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Ferritin up-regulation and transient ROS production in cultured brain astrocytes after loading with iron oxide nanoparticles

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ABSTRACT

To investigate the cellular consequences of a prolonged cellular presence of large amounts of iron oxide nanoparticles (IONPs) as well as the fate of such particles in brain cells, cultured primary astrocytes were loaded for 4 h with dimercaptosuccinate-coated IONPs. Subsequently, the IONP-treated cells were incubated for up to 7 days in IONP-free medium and the cell viability, metabolic parameters and iron metabolism of the cells were investigated. Despite an up to 100-fold elevated specific cellular iron content, IONP-loaded cells remained viable throughout the 7 day main incubation and did not show any substantial alteration in glucose and glutathione metabolism. During the incubation, the high cellular iron content of IONP-loaded astrocytes remained almost constant. Electron microscopy revealed that after 7 days of incubation most of the cellular iron was still present in IONP-filled vesicles. However, the transient appearance of reactive oxygen species (ROS) as well as a strong increase in cellular levels of the iron storage protein ferritin suggest that at least some low-molecular-weight iron was liberated from the accumulated IONPs. These results demonstrate that even the prolonged presence of large amounts of accumulated IONPs does not harm astrocytes and that these cells store IONP-derived iron in ferritin.

1. Introduction

Due to their small size and their magnetic properties, iron oxide nanoparticles (IONPs) are considered for a wide range of therapeutical and biological applications, e.g. as tools for magnetic hyperthermia, as contrast agents in magnetic resonance imaging (MRI), for cell labelling or for targeted drug delivery [1]. IONPs are also considered as a promising tool for neurobiological applications [2,3]. Although they have been shown to enter the brain either by crossing the blood–brain barrier [4] or via the olfactory neuronal pathway [5], little is currently known of the acute or chronic consequences of a presence of IONPs in brain cells. IONPs which have crossed the blood–brain barrier will encounter astrocytes as the first parenchymal brain cells, since these cells almost completely cover the brain capillaries with their endfeet [6]. Astrocytes are of special interest regarding the uptake and metabolism of IONPs, since these cells are known to take up IONPs in vivo [7,8] and in vitro [9–13], and are considered to play an important role in the iron homeostasis of the brain [14]. Astrocytes are the most abundant cell type in the brain and perform a variety of important functions there, including the supply of metabolic nutrients to neurons and the protection of the brain against metal toxicity and oxidative stress [15–17].

The acute consequences of an exposure of cultured astrocytes for a few hours to IONPs have recently been described. Primary viable astrocytes efficiently accumulate IONPs in a time-, concentration- and temperature-dependent manner [9–13]. Fluorescence and electron microscopy have revealed that IONP-exposed astrocytes contain accumulated IONPs in intracellular vesicles, but have also shown that substantial amounts of IONPs are attached extracellularly to the cell membrane [9–11,13]. These observations, as well as the reported reduction of IONP accumulation by endocytosis inhibitors [13], suggest that endocytotic processes are involved in the uptake of IONPs by astrocytes.

IONPs that had been applied acutely to the brain for therapeutical and analytical purposes, such as cancer treatment via hyperthermia or MRI, remain at the sites of administration and are taken up by phagocytic cells and astrocytes, where they are detectable for at least 7 days [7,8]. Although molecular interactions of IONPs with cells and within cells have raised concerns for potential long-term effects of IONPs [18] and of IONP-derived iron [19], the consequences of a prolonged presence of IONPs on the metabolism and functions of brain cells have to our knowledge not been reported so far. To address such questions, we loaded cultured astrocytes with IONPs for 4 h and subsequently monitored the cell

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