PHARMACEUTICS, PREFORMULATION AND DRUG DELIVERY

Encapsulation of Valproate-Loaded Hydrogel Nanoparticles in Intact Human Erythrocytes: A Novel Nano-cell Composite for Drug Delivery

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Received 9 May 2010; revised 10 October 2010; accepted 10 October 2010

Published online 24 November 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.22395

ABSTRACT: A novel drug delivery system possessing prolonged release behavior is introduced to the field of carrier erythrocytes and nanotechnology-based drug delivery. Encapsulation of valproate-loaded nanogels inside human erythrocytes as a novel nanocell composite was the objective of the study to obtain a model novel drug delivery system with an intravenous sustained drug delivery characteristic. "Ionotropic gelation" was used for the fabrication of hydrogel nanoparticles. The nanoparticles obtained were evaluated *in vitro* (particle size, transmitting electron microscopy, zeta potential, Fourier transform infrared spectroscopy, etc.). "Hypotonic dialysis" was used to obtain nanoparticle-loaded erythrocytes. Finally, *in vitro* characterization tests were performed on nanoparticle-loaded erythrocytes. Number- and volume-based sizes, loaded amount (mg), loading ratio (%), and loading efficiency (%) of nanoparticles were, respectively, 61 ± 2 and 74 ± 2 nm, 20.6 ± 1.02 mg, 31.58 ± 1.86%, and 6.86 ± 0.41%. Spherical structure and slightly negative zeta potential of nanoparticles were confirmed. Erythrocytes were loaded by valproate-loaded nanoparticles (entrapment efficiency of 42.07 ± 3.6%). Carrier erythrocytes showed acceptable properties *in vitro* and demonstrated a prolonged drug release behavior over 3 weeks. This approach opens new horizons beyond the current applications of carrier erythrocytes for future investigations. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:1702–1711, 2011

Keywords: ionotropic gelation; chitosan; hydrogel nanoparticles; sodium valproate; carrier erythrocytes; hypotonic dialysis

INTRODUCTION

The first reports on loading the human living cells by therapeutic agents for delivery purposes were published on carrier erythrocytes idea in 1973.[1] Although erythrocytes are the most abundant and readily available cells of the human body, they have gained the highest and the most extensive attention among other cellular carriers for time-controlled or targeted delivery of the drugs and other bioactive agents.[1–7] In fact, the term "carrier erythrocyte" was first introduced in 1979 to describe the drug-loading capacity of the erythrocytes.[1] There are a series of advantages encouraging the wide spread application of erythrocytes in drug delivery, including, mainly, biocompatibility, complete biodegradability, long life span in circulation, possibility of targeted drug delivery to the reticuloendothelial system (RES) organs, relatively simple and inert intracellular environment, ease of handling, and so on.

One of the most important limitations of the carrier erythrocytes in drug encapsulation studies is the rapid escaping of the loaded drug from the carrier-loaded cells, even during the loading procedure, a problem being worse in the case of lipophilic drugs capable of ready diffusion out of the cells. This, in turn, makes a big challenge against obtaining

Abbreviations used: CS, chitosan; TPP, sodium tripolyphosphate; TEM, transmitting electron microscopy; RES, reticuloendothelial system; FTIR, Fourier transform infrared; RBC, red blood cell; PBS, phosphate-buffered saline; OFI, osmotic fragility index.

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Journal of Pharmaceutical Sciences, Vol. 100, 1702–1711 (2011)

carrier cells with desirable drug loading as well as drug release characteristics, that is, high drug encapsulation efficiency with long-term release upon reentry to circulation. Several strategies have been attempted to overcome this problem with the use of membrane cross-linkers being the only successful one.[8–12] This strategy, however, suffers from the inclusion of foreign chemicals in the procedure, particularly, considering that these agents are potentially harmful to carrier cells life span in circulation via making their membranes more rigid compared with the normal cells. We, therefore, evaluated a novel strategy of using drug-loaded nanoparticles, instead of the "bare" drug for encapsulation in erythrocytes to solve the aforementioned problem.

