceptible cells, such as primary CD4 + T lymphocytes, are excessively difficult to transfect with non-viral vectors [9]. Several delivery agents, such as carbon nanotubes [10], polymers [5,11], carbosilane dendrimers [5,6] and liposomes [12,13], have been used to deliver siRNAs into T cell lines and primary peripheral blood mononuclear cells (PBMCs). Although cationic liposomes are commercially available (*e.g.*, Cytofectin™ or Lipofectin™), they unfortunately fail in transfection processes in the presence of serum. Therefore, further studies are needed to design delivery agents that can be efficiently used in intracellular gene therapy applications.

We have demonstrated that water-soluble biocompatible 2G-$[Si\{O(CH_2)_2N(Me)_2^+(CH_2)_2NMe_3^+(I^-)_2\}]_8$ (2G-NN16) dendrimer is efficient transfection material in many cell types, including PBMCs, immortalized lymphoid cell lines and immortalized adherent cell lines (astrocytes and trophoblasts), all of which play a key role in HIV-1 infection. The 2G-NN16 dendrimer was used as therapeutic tools for delivering specific small nucleic acids that modulate the gene expression of target cells [5,8,14]. However, to date, we have not evaluated whether 2G-NN16 dendrimer can be used as efficient transfection agents with primary CD4 T lymphocytes and macrophages. Moreover, we have studied the transfection efficiency and functionality of a newly synthesized second-generation $[G_2O_3(SiONN)_{12}]_{24}^+$ (2G-03NN24) carbosilane

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dendrimer [15]. Since some authors have been demonstrated that molecular weight and surface groups of nanocarriers could be modifying its ability to release proteins or drugs [16,17]; the use of 2G-03NN24 dendrimer has enabled us to compare the effects of its functional groups with those of the functional groups of the 2G-NN16 dendrimer.

We have evaluated the effects of 2G-NN16 and 2G-03NN24 dendrimers on lymphoid and myeloid lineage cells, both of which are involved in HIV-1 infection. CD4 T lymphocytes, the principal target of HIV-1 infection, were chosen as representative cells of the lymphoid lineage; macrophages, the immune system's first line of defense, were chosen as representative cells of the myeloid linage. Macrophages contribute to HIV-1 pathogenesis in mucosal rather than lymphatic tissues. Mononuclear phagocytes exhibit a wide spectrum of polarizations that can change their sensitivity to, and alter the outcome of, HIV-1 infection. Viruses generally induce an M1-like polarization phenotype during the acute phase of infection, and this polarization might alter macrophage susceptibility to HIV-1 infection [18]. Moreover, macrophages are one of the cellular reservoirs of HIV-1 and are responsible for promoting the spread of the virus and hindering the action of antiretroviral drugs, including HAART [19].

Although treatments against HIV-1 infection have mainly targeted HIV-1-encoded enzymes, including reverse transcriptase and protease, new strategies that complement such therapies target the expression of the auxiliary gene Nef, often offering better treatment outcomes [20]. Additionally, Nef has the ability to interact with and recruit host machinery through several functional domains on its primary structure, enhancing viral replication [21,22]. *In vitro*, its expression can increase virus titers by more than two on a logarithmic scale during the early phase of HIV-1 infection. This behavior favors the initial viral spread and onset of AIDS in HIV-1-infected patients [23]. Therefore, we used siRNA-Nef (siNEF) to compare and determine whether these dendrimers could be used as transfection agents in an HIV-1 context.

Accordingly, we rigorously evaluated the effect of 2G-NN16 [24] and 2G-03NN24 [15] dendrimers as vectors for siNEF delivery in primary CD4 T lymphocytes, and we studied whether these cationic carbosilane dendrimers can modify macrophage polarization and induce an inflammatory response.

2. Material and methods

2.1. Dendrimers

Carbosilane dendrimers were synthesized according to described procedures [15,24]. The dendrimers used were 2G-$[Si\{O(CH_2)_2N(Me)_2^+(CH_2)_2NMe_3^+(I^-)_2\}]_8$ and $[G_2O_3(SiONN)_{12}]^{24+}$, which are referred to as 2G-NN16 and 2G-03NN24, respectively. These abbreviations are based on the number of positive charges each dendrimer possesses (Fig. 1). The difference between these two dendrimers is the amount of quaternization of the exterior amine groups on each branch. At pH 7.4 (physiological pH), the two dendrimer variants possess 16 and 24 positive charges, respectively, as shown by ^1H and ^{13}C NMR spectroscopy measurements [23].

