The Journal of Basic & Applied Zoology (2015) **71**, 32–47

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The Egyptian German Society for Zoology

The Journal of Basic & Applied Zoology

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Histopathological and immunological changes induced by magnetite nanoparticles in the spleen, liver and genital tract of mice following intravaginal instillation

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Received 21 January 2015; revised 11 February 2015; accepted 30 March 2015
Available online 16 May 2015

KEYWORDS

Magnetite nanoparticles;
Intravaginal instillation;
Histopathology;
Immunology;
Spleen;
Liver;
Genital tract

Abstract Recently, vaccination against sexually transmitted diseases as well as tumor therapy using new systems such as nanomaterials is a promising strategy. For successful usefulness of magnetite nanoparticles (MNPs) in medical applications through the vaginal route, it is essential to understand their biological fate and cellular toxicity in the animal tissues. This study aims to investigate the biodistribution, histopathological and immunological impacts of MNPs on in the liver, spleen and genital tract tissues of female mice after the intravaginal instillation. MNPs were observed within spleen and liver parenchyma as well as vaginal stroma after 3 days and 2 weeks of the instillation, and completely cleared from the vaginal stroma tissue after 6 weeks. Splenic lymphocytes of treated spleen were characterized with anisokaryosis; anisochromia. Quantitatively, the number of megakaryocyte and lymphocyte nuclei size in the spleen were highly increased after instillation of MNPs. MNPs caused acute inflammation in the liver and tarsal joints dermal. Immunologically, MNPs induced the vaginal secretion IgA and Bcl-2 reactivity in the hepatocytes, the expression of fucose residues and number of $BM8^{+}$ cells in the genital tract tissues after instillation. Our data indicated that MNPs could influence the splenic lymphocytes and hepatocytes as well as the cellular and humoral immune responses in the mice genital tract tissues after 2 weeks of intravaginal instillation. This information could be useful for avoiding the side effects of MNPs

Abbreviations: MNPs, magnetite nanoparticles; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Bcl-2, B-cell lymphoma 2; UEA I, Ulex europaeus agglutinin I; PBS, phosphate buffer saline; IgA, immunoglobulin A; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; DLS, dynamic light scattering; PVDF, polyvinylidene difluoride

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Peer review under responsibility of The Egyptian German Society for Zoology.

http://dx.doi.org/10.1016/j.jobaz.2015.03.003

when used in medical applications. Further investigations about the safety and toxicity of MNPs should be achieved before their use in the medical field.

Introduction

Sexually transmitted diseases such as HIV and Herpes have become extremely widespread all over the world especially in England (Hughes and Field, 2015). In addition to the sexually transmitted diseases, genital tract tumors such as uterine and cervical tumors also have been appeared as the main diseases facing women in the world. Therefore, finding the suitable systems for successful vaccination against sexually transmitted diseases or tumor therapy is the main goal for researchers nowadays. Nanotechnology is one of the most effective systems used to overcome these diseases. It is a promising new field with potential applications in the biological and biomedical fields (Nakamura et al., 2012, 2013). In the biomedical applications, the nanomaterials were applied into the animal model tissues through several routes such as oral, intravenous, or subcutaneous routes, etc. Recently, nanomaterials were used a lot for several applications as vaccine delivery using the vaginal route (Whaley et al., 2010). The most studied nanomaterials with promising potential in the field of biomedical applications are those with magnetic properties (Prodan and Ciobanu, 2013). Recently, Fe_3O_4 magnetite nanoparticles (MNPs) have been widely used for biological applications, such as magnetic resonance imaging therapy (Yallapu et al., 2011), in hyperthermia tumor therapy (Hayashi et al., 2013), in drug delivery systems (Ansari et al., 2014) and molecular detection of biomarkers in biological cells (Haun et al., 2011). It is crucial to widely study the biological toxicity and fate of MNPs to ensure their safety for biological and medical applications. Previously, there was no detailed study that revealed the biological abnormalities or biodistribution caused by MNPs after intravaginal instillation. In a literature, after 3 weeks of intravenous administration of MNPs (193 nm), serum iron levels gradually increased for up to 1 week but levels slowly declined thereafter. Also, MNPs were localized in the liver and spleen parenchyma more than other tissues and cleared gradually after 3 weeks of treatment (Jain et al., 2008). In another study, the biodistribution of intravenously injected MNPs (5 nm) showed 75% of injected dose was found in the spleen and liver at 15 min post-injection. Moreover, 24% of the MNPs remain in liver after 48 hrs post-injection (Shanehsazzadeh et al., 2013). Consequently as reported by Briley-Saebo et al. (2004) MNPs were distributed equally in both liver endothelial and Kupffer cells but not liver parenchyma following intravenous injection in rats. Previous studies also did not reveal any detailed results about biodistribution and fate of MNPs after the intravaginal instillation. Previously, quantum dots and PLGA NPs were observed in the draining lymph nodes and female genital tract tissues after intravaginal instillation (Ballou et al., 2012; Cu et al., 2011).

