The roles of fatty acid on physicochemical properties and improved cancer targeting of fatty acid conjugated albumin nanoparticles

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Introduction

In cancer therapy, patient-friendly nano-systems featuring targeted delivery of anti-cancer agents to the tumor tissues are desirable. This promises to overcome common side effects observed in chemotherapy. Numerous Nano-sized platforms have been rationally designed over the decades as the physiological patterns of cancer cells were better understood.

Several studies have reported a stellar performance of self-assembled nanostructures based on albumin. However, albumin-based NPs formulation still have challenges with stability and toxicity when exposed to biological systems. Additionally, roles of fatty acids on cancer cells have been of interest by many researchers. Therefore, in the present study, we for the first time investigated the roles of different chain lengths of fatty acids when conjugated to albumin for the preparation of nanocarriers for anticancer therapy. The physicochemical properties of fatty acids on and cancer targeting were characterized to establish a new NPs platform for targeting.

Purpose

The hypothesis was that HSA-fatty acid conjugates can maintain the colloidal stability of nanocarriers when exposed to a biological environment and can efficiently encapsulate anticancer drugs for improved efficacy in targeting cancer cells.

Methods

Two different types of fatty acids (short chain, 2-hydroxybutyric acid, C4; long chain, oleic acid, C18:1) were conjugated to albumin via simple 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) coupling reaction. Structural modulations of fatty acid conjugated albumin were identified by FT-IR spectroscopy, MALDI-TOF. Doxorubicin HCl (DOX) was selected as model drug for verifying this system. Additionally, the structures of DOX-loaded fatty acid conjugated ANPs were characterized by Transmission electron microscopy (TEM) equipped with Energy-dispersive X-ray spectroscopy (EDS). In vitro cellular cytotoxicity and uptake of the DOX formulations were evaluated in three cancer cell lines (A549, PANC-1, and HT-29). The statistical significance (p value) of the observed differences was analyzed(*= $p < 0.05$, ** =$p < 0.01$, ***= $p < 0.001$)

Results

The MALDI-TOF and FT-IR spectrums suggested the complex constituted in multi fatty acid-to-one HSA molecule reaction **(Fig. 1)**.

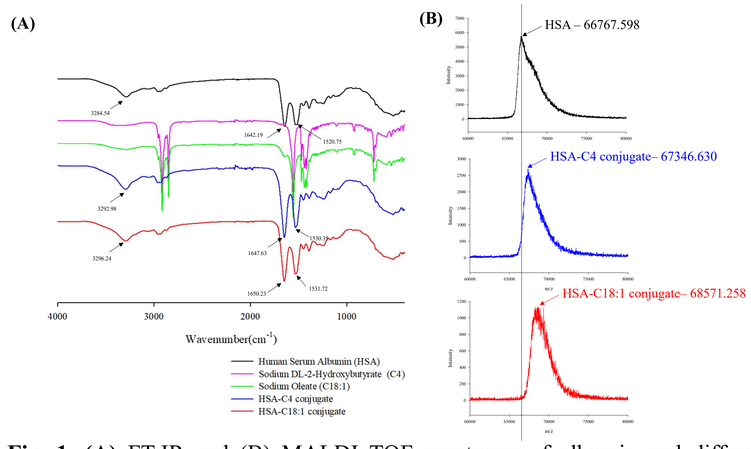

Fig. 1. (A) FT-IR and (B) MALDI TOF spectrums of albumin and different Human serum albumin (HSA)-fatty acid conjugates

Fatty acid conjugated albumin supported the formation of self-assembled structures with an average size of approximately 200 nm and negative surface charge, when incubated with excess DOX in an aqueous solution (**Fig. 2**). Different loading methods affected the physiochemical properties of DOX-loaded NPs (**Table 1**). DOX-loaded fatty acid conjugated to albumin allowed efficient encapsulation of hydrophobic DOX into the core of the self-assembled structure (**Fig. 3**), enabling a sustained release behavior in PBS pH 7.4 medium **(Fig. 4)**.

