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Article

Application of Nanocarrier-Based Targeted Drug Delivery in the Treatment of Liver Fibrosis and Vascular Diseases

Yan Wen [1], Xiaofeng Wu [1], Lilu Wang [1], Hongwei Cai [2,*] and Yawen Wang [3]

[1] The University of Hong Kong, Hong Kong, China
[2] Hangzhou Hengyan Textile Co., Ltd., Hangzhou, China
[3] Carnegie Mellon University, Pittsburgh, Pennsylvania, USA
* Correspondence: Hongwei Cai, Hangzhou Hengyan Textile Co., Ltd., Hangzhou, China

Abstract: Liver fibrosis and vascular diseases are often accompanied by chronic inflammation and excessive extracellular matrix deposition, posing significant clinical challenges. In this study, we developed a poly (lactic-co-glycolic acid) (PLGA)-based nanocarrier system for targeted delivery of anti-inflammatory and antifibrotic agents. The nanocarriers were fabricated via a nanoprecipitation method and functionalized with α-smooth muscle actin (α-SMA) antibodies and vascular cell adhesion molecule-1 (VCAM-1) aptamers to enhance cell-specific targeting. Characterization revealed uniform spherical morphology with a mean particle size of 150 ± 20 nm, increasing to 165 ± 25 nm after ligand modification, and a stable zeta potential of -20 ± 5 mV. Flow cytometry confirmed a conjugation efficiency of 85 ± 5%. Confocal imaging showed 2.3-fold and 2.5-fold increases in uptake by hepatic stellate and vascular endothelial cells, respectively. ELISA results demonstrated significant reductions in TNF-α, IL-6, Collagen I, and Fibronectin levels following treatment with drug-loaded nanocarriers. In vivo studies using C57BL/6 mouse models of liver fibrosis and vascular injury confirmed improved therapeutic efficacy. The nanocarrier group showed marked reductions in ALT (60 ± 6 U/L), AST (80 ± 8 U/L), liver fibrosis area (15 ± 3%), and atherosclerotic plaque (12 ± 2%) compared to controls. No significant adverse effects were observed. These results suggest that ligand-modified PLGA nanocarriers provide a promising strategy for targeted therapy of fibrosis-related diseases improved efficacy and safety profiles.

Keywords: targeted drug delivery; PLGA nanocarriers; liver fibrosis; vascular inflammation; ligand modification; cellular uptake; anti-fibrotic therapy

1. Introduction

Liver fibrosis and vascular diseases are major global health concerns that seriously affect human health [1,2]. Liver fibrosis is a key stage in the progression of chronic liver diseases to cirrhosis, mainly characterized by excessive extracellular matrix (ECM) buildup in the liver, which disrupts normal liver structure and function [3]. Vascular diseases, including atherosclerosis and vascular blockages, result from chronic inflammation of blood vessels and endothelial dysfunction [4]. These conditions eventually lead to narrowing or obstruction of blood vessels, reducing blood supply to organs and tissues. Current treatments for liver fibrosis and vascular diseases have significant limitations [5]. Conventional drug therapies lack selectivity, affecting both diseased and healthy tissues, which leads to severe side effects and restricts the drug dosage and effectiveness [6]. Moreover, many drugs cannot easily penetrate biological barriers, making it difficult to achieve and maintain effective drug concentrations at the target sites [7]. Therefore, a strategy that enables precise drug delivery to diseased areas while improving treatment outcomes and minimizing side effects is urgently needed. Advances in nanotechnology provide new possibilities to address these challenges. Nanocarriers, due to their small size

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(typically 1-1000 nm), high surface area and modifiable surfaces, have shown great promise in drug delivery [8]. With proper design, nanocarriers can target specific cells or tissues, improve drug stability, extend circulation time, and enhance drug distribution in the body. In the treatment of liver fibrosis and vascular diseases, nanocarriers have the potential to deliver anti-inflammatory and antifibrotic drugs with high precision, slowing disease progression and improving therapeutic outcomes.

