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Toxicity, toxicokinetics and biodistribution of dextran stabilized Iron oxide Nanoparticles for biomedical applications

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ABSTRACT

Advancement in the field of nanoscience and technology has alarmingly raised the call for comprehending the potential health effects caused by deliberate or unintentional exposure to nanoparticles. Iron oxide magnetic nanoparticles have an increasing number of biomedical applications and hence a complete toxicological profile of the nanomaterial is therefore a mandatory requirement prior to its intended usage to ensure safety and to minimize potential health hazards upon its exposure. The present study elucidates the toxicity of in house synthesized Dextran stabilized iron oxide nanoparticles (DINP) in a regulatory perspective through various routes of exposure, its associated molecular, immune, genotoxic, carcinogenic effects and bio distribution profile. Synthesized ferrite nanomaterials were successfully coated with dextran (<25 nm) and were physicochemically characterized and subjected to *in vitro* and *in vivo* toxicity evaluations. The results suggest that surface coating of ferrite nanoparticles with dextran helps in improvising particle stability in biological environments. The nanoparticles do not seem to induce oxidative stress mediated toxicological effects, nor altered physiological process or behavior changes or visible pathological lesions. Furthermore no anticipated health hazards are likely to be associated with the use of DINP and could be concluded that the synthesized DINP is nontoxic/safe to be used for biomedical applications

1. Introduction

The revolutionary bloom of nano scale technologies in the present era has set new horizons for the wide exploitation of this unique technology for an ample domain of biomedical applications. However the unique physico-chemical and dimensional characteristics of the nano-sized particles is of great concern as it can interact with the biological system and can scale up to unpredictable manifestations. This growing debate on the risk assessment of nanoparticles has to be addressed to unravel the safety issues to manpower associated with the research, production and end use of the nanoparticles. Nanotoxicology, a science that deals with toxicity studies of nanomaterials (Tiwle, 2012) is particularly appealing as well as demanding in the above context.

Super paramagnetic iron oxide nanoparticles, represents a promising nanoparticle system in nano medicine with numerous clinical as well as theranostic applications such as MRI contrast enhancement, cell targeting and labeling, drug delivery and hyperthermia, cell separation, tissue repairing etc (Laurent and Mahmoudi, 2011). The potential adverse effects of metal and metal oxide nanoparticles are however mitigated/eliminated by appropriate biomimmetic surface coatings (Yu et al., 2012). Dextran ($C_6H_{10}O_5$), a branched polysaccharide, coated Ferrite nanoparticles have been used extensively for health care prospects, including MRI contrast agents, cellular targeting probes, hyperthermia agents etc (Jha et al., 2014). Dextran being hydrophilic and biocompatible aids in the intra cellular uptake of ferrite nanoparticles (Uthaman et al., 2015). Despite the cautious engineering of DINP, their intended application can induce toxicity in biological system, due to the inherent nano scale properties. In the natural milieu, iron homeostasis is well maintained in mammalian cells and biological fluids (Cabantchik, 2014). In excess, the natural iron binding proteins may get saturated allowing free iron to circulate in systemic circulation. This when lodged into internal organs can exacerbate toxic effects due to the production of reactive oxygen species that increases lipid peroxidation with resulting damage to mitochondria and other cellular organelles (Adibhatla and Hatcher, 2010). Iron mediated enhanced oxidative damage to DNA is also reported (Chattopadhyaya and Goswami, 2012). Furthermore,

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absence of an excretory pathway for iron aggravates the toxic effects mediated by iron bioaccumulation (Peto, 2010).

Most intracellular and *in vivo* toxicity of nanoparticles are attributed to their ability of producing excess reactive oxygen species (ROS) (Manke et al., 2013). It has been reported that nanoparticle induced oxidative stress occurs due to the dissolution of iron from iron based nanoparticles which mediates generation of hydroxy and peroxy free radicals from H_2O_2 via the Fenton reaction (Thomas et al., 2009). Excess ROS are markers of oxidative stress and can damage the cells by altering proteins, modulating gene expressions, lipid peroxidation, DNA adduct formations etc finally culminating in pathological conditions (Dalle-Donne et al., 2006). Oxidative stress affecting the cell signaling cascades can either activate apoptotic pathways leading to apoptosis or necrosis (Fulda et al., 2010). It can also get associated with carcinogenic activity of cells. Hence a detrimental outcome is expected through iron nanoparticle interaction at the cellular level. However coating with biocompatible phases ameliorates these effects and hence toxicity evaluation of surface modified iron oxide nanoparticles requires attention.

Toxicity of any nanomaterial is also governed by the route and duration of the exposure. Inhalation, ingestion, injection and dermal contact form the basic exposure routes often encountered by researchers, manufacturing unit people and finally the end users (Ray et al., 2009). Hence a thorough put investigation on the significant biological responses at each exposure routes is needed. Interaction of nanoparticles with immune system is another potential area of research interest. Major consideration in the nanoparticle mediated immunotoxicity is that of inflammatory response and T cell response. Immuno response of DINP interaction needs to be comprehended further, as the primary cells that come in contact with nanoparticles would be the immune cells present in the blood upon systemic absorption. Furthermore, although there are reports that iron oxide particles does not truly cause any direct genotoxic effects (Singh et al., 2010), its consequences at the genetic level due to the indirect action of ROS needs to be elucidated.

