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Evaluation of the cytotoxicity, genotoxicity and mucus permeation capacity of several surface modified poly(anhydride) nanoparticles designed for oral drug delivery

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ABSTRACT

The main concerns with drugs designed for oral administration are their inactivation or degradation in the harsh conditions of the gastrointestinal tract, their poor solubility through the gastrointestinal mucus gel layer, the poor intestinal epithelium permeability that limits their absorption, and their toxicity. In this context, poly(anhydride) nanoparticles are capable of protecting the drug from the harsh environment, reduce the drug's toxicity and, by virtue of surface modification, to enhance or reduce their mucus permeability and the bioadhesion to specific target cells.

The copolymer between methyl vinyl ether and maleic anhydride (commercialized as Gantrez® AN 119) are part of the poly(anhydride) nanoparticles. These biocompatible and biodegradable nanoparticles (NPs) can be modified by using different ligands. Their usefulness as drug carriers and their bioadhesion with components of the intestinal mucosa have been described. However, their toxicity, genotoxicity and mucus permeation capacity has not been thoroughly studied.

The aim of this work was to evaluate and compare the *in vitro* toxicity, cell viability and *in vitro* genotoxicity of the bioadhesive empty Gantrez® AN 119 NPs modified with dextran, aminodextran, 2-hydroxypropyl-β-cyclodextrin, mannosamine and poly-ethylene glycol of different molecular weights.

Results showed that, in general, coated NPs exhibit better mucus permeability than the bare ones, those coated with mannosamine being the most permeable ones. The NPs studied did not affect cell metabolism, membrane integrity or viability of Caco-2 cells at the different conditions tested. Moreover, they did not induce a relevant level of DNA strand breaks and FPG-sensitive sites (as detected with the comet assay).

1. Introduction

Recently, nanoparticles (NPs) have increasingly found practical applications in technology, research and medicine. Their wide use has given rise to a new area of medicine and research, called nanomedicine. In this context, NPs can be utilized in disease prevention, diagnosis, monitoring, treatment (Ahmad et al., 2008; Jain et al., 2011) (e.g. as drug carriers) and mitigation of pain (Mittal et al., 2007). NPs have been defined according to a nanometre scale of size between 0.1 and 100 nm, although in the case of pharmaceutical NPs, the dimension may be higher (De Jong and Borm, 2008).

Oral administration is the most commonly used and accepted route of drug administration. However the main concern is the

Abbreviations: ADEX, aminodextran; DEX, dextran; FBS, fetal bovine serum; FPG, formamidopyridine DNA-glycosylase; GN, gantrez® AN 119; HPBCD, 2-hydroxypropyl-β-cyclodextrine; LDH, lactato deshidrogenasa; MA, mannosamine; MEM, minimum essential medium; NP, nanoparticle; PBS, phosphate buffered saline; PDI, polydispersity index; PEG 0.5, poly-ethylene glycol 500; PEG1, poly-ethylene glycol 1000; PEG2, poly-ethylene glycol 2000; PEG5, poly-ethylene glycol 5000; PEG6, poly-ethylene glycol 6000; PEG10, poly-ethylene glycol 10000; PLGA, poly lactic-co-glycolic acid; ROS, reactive oxygen species; RSG, relative suspension growth; RT, room temperature; SBs, strand breaks; TSG, total suspension growth.

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inactivation or degradation of the drug in the harsh conditions of the gastrointestinal tract. In this context, to prevent rapid presystemic degradation of the drug due to the digestive enzymes of the gastrointestinal tract and the low pH, the NPs are used to enhance the drug's absorption (Hunter et al., 2012).

The poor intestinal epithelium permeability also limits the absorption of drugs (Bernkop-Schnürch, 2013). The diffusion of poorly soluble drugs through the mucus gel layer is crucial to ensure an adequate serum concentration. This mucus layer is secreted by mucosal glands and some cells, such as goblet cells. Its main function is to protect the mucosal tissues (Lai et al., 2009) and it consists of several negatively charged glycoproteins, which form a stable three-dimensional matrix (Friedl et al., 2013). Some strategies currently under investigation aim to solve this problem; an example is the development of slippery-surface NPs (Zabaleta et al., 2012).

Poly(anhydride) NPs have been considered promising platforms for drug delivery and other applications in the treatment of various diseases, such as tuberculosis (Ahmad et al., 2008), bacterial infections (Zaki and Hafaez, 2012) and cancer (Jain et al., 2011), among others. These NPs are biodegradable, surface modifiable to enhance or reduce bioadhesion to specific target cells (Ensign et al., 2012) and capable of sustained drug release. The copolymer between methyl vinyl ether and maleic anhydride (commercialized as Gantrez® AN 119) is an excellent example of the group of poly (anhydride) NPs (Arbós et al., 2002). Their surface can be modified with different ligands in order to alter their physicochemical properties, as well as their distribution *in vivo* (Agüeros et al., 2009; Inchaurraga et al., 2015). It has been demonstrated that Gantrez® AN 119 NPs coated with different ligands have the ability to develop strong bioadhesive interactions with components of the intestinal mucosa (Agüeros et al., 2009, 2010; Arbós et al., 2002, 2004; Porfire et al., 2010; Salman et al., 2005, 2006, 2009; Yoncheva et al., 2005). Salman et al. (2006) observed that Gantrez® AN 119 NPs coated with mannosamine were taken up by Peyer's patches while bare NPs were just localized in the outer layer, probably due to the presence of mannose receptor in this lymphoid tissue. Moreover, Gantrez® AN 119-based NPs are capable of establishing bioadhesive interactions with Caco-2 cells without being internalized (Ojer et al., 2013).