2. Materials and Methods

2.1. Preparation and Characterization of Nanocarriers

Nanocarriers containing anti-inflammatory and antifibrotic drugs were prepared using the nanoprecipitation method [9]. Poly (lactic-co-glycolic acid) (PLGA), a biodegradable polymer, was selected as the carrier material. The drug and PLGA were dissolved in an organic solvent and slowly added dropwise into an aqueous phase containing a surfactant under continuous stirring. The nanoparticles were formed through solvent evaporation. The particle size and zeta potential of the nanocarriers were measured using dynamic light scattering (DLS), while their morphology and structure were examined by transmission electron microscopy (TEM).

2.2. Modification with Targeting Ligands

To enhance targeting ability, specific ligands were selected based on their affinity for receptors highly expressed in liver fibrosis and vascular disease sites. For hepatic stellate cells, which overexpress α-smooth muscle actin (α-SMA), a specific antibody was covalently conjugated to the nanocarrier surface [10]. To target vascular endothelial cells expressing vascular cell adhesion molecule-1 (VCAM-1), an aptamer was chemically linked to the nanocarriers. The modification efficiency was assessed using flow cytometry and immunofluorescence staining.

2.3. In Vitro Cell Studies

Hepatic stellate cells and vascular endothelial cells were cultured to establish in vitro models. The nanocarriers were incubated with the cells, and the CCK-8 assay was used to evaluate their effect on cell viability. Laser confocal microscopy was applied to observe nanocarrier uptake, providing insights into the impact of ligand modification on cellular internalization [11-13]. The levels of inflammatory cytokines and fibrosis-related proteins in the culture supernatant were quantified using an enzyme-linked immunosorbent assay (ELISA) to assess the therapeutic effects of drug-loaded nanocarriers on inflammation and fibrosis.

2.4. Animal Studies

Liver fibrosis and vascular disease models were established in C57BL/6 mice. Liver fibrosis was induced by intraperitoneal injection of carbon tetrachloride (CCl_4), while vascular disease was modeled using a high-fat diet combined with endothelial injury. Mice were randomly assigned to the control, free drug, or nanocarrier treatment groups. Blood and tissue samples were collected at different time points to measure liver function and lipid levels. Histopathological analysis was performed to examine liver and vascular tissue changes. Immunohistochemical staining was used to detect inflammatory cytokines, fibrosis-related proteins and angiogenesis markers, enabling a comprehensive evaluation of the targeting efficiency and therapeutic effects of the nanocarrier system.

3. Results

3.1. Characteristics of Nanocarriers

The prepared nanocarriers had a uniform spherical shape with a narrow size distribution, as shown in Table 1 [13]. DLS measurements showed an average particle size of

150 ± 20 nm and a zeta potential of -20 ± 5 mV. TEM images confirmed a core-shell structure, with the drug evenly encapsulated inside, as shown in Figure 1. After modifying the nanocarriers with targeting ligands, the particle size increased slightly to 165 ± 25 nm, which remained within an acceptable range. The zeta potential showed no significant change, staying at -20 ± 5 mV. Flow cytometry and immunofluorescence staining verified successful ligand attachment, with a modification efficiency of 85 ± 5%, as illustrated in Figure 1. Among 100 randomly selected nanocarriers, 85 ± 5 had targeting ligands successfully conjugated.

Table 1. Physicochemical characteristics of nanocarriers before and after ligand modification.

Nanocarrier Type	Average Particle Size (nm)	Zeta Potential (mV)	Targeting Ligand Modification Efficiency (%)
Unmodified	150 ± 20	-20 ± 5	-
Modified	165 ± 25	-20 ± 5	85 ± 5 (Among 100 randomly selected nanocarriers)

3.2. In Vitro Cell Studies

CCK-8 assay results indicated that nanocarriers at different concentrations (0.1 μg/mL, 1 μg/mL, 10 μg/mL) did not significantly affect the viability of hepatic stellate cells or vascular endothelial cells after 24 hours ($p > 0.05$), as shown in Table 2. These results confirmed that the nanocarriers had good biocompatibility.

Table 2. Cell viability of hepatic stellate and vascular endothelial cells after nanocarrier treatment (CCK-8 assay).

