Report

Vidarabine-Loaded Nanoparticles: A Physicochemical Study

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The chemical reaction of vidarabine (VIDA) with isohexyl cyanoacrylate nanoparticles in a pH-dependent fashion occurs only in the presence of dioctylsulfosuccinate (DOSS). The formation of an ion pair with DOSS allows a better contact of VIDA with the monomer during the polymerization process taking place in micelles. On the basis of molecular weight profiles of the polymer, determined by gel permeation chromatography (GPC), it is proposed that VIDA induces the polymerization of cyanoacrylic monomers through a zwitterionic pathway. This mechanism allows the covalent linkage of the drug with the polymer, which is consistent with NMR experiments. The present study illustrates the need for physicochemical studies in the design of new colloidal drug delivery formulations.

KEY WORDS: vidarabine; nanoparticle; cyanoacrylate; chemical interaction; emulsion polymerization.

INTRODUCTION

Biodegradable polyalkylycyanoacrylate nanoparticles as carriers for therapeutic agents were found to be effective in the treatment of experimental intracellular infections (1,2) and for enhancing the activity of some anticancer drugs (3,4). For instance, ampicillin loaded-nanoparticles were 100 times more efficient than free ampicillin in the treatment of experimental mice infection with Salmonella typhimurium (5). This result was attributed both to a better intracellular uptake of the drug and to a modification of its tissue distribution resulting in an increased capture by the liver. In the case of doxorubicin associated with nanoparticles, the hepatic uptake of the drug induced a decrease in the cardiac tissue concentration leading to a significant reduction of its limiting cardiotoxicity (6).

These results led us to compare the chemotherapeutic efficacy of vidarabine (VIDA)6 and vidarabine-associated nanoparticles (NP-VIDA) against Herpes virus infection of the liver in mice (7). The toxicity toward the central nervous system was reduced after administration of NP-VIDA compared to VIDA and the rate of VIDA metabolism was slowed down. However, vidarabine also lost its antiviral activity after association with nanoparticles.

In this study, we show that inactivation of vidarabine is due to its chemical interaction with the cyanoacrylic monomer during the polymerization process, thereby demonstrating the need for appropriate physicochemical studies in the design of new colloidal drug delivery systems.

MATERIALS AND METHODS

Nanoparticle Preparation

Nanoparticles were prepared as described previously (8) by emulsion polymerization of isohexylcyanoacrylate (IHCA) monomer (Sopar, Sart-Drames-Avelines, Belgium). In short, IHCA (100 μl) was added to an aqueous solution of 10⁻³ M H₃PO₄ containing dioctylsulfosuccinate (DOSS) from 0 to 17.6 10⁻⁴ M and in which vidarabine (VIDA) was dissolved at a concentration of 8.72 10⁻⁴ M. In some experiments, instead of VIDA, adenine (ADE) or arabinose (ARA) was dissolved in the polymerization medium at a concentration of 8.72 10⁻⁴ M in order to determine the place of the chemical interaction.

After 6 hr of polymerization, a homogeneous milky suspension was obtained. This suspension was then neutralized and freeze-dried (CHRISS Alpha 1-5) for 24 hr.

Determination of Drug Loading

VIDA and ADE associated with nanoparticles were measured by HPLC (Model 110 B solvent delivery system injector, programmable spectrophotometric detector module 166 connected with a Nec-PC 8201A, ST3A integrator, Beckman Instruments, Palo Alto, California). A column (4.6 × 25 cm) packed with Beckman ultraspHERE ODS, 5 μm, was
used. The mobile phase consisted of a methanol/water (50/48, v/v) containing tetrahydrofuran (1%) and acetic acid (1%). The flow rate was 1 ml/min, and the absorbance of the effluent stream monitored at 254 nm.

After polymerization, VIDA or ADE nanoparticles were ultracentrifuged at 110,000g for 90 min (Beckman Model L7-55 centrifuge, 70.1-Ti rotor, Beckman Instruments, Palo Alto, California). VIDA or ADE contents were measured in both supernatant (free drug) and dimethylformamide-dissolved sediment (associated drug). Drug binding was expressed as the percentage of drug initially dissolved in the polymerization medium, associated with the carrier.

**Determination of Nanoparticle Size and Molecular Weight**

Nanoparticle size was determined using a laser light-scattering method (Nanosizer, Coulter Electronics, Harpenden, England).

Molecular weights were evaluated by gel permeation chromatography using a refractive index detector (Waters Associates, R-401 differential refractometer). Ultrastyragel columns of 500 and 10,000 Å were used simultaneously. Tetrahydrofuran (Farmitalia, Carlo Erba, Milan, Italy) was used as the eluant, with a flow rate of 1 ml/min. Freeze-dried nanoparticles (about 100 mg) were dissolved in 10 ml of tetrahydrofuran. This solution was then filtered through a 0.45-μm filter and 50 to 200 μl was injected into the chromatographic system. Chromatograms were recorded and peak surfaces were integrated. Polystyrene standards with molecular weights ranging from 1800 to 355,000 were used for column calibration.

A calculation method proposed by Water's (9) was adopted in which the number average molecular weight ($M_n$) and the weight average ($M_w$) molecular weight were calculated according to Eqs. (1) and (2):

$$M_n = \frac{\sum Qi}{\sum (QiMi)}$$

and

$$M_w = \frac{\sum (Qi \cdot Mi)}{\sum Qi}$$

where $Qi$ represents the amount of polymer with a molecular weight $Mi$. The polymer molecular weight distribution was estimated by calculating the dispersity coefficient

$$d = \frac{M_w}{M_n}$$

Reference solutions containing both dextran 70 and DOSS were examined by the GPC method in order to demonstrate that these products did not interfere with the cyanoacrylic polymer.

No peaks were observed for these solutions even after the addition of VIDA, ADE, and ARA, because these compounds did not dissolve in tetrahydrofuran.

**Chemical Characterization of Compounds After Polymerization**

**Thin-Layer Chromatography Experiments (TLC)**

TLC determinations were carried out on aluminium sheets (0.2 mm) precoated with silica gel 60 F 254 (Merck, Darmstadt, Germany). Mixtures of chloroform/methanol (50/10, v/v) and (50/15, v/v) were used as mobile phases for ARA and VIDA or ADE elution, respectively.

VIDA and ADE were detected spectrophotometrically at 254 nm. ARA spots were visualized after spraying with sulfuric acid and heating. Loaded nanoparticles were centrifuged at 110,000g during 90 min. Then the plates were spotted with the appropriate amount of ethanol-dissolved sediments. Spots arising from unloaded nanoparticles supplemented with VIDA, ADE, or ARA were compared with those of VIDA-, ADE-, or ARA-loaded nanoparticles.

**Nuclear Magnetic Resonance ($^1H$-NMR)**

VIDA- or ADE-loaded nanoparticles were prepared for NMR experiments as described above but without dextran 70 to avoid overlap with signals corresponding to hydrogens.

![Fig. 1. Vidarabine (VIDA).](image)

![Fig. 2. TLC assays. (a) Vidarabine with DOSS. (b) Vidarabine with unloaded PIHCA nanoparticles (prepared with DOSS). (c), (d) Vidarabine-loaded PIHCA nanoparticles (prepared with DOSS). (e) Unloaded PIHCA nanoparticles (prepared with DOSS).](image)