Nanotechnology approaches for improved based-drug delivery systems of the immunomodulatory neuropeptide vasoactive intestinal peptide

Rebecca Klippstein[1], *Paula M. Castillo*[1], *Rafael Fernandez-Montesinos* [1], *Ana P. Zaderenko* [2] *and David Pozo* [1]

[1]*CABIMER-Andalusian Center for Molecular Biology and Regenerative Medicine (CSIC-University of Seville, UPO, Junta de Andalucia), Seville, Spain*
[2]*Department of Physical, Chemical and Natural Systems, UPO-University Pablo de Olavide, Seville, Spain*
david.pozo@cabimer.es

In spite of sustained interest in therapeutic applications of vasoactive intestinal peptide (VIP), and the fact that its administration could be largely improved by attachament to functionalized metal nanoparticles, no methods have been described so far to obtain them. The primary aim of the current study was to provide a method for silver nanoparticle conjugation to VIP that would be also useful for tailor-made applications based on nanoparticle multifunctional capabilities (Fernandez-Montesinos et al. 2009). The biological features of VIP are of particular interest to develop multifunctional nanoparticles for innovative therapeutic approaches for human diseases with inflammatory or autoimmune components (Pozo 2008; Delgado et al. 2004). VIP is a 28-aminoacid peptide (His-Ser-Asp-Ala-Leu-Phe-Thr-Asp-Thr-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Met-Lys-Lys-Tyr-Leu-Asn-Ser-Val-Leu-Asn) that was initially isolated from the gastrointestinal tract due to its capacity as a vasodilator (Said and Mutt 1970). VIP was subsequently identified in the central and peripheral nervous systems, and recognized as a widely distributed neuropeptide. VIP exerts its biological functions through interaction with VPAC specific receptors belonging to the class II G protein-coupled receptors. Among its physiological roles, VIP and VPAC receptors have shown their relevance as endogenous factors that regulate inflammatory immune responses and immune tolerance, emerging as a very promising therapeutic factor (Pozo 2003; Pozo and Delgado 2004; Pozo et al. 2007). The mechanisms involved include the deviation towards Th2-driven inflammatory pathways, the specific recruitment and development of Th2 cells, and the peripheral expansion of regulatory T cells (Pozo et al. 2009; Chorny et al. 2005; Gonzalez-Rey et al. 2007). Also, VIP and VPAC receptors are overexpressed in 100% of human prostate cancers (Reubi and Maecke 2008; Reubi 2003). In spite the fact that the structure-function relationships of VPAC receptors are well known, the structure-function relations for VIP are however poorly understood. In this sense, VIP has been shown to have diffuse pharmacophoric domains, with important amino acids all along the peptide for binding to VPAC1 and VPAC2 receptors (Ceraudo et al. 2008). Photoaffinity labelling, molecular dynamic simulation and ligand docking studies have determined that the C-terminal part of VIP from Phe^{6} to Asn^{28} interacts with the N-terminal ectodomain of human VPAC1 receptor. Recently, it has been shown that the N-terminus of VIP also interacts with the human VPAC1 receptor N-terminal domain. VIP was conjugated to tiopronin-capped silver nanoparticles of a narrow size distribution, by means of proper linkers, to obtain VIP functionalized silver nanoparticles with two different VIP orientations Ag@tiopronin@PEG@succinic@[His]VIP and Ag@tiopronin@PEG@VIP[His]. VIP intermediate nanoparticles were characterised by TEM, FTIR, Raman, ^{1}H-NMR and TOCSY. VIP functionalized silver nanoparticles cytotoxicity was determined by LDH release from mixed glial cultures prepared from cerebral cortices of 1-3 days-old C57/Bl mice. Cells were used for LPS stimulation at day 18-22 of culture. Mixed cultures were checked by immunocytochemistry for high enrichment of GFAP and CD68 positive reactive cells, identifying astrocytes and microglia, respectively. Supernatants from mixed glial cells cultures were harvested 24 hours after treatment, and IL-6, TNF-α, and IL-10 production was

determined by ELISA. Two different types of VIP functionalized silver nanoparticles were obtained; both expose the C-terminal part of the neuropeptide, but in the first type VIP is attached to silver nanoparticle trough its free amine terminus Ag@tiopronin@PEG@succinic@[His]VIP while in the second type, VIP N-terminus remains free Ag@tiopronin@PEG@VIP[His]. VIP functionalized silver nanoparticles did not compromise cellular viability and inhibited microglia-induced stimulation under inflammatory conditions. Treatment of primary mixed glial cultures with Ag@tiopronin@PEG@Succinic@[His]VIP or Ag@tiopronin@PEG@VIP[His] nanoparticles with a final concentration of functionalized VIP of 10^{-8}M resulted in an inhibition of LPS-induced production of IL-6 and TNF-α and an increase of IL-10. We have exploited the potential of nanoparticle functionalization as an alternative approach to improve the therapeutic prospect of the endogenous cytokine-like peptide VIP. Our results showed the proof-of-concept for its use, as the chemical synthesis procedure developed to obtain VIP functionalized silver nanoparticles rendered functional products, in terms of biological activity, without any observed cytotoxic effects. The present work provides functional data that demonstrates that VIP can be conjugated to tiopronin-capped silver nanoparticles in two alternative orientations, involving or not the VIP N-terminus, without loss of biological activity. This information is especially valuable for other studies aiming at including VIP in formulations where the possibility of chemical synthesis constraints exists depending on the nanosurface to be functionalized. Our study provides for the first time a proof-of-principle to enhance the therapeutic potential of VIP with the valuable properties of metal nanoparticles for imaging, targeting, and drug delivery. Our study provides for the first time a proof-of-principle to encourage the development of VIP-based nanoparticles that exploit the valuable properties of silver nanoparticles for imaging, targeting, and drug delivery.

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