Toxicology Letters 246 (2016) 17–27

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Toxicity and inflammatory response in Swiss albino mice after intraperitoneal and oral administration of polyurethane nanoparticles

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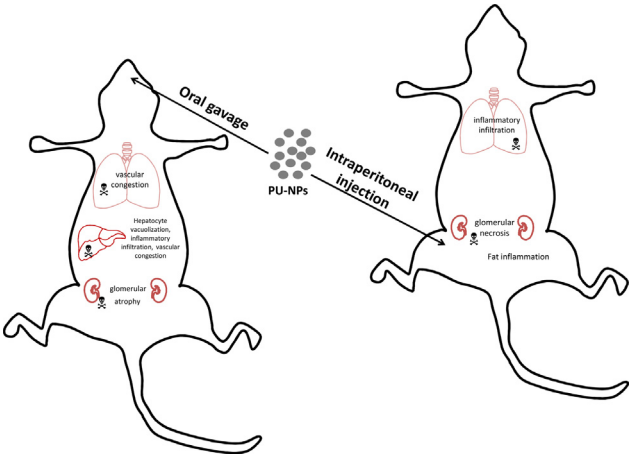
HIGHLIGHTS

- Biocompatibility evaluation of polyurethane nanoparticles *in vivo*.
- Polyurethane nanoparticles induced significant increase in visceral fat accumulation.
- Fat tissue of mice showed diffuse mononuclear inflammatory infiltrate.
- Histopathological assessment evidenced damage in liver, lung and kidney.
- Liver function loss was characterized by hepatic enzymes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 September 2015
Received in revised form 15 January 2016
Accepted 23 January 2016
Available online 25 January 2016

Keywords:
Biocompatibility
Nanoparticles
in vitro
in vivo

ABSTRACT

In this work *in vivo* experiments were conducted in order to characterize the biocompatibility of polyurethane nanoparticles (PU-NPs) after intraperitoneal (i.p.) and oral administration. Additionally, *ex vivo* assays were performed to assess human blood compatibility as well as *in vitro* assays to assess protein binding. Our results indicated that administration of three different concentrations of PU-NPs induced a significant increase in visceral fat accumulation after oral dosing. In addition, fat tissue of mice intraperitoneally treated with the highest concentration of nanoparticles showed diffuse mononuclear inflammatory infiltrate in the fat tissue. Histopathological assessment showed inflammatory infiltrate and hepatocyte vacuolization in the liver, inflammatory infiltration and vascular congestion in the lung and glomerular necrosis in the kidney. Hepatic enzymes related with liver function were significantly increased in both groups of mice treated with PU-NPs. The PU-NPs did not affect the human blood cells number as well as coagulation time but showed a susceptibility to bind in proteins commonly found in

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http://dx.doi.org/10.1016/j.toxlet.2016.01.018
0378-4274/

Inflammation
Toxicity

the blood stream. In addition, increased amounts of pro inflammatory cytokines *in vivo*, as well as *ex vivo* in human cells were observed. Further studies to establish the consequences of long-term exposure to PU-NPs are warranted.

1. Introduction

Nanoparticles are characterized as particles ranging in size between 10 and 1000 nm (Nagarwal et al., 2009; Parveen and Sahoo, 2008) and have been used in pharmaceutical and biomedical applications as biosensors, magnetic resonance, optical detection and drug delivery systems (Agasti et al., 2010; Linkov et al., 2009; Venkataraman et al., 2011). The drug delivery systems are designed to improve the degree and duration of therapeutic exposure at specific target cells or tissues, to overcome the shortcomings of conventional formulations (Qiu and Park, 2001). These systems generally improve bioavailability, sustain release of drugs and/or might protect substances against enzymatic degradation (Ge et al., 2002). Nanotechnology-enabled development is impacting the diagnosis, treatment and prevention of many diseases (Singh and Lillard, 2009).

Polymeric nanoparticles have been studied in the last decades due to their great potential as controlled delivery systems, especially due to their excellent efficiency in endocytosis, high encapsulation properties and compatibility with a diverse number of therapeutic agents (Faraji and Wipf, 2009; Kumari et al., 2010). Polyurethanes have been used in biomedical applications in the last 30 years (Sun et al., 2011) due to synthetic versatility, excellent mechanical properties (Bonzani et al., 2007) and comparative biocompatibility (Laschke et al., 2009; Sun et al., 2011).

