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Preparation and in vitro evaluation of chitosan nanoparticles containing a caspase inhibitor

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Abstract

The aim of this work was to develop a formulation for Z-DEVD-FMK, a peptide which is a caspase inhibitor and has been used in experimental animal studies for a decade. Peptide loaded chitosan nanoparticles were obtained by ionotropic gelation process and Z-DEVD-FMK was quantified by an HPLC method. The influence of the initial peptide concentration on the nanoparticle characteristics and release behavior was evaluated. The CS nanoparticles have a particle diameter (Z-average) ranging from approximately 313–412 nm and a positive zeta potential (20–28 mV). The formulation with the initial peptide concentration of 400 ng/ml provided the highest loading capacity (0.46%) and the highest extent of release (65% at 24 h) suggesting the possibility to achieve a therapeutic dose. According to the data obtained, this chitosan-based nanotechnology opens new and interesting perspectives for anticaspase activity.

Keywords: Chitosan; Nanoparticles; Z-DEVD-FMK; HPLC

1. Introduction

Apoptotic cell death is a prominent phenomenon in neurodegenerative disorder, such as cerebral ischemia. The interleukin converting enzyme (ICE) family of cysteine proteases, now referred to as caspases is a group of apoptosis-regulatory genes that may play a role in ischemic brain injury. Caspase inhibitors attenuate apoptotic cell death during development of ischemia and reduce infarct volume (Thornberrry, 1997; Chen et al., 1998). Z-DEVD-FMK is one of the caspase inhibitors used in the preven-

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tion of apoptotic cell death (Kondratyev and Gale, 2000).

The delivery of hydrophilic drugs to the brain is still a great challenge for the treatment of many brain-related diseases, since hydrophilic drugs cannot cross the blood–brain barrier (BBB). The blood–brain barrier is a very dense biological barrier, and it possesses a unique morphological and physiological characteristic with cerebrovascular endothelial cells, which are tightly connected to each other and supported by glial cells (Ballabh et al., 2004). The BBB represents an insurmountable obstacle for a large number of drugs, including antibiotics, antineoplastic agents, and a variety of central nervous system (CNS)-active drugs, especially neuropeptides. One of the possibilities to overcome this barrier is targeted drug delivery to the brain using nanoparticles (Calvo et al., 2001, 2002; Brigger et al., 2002). Drugs that have successfully been transported into the brain using nanoparticulate drug carrier systems include the hexapeptide dalargin, the dipeptide kytorphin, loperamide, tubocurarine, the NMDA receptor antagonist MRZ 2/576, and doxorubicin (Kreuter, 2001).

Biodegradable nanoparticulate systems have received considerable attention as potential drug delivery vehicles. Chitosan (CS), a polysaccharide known to be a favorable pharmaceutical material because of its biocompatibility and biodegradability, forms an ideal hydrophilic carrier system (Calvo et al., 1997; Mitra et al., 2001; Alonso and Sanchez, 2003). Moreover, chitosan has been shown to be non-toxic and tissue compatible in a range of tests (Aspden et al., 1997). Chitosan nanoparticles are attractive non-viral and cationic carriers for the delivery of peptides, proteins, oligonucleotides, and plasmids (Mao et al., 2001; Janes et al., 2001; Alonso and Sanchez, 2003). They have the capacity to protect sensitive bioactive materials from enzymatic and chemical degradation in vivo and during storage, and to facilitate the transport of charged molecules across the absorptive epithelial cells (Mao et al., 2001).

Chitosan nanoparticles are obtained by the process of ionotropic gelation based on the interaction between the negative groups of the pentasodium tripolyphosphate (TPP) and the positively charged amino groups of CS. This process has been used to prepare CS nanoparticles for the delivery of peptides and proteins (Vila et al., 2004) including insulin (Fernàndez-Urrusuno et al., 1999) and cyclosporine (De Campos et al., 2001).

The aim of this work was to develop a new formulation of the anticaspase peptide, Z-DEVD-FMK based on chitosan nanoparticles for possible targeted delivery to the CNS.

2. Experimental Section

2.1. Materials

The polymer Chitosan Protasan Cl 113 (MW: <150 kD, deacetylation degree: 75–90%) was purchased from FMC Biopolymers (Norway). TPP was supplied by Sigma Chemical Co. (USA). The caspase inhibitor peptide Z-DEVD-FMK, Z-Asp(Ome)-Glu(Ome)-Val-Asp(Ome)-FMK (molecular weight is 668 g/mol) was purchased from Enzyme Systems (USA). Ultrapure water was obtained with MilliQ equipment (Waters, USA).

2.2. Preparation of chitosan nanoparticles

CS nanoparticles were prepared according to the ionotropic gelation process (Calvo et al., 1997; Vila et al., 2004). Blank nanoparticles were obtained upon the addition of a TPP aqueous solution (0.4 mg/ml) to a CS solution (1.75 mg/ml) stirred at room temperature. The formation of nanoparticles was a result of the interaction between the negative groups of TPP and the positively charged amino groups of chitosan. The ratio of chitosan/TPP was established according to the preliminary studies. Z-DEVD-FMK loaded nanoparticles were obtained according to the same procedure and the ratio of chitosan/TPP remained unchanged. Variable amounts of peptide were incorporated to the chitosan solution prior to the formation of nanoparticles in order to investigate the effect of the initial peptide concentration on the nanoparticle characteristics and in vitro release profiles. Nanoparticles were collected by centrifugation at 10,000 rpm on a 10 μl glycerol bed, for 1 h and supernatants were discarded.

2.3. Characterization of the nanoparticles

The morphological examination of the chitosan nanoparticles was performed using a transmission electron microscope (TEM); (CM12 Philips, USA). The samples were resuspended in water and stained with