Exclusive studies on toxicity of dextran stabilized ferrite nanoparticles are limited. It has been shown that dextran coating considerably decreased the iron nanoparticle mediated cytotoxicity in pulmonary artery endothelial cells (Mojica Pisciotti et al., 2014). A comparative study of neurotoxic potential of dextran coated iron-based magnetic nanoparticles reported that coated nanoparticles has no significant effect on synaptic vesicle acidification, glutamate levels as well as synaptic vesicular functions (Borysov et al., 2014). Acute toxicity and irritation studies following subcutaneous injection of a water-based dextran-coated magnetic fluid (dextran-magnetic fluid) in mice concludes that dextran-magnetic fluid is biocompatible and well tolerable (Yu et al., 2008). In a study on the effect of surface coatings on cell behavior and morphology of fibroblasts, it was shown that dextran-magnetite nanoparticles result in decreased proliferation rate and subsequently cell death analogous to that caused by uncoated iron oxide particles (Berry et al., 2003). The cytotoxicity was attributed to the breakdown of the dextran shell exposing bare iron oxide NPs to interact with the cellular systems. Our group has reported the acute dermal and acute oral toxicity studies of dextran coated ferrite nanomaterials (Syama et al., 2014; Mohanan et al., 2014). Furthermore, other than an *in vitro* investigation that demonstrated micronucleus formation in human MCL5 lymphoblastoid cells treated with dextran-coated g-Fe_2O_3 nanoparticles for 24 h (Valdiglesias et al., 2015), genotoxic evaluation of DINP is not been extensively studied. Hence the consequence of DINP interaction at the cellular and molecular level, genotoxic and immuno toxic effects needs to be elucidated in detail before rendering it safe for biomedical application. In the present study, the toxicological profile including toxicokinetics and bio-distribution of an in house synthesized dextran stabilized iron oxide nanoparticles of less than 25 nm particle size is addressed in a regulatory perspective for projecting DINP as a prospective bio-nano material in nanomedicine.

2. Materials and methods

2.1. Synthesis and physicochemical characterization of Dextran stabilized Iron oxide nanoparticles

Iron oxide nanoparticles were synthesized by an alkaline co-precipitation method from heating an aqueous solution of $FeCl_3$ and $FeCl_2{\cdot}4H_2O$ (2:1) in a nitrogen atmosphere at 80 °C followed by precipitation with 3 M NaOH as described elsewhere (Pereira et al., 2012). Physical adsorption of a layer of dextran on to the surface of ferrite nanoparticle was done by stirring of ferrite nanomaterial in a solution of water: ethanol (7:3) containing 3% dextran. Black precipitate obtained is washed with deionized water and lyophilized to get dextran stabilized iron oxide nanoparticles. The synthesized nanoparticle is characterized for its hydrodynamic size by DLS (Malvern Zeta sizer, Nano ZS), particle size by TEM (Hitachi H-7650) component analysis by FTIR (Nicolet 5700 FTIR spectrometer), phase purity analysis by XRD (PAN analytical X'Pert Pro MRD) and TGA analysis(SDT-2960 TA).

2.2. Experimental animals

Albino rats (Wistar), Albino guinea pigs (Hartley) and Albino mice (Swiss) were procured form the Division of Laboratory Animal Sciences, BMT wing, SCTIMST. All the experiments were performed as per the CPSCEA guidelines and in accordance with OECD guidelines after obtaining approval from Institute animal ethics committee prior to the tests. The animals were handled humanely, without hurting or distressing them and with due care for their welfare. Individually ventilated cages were used for the housing of guinea pigs. Rats were housed in anodized cages and mice were grouped in 5 numbers and were housed in ventilated cages. A controlled environmental condition of temperature (22 ± 3° C) and humidity (30–70%) and with a 12 h light and dark cycle was maintained for the animals. Commercially available food and filtered water were given *ad libitum* throughout the experimental period. Guinea pigs in the weight range 300–600 g, albino mice (19–23 g) and rats (200–220 g) were used for the *in vivo* experiments.

2.3. Cytotoxicity assay—In vitro studies using L929 fibroblasts

The cytocompatibility of synthesized DINP was assessed qualitatively by direct contact assay and quantitatively by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay based on established protocol (van Meerloo et al., 2011). Briefly, 96 well plates were seeded at a density of 1×10^4 cells per well with L929 cells and maintained in a 5% CO_2 incubator at 37 °C. After overnight incubation, DINP at a concentration range of 100–800 μg/mL were kept in contact with cells for 24 h. After incubation, the cells were examined under microscope for altered morphology. The cellular response in comparison to negative and positive control was scored as non cytotoxic, mildly cytotoxic, moderate cytotoxic and severely toxic. For quantitative measurement, 25 μL of MTT dye (2 mg/mL) in PBS was added to each well and incubated for 4 h in dark at 37° C. The soluble formazan crystal formed as a result of metabolic reduction of soluble MTT by mitochondrial dehydrogenase is solublized with 200 μL of DMSO and measured at 540 nm in a micro plate reader.