After 2 days of intraperitoneal injection of MNPs (10 nm), the liver showed nuclei atrophy and vacuolar degeneration in hepatocytes. Also, the spleen showed an increase in the monocyte number with chromatin irregularities in the splenic lymphocytes (Prodan and Ciobanu, 2013). Contradictory, after intravenous injection, MNPs (193 nm) did not show any histological abnormalities in the liver, spleen after 7 days of treatment (Jain et al., 2008; Kim et al., 2005). The dose and size of injected NPs can affect the cellular and tissue toxicity in vivo and in vitro. After 2 weeks of intravenous injection of higher dose of titanium dioxide NPs (40 nm), the liver showed hepatocyte degeneration, multifocal lesions, spotty necrosis of liver hepatocytes and portal lymphocyte infiltrations (Xu et al., 2013; Alarifi et al., 2013). In vitro exposure to high concentrations of anionic MNPs results in a dose-dependent diminishing viability and capacity of PC12 cells (Pisanic et al., 2007). Further, MNPs (15 nm, 30 nm) were intravenously injected into mice at a dose of 5 mg/kg, MNPs were not cleared after 1 month and the liver enzymes elevated after 1 month post-injection (Gu et al., 2012). Also, after 2 weeks of oral administration of zinc oxide NPs (120 nm), mice had dose–effect pathological damages in the stomach, liver, heart and spleen (Wang et al., 2008). Moreover, dose-dependent caused some liver damage after 28 days of oral administration of 300 mg of silver NPs (60 nm) (Kim et al., 2008). Titanium dioxide NPs caused splenocyte apoptosis and cytoplasmic vacuolar degeneration after intraperitoneal injection into the mice (Abdel Aziz and Awaad, 2014).

In the past few years, there was no clear study to investigate the cellular and humoral immune responses induced by MNPs especially after intravaginal instillation. Intravenous administration of MNPs increased the number of $CD4^+$ and $CD8^+$ T lymphocytes, interleukin-2, interferon-γ, and interleukin-10 in peripheral blood after 72 h as compared with control (Chen et al., 2010). Moreover, organosilica NPs (100 nm) increased the number of $CD11b^+$ macrophages and IgA^+ cells in the sub-epithelial domes of Peyer's Patches after oral administration. Furthermore, organosilica NPs (925 nm) induced the expression of fucose residues and mucosal IgA on the surface of M cells in the follicle-associated epithelia of Peyer's Patches and increased the number of $33D1^+$ dendritic cells in the sub-epithelial domes of Peyer's Patches.

However, most of previous studies did not elaborate on the different toxicological impacts of MNPs. In this study, mice were treated intravaginally with MNPs for 3 days, 2 weeks and 6 weeks. Mainly, the liver and spleen are the most targeted organs by MNPs or other nanomaterials after intravenous treatment (Hayashi et al., 2013). Additionally, iron has higher affinity to accumulate in the human joints (Nishiya et al., 2003). The direct absorption of MNPs from the genital tract also can affect the immune responses in the genital tract of treated mice. Therefore, the purpose of this study is to evaluate the uptake and biodistribution of MNPs in the liver, spleen and vaginal tissue after intravaginal instillation. Additionally, the detailed cellular and tissue toxicity as well as cellular and humoral immune responses of MNPs were investigated in these tissues. The uptake, biodistribution and the toxicological impacts in cells and tissues caused by