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Research paper

HIV-1 antiviral behavior of anionic PPI metallo-dendrimers with EDA core

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ABSTRACT

The development of novel strategies to prevent HIV-1 infection is of outstanding relevance. Metal complexes of Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+} derived from sulfonated and carboxylated poly(propylene imine) dendrimers with ethylenediamine core were evaluated as tunable antiviral agents against HIV-1. After demonstrating their biocompatibility, specific trends in the antiviral properties were found, related to both the dendritic scaffold (peripheral group, generation) and the bound metal ions (sort, amount). In HEC-1A and VK-2 cell lines, as model of the first barrier against HIV-1 infection, a high preventive inhibitory action was found, which also avoided virus internalization inside cells and inhibited both CCR5 and CXCR4 HIV-1 strains. In peripheral blood mononuclear cells (PBMC), as model of the second barrier, a dual preventive and therapeutic behavior was observed. A rational design of such metallodendrimers opens new avenues for the production of versatile and efficient treatments against HIV-1 infection.

1. Introduction

Due to the raising number of new HIV-1 infections, mainly through sexual transmission [1], an enormous amount of research is being conducted on the pathogenesis, prevention and transmission of this disease. The main objective – the development of a protective vaccine that efficiently controls or eliminates the spread of HIV-1 [2–4] – has not been achieved so far [5–7]. Thus, the basis for controlling the HIV/AIDS epidemic depends on prevention methods and new strategies [8].

A broad range of these new strategies relies on compounds acting on the first steps of HIV-1 infection cycle – i.e. adsorption and fusion of the virus to the cell-, such as topical microbicides [9]. This kind of compounds avoid the interaction between the trimeric protein complex composed of gp120/gp41 on the HIV envelope and the receptors and co-receptors CD4, CXCR5 and CXCR4 and other molecules of the target cell surface [10]. Several compounds interfering in these first steps of the HIV-1 cycle are based on multivalent systems, whose antiviral potency is determined by the amount of active moieties and the scaffold on which these moieties are supported (e.g. polymerized surfactants, micelles, cyclodextrins, dendrimers). Dendrimers have shown promising properties as antiviral agents due to their controlled synthesis and monodisperse architecture and have been mainly functionalized with three types of molecules [11]: carbohydrates [12–19], peptides [20–22] or anionic groups [23]. Dendrimers decorated with anionic moieties interfere in the early stages of viral infection by interacting with the V3 loop of gp120 protein. For example, anionic PAMAM dendrimers have demonstrated to decrease the infection of the cells [24]. Likewise, polylysine dendrimers decorated with carboxylate and sulfonate groups inhibited the entrance of HIV-1 into cells *in vitro*, and in the case of the sulfonated one, acted also on enzymes such as reverse transcriptase and integrase [25,26], as well as sulfonated porphyrins [26]. The SPL7013 polylysine

Abbreviations: HIV-1, Human Immunodeficiency Virus type 1; PBMC, peripheral blood mononuclear cells; ELISA, enzyme-linked immunosorbent assay; CXCR4, C-X-C chemokine receptor type 4; CCR5, C-C chemokine receptor type 5.

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dendrimer (VivaGel), decorated with naphthalene disulfonate moieties showed microbicide activity in vaginal topic use [27], as well as the anionic carbosilane dendrimers developed in our group, which are effective topical microbicides against HIV-1 [28–33].

The design of efficient anti HIV-1 agents has approached some other strategies involving multivalent systems. Complex dendritic architectures which combine different antiviral moieties have been developed. The inclusion of anionic groups in glycodendritic architectures led to efficient antagonists in *in vitro* assays, where the HIV-1 binding was dependent on both carbohydrates and anionic groups. This is the case of PPI dendrimers with randomly sulfated galactose residues [17,34] or other anionic trisaccharides [17], or polylysine dendrimers with sulfated cellobiose [35]. Glycodendropeptides with up to 16 peptides and 9 mannose moieties appear as an attractive molecular tool for fine-tuning the immune response against viral infections [36]. Another approach is based on the inclusion of metal moieties in the dendritic scaffolds, which clearly increase the range of their applications due to the different properties of metals and modulate the activity of dendritic macromolecules where they are anchored [2,37–42]. This is the case of the copper complexes of anionic carbosilane dendrimers previously synthesized in our group. We demonstrated that the peripheral anchoring of the metal improves the antiviral activity against HIV-1 compared to the dendrimer alone [43].

As starting point of this study, we prepared sulfonated and carboxylated-containing N-donor ligands and their corresponding metal complexes based on Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} [44,45]. These complexes presented a dual preventive-therapeutic behavior when treating HIV-1-infected PBMC, but their activity was limited by the low number of anionic moieties. The development of multivalent systems with higher amount of anionic groups could improve such antiviral properties. With these premises, we generated poly(propyleneimine) dendrimers with an ethylenediamino core and multiple carboxylate or sulfonate groups in the periphery [46]. These systems exhibited a specific pattern on the coordination of metal ions, as we demonstrated by means of UV–Vis and EPR spectroscopy using Cu^{2+} as a probe (Fig. 1). The first equivalent is bound in the core of the dendrimer with a CuN_2O_2 geometry (*I Signal* in EPR spectra), while the following equivalents are distributed in the structure in a precise way, mainly with $CuNO_3$ (*II Signal*) and CuO_4 (*III Signal*) geometries depending on the generation and terminal groups nature. Such control over metal distribution on anionic PPI dendrimers pushed us to evaluate the antiviral behavior of the resultant metallodendrimers and its correlation with properties such as the type and amount of metal ions or the generation of the dendrimer. The study was expanded to evaluate diverse metal ions (M = Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+}) at increasing dendrimer:metal (D:M) ratios. Our preliminary *in vitro* results show that these metallodendrimers may act as potential candidates for inhibition of HIV-1 infection.