Cell Type	Control Group (%)	0.1 μg/mL (%)	1 μg/mL (%)	10 μg/mL (%)
Hepatic Stellate Cells	98.5 ± 3.2	97.8 ± 3.5	98.2 ± 3.0	97.5 ± 3.8
Vascular Endothelial Cells	99.0 ± 2.8	98.0 ± 3.3	98.6 ± 3.1	97.9 ± 3.6

Laser confocal microscopy showed that modified nanocarriers had significantly higher uptake in cells compared to unmodified nanocarriers [14,15]. Fluorescence intensity analysis revealed that in hepatic stellate cells, the average intensity increased from 1500 ± 200 a.u. (unmodified) to 3500 ± 300 a.u. (modified), and in vascular endothelial cells, fluorescence intensity increased from 1300 ± 180 a.u. (unmodified) to 3200 ± 280 a.u. (modified), as shown in Table 3. These results confirm that ligand modification improved targeting ability.

Table 3. Fluorescence intensity of nanocarrier uptake in different cell types.

Cell Type	Unmodified Nanocarriers (a.u.)	Modified Nanocarriers (a.u.)
Hepatic Stellate Cells	1500 ± 200	3500 ± 300
Vascular Endothelial Cells	1300 ± 180	3200 ± 280

ELISA results showed that drug-loaded nanocarriers significantly reduced the levels of inflammatory cytokines (TNF-α, IL-6) and fibrosis-related proteins (Collagen I, Fibronectin) in the cell culture supernatant compared to the free drug and control groups, as shown in Table 4.

Table 4. Inflammatory cytokine and fibrosis-related protein levels in vitro (ELISA results).

Protein Name	Control (pg/mL or ng/mL)	Free Drug Group (pg/mL or ng/mL)	Nanocarrier-Delivered Drug Group (pg/mL or ng/mL)
TNF-α	250 ± 20	200 ± 15	120 ± 10
IL-6	300 ± 25	230 ± 20	150 ± 12
Collagen I	500 ± 30	400 ± 25	280 ± 20
Fibronectin	450 ± 35	380 ± 28	250 ± 18

These findings demonstrated that nanocarriers effectively delivered therapeutic drugs and inhibited inflammation and fibrosis progression.

3.3. Animal Study Results

Serum biochemical analysis showed that ALT, AST, total cholesterol (TC), and triglycerides (TG) levels were significantly lower in the nanocarrier-treated group than in the control and free drug groups, as shown in Table 5 [15,16].

Table 5. Serum biomarkers in animal models following different treatments.

Biomarker	Control	Free Drug Group	Nanocarrier Treatment Group
ALT (U/L)	120 ± 10	90 ± 8	60 ± 6
AST (U/L)	150 ± 12	110 ± 10	80 ± 8
TC (mmol/L)	4.5 ± 0.4	3.8 ± 0.3	3.0 ± 0.2
TG (mmol/L)	2.8 ± 0.3	2.2 ± 0.2	1.6 ± 0.1

Histopathological analysis showed that fibrosis and atherosclerotic plaque areas were significantly reduced in the nanocarrier-treated group [17,18], as shown in Table 6. Liver fibrosis area decreased from 35 ± 5% (control) to 15 ± 3%, and vascular plaque area decreased from 28 ± 4% (control) to 12 ± 2%.

Table 6. Histopathological analysis of liver fibrosis and vascular plaque area.

Tissue Type	Control (%)	Nanocarrier Treatment Group (%)
Liver Fibrosis Area	35 ± 5	15 ± 3
Atherosclerotic Plaque Area	28 ± 4	12 ± 2

Immunohistochemical staining further confirmed that inflammatory cytokines, fibrosis-related proteins, and angiogenesis markers were significantly reduced in liver and vascular tissues of the nanocarrier treatment group, as shown in Table 7.

Table 7. Immunohistochemical analysis of protein expression in tissue samples.

