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REVIEW

Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies

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Summary During recent years there has been much interest in the use of nanoparticles for *in vitro* studies as well as for delivery of drugs and contrast agents in animals and humans. To this end it is necessary to increase our understanding of how these particles are taken up and transported within the cells, and to which extent they are metabolized and secreted. In this review we discuss the possibilities, challenges and pitfalls of studying endocytic pathways involved in cellular uptake of nanoparticles. Thus, the use of pharmacological inhibitors, expression of mutated proteins, use of siRNAs and colocalization experiments in such studies are critically evaluated. Although the main focus is on cellular uptake, also aspects of intracellular transport, recycling of nanoparticles to the cell exterior, disturbance of cellular functions, and metabolism of nanoparticles are discussed.

Introduction

Nanoparticles have emerged as promising tools both for basic mechanistic studies of cells and animals, as well as for delivery of drugs or other substances *in vitro* and *in vivo* [1—6]. The rate of uptake and intracellular localization of nanoparticles have been studied by many research groups, and several review articles summarizing the published data are available; see e.g. [7—13]. These reviews reveal that it is difficult to draw general conclusions about how to produce particles for optimal cellular uptake, as the rate and mechanism of uptake turns out to be cell-type dependent

Abbreviations: CME, clathrin-mediated endocytosis; CIE, clathrin-independent endocytosis.

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and vary between nanoparticles with different size, charge, and other surface properties. There are, however, several reports showing that nanoparticles of 20—50 nm are taken up more rapidly than smaller or larger particles [14—16]. Because particles with a positive charge will bind to the negatively charged cell surface, one would expect positively charged particles to be endocytosed more efficiently than negatively charged particles. In fact, a study in HeLa cells with positively and negatively charged nanoparticles of equal size (80 nm) showed a 2-fold higher uptake of the positively charged particles [17]. In contrast, a higher uptake of negatively charged nanoparticles has been reported in HEK cells [18]. As discussed below, many of the conclusions drawn about cellular uptake of nanoparticles need to be re-evaluated in light of the present knowledge of endocytic mechanisms.

Cell-type specific variation in handling of internalized particles can be expected, and significant differences in intracellular sorting, trafficking and localization of nonconjugated quantum dots (QDs) have been reported in three closely related human prostate cancer cells [19]. It is clear that for delivery of nanoparticles to heterogeneous tumours, differences in cellular uptake and sorting can have significant implications. Importantly, the polyvalent surface of nanoparticles may induce cross-linking of cellular receptors, start signalling processes, induce structural alterations at the cell surface, and interfere with normal cell function [15,20]. Moreover, when studying cellular uptake of nanoparticles one should keep in mind that the rate of endocytosis may also depend on the cell density [21,22].

So far most focus has been on uptake of nanoparticles into non-polarized cells. Importantly, polarized cells can have different endocytic mechanisms on the apical and basolateral pole [23]. Thus, a nonpolarized epithelial cell cannot be expected to correctly reflect the complexity found in epithelial cell layers where clathrin-independent endocytosis (CIE) is selectively regulated at the apical side and caveolae can be found exclusively at the basolateral side [23].

There is still a lot to learn about cellular uptake and intracellular transport of nanoparticles in order to interpret data from *in vitro* studies and to improve the *in vivo* use of the particles. Also, the recent report that caveosomes is an artifact in cells overexpressing caveolin [24] is important for re-interpretation of data already published regarding intracellular localization and degradation of nanoparticles.

In this article we present a summary of the present knowledge of different endocytic mechanisms and we describe how involvement of the various endocytic pathways in uptake of nanoparticles can be studied, including the pitfalls in performing such studies. We also shortly discuss some aspects of intracellular transport of particles, recycling to the cell exterior, metabolism and disturbances of cellular processes caused by nanoparticles.

Endocytic mechanisms

Cells use endocytosis for uptake of nutrients, down-regulation of growth factor receptors and as a master regulator of the signalling circuitry. There are several different types of endocytosis, all based on formation of intracellular vesicles following invagination of the plasma membrane or ruffling giving rise to larger vesicles [25—28]. Phagocytosis (''cell eating'') is used for uptake of large particles such as bacteria, and is the first step in uptake and degradation of particles larger than 0.5 μm. Pinocytosis (''cell drinking'') is used to internalize fluid surrounding the cell, implying that all substances in the fluid phase area of invagination are taken up simultaneously. There are multiple types of endocytic pathways distinguished by specific molecular regulators as shown in Fig. 1. The clathrin-mediated endocytosis (CME) is by far the best studied of these mechanisms and was for a long time believed to be the only endocytic mechanism in addition to phagocytosis and macropinocytosis. However, during the last 20 years several mechanisms of CIE have been described [25,28,29]. These include dynamin-dependent mechanisms (RhoA and caveolin-caveolae/lipid raft dependent) and dynamin-independent mechanisms (Cdc42 dependent and Arf6 dependent). The Cdc42/Arf1 dependent uptake has by some authors also been called the CLIC/GEEC pathway (CLIC, clathrin-independent carrier; GEEC, GPI-AP (glycosyl-phosphatidylinositol-anchored proteins) enriched early endosomal compartment) [28]. Depending on the receptor studied, the so-called ''receptor-mediated endocytosis'' can involve various mechanisms of endocytosis and should therefore not be used synonymously with clathrin-mediated endocytosis as one sometimes can see in the literature. One can expect an increase in complexity not only when it comes to the number of endocytic mechanisms but also regarding their regulation by signalling.

In a major part of the literature on cellular uptake of nanoparticles the discussion is restricted to clathrin-mediated and caveolae-mediated endocytosis in addition to phagocytosis and macropinocytosis. However, the con-

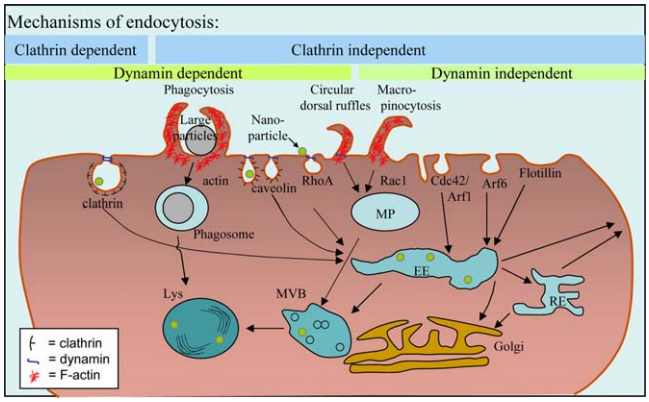

Figure 1 Model of endocytic mechanisms and intracellular transport. Nanoparticles (green dots) and other substances taken up by endocytosis are enclosed within the early endosomes (EE), phagosomes or macropinosomes (MP). These vesicles with particles then mature down the degradative pathway and become multivesicular bodies/late endosomes (MVB) which fuse with lysosomes (Lys). Alternatively, the nanoparticles may be transported back to the cell surface either directly from EE or through the recycling endosomes (RE). The pH drops gradually from the cell surface to lysosomes where pH is 4.0—5.5. The lysosomes contain proteases and other enzymes that degrade most biological substances.