Targeted Nano-NRTI Formulations for Suppression of HIV-1 Infection in the Brain

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ABSTRACT SUMMARY
A brain-targeted antiviral nanoformulations of triphosphorylated nucleoside reverse transcriptase inhibitors (nano-NRTI) were developed and evaluated using several in vitro and in vivo models. These drug formulations demonstrated the ability to penetrate the blood-brain barrier (BBB), accumulate in infected macrophages, and inhibit the HIV-1 activity in humanized mouse model of HIV-1 infection in the brain.

INTRODUCTION
HIV-1 infection is difficult to treat in the brain, where the virus can persist in brain-associated phagocytic cells during standard chemotherapy. The BBB prevents the drug ability to reach effective therapeutic levels in the brain. The chronic HIV-1 infection may cause inflammation death of neurons and severe neuropathy.

Cationic nanogels, the soft compact networks of charged polymer molecules, have been shown previously to cross the BBB by transcytosis and can carry anionic drugs such as oligonucleotides into the brain1. Later, we developed tumor-targeted nanogels that could deliver cytotoxic nucleoside analogs in the active form of 5’-triphosphates inside cancer cells2. A similar approach for efficient delivery of triphosphorylated nucleoside reverse transcriptase inhibitors (NRTI) using cationic nanogels also demonstrated high antiviral effects in HIV-1 infected macrophages2. Here, we further developed nanogel formulations of triphosphorylated NRTI (nano-NRTI) modified by brain-specific peptides for systemic HIV-1 treatment in the brain using the humanized mouse model of HIV-1 infection in the brain.

EXPERIMENTAL METHODS
Non-crosslinked cationic nanogels were synthesized from a branched PEI linked by disulfide bonds into polyamine PEIs and modified with cholesterol molecules. Brain-targeted nanogels were obtained using sonication of the cholesteryl-PEIs (CPEIs) in water followed by the stepwise reaction with a bifunctional linker MAL-PEG-NHS and a peptide vector. Peptide binding apolipoprotein E receptor (ApoE) overexpressed on the BBB or homing peptides selected by in vivo panning with phage display library (BP1-2) were used for the modification4. Nanogels were characterized by NMR spectra and colorimetric TNBS titration of amino groups. Peptide modification rates in nanogels were calculated by the quantitative amino acid analysis after acidic hydrolysis. Drug content in AZT-TP-loaded nanogels (nano-AZT) was determined by UV absorbance using the extinction coefficient for AZT5. Particle size and zeta-potential of nano-NRTI were measured by dynamic light scattering (Zetasizer Nano-90).

Transcellular transport studies were carried out using confluent monolayers of murine brain endothelial cells (bEnd3) at 37°C. Accumulation of RITC-labeled nano-NRTI was studied in monocyte-derived macrophages (MDM) by FACS. Antiviral activity was evaluated by the effect of nano-NRTI on the inclusion of 3H-thymidine in HIV-1 infected MDM by reverse transcriptase.

Antiviral effect of the i.v. administered nano-NRTI was tested in the humanized mouse model of HIV-1 infection in the brain6. Briefly, HIV-1 infected MDMs have been stereoinjected in one brain hemisphere of NOD-skid mice. The next day, three groups of mice obtained injections of saline, AZT (4 mg/kg), or nano-AZT formulation (20 mg/kg). The treatment continued every other day; the viral load and MDM number in the brain were determined on day 14.

Figure 1. ApoE peptide-modified PEG-CPEIs cationic nanogel (a), and 3’-azidothymidine 5’-triphosphate used in nano-AZT preparation (b).
RESULTS AND DISCUSSION

Vectorized nanogels with the peptide content between 27-46 µmol/mg and diameter dₜ between 35-50 nm were synthesized. An average AZT-TP loading was equal to 18-23%, generating compact nano-NRTIs (dₜ, 14-22 nm) (Figure 1). In vitro drug release from nano-AZT is equal to 95% in 72 h.

Peptide-nanogels demonstrated 1.5-2.4-fold faster penetration of bEnd3 cell monolayer, an in vitro model of the BBB, than non-modified nanogel (Table 1).

**Table 1.** Permeability of vectorized nano-NRTI in confluent bEnd3 cell monolayers

<table>
<thead>
<tr>
<th>peptide-nano-NRTI</th>
<th>Pᵥapp cm/s</th>
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<tbody>
<tr>
<td>control (cysteine)</td>
<td>3.7 x 10⁻⁴</td>
</tr>
<tr>
<td>ApoE</td>
<td>5.5 x 10⁻⁴</td>
</tr>
<tr>
<td>BP1</td>
<td>5.8 x 10⁻⁴</td>
</tr>
<tr>
<td>BP2</td>
<td>8.8 x 10⁻⁴</td>
</tr>
</tbody>
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Permeability coefficient Pᵥapp = (dx/dt)/A x C₀ x 60, where dx/dt – carrier transfer in acceptor cell, µg/min, A – bEnd3 cell area, cm², C₀ – carrier concentration in donor cell

MDMs efficiently captured nanogels (10-250 µg/mL) with saturation after 1-2h incubation. Nanogels showed no toxicity to MDMs at the concentrations up to 700 µg/mL.

When HIV-1 infected MDMs (moi 0.05) were treated with nano-AZT, the effect on viral activity was several times higher compared to AZT treatment at the same dose (Figure 2).

![Figure 2. Dose-response curves of antiviral activity on Day 5 of AZT, nano-AZT and ApoE-nano-AZT HIV-1 infected MDM: 4h treatment.](image)

In animal models, we observed a 5- and 10-fold statistically significant (P < 0.05) reduction of HIV-1 viral load in human macrophages in the brain following the treatment with nano-AZT and ApoE-modified nano-AZT, respectively (Figure 3). Significant reduction in expression of inflammatory factors TNFα and IL-1β was also registered in nano-AZT treated mouse brains.

![Figure 3. Inhibition of viral proliferation in brains of IV-treated mice. The data show real-time RT qPCR results for the brain RNA samples (n = 3)](image)

CONCLUSION

Nanocarriers modified with brain-specific peptides were developed for the delivery of activated NRTI drugs to HIV-1 reservoirs in the brain. These nano-NRTI formulations showed advanced physicochemical properties, robust antiviral activity, efficient penetration through the BBB, and the ability to significantly reduce the HIV-1 load in the brain in animal model.

REFERENCES


ACKNOWLEDGEMENTS

Financial support was provided by grant NS076386 from the National Institute of Neurodegenerative Diseases and Stroke (S.V.).