Tissue Type	Protein Name	Control	Free Drug Group	Nanocarrier Treatment Group
Liver	TNF-α	1.00 ± 0.08	0.75 ± 0.06	0.40 ± 0.04
Vascular	VCAM-1	1.05 ± 0.09	0.80 ± 0.07	0.50 ± 0.05
Liver	Collagen I	1.20 ± 0.10	0.90 ± 0.08	0.60 ± 0.06
Vascular	VEGF	1.10 ± 0.09	0.85 ± 0.07	0.65 ± 0.05

These results confirmed the improved targeting and therapeutic effects of the nanocarrier system. Additionally, throughout the study, no significant adverse effects, such as abnormal weight loss, lethargy, or hair loss, were observed in the nanocarrier-treated group (n = 50), suggesting that the nanocarriers effectively reduced systemic drug toxicity.

4. Discussion

This study successfully developed a nanocarrier-based targeted drug delivery system for the treatment of liver fibrosis and vascular diseases. Through precise design, the

nanocarrier exhibited strong targeting ability, good biocompatibility, and effective tissue penetration. Both in vitro and in vivo results consistently demonstrated that the system efficiently transported anti-inflammatory and anti-fibrotic drugs to diseased sites, significantly slowing disease progression while reducing systemic side effects [19,20]. The choice of carrier material and targeting ligand plays a key role in nanocarrier design. PLGA, a biodegradable polymer with good biocompatibility and high drug-loading capacity, has been widely used in drug delivery systems. Surface modification with specific targeting ligands allows the nanocarrier to accurately recognize and bind to diseased cells or tissues, thereby improving drug delivery efficiency [21]. However, further optimization of design parameters is required. Adjusting particle size, surface charge and ligand density can help enhance stability, extend circulation time, and improve targeting specificity [22]. Despite these promising results, challenges remain for clinical application. Issues such as scalability, quality control and safety assessment need to be addressed [23]. Additionally, the interaction between nanocarriers and biological systems is not yet fully understood [24]. Further research is required to clarify their metabolism, distribution, and potential toxicity in vivo [25]. A deeper understanding of these aspects will provide a strong foundation for the clinical translation of nanocarrier-based drug delivery systems.

5. Conclusion

In this study, we developed a PLGA-based nanocarrier system for the targeted delivery of anti-inflammatory and antifibrotic drugs. The nanocarriers were prepared using a nanoprecipitation method and showed stable physicochemical properties, with an average particle size of 150 ± 20 nm and a zeta potential of -20 ± 5 mV. After surface modification with α-SMA antibodies and VCAM-1 aptamers, the particle size slightly increased to 165 ± 25 nm, and the ligand conjugation efficiency reached 85 ± 5%, indicating successful targeting modification. Cellular uptake experiments showed that ligand-modified nanocarriers were more efficiently internalized by hepatic stellate cells and vascular endothelial cells. Fluorescence intensity increased from 1500 ± 200 to 3500 ± 300 in hepatic stellate cells and from 1300 ± 180 to 3200 ± 280 in endothelial cells. These results confirmed that surface modification significantly improved the targeting ability. In addition, ELISA analysis demonstrated that nanocarrier-delivered drugs led to a more pronounced reduction in TNF-α, IL-6, Collagen I, and Fibronectin levels than free drugs or controls, confirming the therapeutic effect in vitro. In vivo, the nanocarrier-treated group showed improved liver function and reduced blood lipid levels. Serum ALT and AST decreased to 60 ± 6 U/L and 80 ± 8 U/L, respectively, while TC and TG dropped to 3.0 ± 0.2 mmol/L and 1.6 ± 0.1 mmol/L. Histological analysis showed that liver fibrosis area decreased from 35 ± 5% to 15 ± 3%, and vascular plaque area decreased from 28 ± 4% to 12 ± 2%. Immunohistochemistry confirmed the lower expression of TNF-α, VCAM-1, Collagen I, and VEGF in treated tissues. No obvious toxicity or side effects were observed during the entire study.

These findings indicate that the nanocarrier system can effectively improve drug accumulation at disease sites and enhance therapeutic effects while minimizing systemic toxicity. Although the results are promising, further research is needed to evaluate large-scale production, long-term safety, and the in vivo fate of the nanocarriers. Future optimization of particle size, surface charge, and ligand density may help improve targeting accuracy and biological stability. These efforts will support the potential clinical application of nanocarrier-based drug delivery systems in the treatment of liver fibrosis and vascular diseases.

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