Liposomes: Emerging Nanoparticles for Treating Pyelonephritis caused by Invasive Uropathogenic *Escherichia coli*

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Abstract

Urinary tract infections (UTIs) are among the most common ailments across the U.S., affecting millions of people yearly. UTIs are often caused by strains of uropathogenic *Escherichia coli* (UPEC), some of which are invasive in nature. Invasive UPEC can ascend the bladder and colonize the kidneys, thereby resulting in pyelonephritis. Antibiotics are routinely prescribed to treat UTIs but can be ineffective in eliminating invasive pathogens, thereby contributing to recurring infections and antibiotic resistance. Therefore, novel and innovative strategies are essential to treat infections caused by intracellular UPEC. Liposomes are inert and biocompatible nanoparticles that can be used to encapsulate a wide variety of molecules, including antimicrobial agents. The goal of the current study is to investigate the efficacy of ciprofloxacin-encapsulated liposomes to treat pyelonephritis caused by invasive UPEC. We hypothesize that biocompatible ciprofloxacin-encapsulated liposomes will reduce the bacterial load in kidney cells infected with UPEC. To test our hypothesis, the human kidney HK-2 cell line was used as the host and the uropathogenic *E. coli* strain CI5 was used as the pathogen. Infected HK-2 cells were treated with different concentrations of ciprofloxacin-encapsulated liposomes and our results indicate that there was a decrease in the number of intracellular UPEC found in infected HK-2 kidney cells. Our findings suggest that further investigation is warranted to evaluate the therapeutic effects of liposomes in treating pyelonephritis caused by intracellular uropathogens.

Introduction

Urinary tract infections (UTIs) are the second most common ailments in humans. They are caused by uropathogens like bacteria, that can colonize the bladder, ureters and kidneys. UTIs cause significant morbidity and more than $2 billion are spent annually in treatment in the US alone [1, 2]. Bladder infections are the most common UTIs, however, some invasive uropathogens can infect the kidneys by ascending through the urinary tract. The resulting pyelonephritis is harder to treat and may result in kidney damage and septicemia. Uropathogenic *Escherichia coli* (UPEC) are the primary causative agent in more than 80% of UTIs [3]. Currently, a range of antibiotics are prescribed to treat UTIs. However, some invasive UPEC are able to evade the effects of antibiotics and internalize into tissues, provoking recurrent or chronic infections. A critical need for new strategies is in required to treat UTIs caused by invasive uropathogens.

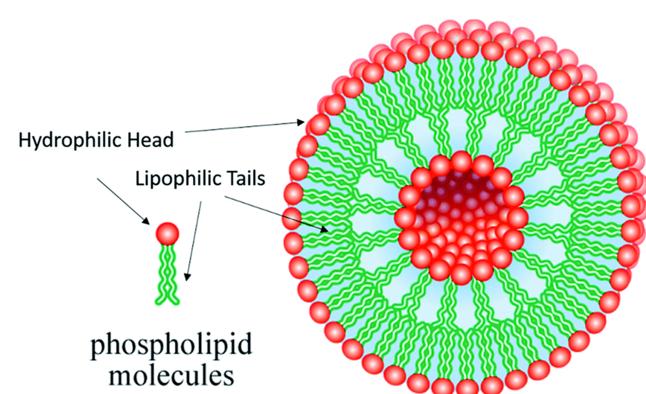

Figure 1: Structure of liposome[4]

Type of liposome nanoparticle	Mean diameter (nm)	Zeta-potential (mV)	Polydispersity index
Empty liposome	193.28	-1.67	0.16
Ciprofloxacin-encapsulated liposome	207.27	-5.98	0.08
Coumarin-liposome	132	-16.7	0.1

Table 1: Properties of liposome synthesized

Liposomes are spherical nanoparticles with an aqueous core surrounded by a lipid bilayer. The aqueous core can be used to carry antimicrobial drug compounds, thereby making liposomes efficient drug delivery systems [4,5]. In the current study, we hypothesize that biocompatible ciprofloxacin-encapsulated liposomes will reduce the bacterial load in kidney cells infected with UPEC. To test this hypothesis, we used an in vitro model of UTI pathogenesis that consists of the human kidney cell line HK-2 and the invasive uropathogen *Escherichia coli* strain CI5. Different types of liposomes were synthesized and characterized (table 1). We evaluated the cytotoxicity of these liposomes in the HK-2 cell line followed by the uptake and binding of liposome nanoparticles by the HK-2 cell line. We also compared the microbicidal efficacy of liposomes and antibiotics on infected HK-2 cells. These studies will enable us to determine if liposome nanoparticles can be used as a therapeutic to treat pyelonephritis.

