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Research paper

In vivo toxicological evaluation of polymeric nanocapsules after intradermal administration

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ABSTRACT

Polymeric nanocarriers have shown great promise as delivery systems. An alternative strategy has been to explore new delivery routes, such as intradermal (i.d.), that can be used for vaccines and patch-based drug delivery. Despite their many advantages, there are few toxicity studies, especially *in vivo*. We report a safety assessment of biodegradable poly(ε-caprolactone) lipid-core nanocapsules (LNC) with a mean size of 245 ± 10 nm following single and repeated intradermal injections to Wistar rats. Suspensions were prepared by interfacial deposition of polymer. The animals (n = 6/group) received a single-dose of saline solution (1.2 ml/kg) or LNC (7.2×10^{12} LNC/kg), or repeated-doses of two controls, saline solution or Tween 80 (0.9 ml/kg), or three different concentrations of LNC (1.8, 3.6, and 5.4×10^{12} LNC/kg) for 28 consecutive days. Clinical and physiological signs and mortality were observed. Samples of urine, blood, and tissue were used to perform toxicological evaluation. There were no clinical signs of toxicity or mortality, but there was a slight decrease in the relative body weights in the Tween 80–treated group ($p < 0.01$) after repeated administration. No histopathological alterations were observed in tissues or significant changes in blood and urinary biomarkers for tissue damage. Mild alterations in white blood cells count with increases in granulocytes in the Tween-80 group ($p < 0.05$) were found. Genotoxicity was evaluated through the comet assay, and no statistical difference was observed among the groups. Therefore, we conclude that, under the conditions of these experiments, biodegradable LNC did not present appreciable toxicity after 28 consecutive days of intradermal administration and is promising for its future application in vaccines and *patch-based devices* for enhancing the delivery of drugs.

1. Introduction

Nanoparticles (NPs) have attracted increasing attention as carriers of preventive, therapeutic, and/or diagnostic agents in clinical medicines [1,2]. Polymeric nanoparticles formulated from biodegradable polymers are being widely explored as carriers for controlled delivery of different agents including proteins, peptides, plasmid DNA (pDNA), and low molecular-weight compounds [3–6]. Lipid-core nanocapsules (LNC) are composed by a dispersion of solid and liquid lipids in the core enveloped by a polymer wall [7]. Several studies reporting the use of LNC prepared with poly(ε-caprolactone) (PCL) stabilized with Tween 80 have shown great potential as drug delivery systems for topical, oral, or systemic applications [8–12]. PCL is degraded by hydrolysis of its ester linkages in physiological conditions, such as in the human body, and has therefore received a great deal of attention for use in drug delivery [5]. PCL also has the ability to form compatible blends with other polymers, which can affect the degradation kinetics, facilitating tailoring to fulfill desired release profiles [13].

The potential to spare dose by intradermal administration (i.d.) was suggested over 25 years ago and has received renewed

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attention recently [14]. This attention has been drawn to new types of delivery methods, such as needle–syringe, liquid jet injectors, biolistic particle injection, and microneedle arrays/patches [15]. The skin, in particular, is a potent immunostimulatory tissue, and it is populated with numerous highly potent resident antigen-presenting cells (APCs) called Langerhans' cells and dermal dendrocytes that play a vital role in the induction of immune response [6,14,16]. Intradermal route is an additional way to administer drugs. Recently, studies have shown advances in delivery of vaccine that is expected to induce an adaptive immune response for a broad range of infectious diseases and cancers [6,17].

Various approaches have been tested in recent years. One of these is the development of nanoparticles prepared from biodegradable and biocompatible polymers that acts as a vaccine delivery system to induce both humoral and cellular immune responses showing great promise [18]. According to Akagi et al. [6], nanoparticles for controlled delivery systems can deliver either the antigens or adjuvants to the desired location at predetermined rates and durations to generate an optimal immune response. They also pointed out that the carrier had potential advantages of protecting the vaccine from degradation until it is released and reducing the systemic side effects and the possibility of co-encapsulating multiple antigenic epitopes or both antigen and adjuvant in a single carrier. Thus, nanotechnology-based antigen delivery is developing as a vaccine strategy owing to its dose-sparing and prolonged antigen-presentation features [19].

Furthermore, when co-formulated in micro- or nanoparticles, foreign proteins are much more effective in eliciting immune responses than as a plain protein solution [20–22]; as a consequence, several types of particulate systems have been studied for vaccine delivery, including liposomes, oil-in-water emulsions, virus like particles, and polymeric carriers [20,22–24]. Recently, Verheul et al. [24] studied trimethyl chitosan-hyaluronic acid nanoparticles (TMC) as an intradermal vaccine carrier system with superior antibody titers against ovalbumin and diphtheria toxoid (DT) compared to the plain antigens.