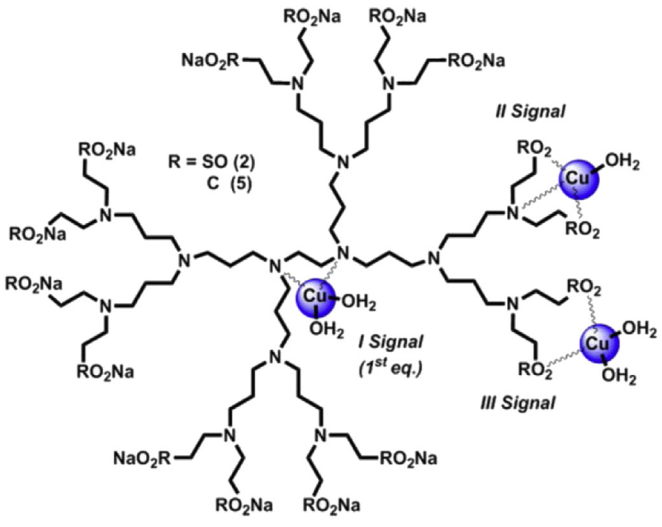

Fig. 1. Metal-coordination ability of the dendrimers. Second generation dendrimer model structure and metal coordination points.

2. Materials and methods

2.1. Samples preparation

The previously developed PPI dendrimers with peripheral sulfonate and carboxylate groups [46] were used to prepare metallodendrimers with Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+}, at different dendrimer:metal (D:M) ratios (G1, ratios 1:1, 1:3 and 1:5; G2, ratios 1:1, 1:5 and 1:9; and G3, ratios 1:1, 1:9 and 1:17). The stock solutions of dendrimers (*stock D*: 800, 400, 200 and 40 μM) and the different metal salts (*stock M*: 4 mM) were prepared by dissolution of the solids in sterile water, while those of metallodendrimers (*stock MD*) were prepared by mixing the right amounts of *stock D* (800 μM) and *stock M* (Table S1, ESI), and stirring the mixture at room temperature for 4 h. Due to the hygroscopic properties of the isolated metallodendrimers, oily solids difficult to handle, the samples were prepared and maintained in solution. However, the degree of metal coordination to the dendritic macromolecule was also evaluated by nanofiltration of the mixtures against dialysis membranes of 500 g/mol cut-off with water. No free metal ions in the washing solutions were detected by UV–Vis and ^{1}H NMR. Control solutions of the dendrimers (*control D*) and the metal salts (*control M*) were prepared at 182 μM and 3.1 mM, respectively. They were added in different volumes depending on each experiment. For biocompatibility assays, cells were treated with 5 μL of each dendrimer, at final concentrations of 1, 5, 10 and 20 μM. In the case of the metallodendrimers, all experiments were performed with the amounts indicated in Table S1 (ESI), to final concentrations of 10 μM.

2.2. Biomedical assays

2.2.1. Primary cells and cell lines

Blood samples were obtained from healthy anonymous donors from transfusion centers of Madrid (Spain), following national guidelines. Peripheral blood mononuclear cells (PBMC) were isolated on a Ficoll-Hypaque density gradient (Rafer, Zaragoza, Spain) following the current procedures of Spanish HIV BioBank [47]. PBMC were cultured in RPMI-1640 medium (Gibco, Paisley, UK) containing 10% heat-inactivated FBS, 1% (2 mM) L-glutamine and antibiotic cocktail (1% ampicillin, 1% cloxacillin and 0.32% gentamicin; Sigma, St-Louis, MO, USA) at 37 °C in a 5% CO_2 atmosphere. Prior to compounds treatment, PBMC were activated for 48 h with 1 μg/mL of phythemagglutinin (PHA, Remel, Santa Fe, USA) and 60 U/mL of recombinant interleukin-2 (IL-2, Bachem, Bubendorf, Switzerland).

Hec-1A cells (epithelial cell line, derived from human endometrial adenocarcinoma, uterus mucosa carcinoma) and VK-2 (epithelial cell line, derived from human vagina mucosa) were obtained through ATCC. Hec-1A were grown in McCoy's 5A Medium Modified (Biochrom AG©, Berlin, Germany), supplemented with 10% of FBS, 2 mM L-glutamin (ICN Pharmaceuticals, CostaMesa, CA, USA) and antibiotic cocktail (1% penicillin/streptomycin). VK-2/E6E7 were grown in Keratinocyte-Serum Free Medium (GIBCO-BRL 17005-42) with human recombinant EGF (0.1 ng/ml), bovine pituitary extract (0.05 mg/ml, Sigma–Aldrich)