Results

HK-2 cells are invaded by *E. coli* CI5 and respond by producing pro-inflammatory molecules

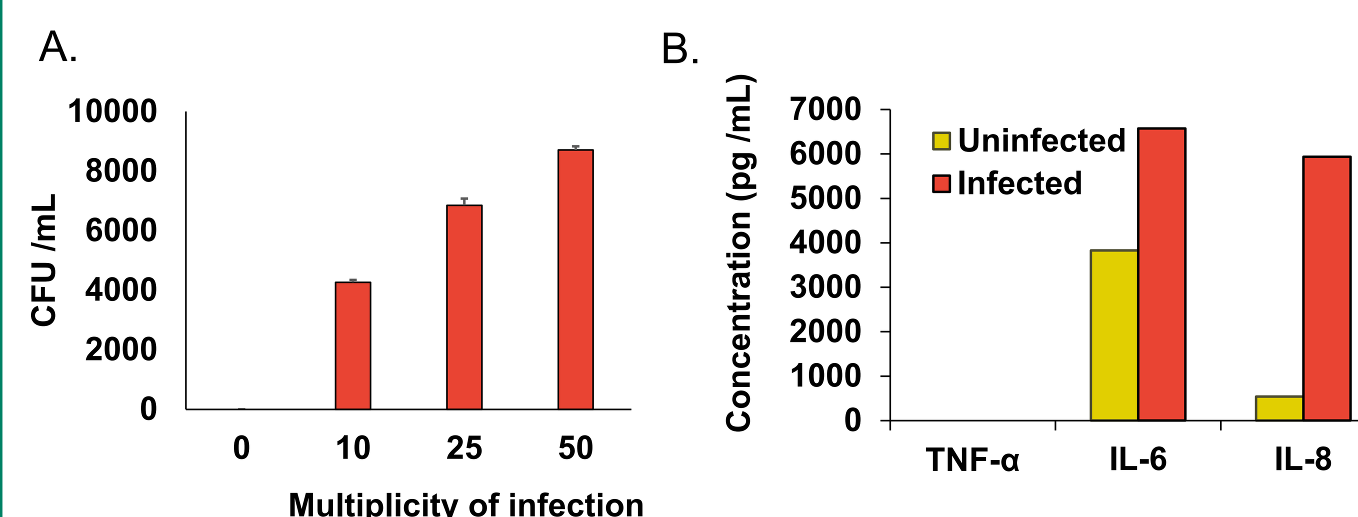

Figure 2: (A) HK-2 cells were infected with different MOI of *E. coli* CI5 strain followed by treatment with gentamicin. HK-2 cells were lysed and the colony forming units (CFU) of bacteria were enumerated and depicted in the graph . (B) Secretion of pro-inflammatory molecules from infected HK-2 cells was evaluated in culture supernatants by ELISAs.