In recent years, significant efforts have been devoted to use the potentials of nanotechnology in drug delivery because it offers a suitable means of delivering small as well as large therapeutic molecules or even genes for either targeted or time-controlled purposes.[13] The nanometer size ranges of these drug carriers offer certain advantages for drug delivery, the most profound of them being long circulation time, and the possibility of selective penetration into tissues through capillary walls, epithelial linings (e.g., liver), and being taken up by the cells.[14]

Hydrogel nanoparticles are a family of particulate polymeric nanostructures (recently referred to as nanogels[1]), being the point of convergence of the beneficial characteristics of nanoparticles, as described, and hydrogels, mainly hydrophilicity, flexibility, versatility, high water absorptivity, and biocompatibility.[15] Besides the commonly used synthetic polymers, active research is focused on the preparation of nanogels using naturally occurring hydrophilic polymers. Claiming benefit from these kinds of polymers, chitosan (CS)-based nanoparticles are the most widely studied one owing to their remarkable advantages, which are beyond the scope of this article. CS, the deacetylated derivative of chitin, is a US Food and Drug Administration-approved natural gel-forming polycationic polymer with remarkable advantageous properties for biomedical applications, including, among others, biocompatibility, biodegradability, biosafety, and the capability of forming tailor-made nanoparticles for different purposes.[16–19]

In this study, CS-based nanogels were prepared, loaded by the widely used antiepileptic drug sodium valproate, a model molecule with high cell-escaping characteristics during the erythrocyte encapsulation procedure, optimized, and characterized *in vitro* and, then, the prepared nanocarriers were encapsulated in human intact erythrocytes by hypotonic dialysis method. Finally, the prepared nanogel-loaded erythrocytes were characterized *in vitro* for their drug loading, drug release, and survival-indicating characteristics.

MATERIALS AND METHODS

Materials

Chitosan (art. no. 212f498-89 were purchased locally. Sodium valproate was kindly donated by Rouz Darou Pharmaceutical Co. (Tehran, Iran). Dialysis tubing (cutoff 12 kDa; Viskase, Darien, IL, USA) was purchased locally. Other chemicals and solvents were from chemical laboratory and high-performance liquid chromatography (HPLC) grades, as needed, and were prepared locally.

Preparation of Valproate-loaded Nanopaticles

Valproate-loaded nanopaticles were prepared by the ionotropic gelation method, which is based on the ionic interaction between the polycationic polymer and the sodium tripolyphospahte (TPP).[15] The method was optimized by the Taguchi factorial design and the optimized setting was used throughout the study for preparation of nanogels. Briefly, the aqueous solution of sodium acetate with a concentration of 5.54% (w/v, 0.67 M) was prepared and the pH of the solution was adjusted to 5.5 using glacial acetic acid. Then, to this buffer solution, adequate amount of CS was added and mixed thoroughly at 2000 rpm for 2 h to obtain the final concentration of 0.3% (w/v). To prepare the counterion solution, TPP (2.5% w/v) and sodium valproate (25 mg/mL) were dissolved in distilled water. The dropwise addition of the anion/drug solution to CS bufferic solution with a volume ratio of 1:4 over 2 min at ambient temperature under continuous stirring (2000 rpm) and leaving the mixture to be stirred for 20 min resulted in our desired nanoparticles. Nanoparticles were separated from the rare out-of-range particles by light centrifuging at 3000g for 10 min.

In Vitro Characterization of Valproate-Loaded Nanoparticles

Loading Parameters of Valproate in Nanoparticles

The loaded amount of valproate in nanoparticles was easily determined as follows:

To 1 mL of final nanoparticle suspension, 0.015 mL of perchloric acid ($HClO_4$, 70%) was added to destruct the nanoparticles. The sample was then vortexed for 1 min and subjected to chromatographic analysis to determine the drug concentration. The result is defined as total drug concentration. On the contrary, a further 1-mL aliquot of the nanoparticle suspension was filtered through a 50-nm filter (Millipore, Bedfored, Massachusetts) and after adding 0.015-mL $HClO_4$ (70%) to the filtrate and vortex mixing for

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