2.2. Primary cell cultures

Human peripheral blood mononuclear cells (PBMCs) were isolated from the buffy coats of healthy donors on a Ficoll-Hypaque density gradient (Rafer) according to standard procedures of Spanish HIV HGM BioBank [25]. CD4 T cells and monocytes were purified using immunomagnetic CD4 and CD14 microbeads (Miltenyi Biotec), respectively. Purified CD4 T cells were stimulated for 48 h with 2 μg/mL of phytohemaglutinine (PHA) (Remel) and 50 IU/mL of interleukin 2 (IL-2) and seeded in RPMI 1640 medium (Sigma) with 10% fetal bovine serum (FBS), 1% L-glutamine, and an antibiotic mix (125 μg/mL ampicillin, 125 μg/mL cloxacillin and 40 μg/mL gentamicin; Sigma) in a 5% CO_2 environment at 37 °C. Monocytes were cultured at 0.5×10^6 cells/mL for 7 days in RPMI medium supplemented with FBS using same conditions. Here though, the RPMI contained recombinant human granulocyte macrophage colony stimulating factor (rh GM-CSF; ImmunoTools SL) so that monocyte derived macrophages could be generated.

2.3. MTT assay

Cellular mitochondrial metabolism was evaluated by detecting the reduction of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium-bromide (MTT) as an indicator of dendrimer-induced toxicity. Cells were treated with different concentrations of dendrimers (5–15 μM) or dendriplexes containing different types of siRNA. After 24 h, MTT was added to the cultured cells. After 4 h, the reaction was stopped by adding DMSO, and the sample plate was evaluated using a plate reader.

2.4. Proliferative assay

CD4 T lymphocytes were treated with 2G-NN16 dendrimers (5 μM) or 2 μg/mL PHA (Remel) at different concentrations for 48 h. Cells were pulsed with 1 mCi of [3H] thymidine (PerkinElmer) for 16 h in culture and then harvested in glass fiber filters (PerkinElmer) using an automatic cell harvester. Radioactivity incorporation was measured with a 1450 MicroBeta Luminescence Counter (PerkinElmer).

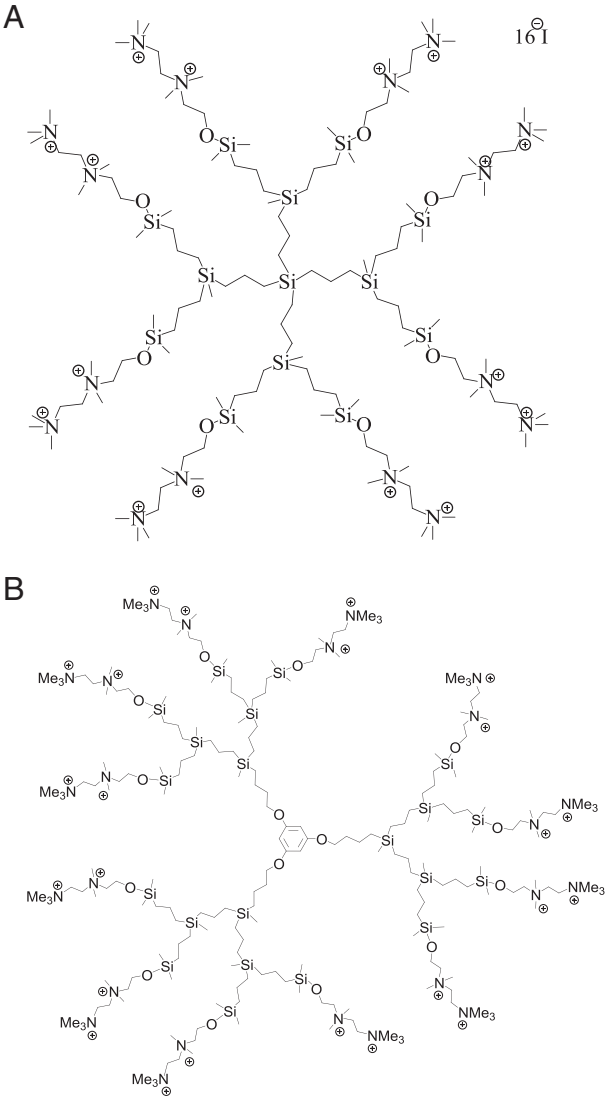

Fig. 1. Schematic representation of dendrimer structure. 2G-NN16 (A) and 2G-03NN24 (B) carbosilane dendrimers.