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Research paper

Biodistribution of orally administered poly(lactic-co-glycolic) acid nanoparticles for 7 days followed by 21 day recovery in F344 rats

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ABSTRACT

The aim of this research was to assess biodistribution of orally administered poly(lactide-co-glycolide) acid nanoparticles (PLGA NPs) in rats and investigate the excretion of PLGA nanoparticles after administration has ended. The experiment was divided into 2 phases. In phase I, F344 rats were orally administered fluorescently tagged PLGA nanoparticles daily (3 mg/day) for 7 days, followed by a mass balance analysis which was performed on tissues of interest to determine NP biodistribution. In phase II, after 7 days of oral exposure, rats were no longer administered PLGA NPs, and amount of NPs excreted was measured each week for 3 weeks. At day seven, the last day of the nanoparticle exposure period, over half of the daily administered PLGA NPs were excreted. Among the nanoparticles recovered from the tissues, the majority was recovered in the intestines (23.4% daily dose), followed by the liver (11.4% daily dose), kidney (5.5% daily dose), spleen (2.5% daily dose), lung (2.0% daily dose), brain (1.0% daily dose), plasma (0.7% daily dose), and heart (0.2% daily dose), respectively. During phase II, the amount of NPs in the feces declined from the maximum excretion on day 7 (58.3% daily dose) to the minimum value on day 28 (6.7% daily dose), 3 weeks after NP administration ended. Little change in nanoparticle excretion was observed between day 21 and day 28, indicating the baseline had been reached. The findings are significant for understanding biodistribution and excretion of orally administered PLGA NPs and are relevant to their application in food, agriculture, and medicine.

1. Introduction

Polymeric nanoparticles have been developed as viable drug delivery systems for treatment of diseases ranging from Alzheimer's disease to cancer (Fonseca-Santos et al., 2015; Xiao et al., 2015; Joshi et al., 2014). Due to their flexibility, biodegradability, and relatively low levels of toxicity, polymeric nanoparticles may be preferred to other types of nanoparticles as drug delivery systems (Navarro et al., 2016). Knowledge regarding the biodistribution of nanoparticles *in vivo* is critically important for determining their efficacy, as well as toxicity. Biodistribution of polymeric nanoparticles has been determined for intravenous (i.v.) (Mohammad and Reineke, 2013), oral (Semete et al., 2012; Khalil et al., 2013), intraperitoneal (Semete et al., 2010a), topical (Zhang et al., 2013) and intranasal (Sharma et al., 2014) administration. The techniques applied for tracking nanoparticles *in vivo* fall mainly into two categories, namely: fluorescent methods (Semete et al., 2010a; Semete et al., 2010b) and radioactive labelling (Arora et al., 2012; Llop et al., 2015). Studies dedicated to tracking nanoparticles *in vivo* after oral delivery are relatively sparse compared to those using intravenous administration (Bazile et al., 1992; Leray et al., 1994; Esmaeili et al., 2008; Shan et al., 2009; Tosi et al., 2010; Yadav et al., 2011), and many are focused on inorganic nanoparticles, such as zinc oxide (Cho et al., 2013; Baek et al., 2012; Lee et al., 2012) and silver (Hadrup et al., 2012; Kim et al., 2008; Loeschner et al., 2011). Studies that analyzed the biodistribution of nanoparticles following intravenous administration showed high concentrations of nanoparticles distributed to reticuloendothelial organs, such as the spleen and bone marrow, as well as the liver, kidneys, and intestines (Leray et al., 1994; Esmaeili et al., 2008; Shan et al., 2009). Studies focused on the oral administration of metallic nanoparticles indicate that, while some metallic nanoparticles such as titanium oxide are not well absorbed in the GI tract (Cho et al., 2013), others such as zinc oxide and silver are well absorbed and tend to concentrate in the intestines, liver, and kidneys (Baek et al., 2012; Lee et al., 2012; Hadrup et al., 2012; Kim et al., 2008; Loeschner et al., 2011).

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While only mild to no toxicity was observed following the oral administration of silver nanoparticles (Hadrup et al., 2012; Kim et al., 2008), much greater toxicity was observed for zinc oxide (Li et al., 2012).

Oral delivery of drugs with polymeric nanoparticles is particularly of interest, because it overcomes limitations such as poor stability, low mucosal permeability, and low solubility of the drugs in gastric fluids (Joshi et al., 2014; Murugeshu et al., 2011). Oral drug delivery is the most preferred method of drug delivery due to patient compliance, cheaper production cost, and the potential for many drugs to be taken at home that are typically administered in a hospital or clinic setting (Yun et al., 2013). Furthermore, using nanoparticles to administer drugs orally has the potential to bypass adverse effects associated with IV delivery (Mei et al., 2013).

Poly(lactic-co-glycolic) acid (PLGA) is a biodegradable polymer that has seen extensive use in research and medicine due to its biocompatibility (Makadia and Siegel, 2011a). Common applications of PLGA include its use as material for sutures and implantable scaffolds as well as its use as a vehicle for drug delivery. PLGA is of particular interest for drug delivery due to its ability to biodegrade, and it's versatility; by adjusting the ratio of its components, lactic acid and glycolic acid, and conjugating it to copolymers and other molecules the adhesion, diffusion, and distribution properties of the delivery systems can be modulated (Makadia and Siegel, 2011a). The available literature on the mass balance of orally administered PLGA nanoparticles is limited, especially for cases where nanoparticle administration is carried out for a certain time followed by a recovery period when no nanoparticles are incorporated into the diet. Instead, most studies focused on polymeric nanoparticles address the efficacy of nanodelivered drugs for the treatment of diabetes, cancer, and other diseases (Jin et al., 2009; Malathi et al., 2015), without addressing the biodistribution and elimination of the nanoparticles. In general, the studies that do focus on the biodistribution of polymeric nanoparticles involve a single dose (Leray et al., 1994) or repeated doses of nanoparticles varying from 7 [11, 33, 34] to 21 days (Navarro et al., 2016). The recovery time of the tissues to the baseline after a long exposure to a nanoparticle treatment is not currently addressed in the available literature, to the authors' knowledge.