Drug-encapsulated liposomes are not cytotoxic to HK-2 cells

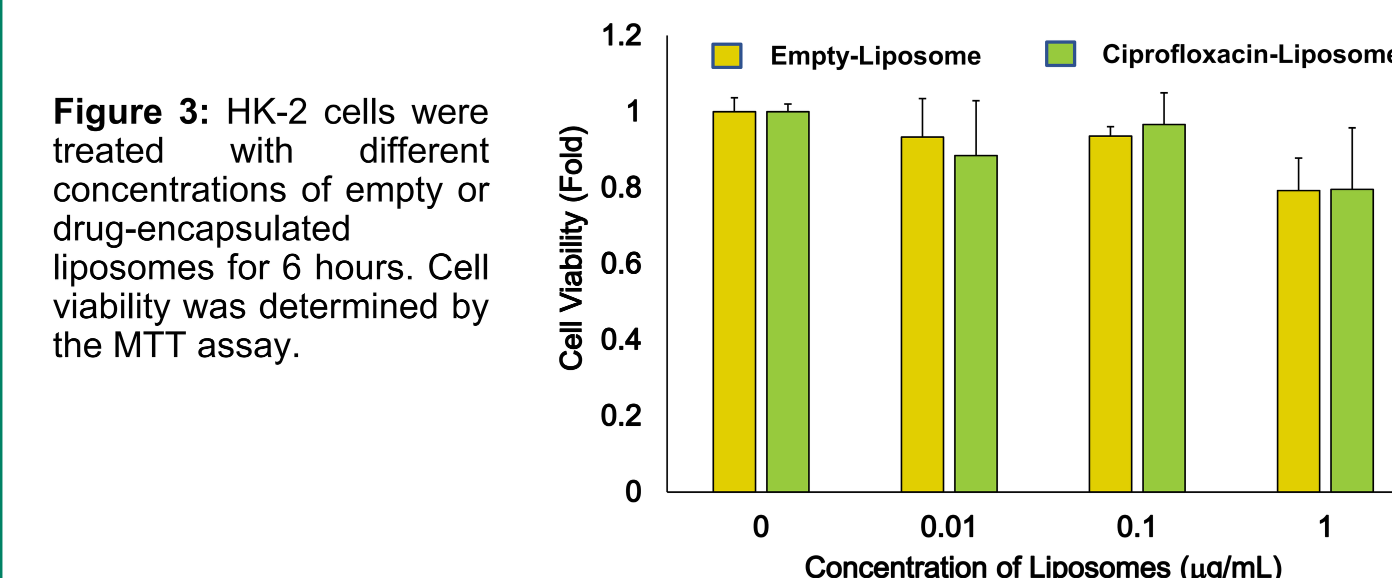

Figure 3: HK-2 cells were treated with different concentrations of empty or drug-encapsulated liposomes for 6 hours. Cell viability was determined by the MTT assay.

Liposomes bind to HK-2 cells

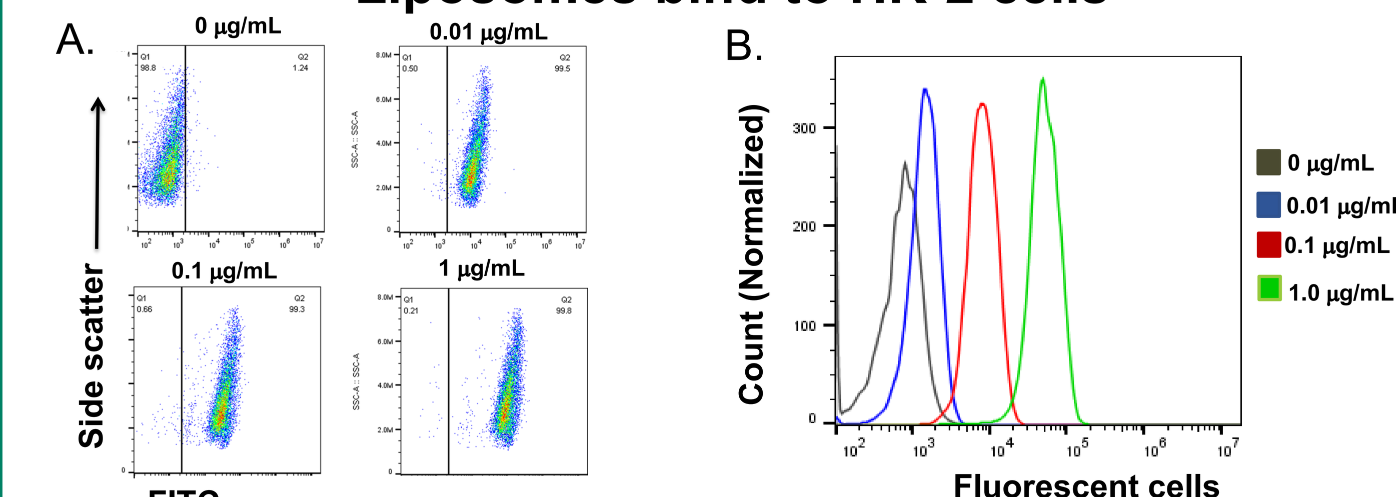

Figure 4: HK-2 cells were treated with coumarin-labeled liposomes and analyzed by flow cytometry. Binding of liposomes to HK-2 cells is shown as scatter plots (A) and histogram (B).

