International Journal of Biological Macromolecules 88 (2016) 146–155

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Cytotoxicity and genotoxicity of a trypanocidal drug quinapyramine sulfate loaded-sodium alginate nanoparticles in mammalian cells

Anju Manuja[a,*], Balvinder Kumar[a], Meenu Chopra[a], Anshu Bajaj[a], Rajender Kumar[a], Neeraj Dilbaghi[b], Sandeep Kumar[b], Sandeep Singh[a,b], T. Riyesh[a], Suresh C. Yadav[a]

[a] National Research Centre on Equines, Sirsa Road, Hisar 125001, Haryana, India
[b] Department of Bio & Nanotechnology, Guru Jambheshwar University of Science & Technology, Hisar 125001, Haryana, India

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 16 March 2016
Accepted 17 March 2016
Available online 18 March 2016

Keywords:
Trypanosoma
Nanomedicine
Genotoxicity
Cytotoxicity
Oxidative stress

ABSTRACT

We synthesized quinapyramine sulfate loaded-sodium alginate nanoparticles (QS-NPs) to reduce undesirable toxic effects of QS against the parasite *Trypanosoma evansi*, a causative agent of trypanosomosis. To determine the safety of the formulated nanoparticles, biocompatibility of QS-NPs was determined using Vero, Hela cell lines and horse erythrocytes in a dose-dependent manner. Our experiments unveiled a concentration-dependent safety/cytotoxicity (metabolic activity), genotoxicity (DNA damage, chromosomal aberrations), production of reactive oxygen species and hemolysis in QS-NPs treated cells. Annexin-V propidium iodide (PI) staining showed no massive apoptosis or necrosis. However, at very high doses (more than 300 times than the effective doses), we observed more toxicity in QS-NPs treated cells as compared to QS treated cells. QS-NPs were safe at effective trypanocidal doses and even at doses several times higher than the effective dose.

1. Background

Trypanosomosis caused by *Trypanosoma evansi* is an economically important disease of livestock. It has been recorded worldwide and is a major constraint to livestock productivity in Asia, Africa and South America. It affects a wide range of domestic and wild animals [1–4]. The parasite has also been reported to jump species and infect human [5]. Quinapyramine sulfate an aminoquinaldine, is a highly effective drug against this parasite. Therapeutic doses of the drug induce severe anaphylactic reaction in animals. In order to reduce undesirable toxic effects quinapyramine sulfate loaded-sodium alginate nanoparticles (QS-NPs) were synthesized in our lab. These QS-NPs were highly effective and were able to clear the parasites at much lower concentrations *in vitro* as well as *in vivo* in the mouse model [6]. We have shown that the survivability rate of 100% was obtained in the groups of mice receiving the recommended dose of conventional drug QS @ 5 mg/kg body weight and QS-NPs given @ 0.73 mg QS/kg body weight [6].

The small size nanoparticles allow the drug to cross biological barriers and enhance the efficacy due to increased surface to volume ratio. At the same time access of these particles into the cells and various cellular compartments including the nucleus can be toxic to the host. The nanoparticles may penetrate the cell and influence the cellular respiration through inactivating the essential enzymes by forming complexes with the catalytic sulfur of thiol groups in cysteine residues [7] and through the production of toxic radicals such as superoxide, hydrogen peroxide, and hydroxyl ions. The generation of reactive oxygen species (ROS) by cultured cells upon exposure to nanoparticles is also quite a common phenomenon. Nanoparticles if administered above a certain concentration exhibit genotoxic and cytotoxic effects which include DNA damage, chromosomal aberrations, and cell death [8]. Therefore, there is a need to address safety issues that are likely to arise from these applications. Establishing the entire toxicological profile is thus indispensable for proving the safety of nanoformulations. The dose dependent toxicity studies of nanoformulations are required for safe delivery. The present investigation aims to determine the safety of the QS-NPs before therapeutic applications against *T. evansi* in animals. The dose dependent toxicity studies, like metabolic activity of cells, generation of reactive oxygen species, DNA damage and apoptosis studies of QS-NPs using mammalian cell lines were performed.

* Corresponding author.
E-mail address: amanuja@rediffmail.com (A. Manuja).