The aim of this study was to quantify the biodistribution and excretion of PLGA nanoparticles after 7 days of oral administration in F344 rats, and to analyze nanoparticle excretion in animals exposed to nanoparticles for 7 days after a recovery period of 7, 14 and 21 days. To achieve this aim, nanoparticles were covalently linked to a fluorophore, namely, tetramethylrhodamine-5-isothiocyante (TRITC), which allowed nanoparticle tracking *in vivo* in tissues, blood, feces and urine at various time points.

2. Material and methods

2.1. Reagents

Poly(lactide-co-glycolide acid)(PLGA) 50:50, (MW: 30,000 to 70,000 g/mol). Poly-vinyl alcohol (PVA) 87–89% hydrolyzed (31,000–50,000 g/mol), -[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate) (HATU), *N*-Boc-ethylenediamine, *N*,*N*-Diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), triethylamine, tetramethylrhodamine-5-isothiocyanate (TRITC), and dichloromethane (DCM) were purchased from Sigma-Aldrich (St. Louis, MO). Acetone, ethanol, ethyl acetate were HPLC grade (Mallinckrodt Baker, Pittsburgh, NJ).

2.2. PLGA-TRITC nanoparticles synthesis & residual PVA measurement

The attachment of TRITC to PLGA was performed using uronium salt chemistry, following the protocol previously described by Navarro et al. (Navarro et al., 2016). The polymeric nanoparticles were synthesized by an emulsion evaporation technique described by Astete and Sabliov (Astete and Sabliov, 2006) and by Murugeshu et al. (Murugeshu et al., 2011). The purification was performed by dialysis (MWCO 100,000 g/mol) with regenerated cellulose membranes (Spectrum Laboratories, Rancho Dominguez, *CA*) for a period of 2 days. Then, polymeric nanoparticles were dried using a freeze drier for 2 days (Labconco, Kansas City, MO). The final dry product was stored at 4 °C for characterization and animal studies. The quantification of residual PVA was done by a colorimetric method (Zigoneanu et al., 2008), detailed by Navarro et al. (Navarro et al., 2014). After preparation, the standard curve and samples were read using a spectrophotometer (Genesis 6, Thermo Scientific, Asheville, NC) at 690 nm.

2.3. Morphology, particles size, size distribution, and zeta potential measurements

A method detailed by Navarro et al. (Navarro et al., 2014) was used to determine NP morphology. Transmission electron microscopy (TEM) pictures were taken with a JEOL 1400 (Jeol USA Inc., Peabody, MA). The image was taken on a sample of nanoparticle powder re-suspended in water, immediately following freeze-drying step. Uranyl acetate (2%) was added to the sample as contrast agent.

Nanoparticles size, distribution and zeta potential were measure with a Malvern Zetasizer Nano ZS (Malvern Instrument Ltda., Worcestershire, UK). Zeta potential was measured using a 10 mM NaCl solution at pH 7.2; both parameters, size and zeta potential measurements were done at a nanoparticle concentration of 200 μg/ml.

2.4. In vivo biodistribution of fluorescently tagged PLGA nanoparticles

Male F344 rats (200–300 g) were used for the *in vivo* studies. At the time of the experiment, the average age of the rats was 12 weeks. The animals were acclimated for one week prior to the start of the study. Animals were on a 12-hour light/dark cycle and randomly divided according to the previous study (Navarro et al., 2014). Rats ($n = 8$) were housed, 2 per cage, and provided feed (Lab Diet 5001, St. Louis, MO) and water *ad libitum*. Four animals were gavaged daily with a solution of fluorescently tagged PLGA nanoparticles (3 mg/ml) suspended in 1 ml PBS (pH 7.4). A typical dose of drug loaded polymeric nanoparticles is 10 mg/kg body weight, which is why 3 mg/ml was chosen as the daily dose for rats. The control group consisted of four rats which were daily gavaged with PBS (pH 7.4). An additional three rats were euthanized after acclimation and used to prepare fluorescent standard curves by measuring fluorescence as a function of nanoparticle concentration, separately, in the tissues of interest: brain, intestine, kidney, liver, and spleen. The tissues recovered from treated animals were stored at −80 °C until processing. Each tissue was homogenized in PBS and digested with 1 M NaOH, following a protocol described by Yin et al. (Yin et al., 2007). Finally, samples were centrifuged for 10 min and the fluorescence was measured in the collected supernatant following a protocol previously described by Navarro et al. (Navarro et al., 2014). The amount of nanoparticles distributed in each tissue was calculated based on the standard curve for that individual tissue.

On day 7, blood was collected as was previously described (Navarro et al., 2014) and centrifuged to separate the plasma fraction at 2800*g* for 10 min at room temperature using a Sorvall Legend Mach 1.6R centrifuge (Thermo Scientific, Waltham, MA). The plasma samples were kept at −80 °C until use. The collected plasma was used to quantify the fluorescent PLGA NPs. The amount of fluorescence was determined in plasma based on the standard curves previously prepared. The nanoparticle concentration in plasma was expressed as % dose based on the assumption that an adult male rat of 250 g contains 6.4 ml of blood and 4.2 ml of plasma for every 100 g of body weight (Plapied et al., 2011). The amount of fluorescent nanoparticles excreted in urine and feces on day 7 was also determined for the biodistribution study in rats ($n = 4$).