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# Liposome encapsulated berberine treatment attenuates cardiac dysfunction after myocardial infarction

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ABSTRACT

Inflammation is a known mediator of adverse ventricular remodeling after myocardial infarction (MI) that may lead to reduction of ejection fraction and subsequent heart failure. Berberine is a isoquinoline quarternary alkaloid from plants that has been associated with anti-inflammatory, anti-oxidative, and cardioprotective properties. Its poor solubility in aqueous buffers and its short half-life in the circulation upon injection, however, have been hampering the extensive usage of this natural product. We hypothesized that encapsulation of berberine into long circulating liposomes could improve its therapeutic availability and efficacy by protecting cardiac function against MI *in vivo*. Berberine-loaded liposomes were prepared by ethanol injection and characterized. They contained 0.3 mg/mL of the drug and were 0.11 μm in diameter. Subsequently they were tested for IL-6 secretion inhibition in RAW 264.7 macrophages and for cardiac function protection against adverse remodeling after MI in C57BL/6J mice. *In vitro*, free berberine significantly inhibited IL-6 secretion ($IC_{50}$ = 10.4 μM), whereas encapsulated berberine did not as it was not released from the formulation in the time frame of the *in vitro* study. *In vivo*, berberine-loaded liposomes significantly preserved the cardiac ejection fraction at day 28 after MI by 64% as compared to control liposomes and free berberine. In conclusion, liposomal encapsulation enhanced the solubility of berberine in buffer and preserves ejection fraction after MI. This shows that delivery of berberine-loaded liposomes significantly improves its therapeutic availability and identifies berberine-loaded liposomes as potential treatment of adverse remodeling after MI.



## 1. Introduction

Acute myocardial infarction (MI) after coronary occlusion leads to scarring of the left ventricle that can induce adverse left ventricular remodeling, which may lead to congestive heart failure (CHF) [1]. CHF is a rapid growing pathology in which the heart cannot supply enough blood to the body resulting in severe fatigue, breathlessness and ultimately death [2]. Despite advances in the treatment of CHF over the last decades, mortality and readmission remains high [3]. Left ventricular remodeling is a complex inflammatory process involving the innate immune system with infiltration of immune cells, in particular macrophages, matrix degradation and scarring [4,5]. Although it is generally

*Abbreviations:* BB, berberine chloride; BB-lip, berberine liposomes; CAD, charged aerosol detector; CHF, congestive heart failure; DLS, dynamic light scattering; HBS, HEPES buffered saline; HF, heart failures; LPS, lipopolysaccharide; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PBS, phosphate buffered saline; RT, room temperature.

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accepted that inflammation plays crucial roles in adverse left ventricular remodeling and subsequently CHF [6], there is no evidence-based therapy focused on reducing inflammation yet.

*Berberis* spp. are widely distributed plants from the Berberidaceae family [7,8], from which berberine, a small fluorescent isoquinoline quarternary alkaloid [9,10] (Fig. 1), can be isolated [8,10]. Both berberine and the Berberis plants have a long history in Middle Eastern [11], Ayurvedic [10], Chinese [7] and Native American [9] traditional medicine. Berberine has been indexed in the Medical Subject Headings (MeSH) since 1975 [12] and nowadays has gained considerable attention from the Western scientific community [10]. Beneficial properties such as anti-inflammatory, anti-microbial, anti-diarrheal [9], anti-oxidative [9,13], vasorelaxant [9,11] and cholesterol lowering [11] effects have been ascribed to this natural product.

Importantly, long term and high dose berberine treatment has been reported to improve cardiac function [3,7] and to exert cardioprotective effects [14] in murine heart failure models. Furthermore, berberine was shown to improve survival in patients with CHF when given orally or intraperitoneally [15]. Clinical use of berberine, however, has been greatly impeded by its limited bioavailability due to the low absorption rate in the intestine (<5%) and excretion by P-glycoprotein and multidrug resistance associated protein-1 [16].

In this study, we investigated the use of berberine to reduce adverse remodeling and protect heart function in a mouse MI model. To overcome the low bioavailability of free berberine (BB) in the affected heart, berberine was loaded in long circulating liposomes (BB-lip) and administered intravenously. Due to locally enhanced vascular permeability in the affected regions of the heart [17], liposomes are expected to passively target the inflammatory site and subsequently release the drug after uptake by macrophages present in the region. This process would improve local drug delivery that may improve the therapeutic effect and reduce potential adverse effects by decreasing systemic exposure to berberine,

