International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 1519-1523
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Human Paraoxonase-1: Functional Insights Within PLGA-Based Nanoparticles

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Received: 12 Jan 2019 / Accepted: 6 Mar 2019 / Published online: 1 Apr 2019
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Abstract

Human serum paraoxonase-1 (PON1) is a calcium dependent interfacial activated membrane protein associated with high density lipoproteins (HDL) play significant role in fundamental biological processes. This study determines the feasibility of formulating PON1 loaded solid lipid nanoparticles for developing a surrogate for the HDL associated PON1 in the form of PLGA (Poly Lactic glycolic acid) based solid lipid nano formulations. Studies were made on the preparation of h-PON1(human Paraoxonase-1) loaded solid lipids nano formulations with PLGA and all activities of PON-1 like lactonase, aryl esterase and paraoxonase were performed and PON1 did not lose its activity in various steps of nanoparticle preparation. Nanoparticulated PLGA based PON1 showed the highest activities.

Keywords

Paraoxonase-1, PLGA Nanoparticles, Drug Delivery, Nano formulations, Enzyme Activity.

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INTRODUCTION

Serum paraoxonase 1 (PON1) is a Ca2+-dependent mammalian enzyme, synthesized in the liver present in the plasma, and have broad range of hydrolytic activities that can be grouped under three categories: lactonase, aryl esterase and phosphotriesterase.[1,2,3,4,5] The lactonase activity of PON1 is responsible for the anti-oxidant properties and it has been significant connection with anti-atherogenic properties against macrophage foam cell formation, it modulates atherosclerotic lesion development,[6] attenuation of cholesterol and oxidized lipid influx, inhibition of macrophage cholesterol biosynthesis and stimulation of macrophage cholesterol efflux[7,8,9,10,11,12] while its phosphotriesterase activity imparts an important role to the enzyme in natural defense against various organophosphates (OPs) intoxication, including nerve gases[13,14,15] However, recent reports have suggested that PON1 and the other PONs are in fact lactonases, catalyzing both hydrolysis and formation of a variety of lactones[16,17,18] The structure-reactivity studies have also established that the native activity of PON1 is lactonase,[19,20] while other activities, e.g. arylesterase and paraoxonase are now being considered as merely promiscuous activities of PON1 which are not shared by other family members (e.g. PON2 and PON3).

PON1 has potential antioxidant properties as demonstrated in several studies and playing very crucial role in various disease conditions in human beings, but due to their short half-life, in stability and delivery problems, the complete therapeutic potential may not be possible with PON1 peptides.[21] Novel drug delivery systems for interfacially activated membrane proteins are still not yet

resolved.[21,22] The present study demonstrated preparation of PLGA based PON1 loaded solid lipid nanoparticle, understanding the activity of PON1 when formulated as solid lipid nanoparticles and paid attention on freeze drying of purified human PON1, The particle size of the h-PON1 loaded solid lipid nanoparticles and their combinations were measured by using SEM, AFM and TEM, their images were showed to be similar size of nanoparticle and were found to be spherical in shape, nano range from all the images in all instances.

Materials:

Cholesterol, dextrose, ethyl acetate, glucose, glycerol, maltose, mannitol, Poly Lactic glycolic acid, Poly vinyl alcohol. calcium chloride, ethylene diamine tetra acetic acid (EDTA), trizma hydrochloride (Tris-HCl), M-cresol purple indicator, tergitol NP-10 nonionic, paraoxon-ethyl pestanal, γ-nonanoic lactone, imidazole, bicine buffer, phenyl acetate, protease inhibitor cocktail, sodium chloride, sodium dodecyl sulfate (SDS) and all the other chemicals used were purchased from sigma aldrich and were of analytical grade.

METHODOLOGY

Freeze drying of purified human PON1 (h-PON1)

Purified human PON1 was freeze dried by using Scanvac cool safe freeze dryer. The freezer was turned on (Scanvac cool safe) 3 hours before use to achieve -110^{0}C. Weigh and measured volume of sample with excipients (1% Trehalose 1% maltose) and was in RB (Round bottom flask). Then sample was frozen by direct submersion in a low temperature bath or freezing chamber using methanol (-110^{0}C). The pre-frozen product was attached to the manifold. All the rubber valves of manifold were closed. After the sample was adequately frozen in the condenser at a temperature of -110^{0}C vacuum (0.1-0.6 m bar) was created in the drying chamber using vacuumed pump. It continued until the entire frozen matrix appeared dry/powdered form. Then the freeze-drying process was stopped by closing the vacuum valve or rubber valve and removing the RB flask from the adaptor.

