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In vitro toxicity assessment of oral nanocarriers☆

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ABSTRACT

The fascinating properties of nanomaterials opened new frontiers in medicine. Nanocarriers are useful systems in transporting drugs to site-specific targets. The unique physico-chemical characteristics making nanocarriers promising devices to treat diseases may also be responsible for potential adverse effects. In order to develop functional nano-based drug delivery systems, efficacy and safety should be carefully evaluated. To date, no common testing strategy to address nanomaterial toxicological challenges has been generated. Different cell culture models are currently used to evaluate nanocarrier safety using conventional *in vitro* assays, but overall they have generated a huge amount of conflicting data. In this review we describe state-of-the-art approaches for *in vitro* testing of orally administered nanocarriers, highlighting the importance of developing harmonized and validated standard operating procedures. These procedures should be applied in a safe-by-design context with the aim to reduce and/or eliminate the uncertainties and risks associated with nanomedicine development.

Contents

Abbreviations: FDA, Food and Drugs Administration; PAMPA, Parallel artificial membrane permeability assay; FAE, Follicle-associated epithelium; TEER, Trans-epithelial electrical resistance; NPs, Nanoparticles; EFSA, European Food Safety Authority; μCCA, Microscale cell culture analog; GIT, gastrointestinal tract; ADME, Absorption, distribution, metabolism and excretion; OECD, Organisation for Economic Co-operation and Development; ISO, International organization for standards; ASTM, American society for testing and materials; NCI, National Cancer Institute; NCL, Nanotechnology Characterization Laboratory; ECVAM, European Center for the Validation of Alternative Methods; SOPs, Standard Operating Procedures.

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1. Introduction

Nanotechnology deals with the manipulation of matter at atomic and molecular level in order to alter its properties, offering new and innovative products for daily life [1]. Medical applications of nanotechnology refer to nanomedicine, which is aimed at diagnosing, treating and preventing acute and chronic diseases including cancer, cardiovascular and infectious diseases. Small size, increased surface area, reactivity and proper functionalization confer to nanomaterials unique properties such as the ability to cross biological barriers and reach specific tissues and cells [2]. The European Science Foundation identified five main areas of nanomedicine: (i) analytical techniques and *ex vivo* diagnostic tools; (ii) nanoimaging; (iii) nanomaterials and nanodevices; (iv) novel therapeutics and drug delivery systems; and (v) translation from bench to clinic, including industrial scale-up, validation and regulation, and safety and efficacy evaluation [3,4]. The majority of commercial nanotechnology-based products for *in vivo* applications are composed of "soft" nanostructures encompassing liposomes, micelles, emulsions, dendrimers, and other polymeric and protein nanostructures. Biodegradable "soft" platforms are preferred for therapeutic delivery applications, as it appears looking at FDA-approved "soft" nanostructures. Examples of approved drug delivery systems are Abraxane® (albumin protein-bound paclitaxel to treat metastatic breast cancer), Doxil® (pegylated-stabilized liposomal doxorubicin for the treatment of ovarian cancer), DaunoXome® (non-pegylated liposomal daunorubicin for curing HIV-associated Kaposi's sarcoma), and Copaxone® (Glatiramer acetate – copolymer composed of L-glutamic acid, L-alanine, L-lysine, and L-tyrosine – indicated to treat multiple sclerosis) [1,5]. "Hard" nanostructures, such as iron oxide, gold, silver, or ceramic, are mainly developed for medical imaging and theranostics [6]. Approved "hard" nanostructures are mostly composed of superparamagnetic iron oxide as the contrast agents Endorem®, Resovist® and Feridex® [7].