Gantrez® AN 119-based NPs, as biodegradable and biocompatible NPs, are considered to be of low or no toxicity to the organism (Landsiedel et al., 2012). Consequently, fewer studies have focused on potential adverse effects of these types of nanomaterials. However, bioadhesive NPs can affect membrane stability either directly (physical damage) or indirectly (oxidation) which can lead to apoptosis and finally, cell death. Actually, oxidative stress has been established as one of the crucial factors determining the toxicity of several NPs (Ahmad et al., 2012; Kumar et al., 2011; Nel et al., 2006). Usually, the oxidative stress is generated by an increase in intracellular reactive oxygen species (ROS), highly reactive molecules that can react with cell biomolecules including the DNA.

There are many studies that support the usefulness of this type of NPs as drug carriers (Agüeros et al., 2010; Arbós et al., 2004; Salman et al., 2009); however, the toxicity and the mucus permeation capability have not been thoroughly investigated. In this study the *in vitro* toxicity, genotoxicity and the mucus permeability of empty Gantrez® AN 119 NP modified with dextran (DEX), aminodextran (ADEX), cyclodextrin (HPBCD), mannosamine (MA) and poly-ethylene glycol (PEG) as ligands are evaluated. DEX and ADEX are known to be used as a stabilizing coating material to protect metal NPs from oxidation and improve their biocompatibility (Easo and Mohanan, 2013). DEX coating has been described as a muco-penetration enhancer across the intestinal mucus barrier (Beloqui et al., 2014) but it produces an uncommon but significant acute renal failure (Brooks et al., 2001). Several studies have shown that NPs based on HPBCD reduce their toxicity as well as improves the permeability of drugs (Nagai et al., 2014; Jaiswal et al., 2015; Wu et al., 2013). Furthermore, it has been demonstrated that some HPBCD derivatives do not exert adverse effects in humans after oral or intravenous administration (Stella and He, 2008). Mannose and its derivatives are interesting surface ligands due to their capability to link with the mannose receptors, highly expressed in the cells of the mucosal immune system (*i.e.* macrophages and dendritic cells) (Carrillo-Conde et al., 2011). Similarly, PEG coating of NPs surfaces has been demonstrated to be an effective strategy to ensure rapid NP transport through the mucus (Li et al., 2015; Liu et al., 2013).

Therefore, the aim of this study was to evaluate and compare the *in vitro* toxicity, cell viability and genotoxicity of the bioadhesive empty Gantrez® AN 119 NP modified with DEX, ADEX, HPBCD, MA and different PEG as ligands in human colon cell lines. Moreover, the diffusion capability through gastrointestinal natural mucus was evaluated using an *in vitro* transwell diffusion technique. All these determinations will help in the selection of the most promising NP formulation for oral drug delivery.

2. Material and methods

2.1. Materials

The copolymer of methyl vinyl ether and maleic anhydride (Gantrez® AN 119; Mw: 200000) was provided by Ashland (Barcelona, Spain). Poly(ethylene glycol) 2000 (PEG2), poly(ethylene glycol) 6000 (PEG6), poly(ethylene glycol) 10000 (PEG10) were provided by Fluka (Switzerland). 2-hydroxypropyl-β-cyclodextrin (HPBCD) and Dextran (DEX, Mw 70000) were provided by Sigma-Aldrich (Steinheim, Germany). Mannosamine (MA) was purchased from Sigma (Spain). Aminodextran (ADEX, Mw 70000) was obtained from Invitrogen (Spain). Lumogen® Red F 305 was supplied by BASF (North America).

Acetone was obtained from VWR Prolabo (Fontenay-sous-Bois, France). Deionized water (18.2 Ω resistivity) was prepared by a water purification system (Wasserlab, Pamplona, Spain). Nitrogen gas (ultra-pure, >99%) was produced using an Alltech nitrogen generator (Ingeniería Analítica, Barcelona, Spain).

2.2. Preparation of NPs

In this study seven types of modified NPs were produced and evaluated. In all cases, NPs were prepared from an acetone phase containing the copolymer of methyl vinyl ether and maleic anhydride (Gantrez® AN 119) and a hydrophilic compound, by a desolvation procedure with a mixture of ethanol and water. The following hydrophilic compounds were employed to produce the different types of NPs: ADEX and DEX, HPBCD, MA and PEG2, PEG6 and PEG10. The resulting NPs were purified by tangential filtration (VivaSpin®20 300,000 MW.C.O., Vivascience, Sartorius group, Hannover, Germany) and, finally, dried by spray-drying. For this purpose, the following parameters were selected: inlet temperature of 90 °C, outlet temperature of 60 °C, spray-flow of 600 L/h, and aspirator at 100% of the maximum capacity.

For the preparation of NPs containing dextrans (Porfire et al., 2010), 400 mg of the copolymer (Gantrez® AN 119) were dissolved in 20 mL acetone containing either ADEX (1.2 mg) or DEX (80 mg). Then, NPs were obtained by the addition of 40 mL of an ethanol: water mixture (1:1, v/v) and the mixture was incubated at room temperature (RT) under magnetic agitation for 30 min. The organic solvents were eliminated under reduced pressure evaporation and the resulting nanosuspension was purified by tangential filtration using Vivaspin (3000 rpm, 5 min, 4 °C). Finally, the NPs were