Preparation of h-PON1 loaded solid lipid nanoparticles

PON1 loaded PLGA nanoparticles were prepared by the emulsion-diffusion-evaporation method with modifications. PON1 was dispersed in glycerin, which is used with a view to stabilize the enzyme. The aqueous phase is generally water containing a stabilizer. In this experiment, pH 8.0 buffer with 1mM $CaCl_2$, 0.01% tergitol was used. The buffer was prepared by adding 1mM $CaCl_2$, 0.01% tergitol, and 10% glycerin to Tris HCl 50 mM (Trizma hydrochloride) reagent grade. The prepared buffer was used to solubilize 1% PVA which acts as the stabilizer. Freeze dried PON1, was added to 0.3 ml glycerin and stirred for 5 min. This solution was added to 2 ml ethyl acetate solution containing 50 mg PLGA. The organic phase was stirred using a magnetic stirrer for at least 15 min. In a separate vial 50 mg PVA was dissolved in 5 ml buffer solution. While homogenization, using tissue homogenizer, the solution containing PON1 and PLGA was added in a drop wise manner to 5ml of 1% PVA in buffer solution. Homogenization was carried out at 15,000 rpm for 2 min. Later, the formed emulsion was added to 20 ml buffer solution on the magnetic stirrer, stirring at 800 rpm. To facilitate complete diffusion followed by evaporation of the organic solvent stirring was continued for at least 15 h. Then, the nanoparticles were centrifuged at 15,000 g for 5 min and the pellet was washed with the buffer solution and the supernatant, which contains unbound stabilizer, was discarded. The pellet was redispersed in the required amount of buffer solution for characterization.

CHARACTERIZATION OF NANOPARTICLES

Atomic force microscopy (AFM)

The surface morphology of solid lipid nanoparticles was analyzed using AFM Park system Model: XE-70 that was attached to a Nikon eclipse TE 2000-S microscope. The system was run by nanoscope software[21].

Scanning Electron Microscope (SEM)

In this study, images from ZEISS scanning electron microscope (SEM) for surface topography studies. h-PON1 loaded PLGA solid lipid nano particles were observed under a ZEISS scanning electron microscope (SEM).

RESULT AND DISCUSSION

Activity measurements of h-PON1 during various steps of solid lipid nanoparticles (SLN) preparation

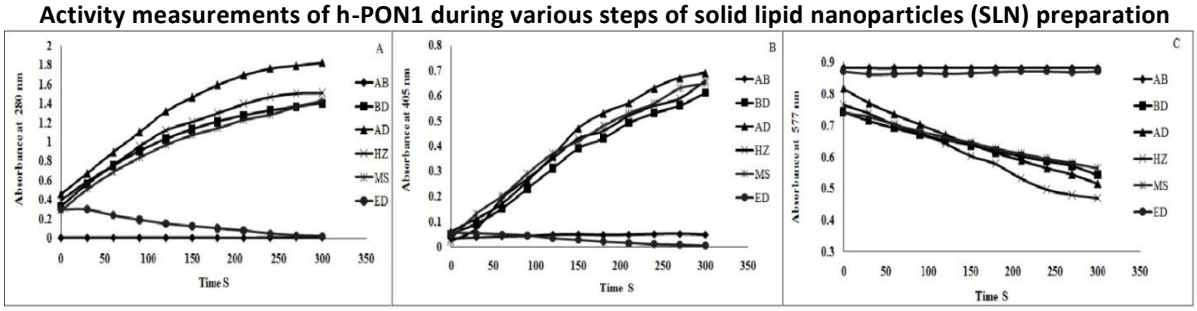

Figure 1: A: Phenyl acetate activity[22] of PON1 in various steps of PLGA SLN preparation. AB-Activity buffer; BD-Before freeze drying; AD-After freeze drying at -110°C with 0.1 % trehalose and 0.1% maltose; HZ-After homogenization at 5000 rpm/2 min; MS- Final step of nanoparticle process after overnight magnetic stirrer; ED-PON 1 with EDTA.

B: Paraoxonase activity[22] of PON1 in various steps of PLGA SLN preparation. AB- activity buffer; BD-before freeze drying; AD-After Freeze drying at -110 °C with 0.1 % trehalose and 0.1% maltose; HZ-After homogenization at 5000 rpm/2 min; MS- Final step of nanoparticle process after overnight magnetic stirrer; ED-PON 1 with EDTA.

C: Lactonase activity[22] of PON1in various steps of PLGA SLN preparation. AB-Activity buffer; BD-Before freeze drying; AD-After Freeze drying at -110°C with 0.1 % trehalose and 0.1% maltose; HZ-After homogenization at 5000 rpm/2 min; MS-Final step of nanoparticle process after overnight magnetic stirrer; ED-PON 1 with EDTA.

Activity of PON1 has been measured during various steps of the PLGA solid lipid nanoparticle (SLN) preparation process. After purification the enzyme has been freeze dried until the nanoparticle preparation process has been started. The activity of the enzyme with respect to paraoxonase, arylesterase and lactonase has been studied before freeze drying and after freeze drying. There was no difference in activities of the enzyme before and after freeze drying (Fig. 1A, 1B & 1C). The activities were also tested from the samples collected during various steps of the nanoparticle preparation process viz, after homogenization and after overnight stirring step for the removal of organic solvent from the nanoparticle preparation. All the results indicate that the enzyme PON1 retained all the three activities during this process (Fig. 1A, 1B & 1C).