Drug-encapsulated liposomes decrease the bacterial load in HK-2 cells

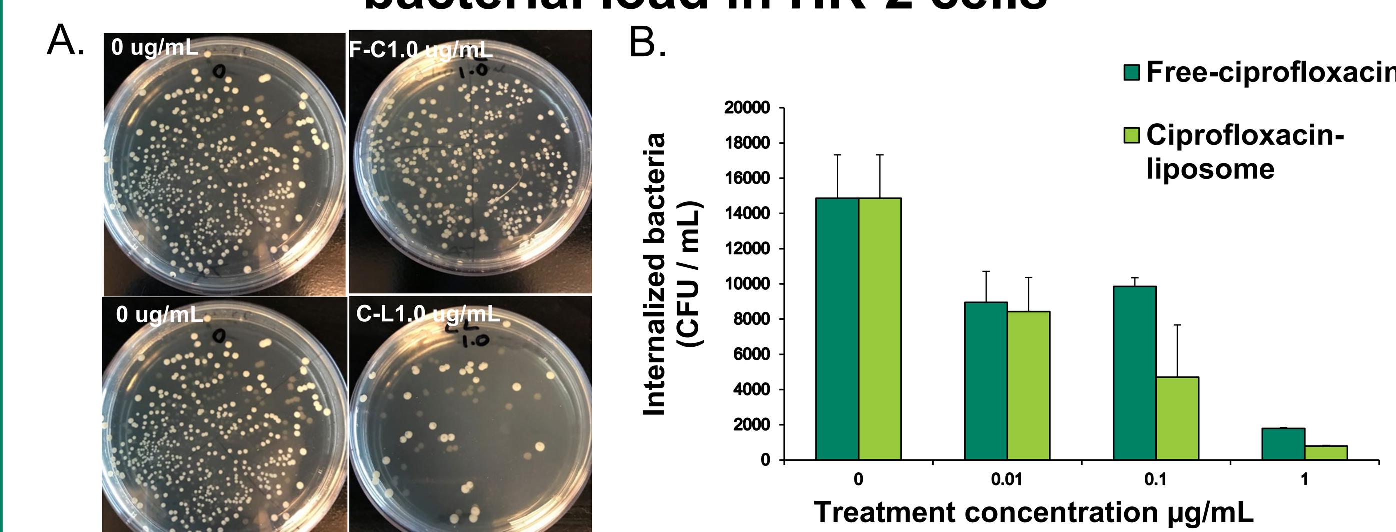

Fig 5: HK-2 cells were infected with *E. coli* CI5 at MOI of 1:50, followed by gentamicin treatment to kill extracellular bacteria, followed by incubation with either ciprofloxacin–bearing liposomes or free ciprofloxacin. Intracellular bacteria were enumerated. Representative LB agar plates (A) and quantification of CFU/mL are shown (B).

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Methods

Liposome synthesis and characterization: Liposomes were prepared by hydration of a lipid film followed by the extrusion method. Encapsulation of ciprofloxacin (2 mg/100 mg lipid concentration) into the liposomes was carried out using pH-gradient loading at 37°C for 1 h. Liposome were characterized for size, polydispersity index and zeta potential using a dynamic light scattering instrument.
Handling of HK-2 cells: The human kidney proximal tubule cell line, HK-2, was grown in media comprised of Keratinocyte serum-free media supplemented with bovine pituitary extract, human epidermal growth factor, and 2% fetal bovine serum. The cells were incubated in at 37°C in a humidified environment containing 5% CO_2. The cells were passaged routinely upon achieving 85-90% confluency.
Invasion Assays: HK-2 cells were infected with the *E. coli* CI5 strain (generously gifted by Dr. Soman Abraham from Duke University) at different MOI for 1.5 hours. The cells were washed with serum free media and treated with 100 µg/mL of gentamicin for 1 hour to kill all the extracellular bacteria. HK-2 cells were treated with liposomes and ciprofloxacin antibiotic for 3 hours. The cells were lysed with 1% Triton X-100, lysates were plated on sterile L-agar plates and incubated at 37°C overnight. The number of colony forming units were enumerated after 24 hours. For ELISAs, the cells were washed after gentamicin treatment and incubated for 24 hours. The media was collected and frozen at -80°C.
ELISA: A sandwich ELISA was performed using the TNFα, IL-6 and IL-8 ELISA kit from Biolegend according to the manufacturer's instructions.
Cytotoxicity Assay: HK-2 cells were treated with different concentrations of liposomes for 6 hours. MTT was added to the cells 1 hour prior to the incubation period. After the incubation period, cells were washed with 1X phosphate buffered saline and solubilized using dimethyl sulfoxide. The resulting formazan that was produced was measured spectrophotometrically at 595 nm.
Flow Cytometry: HK-2 cells were treated with different concentrations of fluorescent liposomes. The cells were washed using 1X PBS and analyzed for liposome binding by flow cytometry using the Cytoflex flow cytometer.

Conclusion

Our findings demonstrate that the human kidney cell line HK-2 is a suitable model for studying interactions between the host and invasive uropathogens that cause pyelonephritis. UPEC are able to internalize into HK-2 cells and induce the production of pro-inflammatory molecules. Treatment of HK-2 cells with drug-encapsulated liposome nanoparticles revealed that liposomes are not toxic to HK-2 cells at all concentrations tested. Additionally, by using fluorescent liposomes, we were able to determine that liposomes can interact with HK-2 cells in a dose dependent manner. Treatment of infected HK-2 cells with drug-encapsulated liposomes showed that there was a concentration dependent decrease in the number of intracellular UPEC found in infected HK-2 kidney cells. These findings suggest that liposomes are good drug delivery candidates for the treatment of pyelonephritis caused by intracellular pathogens.

References

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