



Development of novel nanoparticles shelled with heparin for berberine delivery to treat *Helicobacter pylori*

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ABSTRACT

Various approaches have been proposed to overcome the unpleasant side-effects associated with antibiotic treatment for *Helicobacter pylori*. The limited effectiveness of such approaches has forced researchers to consider alternative strategies to eliminate *H. pylori* infection. The plant alkaloid berberine is known to significantly reduce proliferation of *H. pylori*. To localize berberine to the site of *H. pylori* infection, this study developed a novel nanoparticle berberine carrier with a heparin shell. Analysis of a simulated gastrointestinal medium indicated that the proposed in vitro drug carrier system effectively controlled the release of berberine, which interacted specifically with the intercellular space at the site of *H. pylori* infection. Furthermore, the prepared nanoparticles significantly increased the suppressive effect of berberine on *H. pylori* growth while efficiently reducing cytotoxic effects in *H. pylori*-infected cells.

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1. Introduction

Gastric ulcer disease is a global health concern because of the considerable economic burden associated with its high morbidity and mortality rates [1]. *Helicobacter pylori* infection is considered to be a primary risk factor for the development of gastric ulcer and gastric cancer [2]. The bacterium *H. pylori* produces the enzyme urease, which is able to hydrolyze urea to ammonia and bicarbonate in order to neutralize the acidic pH of the stomach environment. It has been reported that the normal gastric pH of approximately 1–3 can be elevated to about 4.5–7.0, thus creating an environment conducive to *H. pylori* colonization [3]. Moreover, the microorganism mainly exists deep within the gastric mucus layer, where it adheres to gastric epithelial cells through a variety of adhesion-like proteins [3,4]. In order to effectively eradicate *H. pylori* infection the therapeutic agent must be able to penetrate the gastric mucus layer and maintain a concentration sufficient for antibacterial activity at the infected site for a suitable length of time. The most widely recommended regimen includes a triple therapy which combines various antibiotics (amoxicillin, clarithro-

mycin, and metronidazole) and a proton pump inhibitor administered over a period of 2 weeks [5]. However, the occurrence of unpleasant side-effects, such as a metallic taste in the mouth, diarrhea, and nausea, may cause the patient to interrupt the prescribed course of antibiotics, thus promoting the development of bacterial resistance [6]. This situation has forced researchers to look for alternative strategies to treat and eradicate *H. pylori* infection.

Several herbal medicines have been tested for their potential antibacterial activity against *H. pylori* in vitro and in clinical studies as possible candidates for use in modified eradication therapies [2,7]. Berberine (structure shown in Fig. 1a) is an isoquinoline quaternary alkaloid derived from a number of species of the barberry plant, including *Berberis aristata* and *Coptis chinensis* [8]. Berberine has been used for over 2000 years in traditional Eastern medicine to treat gastro-enteritis and secretory diarrhea and is also effective in the prevention and treatment of diarrheal illness [9]. Recent pharmacological studies have demonstrated that berberine is able to exert inhibitory effects on the proliferation capacity of *H. pylori* and the activity of *H. pylori* N-acetyltransferase [10]. Thus, berberine could act to reduce the growth of *H. pylori* in infected individuals, promote antineoplastic activity against gastric cancer cells and protect the gastric mucosa from damage [11–13]. However, it is unknown whether berberine may be able to readily penetrate the epithelium. Therefore, this study developed a site-specific drug delivery system that not only facilitates penetration of the stomach