The objectives of nanomedicine in drug delivery consist of: (i) improving solubility and bioavailability of hydrophobic drugs; (ii) improving the circulatory presence of drugs, *e.g.* avoiding the breakdown of orally administered drugs before reaching therapeutic site; (iii) reducing side effects by decreasing administered doses; (iv) targeting drugs to specific tissues and cells as well as individual pathogens or biomolecules; and (v) controlling drug release [8]. Despite the wide development and use of nanotechnology-based medical applications, the potential health risks associated to nanomaterials are often ignored [9]. Nanomaterials used for medical applications are indeed intentionally administered to patients, and nano-specific side effects should be carefully evaluated. Nanotoxicology is defined as the study of adverse effects of nanomaterials on living organisms and ecosystems, including the prevention and amelioration of such adverse effects. Nanotechnology, nanomedicine and nanotoxicology are complementary disciplines aimed at the improvement of human life, weighing benefits and risks [3]. Risk is function of hazard and exposure, and the generally accepted approach consists of hazard identification and characterization, exposure assessment and risk characterization [2]. Hazard characterization is achieved by toxicity testing of nanomaterials using *in vitro* and *in vivo* assays and establishing a dose–response relationship, corresponding to the likelihood of adverse health effects at varying degrees of exposure [2,3]. Exposure is a measure of the amount of substance that enters into contact with the target, *i.e.* the patient. Exposure to pharmaceuticals is direct, since they are administered in a well-known dose *via* specific exposure route such as oral, transdermal, intravenous, and inhalation. Exposure can be external (administration dose) or internal (dose in plasma or target organ/tissue).

The oral route is considered the most common and accessible administration way of drug formulations due to good patient compliance and low costs [10]. Conversely from the other exposure routes characterized by a well-defined and constant biochemical environment, oral administration of drugs implicates their exposure to extremely different biological and chemical conditions, in terms of pH, ionic strength, enzymes and exposure to intestinal microbiota, mining their stability (Fig. 1). The harsh conditions of gastrointestinal tract can lead to drug instability and degradation, with a decrease in bioaccessibility and bioavailability. Nanotechnology represents a good opportunity to overcome these problems, generating delivery systems able to protect drugs from the unfavorable gastrointestinal environment, favoring a controlled release and targeting of drugs, and increasing bioaccessibility and bioavailability. Strategies combining nanocarrier passive targeting to injury sites through enhanced permeability and retention effect and ligand-mediated or receptor-targeted active targeting are continuously developed to improve drug bioavailability [11]. In passive targeting, nanocarriers can be passively extravasated through leaky endothelium, allowing their accumulation in the tumor region and the release of drug in the extracellular matrix [12,13]. Therapeutic efficacy of drugs can also be improved by using active targeting, which consists of ligand incorporation on nanocarrier surface that binds to cell surface receptors. In this case, the presence of leaky vasculature is not required [12,13]. Compared with passive targeting, active targeting aims at reducing side effects acting on diseased cells only. Even if patient safety and morbidity enhancements were achieved using drug-loaded nanocarriers (*i.e.* Doxil® and Abraxane®), they still offer marginal improvements over conventional formulations [14,15]. Design of multifunctional nanoparticles, able to properly negotiate with biological barriers sequentially encountered before reaching the site of interest, seems to be a promising approach to deal with this problem.

The purpose of this review is to describe *in vitro* strategies for assessing toxicity of newly developed nanocarriers for oral administration of drugs, taking into consideration potentialities and limits. This review raises the urgent need to develop validated standard operating procedures in order to produce reliable and comparable data for the safety evaluation of nano-based formulations in a Safe-by-Design context.

2. Cell culture models for toxicity assessment of oral nanocarriers

The growing interest in developing nanotechnology-based drug delivery systems needs models to screen and predict their efficacy and safety. Cell culture models are valuable systems to predict interactions between drug delivery systems and gastrointestinal tract, providing information on molecular mechanisms underlying absorption and toxicity. Moreover, cell culture-based approaches permit to select the most suitable formulations for further *in vivo* testing, reducing time and costs and being in compliance with 3Rs principle (Replacement, Reduction and Refinement) [16]. Cell culture models are suitable to study acute, delayed and repeated toxicity. The most common *in vitro* models to study molecule permeability are the parallel artificial membrane permeability assay (PAMPA) [17] and the cell-based Caco-2 systems [18]. While PAMPA assay is used to evaluate passive permeability, the Caco-2 cell systems are used to measure passive and active permeability. Caco-2 cells allow to simultaneously evaluate efficacy and safety of delivery systems, investigating their mode of action (Fig. 2).