Nanoparticle-mediated brain delivery of a tetracationic porphyrin with potent anti-prion activities

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Project objectives

Prion diseases are caused by the conformational conversion of the cellular prion protein (PrP^{C}) into a misfolded isoform (PrP^{Sc} or prion) that propagates by inducing misfolding of native PrP^{C}. Possible therapeutic strategies include downregulating PrP^{C} level, thus reducing the substrate for PrP^{Sc} formation, or blocking the process of PrP^{C} to PrP^{Sc} conversion.
We identified a tetracationic porphyrin (VA01) which does both. VA01 potently inhibits PrP^{Sc} replication in cell-free reactions (PMCA), and in prion-infected cells and organotypic cerebellar cultures (COCS). When injected in mice a fraction of VA01 crosses the blood-brain barrier (BBB) and reaches the brain, but its brain concentrations are too low to achieve robust and consistent anti-prion effects.
The overall aim of this projects was to use functionalized nanoparticles (NPs), which are very small spherical shuttles able to cross the BBB, to deliver VA01 to the brain. Specifically, we aimed: 1) to study the ability of VA01-loaded NPs to reach the brain of mice after peripheral injection; and 2) to carry out efficacy tests in prion-infected mice.

Accomplishments

We optimized the protocols to efficiently load VA01 into PLGA [poly(lactide-co-glycolide)] NPs modified with the g7 glycopeptide, which in previous studies had shown a remarkable ability to cross the BBB and deliver drugs to the brain. VA01-NPs had similar anti-prion activities as the free VA01 when tested *in vitro*: they down-regulated PrP^{C} in neuronal cultures and decreased PrP^{Sc} levels in prion-infected COCS. Surprisingly, however, VA01-NPs exhibited poor BBB passage *in vivo*. Studies in mice indicated that the VA01-NPs accumulated mainly in the kidney, spleen and liver. This could be due to some VA01 present on the external surface of the NPs interfering with their ability to penetrate the brain. To overcome this problem, we tried a number of different approaches, such as extensively washing the VA01-loaded NPs to get rid of residual VA01 bound to their surface, and changing the loading procedure in order to chemically bind the brain-targeting g7 glycopeptide to the NPs after loading them with the porphyrin. However, none of these approaches improved the NP ability to deliver VA01 to the brain.

Conclusions

VA01-NPs retain the anti-prion properties of the free VA01 *in vitro*, but unfortunately they do not cross the BBB as efficiently as expected, failing to deliver VA01 to the brain.

Next steps

We are investigating the mechanism by which VA01 interferes with the g7-PLGA NP ability to cross the BBB. This will allow us to devise a methodology to solve this problem. We would also like to test other types of NPs, which use different brain-targeting peptides or are made of a different material.

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