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Disturbance of ion environment and immune regulation following biodistribution of magnetic iron oxide nanoparticles injected intravenously

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HIGHLIGHTS

- In this study, we used magnetic iron oxide nanoparticles (M-FeNPs).
- Mice received a single injection of M-FeNPs via the tail vein.
- At 13 weeks post-injection, M-FeNPs the most distributed in the spleen.
- M-FeNPs altered levels of redox response-related elements in tissues.
- M-FeNPs disturbed ion homeostasis in tissues.
- M-FeNPs attenuated expression of antigen presentation-related proteins.

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ABSTRACT

Although it is expected that accumulation of metal oxide nanoparticles that can induce redox reaction in the biological system may influence ion homeostasis and immune regulation through generation of free radicals, the relationship is still unclear. In this study, mice received magnetic iron oxide nanoparticles (M-FeNPs, 2 and 4 mg/kg) a single via the tail vein, and their distribution in tissues was investigated over time (1, 4, and 13 weeks). In addition, we evaluated the effects on homeostasis of redox reaction-related elements, the ion environment and immune regulation. The iron level in tissues reached at the maximum on 4 weeks after injection and M-FeNPs the most distributed in the spleen at 13 weeks. Additionally, levels of redox reaction-related elements in tissues were notably altered since 1 week post-injection. While levels of K^+ and Na^+ in tissue tended to decrease with time, Ca^{2+} levels reached to the maximum at 4 weeks post-injection. On 13 weeks post-injection, the increased percentages of neutrophils and eosinophils, the enhanced release of LDH, and the elevated secretion of IL-8 and IL-6 were clearly observed in the blood of M-FeNP-treated mice compared to the control. While expression of antigen presentation related-proteins and the maturation of dendritic cells were markedly inhibited following distribution of M-FeNPs, the expression of several chemokines, including CXCR2, CCR5, and CD123, was enhanced on the splenocytes of the treated groups. Taken together, we suggest that accumulation of M-FeNPs may induce adverse health effects by disturbing homeostasis of the immune regulation and ion environment.

1. Introduction

The Organization for Economic Co-operation and Development (OECD) established the OECD Working Party on Manufactured Nanomaterials to promote international co-operation on the human health and environmental safety aspects of manufactured

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nanoparticles in 2006, and at its 7th meeting, it listed 13 high-priority nanomaterials for study (www.oecd.org., 2010). Among them, magnetic iron oxide nanoparticles (M-FeNPs) are attracting considerable interest in medical fields with objectives, such as a contrast agent of magnetic resonance imaging, drug delivery, and hyperthermia of cancer (Antonelli et al., 2013; Rosen et al., 2012; Wahajuddin and Arora, 2012; Sun et al., 2011; Hilger and Kaiser, 2012).

Nanoparticles exhibit different physical, chemical, optical, electrical, catalytic, and mechanical properties with the larger particles due to their unique manufacturing process, and their behavior, distribution, persistence, and bioaccumulation in humans depend on their interactions with human body components (Hagens et al., 2007; Monopoli et al., 2012; Nel et al., 2009; Sayes et al., 2007; Wittmaack, 2007; Yang et al., 2015). For example, on entering the bloodstream, nanoparticles can interact with blood components such as proteins, platelets, and red and white blood cells, and their effects within biological systems may be altered remarkably by the formation of the protein corona. In addition, some researchers suggested that nanoparticles were observed inside red blood cells (RBC) despite the fact that erythrocytes do not have phagocytic receptors (Geiser et al., 2005; Rothen-Rutishauser et al., 2006). This means that nanoparticles be able to cross the cell membrane via another process, such as electrostatic attraction or ion channels, as well a general foreign body uptake process, such as phagocytosis and endocytosis.