## 2. Materials and methods

### 2.1. Chemicals

Berberine chloride, lipopolysaccharide from *Escherichia coli* 055:B5, cholesterol and penicillin/streptomycin, sulfuric acid, triethylamine, methanol LC-MS grade (Fluka Analytical) and resazurin sodium salt were purchased from Sigma-Aldrich Chemie BV, The Netherlands and used without further purification. Albumin bovine fraction V, sodium chloride and 1-step ultra TMB ELISA substrate were obtained from Fisher Chemical, Thermo Scientific, BV, The Netherlands. IL-6 cytoset was acquired from Life Technologies BV, The Netherlands. Prednisolone disodium phosphate was obtained from Fagron BV, The Netherlands. Tween 20 and HEPES were purchased from Acros Organics, Belgium. RPMI-1640 without L-glutamine and L-glutamine 200 mM from PAA Laboratories GmbH, Germany. Fetal bovine serum (FBS) was purchased from Lonza, Belgium, dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphoethanolamine-poly ethylene glycol 2000 (DSPE-$PEG_{2000}$) were obtained from Lipoid GmbH, Germany. Ethanol absolute, ACS, ISO, Reag. Ph Eur was purchased from Merck Millipore B.V., Amsterdam, The Netherlands. MilliQ water was obtained from a Merck Millipore Q-POD. Medetomidine and atipamezole were purchased from Pfizer Animal Health, Exton, PA, USA. Midazolam was purchased from sciencelab.com, Texas, USA, fentanyl from Pfizer Pharmaceuticals Group, New York, USA, flumazenil from Sagent Pharmaceuticals, Illinois, USA, buprenorphine from Hospira Inc., Illinois, USA, and isoflurane from Baxter, Singapore.

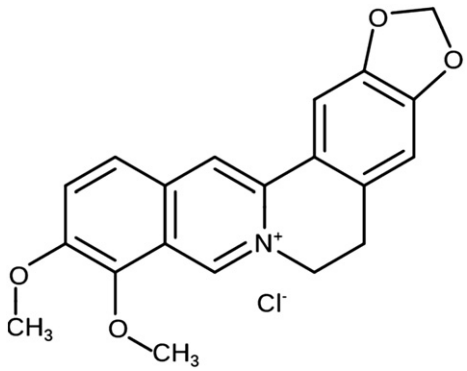


**Fig. 1.** Molecular structure of the isoquinoline quarternary alkaloid berberine chloride.

### 2.2. BB-lip preparation

Liposomes were prepared by the 'ethanol injection method' [18]. Briefly, 734 mg DPPC, 226 mg DSPE-$PEG_{2000}$ and 209 mg cholesterol (molar ratio: 1:0.08:0.28) were dissolved in 1 mL ethanol absolute while stirring on a hot plate. BB, with a final concentration of 3 mg/mL, was dissolved in 9 mL HEPES buffered saline (HBS, 10 mM HEPES with 150 mM NaCl) at pH 7.4 while stirring on a hot plate. Once both solutions were clear, the lipids were rapidly injected into the BB solution using a preheated 5 mL syringe (BD Plastipak, Ireland) through a 18 gauge needle (BD Microlance 3, Ireland). The BB-lipid solution was extruded multiple times at 70 °C through a final membrane pore size of 0.1 μm (Nuclepore Track-Etch Membrane, Whatman) using a thermostat pump (Polystat 36, Fisher Scientific, The Netherlands) coupled to an extruder (Lipofast LF-50, Avastin, Germany). Subsequently, the BB-lip solution was dialyzed against HBS buffer at pH 7.4 for two days, changing buffer four times, to remove ethanol and free BB. To remove the final free BB, BB-lips were cleaned using a PD10 Sephadex G-25M column (GE Healthcare, UK). BB-lips were stored at 4 °C until further usage. Control liposomes (control-lip) without BB were prepared in the same way. Cyanine 5.5 liposomes (Cy5.5-lip) were prepared as described by Lobatto et al. [19].

### 2.3. Size determination of BB-lip

The diameter of the BB-lip was determined using dynamic light scattering (DLS, Malvern Instruments). Samples were diluted 100 times until the solution was only slightly yellow and measured at 20 °C using a 173° scattering angle. Intensity results were selected and the diameter and polydispersity index (pdi) of BB-lip was calculated using the Zetasizer Software (version 7.02, Malvern).

### 2.4. BB concentration in BB-lip and release in plasma

BB concentration in BB-lip was determined using the UV-VIS program of a nanodrop (NanoDrop ND-1000 spectrophotometer, Fisher Scientific, The Netherlands). A standard calibration curve of 10, 25. 50, 75 and 100 μg/mL of BB in water was used to determine the concentration in BB-lip. BB-lip solution was diluted 1:10 and 1:20 in methanol to dissolve the lipids. For lipid background controls, control liposomes were diluted in methanol (1:10, 1:20) and control liposomes were spiked diluted with methanol, with a final concentration of 25 μg/mL BB. Furthermore, solvent controls included water (blank), HBS and methanol. Absorption was measured at 346 nm and the concentration of BB in BB-lip was deduced from the standard curve.

For release experiments, BB-lip 100 μg/mL final concentration were placed in a dialysis bag with a molecular weight cut-off of 300 kDa in a 20× larger volume of human plasma for 24 h at 37 °C. Released BB was determined in the plasma compartment from a standard curve also prepared in plasma that was diluted in water as described above.