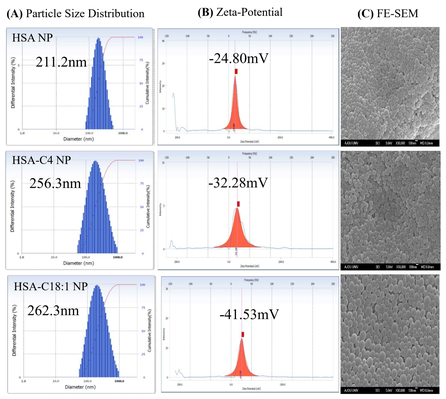

Fig. 2. Morphological analysis of different ~~plain~~ HSA-based NPs

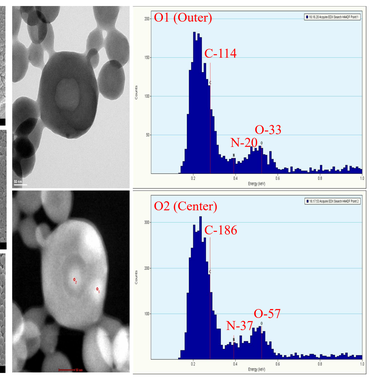

Fig. 3. TEM/EDS analysis of DOX-loaded HSA-C18:1 NPs

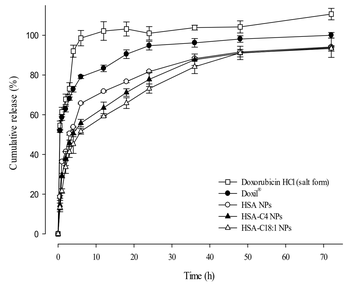

Fig. 4. In vitro release profiles of DOX-loaded NPs at pH 7.4 PBS buffer medium

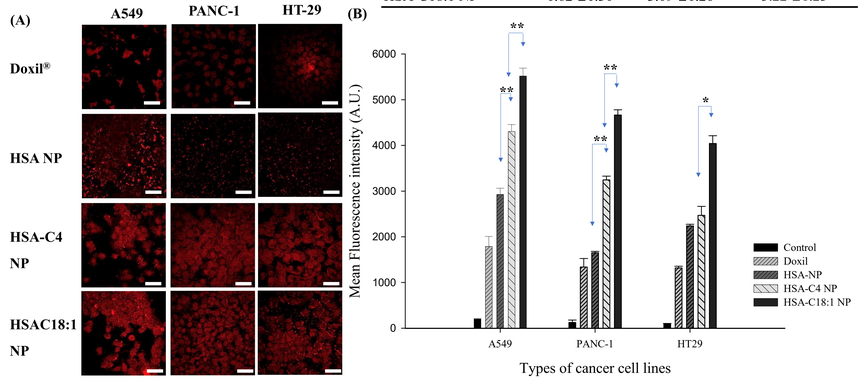

Fig. 5. In vitro cellular uptake studies of DOX-loaded NPs

Table 1. Physiochemical properties of different DOX-loaded NPs according to loading methods

Methods	Sample Type	Particle size (nm) / PDI	Zeta potential (mV)	Loading content (%)	Loading efficiency (%)
In process	HSA NP	433.07 ± 34.53 / 0.057 ± 0.036	-16.34 ± 2.04	28.81 ± 0.07	80.17 ± 1.46
	HSA-C18:1 NP	199.53 ± 6.28 / 0.133 ± 0.035	-38.89 ± 0.30	4.27 ± 0.10	11.87 ± 0.27
Incubation	HSA NP	169.78 ± 44.17 / 0.066 ± 0.022	-17.22 ± 1.80	21.81 ± 0.17	55.51 ± 6.64
	HSA-C18:1 NP	118.38 ± 87.46 / 0.237 ± 0.120	-48.17 ± 0.99	37.53 ± 0.14	91.14 ± 2.53

Table 2. Comparison of IC50 values (μg/mL) in various DOX-loaded NPs against cancer cells

Samples	Types of cancer cell lines		
	A549	PANC-1	HT-29
Doxil®	24.20 ±1.28	17.22 ±1.37	20.73 ±1.31
HSA NP	8.13 ±1.18	11.37 ±1.21	14.39 ±1.34
HSA-C4 NP	7.39 ±1.19	5.37 ±1.30	9.64 ±1.16
HSA-C18:1 NP	1.62 ±1.30	3.09 ±1.26	3.22 ±1.23

DOX-loaded fatty acid conjugated ANPs showed an increased cytotoxic effects *in vitro*. Specifically, *in vitro* cytotoxicity studies with three cancer cell lines (A549, HT-29, and PANC-1) indicated that DOX-loaded C18:1 conjugated albumin have distinctive cytotoxic effects compared to Doxil® (**Table 2**). Confocal microscopy and flow cytometry exhibited that the cellular uptake of DOX-loaded fatty acid conjugated ANPs was varied by the different chain lengths of fatty acids **(Fig. 5)**.

Conclusions

A novel HSA-fatty acid conjugates was synthesized. This nanocarrier is stable when exposed to a biological environment and can be loaded with hydrophilic anticancer drugs. This new carrier system improves the targeting efficiency and drug accumulation in cancer cells. Fatty acid albumin conjugates are promising new drug carriers in cancer therapy.

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