The applications of nanoparticles in medicine can represent another exposure route for nanoparticles besides typical exposure pathways, including inhalation, oral, and the skin (Yah et al., 2012). For example, nanoparticles can be injected directly into the body to enhance the contrast in medical imaging or for drug delivery applications, and wear from implanted biomaterials can inadvertently occur in the body (Shinohara et al., 2014; Wang et al., 2009). Additionally, the toxicity of nanoparticles depends on the internal dose (the dose of nanoparticles that reaches the systemic circulation and organs and tissues) rather than the external dose (the total dose of nanoparticles applied via typical exposure pathways) (Hagens et al., 2007; Braakhuis et al., 2014; Oberdörster et al., 2005). Therefore, identification of the target organ for accumulation is essential for the safe and reliable use of nanotechnology in consumer products, food, medicine, and other applications. In this regard, the liver and spleen have been identified as target organs of intravenously injected nanoparticles in some studies, and macrophages have been indicated as the key player in systemic circulation and for biodistribution of nanoparticles (Baratli et al., 2014; Fraga et al., 2014; Fujihara et al., 2015; Fabian et al., 2008; Yang et al., 2015), although a part of the individual nanoparticles can move freely within the body (Park et al., 2014a,b). Furthermore, some researchers recently reported an immunotoxic response following exposure to nanoparticles (Seydoux et al., 2014; Tkach et al., 2013; Altaf and Revell, 2013; Park et al., 2015). However, it remains unclear whether bioaccumulation in these tissues contributes directly to the immunotoxic response observed. Additionally, metal oxide nanoparticles can interact with receptors on the membrane repeating redox reactions in the body (Liu et al., 2006; Park et al., 2014c), in this process, metal oxide nanoparticles can influence the absorption and excretion of trace elements in the body. Furthermore, some metal oxide nanoparticles can be dissolved and ionized in our body, therefore we can guess that metal oxide nanoparticles may affect the intracellular ion environment in our body (Utembe et al., 2015). However, to date, there are very few reports on the effect of nanoparticles on the ion environment in vivo. Herein, we hypothesized that accumulation of M-FeNPs may induce adverse health effects by disturbing homeostasis in the function of immune system and ion environment. For this, we injected a single M-FeNPs (2 and 4 mg/kg) via the tail vein of mice considering that a 20 mg/kg dose is toxic for humans (Velez and Delaney, 2006; Yang et al., 2015), and then investigated biodistribution with time. In addition, we identified changes in the immune regulation and ion homeostasis following tissue accumulation of M-FeNPs.

2. Material and methods

2.1. Preparation and characterization of M-FeNPs

As described previously, M-FeNPs were manufactured by thermal decomposition method of precursors in the organic phase (Park et al., 2014a,b). Briefly, M-FeNPs, 2 mmol iron oleate, 1 mmol oleic acid, and 10 g 1-octadecene (Sigma–Aldrich, St. Louis, MO, USA) were loaded into a 50 mL, 3-necked flask and degassed under vacuum conditions at 100 °C for 1 h (Park et al., 2004). The temperature was raised to 320 °C, and the mixture was stirred for 30 min under an N_2 atmosphere prior to cooling to room temperature (RT). Iron oxide nanocrystals were precipitated from the reaction solution by adding isopropanol and were separated by centrifugation. Nanocrystals were dispersed in tetrahydrofuran (THF), followed by the addition of phospholipid. After evacuation of THF, the mixture was incubated at 50 °C under vacuum conditions for 10 min. Water was added, and unbound phospholipid was eliminated by dialysis. The same steps were performed without iron compounds for the vehicle control. M-FeNPs were spherical type (Supple 1). The surface charge on M-FeNPs prepared and suspended in FBS was -36.8 ± 2.3 and -9.4 ± 0.02 mV,

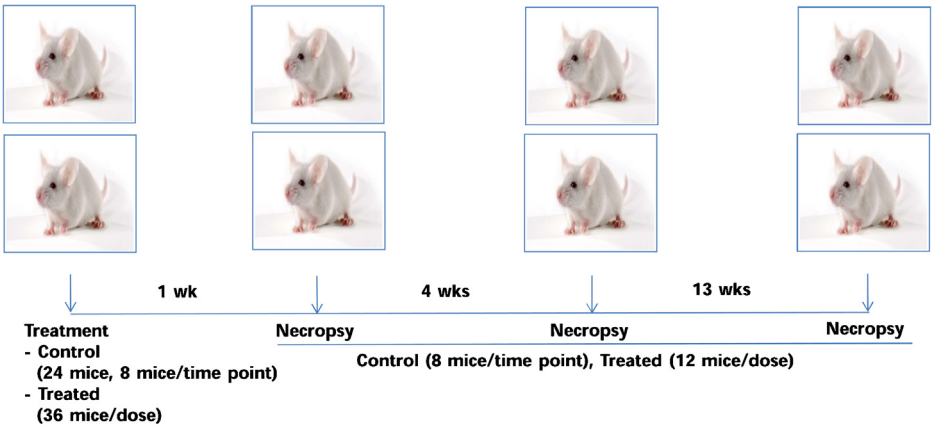

Fig. 1. An experimental design.