http://dx.doi.org/10.1016/j.ijbiomac.2016.03.034
0141-8130/

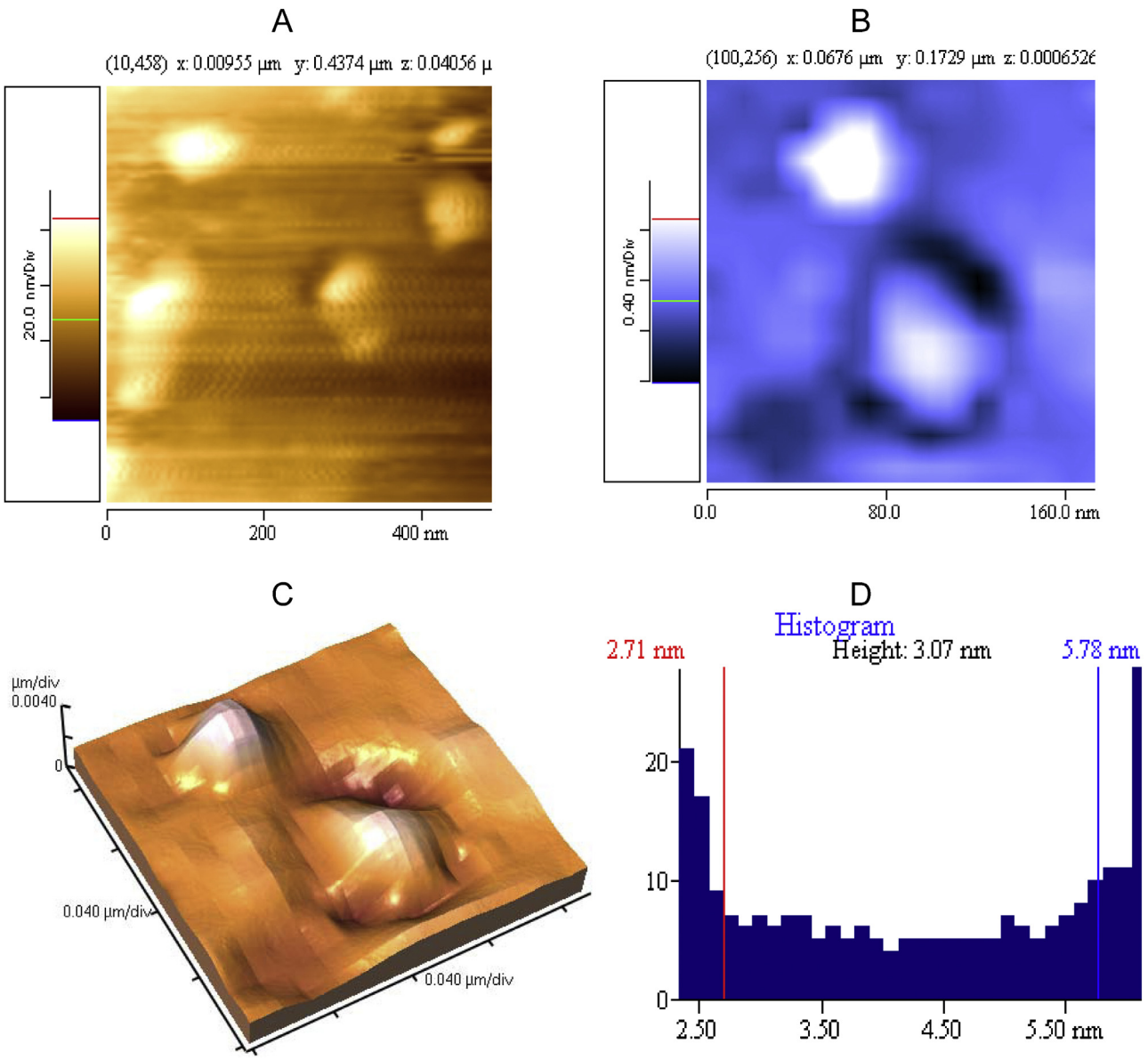

Fig. 1. Images of quinapyramine sulfate-loaded sodium alginate nanoparticles.
(A) QS-NPs viewed under AFM. (B) AFM image encapsulated QS-NPs. (C) Three-dimensional view of AFM image. (D) Size distribution of QS-NPs.

2. Experimental

2.1. Materials

Chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich Chemicals Private Ltd. (St. Louis, USA). Dioctyl sodium sulphosuccinate (AOT) was purchased from Central Drug House Private Ltd. (New Delhi, India). The Vybrant® apoptosis assay kit and Comet slides were from Invitrogen, Life Technologies, (Carlsbad, CA) and Chromous Biotech, (Bangalore, India). All the solvents used in the study were of analytical grade.

2.2. Preparation and characterization of nanoformulation

QS-NPs were synthesized using double emulsion cross-linking method as described previously [6]. The primary emulsion was prepared by emulsifying 1.0% w/v aqueous sodium alginate solution containing quinapyramine sulfate into dioctyl sodium sulfosuccinate (AOT) solution in 15% w/v methylene chloride. It was further emulsified into aqueous polyvinyl alcohol (PVA) solution by sonication over ice bath to form a secondary water-in-oil-in-water emulsion. QS-NPs were recovered by centrifugation at 15,000*g*, washed two times with deionized water to remove PVA, untrapped drug and resuspended in water. D-Mannitol (5%, w/v) was added as cryoprotectant, sonicated using a probe sonicator (on pulse 15 s and off pulse 20 s) for proper homogenization of drug and lyophilized at −90 °C and 0.0010 mbar pressure. Formulated nanoparticles were dispersed in deionized water at a concentration of 1 mg/ml. Atomic force microscopy (AFM) was perfomed to study the surface morphology and size distribution. The AFM images were captured with Veeco di-CP-II. AFM microscope (Veeco, NY, USA) operating in tapping non-contact mode.

2.3. Cell culture

Vero cell line (African green monkey kidney cell line) was obtained from the Veterinary Type Culture Collection (VTCC),