However, despite the great and promising utilization of nanoparticles in different areas, their use has been associated with generation of oxygen reactive species (Silva et al., 2012), inflammation (Hussain et al., 2009; Park and Park, 2009), mitochondrial damage (Hiura et al., 2000; Upadhyay et al., 2003), carcinogenic and mutagenic effects (Borm et al., 2006; Donaldson et al., 2005; Savolainen et al., 2010). Currently, there is insufficient understanding of the relationship between nanostructured material properties and their biological or environmental impact (Yokel and MacPhail, 2011).

The interaction of nanoparticles with the human body is related to their physical and chemical properties such size, shape, porosity, surface charge, surface area and chemical composition (Oberdorster et al., 2005; Shinde et al., 2012). Nanoparticles prepared with the same components exhibit distinct toxicological responses due to differences in these parameters (Elsaesser and Howard, 2012; Shinde et al., 2012).

In our previous work, ~130 nm lipid nanoparticles induced hematological changes and inflammatory responses in mice (Silva et al., 2013), despite many other studies indicating good biocompatibility of analogous nanocarriers (Chattopadhyay et al., 2007; Joshi and Müller, 2009; Kwon et al., 2008; Müller et al., 2006; Pardeike et al., 2009; Wang and Thanou, 2010). Similarly, the potential toxicity of titanium dioxide nanoparticles has been demonstrated *in vitro* and *in vivo* (Chen et al., 2009; Hussain et al., 2009; Pujalté et al., 2011; Zhang et al., 2010), but are still considered by some investigators to be promising materials for various applications including nanomedicine (Devanand Venkatasubbu et al., 2013; PEN, 2009).

The aim of this work was to investigate possible toxic consequences of polyurethane nanoparticles (PU-NPs) in mice according to the route of administration and doses applied. In addition, ex vivo approaches using human blood were applied as a surrogate to estimate PU-NPs effects to hematologic human system.

2. Materials and methods

2.1. PU-NPs preparation and characterization

The preparation and characterization of PU-NPs is described in (Zanetti-Ramos et al., 2006). Briefly, a natural triol, diisocyanate and olive oil were added in a solution of tween 80 under stirring at room temperature. The polyurethane were obtained after homogenization of the preparation components at 7200 × *g* for 15 min using Ultra-Turrax® T18 (IKA® Germany), followed by mechanical stirring at 60 °C for 4 h.

In order to evaluate the physico-chemical properties of the pristine nanoparticles, size and surface charge were determined by dynamic light scattering and laser-Doppler anemometry, using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK), equipped with 173° scattering angle, as previously described (Silva et al., 2012). PU-NP shape was evaluated by transmission electron microscopy (TEM) also as previously described (Silva et al., 2012). X-ray diffraction (XRD) was used for the structural characterization of liquid (inside a capillary) and powder (from lyophilization of aqueous solutions) PU-NPs. XRD measurements were conducted using a PanAnalytical Xpert Multi-purpose diffractometer (Cu-Kα radiation, λ = 0.15418 nm), in Bragg–Brentano configuration with an X'Celerator detector.

2.2. Evaluation of protein interaction

Assessment of protein interaction with PU-NPs was performed as previously described (Liptrott et al., 2014). PU-NPs (2.5 mg/ml) were incubated for 0, 2, 6 and 24 h with fluorescently labeled recombinant fibrinogen (Alexa-fluor 647), albumin (BODIPY) and transferrin (Alexa-fluor 488) (Life Technologies, Paisley, UK), in a final concentration of 100 μg/ml. Samples were analyzed by flow cytometry (MacsQuant, Miltenyi Biotec Inc., Surrey, UK), according to forward scatter and side scatter characteristics (FSC/SSC) as well as fluorescence.

2.3. In vivo *toxicological assays*

Animals were maintained and handled in accordance with the Principles of Animal Care and procedures were previously approved by the Ethics Committee for Animal use (01/2012). Forty-eight male swiss albino mice (6–8 weeks old) were maintained at 23 ± 2 °C with relative humidity of 50–60% under a 12:12 h light:dark cycle with food and water *ad libitum*. Mice were matched for body weight (25 to 30 g) prior to performing the experimental procedures. In the study, animals were divided into eight groups according to the dose and route of administration ($n = 6$), comprising two control groups of oral gavage and intraperitoneal (i.p.) route, which received only the vehicle (saline) and six experimental groups that received three different concentrations of PU-NPs (2 mg/kg, 5 mg/kg and 10 mg/kg) by oral gavage and i.p. routes. According to the suggested by the Organization for Economic Co-operation and Development (OECD) Guideline, the doses used to investigate the toxicity of chemicals must be between 5 to 5000 mg/kg. We choose the maximum dose of 10 mg/kg based on the maximum volume possible to administer to the animal by i.p. and oral pathway (Morton et al., 2001). The