Monitoring the intracellular transfer of chitosan nanoparticles by transmission electron microscopy

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Chitosan-based nanoparticles (chiNPs) are biocompatible polymeric drug carriers; they are able to prolong drug activity by stabilizing and modulating the release of the encapsulated agent, which allows its delayed delivery after NP administration (1). Due to their polycationic nature, chiNPs easily interact with the cell membrane thus rapidly crossing endothelial linings and entering cells; they are also able to cross the blood brain barrier (2).

Based on these features, we choose chiNPs as promising drug delivery systems for targeting the hypometabolizing D-Ala(2)-D-Leu(5)-enkephalin (DADLE) to the central nervous system (3). The induction of a hypometabolic state is of potential interest for surgical procedures, for preserving organs for transplantation, or for neuro- and cardio-protection. However, in the attempt to design a suitable drug delivery strategy, preliminary studies on target cells are required to elucidate the uptake mechanisms of NPs and their intracellular fate, with special reference to their degradation pathway.

Transmission electron microscopy is a valuable tool to investigate the intracellular trafficking pathway of NPs and clarify their interaction with organelles; however, due to their moderate homogeneous electron density, chiNPs are almost indistinguishable from the cytosolic milieu. To overcome this difficulty, cytochemical and immunocytochemical approaches were used to make chiNPs unequivocally detectable at the ultrastructural level. Neuronal cultured cells were administered FITC-labelled chiNPs (Fig. 1), and then submitted to DAB photo-oxidation. The resulting reaction product was easily visualized after osmication in epoxy resin-embedded samples (Fig. 2), revealing that chiNPs are internalized by endocytosis and can escape endosomes thus avoiding lysosomal degradation. In acrylic resin-embedded samples DAB precipitates were also visible without osmication; these specimens proved to be optimal for combining DAB photo-oxidation with immunoelectron microscopy, thus allowing the precise identification of the chiNP degradation sites (4).

DADLE-loaded chiNPs were also recognized in neuronal cultured cells by immunogold labeling with an anti-enkephalin antibody and this approach also allowed to label DADLE molecules released from chiNPs into the cytoplasm (Fig. 3) (5).

Preliminary tests ex vivo envisaged promising application of these ultrastructural techniques to also detect chiNPs in explanted tissues and organs, after systemic administration.


Acknowledgement: This work and BC fellowship were funded by Fondazione Cariverona, project Verona Nanomedicine Initiative. MC and VG are PhD students (at the University of Verona and Pavia, respectively).
Fig. 1: Fluorescence microscopy: FITC-labelled chiNPs are distributed in the cytoplasm of a neuronal cell. DNA was stained with Hoechst 33258. Bar, 10 µm.

Fig. 2: Transmission electron microscopy (epoxy resin embedding): a finely granular electron dense product is evident in an endosome-enclosed FITC-labelled chiNP after DAB photo-oxidation. Bar, 150 nm.

Fig. 3: Immunoelectron microscopy (acrylic resin embedding): a chiNP is labelled with the anti-DADLE antibody; some labelling also occurs in the cytosol. Bar, 150 nm.