In Vivo Investigation of Thiomer–Polyvinylpyrrolidon Nanoparticles Using Magnetic Resonance Imaging

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ABSTRACT: This study focused on the investigation of the permeation enhancing effects of a stomach targeted, nanoparticulate drug delivery system. The polyacrylic acid–cysteine/polyvinylpyrrolidon nanoparticles were loaded with the magnetic resonance imaging (MRI) contrast agent diethylenetriaminepentaacetic acid gadoliniu-m(III)dihydrogen salt (Gd-DTPA). Average particle size was determined to be 130 nm and the optimum for stability was found to be below a pH of 4.5. *In vitro* permeation studies were performed on rat gastric mucosa and revealed an eightfold increase in Gd-DTPA uptake when incorporated in the nanoparticles compared to evaluation in the presence of unformulated polyacrylic acid–cysteine. *In vivo* investigations with rats were performed via the noninvasive MRI method in order to track the nanoparticles way through the gastrointestinal tract. When Gd-DTPA was administered orally as nanoparticulate suspension, an increased MRI signal in the urinary bladder was detected after 34 min, providing evidence for systemic uptake and renal elimination of the contrast agent. As control experiments with Gd-DTPA only or in combination with unformulated polyacrylic acid–cysteine revealed no MRI signal increase at all, the significant permeation enhancing effect could be identified based on the nanoparticulate formulation. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:2008–2017, 2010

Keywords: nanoparticles; oral drug delivery; absorption enhancer; polymeric drug delivery; renal excretion

INTRODUCTION

Oral drug delivery is still the favored method of drug administration because of apparent advantages such as high patients compliance, convenient, and painless application, where no medical help is needed and good possibilities for modification of drug release. In pharmaceutical technology, one of the main goals is to increase drug uptake from the gastrointestinal mucosa to reduce the frequency of medication and consequential side effects. During the last few years, a new field, offering undreamed-of possibilities, has been introduced in pharmacy: the nanotechnology. Nanoparticulate delivery systems offer the advantage of a deeper interpenetration into the mucus gel layer, enabling

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direct contact of the multifunctional polymer and the included drug itself at the site of absorption.[1] Multifunctional polymers are modified basic polymers like polyacrylic acid or chitosan, which are further developed by introduction of physiologically active compounds, allowing modification and improvement of drug absorption.

Therefore, it was the aim of this study to test a stomach targeted nanoparticulate drug delivery system, prepared with such a multifunctional polymer, on its resorption enhancing facilities *in vitro* as well as *in vivo*. Stomach targeted delivery systems are of particular interest for drugs that (i) are locally active in the stomach,[2] (ii) have an absorption window in the stomach,[3] (iii) are unstable in the intestinal or the colonic environment,[4] or (iv) exhibit low solubility at high pH values.[5]

For our purpose, nanoparticles were synthesized on the basis of the well-characterized and strongly permeation enhancing polymer, polyacrylic acid–cysteine (PAA–Cys).[6,7] Besides the permeation enhancing properties of PAA–Cys, the thiolated polymer is able to reduce enzymatic degradation[8,9] and exhibits strong mucoadhesive properties.[10–12] For the formation of particles, polyvinylpyrrolidon (PVP) was chosen as template polymer, as reported by Chun et al.[13] Interpolymer complexes were formed by hydrogen bonds between the carboxyl groups of PAA–Cys and the carbonyl groups of PVP,[13] sparing the bioactive sulfhydryl groups of the PAA–Cys. As model compound for *in vitro* and *in vivo* studies in rats, a magnetic resonance imaging (MRI) contrast agent diethylenetriaminepentaacetic acid gadolinium(III)dihydrogen salt hydrate (Gd-DTPA)] was incorporated into the nanoparticles. Gd-DTPA was chosen on the one hand to visualize the pathway of nanoparticles *in vivo* via MRI and on the other hand, because Gd-DTPA is an excellent example for drugs which are very poorly resorbed from the gastrointestinal tract.[14]

Studies performed so far with nanoparticles loaded with different paramagnetic contrast agents mostly investigated the behavior of nanoparticles having been injected intravenously,[15–17] but for oral administration, animal studies are missing. After oral application of the nanoparticulate delivery systems, rats were investigated by means of standard T1 weighted MRI and quantitative T1 mapping. In order to investigate the *in vivo* results gained from the MRI visualization *in vitro*, permeation studies on rat gastric mucosa were performed. Graphite furnace-atomic absorption spectroscopy (GF-AAS) was used for analyzing the *in vitro* permeation behavior of Gd-DTPA.[18]

MATERIALS AND METHODS

Materials

Polyacrylic acid (100 kDa, 35% (w/w) solution in water), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), L-cysteine hydrochloride, and Gd-DTPA were purchased from Sigma–Aldrich (Vienna, Austria). PVP (Kollidon 12 PF, 2500 Da) was kindly donated from BASF (Ludwigshafen, Germany).

Synthesis and Characterization of Thiolated Polyacrylic Acid

PAA–Cys conjugate of 100 kDa molecular weight was synthesized according to a method described previously.[10] In brief, PAA was diluted to a final concentration of 1% (m/v) with distilled water. The pH was adjusted to 6 by the addition of 1 M NaOH and an aqueous EDAC solution was added in a final concentration of 100 mM to activate the carboxylic acid moieties of PAA. After 15 min incubation under stirring at room temperature, an aqueous 10% (m/v) L-cysteine hydrochloride solution, in which pH has been adjusted to 6, was added. The reaction mixture was then stirred for 3 h at room temperature. Subsequently, the resulting PAA–Cys conjugate was purified by dialysis two times against 0.2 mM HCl solution in deionized water, then two times against 0.2 mM HCl containing 5% NaCl, and finally again two times against 0.2 mM HCl. After dialysis, the thiomer was freeze-dried at -70°C and 7 mbar (Benchtop 2K, VirTis, Gardiner, NY) and stored at room temperature until further use. The amount of thiol groups on the PAA–Cys conjugate was determined via Ellman's reagent (5,5′-dithiobis(2-nitrobenzoic acid)) as described previously.[11]

Synthesis of Gd-DTPA-Loaded Nanoparticles

The preparation method for the PAA–Cys/PVP nanoparticles was adopted from Chun et al.[13] and modified for our purpose. PAA–Cys was dissolved in distilled water at a final concentration of 0.05% (m/v). Then, 3 mL of an aqueous Gd-DTPA solution (10%, m/v) was added. In the next step