藥物奈米載體於治療胃潰瘍之應用：體外與體內實驗 (3/3)

Application of Nanoparticles Used for Gastric Ulcer Therapy: *In vitro and In vivo* Studies (3/3)

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摘要

消化性潰瘍為一種普見的消化系統疾病，以胃幽門螺旋桿菌感染情況最嚴重。臨床上對抗胃幽門螺旋桿菌以抗生素藥物為主，但此藥物需長時間服用以達到消滅胃幽門螺旋桿菌。本研究希望結合奈米技術在消化性潰瘍的治療，探討包覆不同藥物載體對消滅胃幽門螺旋桿菌之效果與影響。黃連素是黃連的生物鹼，可抑制胃幽螺旋桿菌增殖的作用，本研究計畫第三年為形成岩藻醣奈米載體保護黃連素不被胃酸破壞，並標的幽門螺旋桿菌達到黃連素有效治療胃幽門螺旋桿菌效果。

Abstract

Helicobacter pylori is considered to be an important etiological factor in gastric ulcer disease. Berberine is a main alkaloid of Coptis chinensis, treat gastrointestinal infection and exert inhibitory effects on the proliferation of *H. pylori*. The adhesions of *H. pylori* are a result of surface-associated antigens capable of recognizing specific carbohydrate fucose receptors of mucosal epithelial cells. The fucose-conjugated nanoparticles (NPs) were developed to protect berberine from destruction by gastric acids and to assist its infiltration into the mucus layer to specifically target *H. pylori* via receptor-mediated uptake. Drug carrier system effectively controls the release of berberine, which interacts specifically at the site of *H. pylori* infection. *In* vivo study, with berberine-loaded fucose-conjugated NPs, a more complete *H. pylori* clearance effect was observed, and *H. pylori*-associated gastric inflammation in an infected animal model was effectively reduced.

Introduction

The *H. pylori* colonize the human gastric mucus layer and adheres to the surface epithelial cells through a variety of adhesin-like proteins [1]. Clarithromycin or metronidazole combined with amoxicillin and a proton pump inhibitor is the most frequently used regimen for first-line *H. pylori* therapy for symptomatic patients [2]. Furthermore, the occurrence of unpleasant side effects, such as a metallic taste in the mouth, diarrhea, and nausea, may cause the patient to discontinue [3]. Coptis chinensis (Huanglian in Chinese), a traditional Chinese medicinal herb. Berberine, a main alkaloid of Coptis chinensis, has been studied. It can be used to treat gastrointestinal infections and inhibit the proliferation of *H. pylori* [4]. A main concern was the possibility of topical administration of the spectrum drug through direct bind to and site-specific contact with *H. pylori*, and thus its ability to increase the value of *H. pylori* growth inhibition. We developed fucose-conjugated NPs as vehicles for berberine delivery that specifically target *H. pylori* through receptor-mediated uptake. The prepared NPs are thought to infiltrate the mucus layer, directly contacting the region of *H. pylori* on the gastric epithelium. At the site of infection, the prepared NPs become unstable because of their pH sensitivity and release berberine to act locally on *H. pylori* (Fig. 1).

Materials and methods

Preparation of fucose-conjugated NPs

Fucose-conjugated NPs were prepared using a simple ionic gelation method with magnetic stirring at room temperature.

Immunohistochemistry staining

The tissue section was also subjected to immunohistochemical analyses using the rabbit polyclonal *H. pylori* antibody and the NovoLink polymer detection system.

Results and Discussion

Characterization of fucose-conjugated NPs

We produced fucose-conjugated chitosan using a reductive alkylation reaction as a result of the reaction of fucose with the amine groups on chitosan. The link between chitosan and fucose was confirmed by ^{1}H-NMR spectroscopy (Fig. 2). The spectrum of the fucose-chitosan conjugate show that the CH_3-6 signal at 1.27 remained intact with fucose. The signal observed between 2.78 to 3.10 ppm corresponded with the NCH of C-2 in chitosan and NCH_2 of C-1 in fucose moiety. The signals between 3.48 and 4.12 ppm ascribed to H-3 to H-6 of chitosan overlapped with H-2 to H-5 of fucose.

Berberine release profiles of berberine-loaded fucose-conjugated NPs

The pH responses of the berberine-loaded fucose-chitosan/heparin NPs were examined by TEM for their morphology and release profiles (Fig. 3). At a pH 1.2, some of the $–COO^-$ groups on heparin became protonated (–COOH) and resulted in an electrostatic interaction between fucose-chitosan

and heparin was therefore relatively weak due to berberine release 34.9 ± 4.9% (for 120 min). Outside this pH range (e.g., pH 7.0), the NPs became unstable, broke apart, and then released berberine from the NPs. This is because at pH 7.0, chitosan is deprotonated, causing the collapse of the NPs [5].