The increasing manufacturing and use of nanomaterials, specifically in the form of NP, for diverse biomedical applications have raised serious concerns about their safety for human health [25]. Owing to their small size, the surface area-to-mass ratio of nanoparticles is high, and a large fraction of reactive electrons is exposed at the surface. This has been proposed to increase toxicity [26]. Based on these, Oberdörster defined nanotoxicology as the science of engineered nanodevices and nanostructures that deals with their effects on living organisms, which are gaining increasing importance with the growth of nanotechnological applications [25]. It is also proposed that nanotoxicology will develop and implement "nano-specific" protocols in order to investigate and gain the knowledge necessary to determine the potential toxicity of the plethora of different NPs [27].

Although there are a number of reports regarding toxicity evaluation of inorganic nanoparticles, knowledge on biodegradable nanomaterials, which have always been considered safe, is still limited. Little is known about the potential toxicity of nanoparticles used in targeted drug delivery and gene therapies in terms of human health [28]. The various interactions of NP with fluids, cells, and tissues need to be considered starting at the portal of entry and then via a range of possible pathways toward target organs. The potential for significant biological response at each of these sites requires investigation [29]. Before studying the potential effect of LNC for vaccine delivery, we decided to evaluate their safety after intradermal administration.

Therefore, the aim of this study was to investigate the possible toxic effects of LNCs after repeated-dose treatments in an animal model. The treatment involved intradermal injection of LNCs and a nonionic surfactant – Tween 80 – used for stabilization of the LNC in the formulation. Systematic toxicological evaluation of these lipid-core nanocapsules is important for its future application in vaccines and *patch-based devices* for enhancing the *delivery* of drugs.

2. Materials and methods

2.1. Chemicals and reagents

Span 60® (sorbitan monostearate) was supplied by Sigma–Aldrich (Strasbourg, France) and poly(ε-caprolactone) (PCL, M_W = 50,000) was supplied by Capa (Toledo, Ohio). Caprylic/capric triglyceride (CCT) and Tween 80 (polysorbate 80) were obtained from Delaware (Porto Alegre, Brazil). All other chemicals and solvents used were of analytical or pharmaceutical grade. All reagents were used as received.

2.2. Lipid-core nanocapsules preparation

Lipid-core nanocapsules were prepared as previously reported [7,30]. Briefly, an organic solution composed of PCL (0.1 g), sorbitan monostearate (0.038 g), CCT (0.16 g), and acetone (27 mL) was stirred at 40 °C until dissolution of all components. In a separate flask, polysorbate 80 (0.77 g) was added into water (53 mL) at room temperature. Then, the organic phase was injected into the aqueous phase using a funnel and magnetic stirring. The turbid solution was stirred 10 min at room temperature. The acetone was eliminated and the formulation concentrated to approximately 9.5 mL in a rotatory evaporator at 40 °C under reduced pressure. The volume was adjusted to 10 mL using a volumetric flask.

2.3. Physicochemical characterization of the lipid-core nanocapsules

Mean particle size, polydispersity index (PDI), zeta potential, and pH were determined as previously described [7]. Particle size, polydispersity index, and zeta potential of the formulation were determined using a Zetasizer®nano-ZS ZEN 3600 model (Malvern, UK). The samples were diluted without previous treatment in water (MilliQ®) (particle size) or in 10 mmol L^{-1} NaCl aqueous solution (zeta potential).

To the calculus of the particle number density (N), the mean diameters obtained by dynamic light scattering were used. Number of particles per milliliter (particle number density, N) was determined to access the surface area of the nanocapsules (S) in a determined volume of suspension according to Jäger et al. [30].

The pH value of the formulation was directly determined without sample treatment using a potentiometer (Micronal B-474). Morphology and structure of the resulting LNC were observed with transmission electron microscope (TEM). Sample was diluted and deposited on specimen grid (Formvar-Carbon support film, Electron Microscopy Sciences). Subsequently, they were negatively stained with uranyl acetate solution (2% w/v). The analyses were carried out using a transmission electron microscope (TEM; JEM 1200 Exll) operating at 80 kV.

2.4. Animals and treatments

Male Wistar rats weighing 270 ± 60 g, aged 6–8 weeks obtained from Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS), were used. The rats were housed in polycarbonate cages with steel-wire tops (four animals per cage) at room temperature (22 ± 0.1 °C) and humidity (50 ± 10%) with a 12 h light/dark cycle, receiving standard food and water *ad libitum*. The studies were conducted by randomly distributing the rodents into groups of six rats each. For urine analysis, the animals were housed

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