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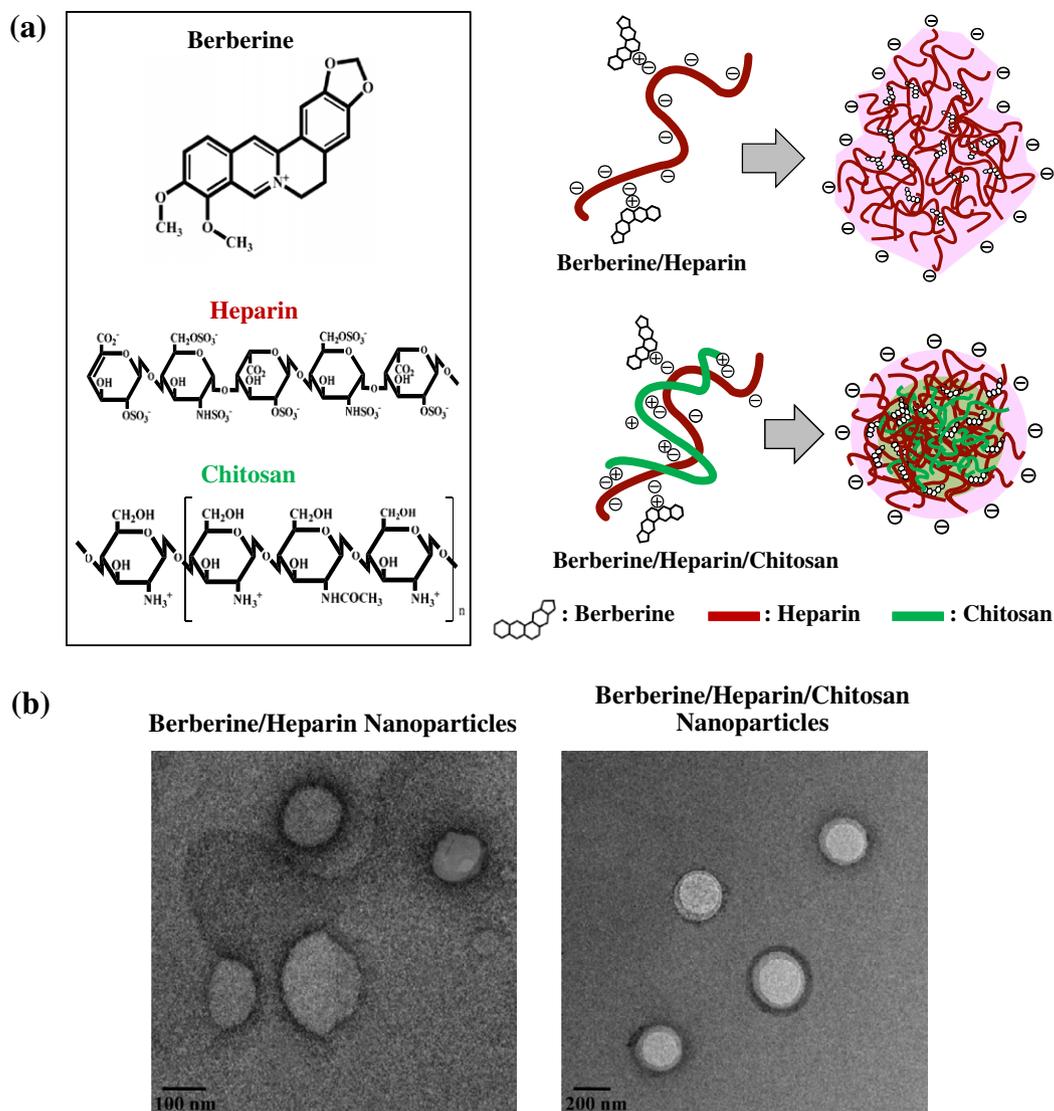


Fig. 1. (a) Schematic illustrations of the internal structures of berberine/heparin nanoparticles and berberine/heparin/chitosan nanoparticles. (b) TEM micrographs of the berberine/heparin nanoparticles and berberine/heparin/chitosan nanoparticles.

mucus layer, but also delivers a sufficient berberine concentration to eradicate *H. pylori* infection.

To localize berberine at the *H. pylori* infection site on the gastric epithelium, where it may improve the efficacy of concomitantly administered anti-*H. pylori* agents, a novel nanoparticle with an unfractionated heparin shell was employed as a drug delivery system. The polymeric chain of unfractionated heparin sodium salt (mean molecular weight (MW) 15 kDa), which is a polyanionic mucopolysaccharide, is composed of repeating disaccharide units of D-glucosamine and uronic acid linked by 1 → 4 interglycosidic bonds. The uronic acid residue may be either D-glucuronic acid or L-iduronic acid [14,15]. Recently heparin, a well-known anticoagulant, has been reported to have the ability to bind to cell receptors and accelerate gastric ulcer healing, which is associated with mucosal regeneration, proliferation, and angiogenesis [16]. We found that heparin crosslinked with berberine appears to have a heterogeneous size distribution with an oval donut shape (Figs. 1b and 2). To form a more suitable complex, we employed chitosan, a cationic polysaccharide known to be non-toxic, bioabsorbable, and biocompatible [17]. Chitosan has also been shown to adhere to and open tight junctions between epithelial cells and to increase the permeability of epithelial cell monolayers [18].

When blended with heparin the resulting chitosan–heparin complex was determined to be spherical in shape with a relatively homogeneous size distribution (Fig. 1).

In this study we have prepared nanoparticles composed of berberine, heparin, and chitosan at various weight ratios and examined their physico-chemical characteristics by Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and dynamic light scattering. We also investigated the characteristics of in vitro release of berberine from the prepared nanoparticles and examined the in vitro growth inhibition of *H. pylori* isolates. The effect of the nanoparticles and their mechanisms of interaction with a human gastric carcinoma epithelial cell line (AGS) [19] were investigated by confocal laser scanning microscopy (CLSM).

2. Experiments and protocols

2.1. Materials

Chitosan (MW 50 kDa), approximately 85% deacetylated, was obtained from Koyo Chemical Co. Ltd (Japan). Heparin (MW 15 kDa,

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