Relationship between *H. pylori* and berberine-loaded NPs co-cultured with AGS cells

The AGS cell monolayers were incubated with fluorescent berberine loaded in Cy3-fucose-chitosan/FA-heparin NPs and observed by CLSM to ascertain whether the NPs and berberine were co-localized and interacted at the same location of intercellular spaces of *H. pylori* infected sites at different depths (green arrows) (Fig 4). Chitosan, a cationic polysaccharide, is derived from chitin by alkaline deacetylation, and has the special feature of adhering to the mucosal surface [6]. The berberine-loaded NPs produced an intense fluorescence that emanated from deep within the cells, indicating that the NPs were capable of carrying berberine to AGS cell monolayers infected with *H. pylori*.

Immunohistochemistry staining in *H. pylori* infected mice

The immunostained slide of the gastric tissue biopsy of infected mice treated with deionized water showed a high level of bacterial colonization on top of the mucous layer, and epithelial cells with some bacteria penetrated into the gastric glands (red arrows). On the contrary, the density of bacteria in the gastric tissue biopsy in infected mice treated with the berberine-loaded fucose-conjugated NPs was obviously less than the one observed on the slides of other groups (red arrows) (Fig 5).

Conclusions

The chemical structure characterized by indicate that the fucose-conjugated NPs composed of fucose-chitosan and heparin have been prepared successfully. The fucose-conjugated NPs with pH-responsive characteristics can protect berberine from destruction by gastric acids, allowing the drug to infiltrate the mucus layer, and come into contact with *H. pylori* through receptor-mediated uptake, and significantly enhance the suppressive effect of berberine on H. pylori growth.

References

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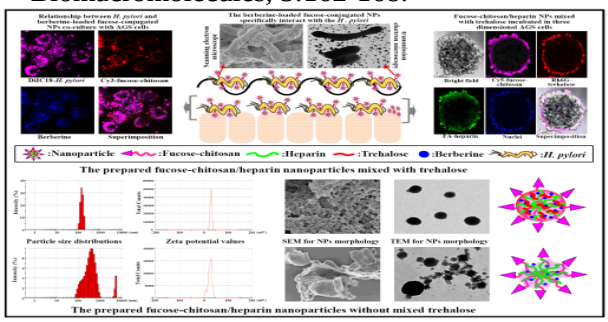

Fig. 1. Representation of berberine-loaded NPs and the strategy for eradicating *H. pylori* using NPs.

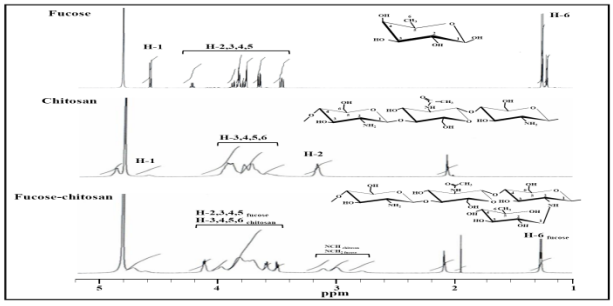

Fig. 2. ^{1}H NMR spectra of fucose-chitosan.

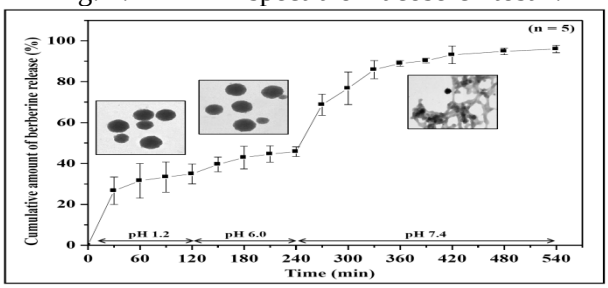

Fig. 3. TEM micrographs of NPs and *in vitro* release profiles of berberine from NPs.

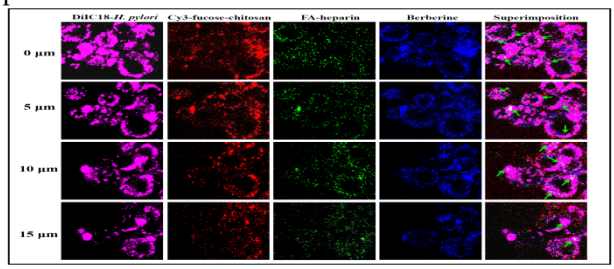

Fig. 4 Fluorescent images of AGS cell monolayers infected with *H. pylori* and incubated.

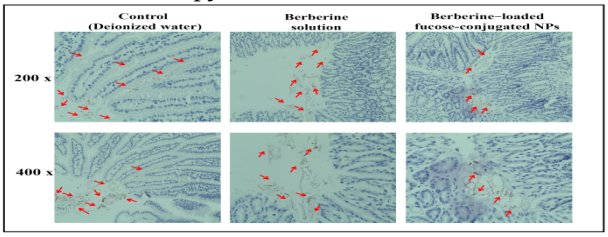

Fig. 5. Immunohistochemical staining analysis of a *H. pylori*–infected mouse treated with NPs