Preparation and characterization of h-PON1 loaded PLGA SLN by AFM (Atomic force microscopy) and SEM (Scanning electron microscopy):

PON1 loaded PLGA solid lipid nanoparticles have been successfully prepared using various lipids and their combinations. The choice of a particular method for encapsulation of a substance in a colloidal carrier is most determined by the solubility characteristics of the active ingredient as well as the lipid. Pharmaceutical compounds are soluble in either aqueous or non-aqueous solvents, which facilitate incorporation of these compounds into the nanoparticles when the emulsification technique is adopted. A modified emulsion-diffusion-evaporation process was optimized to obtain PON1 loaded PLGA nanoparticles and most importantly to get active enzyme loaded into the nanoparticles.[27] Several modifications were carried out in the preparation process like controlling the temperature while emulsification at 4°C, reducing the homogenization speed from 15,000 to 5,000 rpm and using different types of surfactants like didodecyl dimethyl ammonium bromide (DMAB) and PVA. However, none of these modifications could preserve the enzyme activity. It was understood from the literature that the pH, ionic strength and solvents have definite roles in the stabilization of PON1 in PLGA nanoparticles. Therefore, considering these facts, a suitable buffer was used instead of water in various steps of the preparation process of nanoparticles. This strategy resulted in nanoparticles without complete loss of the enzyme activity, which was confirmed by analyzing the enzyme present in the supernatant. The literature suggests the use of double emulsion process for preparation of most of the enzyme loaded nanoparticles. However, in single emulsification process, with the use of a co-solvent, suitable conditions like pH and ionic strength for a specific enzyme will prevent the loss of enzyme activity. The present method provides extended conditions for the enzyme to remain stable[28,31,32, 33.]

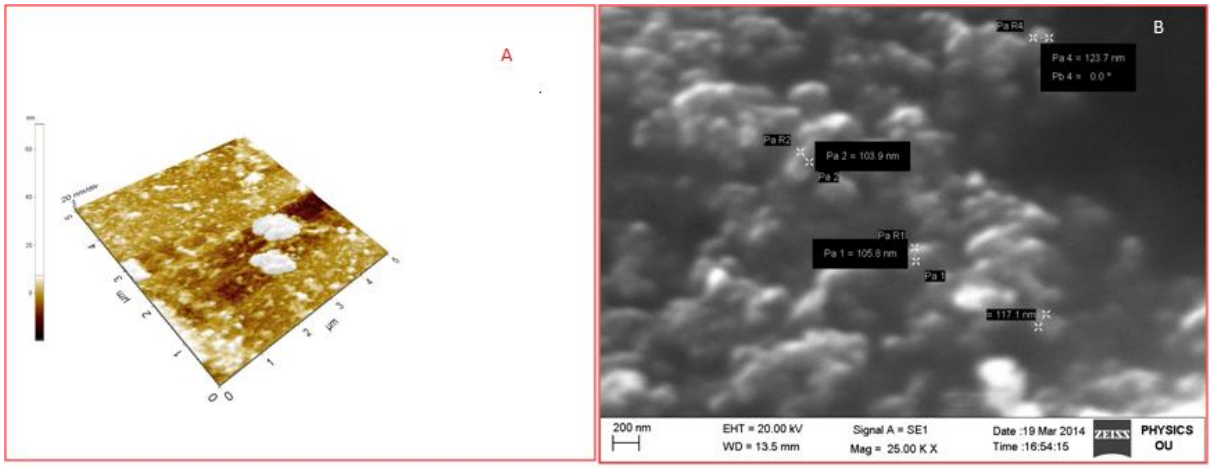

Fig: 2 A. AFM. B. SEM images of h-PON1 loaded PLGA solid lipid nanoparticle

The particle size of the PON1 loaded solid lipid nanoparticles prepared from PLGA combinations were listed in table 1. The AFM and SEM, images showed similar size of nanoparticle were found to be spherical from all the images in all instances (Figures 2.A. AFM. B. SEM images)

Table 1: Particle sizes of the PON1 containing solid lipid nanoparticles prepared by using PLGA

Types of nanoparticles	Size (nm)
PON1 PLGA NANOPARTICLES (PON1 PLGA)	254 ±20

CONCLUSIONS

Purified freeze-dried h-PON1 did not lose its activity in various steps of nanoparticle preparations**.** The particle size of the h-PON1 loaded PLGA solid lipid nanoparticles prepared with PLGA. The AFM and SEM, images showed similar size of nanoparticle were found to be spherical from all the images in all instances. Nanoparticulate PON1 loaded PLGA nanoparticles show highest lactonase, arylesterase and paraoxonase activities of PON1 compared to normal PON1.

Acknowledgements

The authors are grateful for the support provided by Zaney Pharma Innovations. Hyderabad, India for providing instrumentation facility.

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