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Design and *in vitro* evaluation of a novel poly(methacrylic acid)/metronidazole antibacterial nanogel as an oral dosage form

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ABSTRACT

To overcome the undesirable side-effects of metronidazole (MTZ), ethylene glycol dimethacrylate is used as the cross-linker, and a series of poly(methacrylic acid) (PMAA) nanogels were prepared to load the MTZ. We investigated the morphology, size, *in vitro* release property in the simulated gastrointestinal medium, long-term antibacterial performance against *Bacteroides fragilis*, cytotoxicity, stability and activity of this novel MTZ/PMAA nanogel. The results indicate that the MTZ/PMAA nanogel sustained the release of MTZ in long-term antibacterial activity in the simulated gastrointestinal medium. This MTZ/PMAA nanogel exhibits less cytotoxicity than MTZ alone, suggesting that MTZ/PMAA nanogel is a more useful dosage form than MTZ for mild-to-moderate Clostridium difficile infections. The novel aspects of this study include the synthesis of a nanogel and the three-phase study of the release profile, which might be useful for other researchers in this field.

1. Introduction

Metronidazole (MTZ, CAS: 443-48-1, Fig. S1) is 5-nitroimidazole-based drug with highly antibiotic and anticoccidial activity [1]. MTZ is useful for the treatment of systemic anaerobic infections, including those caused by *Bacteroides fragilis.* Common adverse drug reactions (≥1% of patients) associated with systemic metronidazole therapy include the following: nausea, diarrhoea and/or a metallic taste in the mouth. Intravenous administration is commonly associated with thrombophlebitis. Infrequent adverse effects include the following: hypersensitivity reactions (rash, itch, flushing and fever), headache, dizziness, vomiting, glossitis, stomatitis, dark urine, and/or paraesthesia [2]. High dose and/or long-term systemic treatment with metronidazole are/is associated with the development of leukopenia, neutropenia, increased risk of peripheral neuropathy and/or CNS toxicity [2].

To increase patient compliance and overcome undesirable side effects, MTZ could be entrapped in a biodegradable nanoparticle for sustained delivery and designed as a prodrug [3], gel [4–8] or nanofiber [9–11]. These systems have been proposed to achieve a controlled and low-release rate of the drug to ensure a constant *in vivo* drug concentration for an extended time while preventing harmful side effects and deficits [12,13].

In recent years, nanogel represents a promising class of soft materials for drug delivery and controlled release of bioactive molecules [14–16]. It exhibits several attractive features over other particulate delivery systems including stability, ease of synthesis, and good control over particle size. The nanogel based on poly(methacrylic acid) (PMAA) has been extensively explored for drug delivery and tissue engineering. Argentiere et al. [17] prepared a pH stimuli–response nanogel based on PMAA for the uptake and controlled release of a bioactive molecule. Shi et al. [18] prepared a uniform molecularly imprinted PMAA nanosphere for the loading and sustained release of gatifloxacin. Pan et al. [19] prepared a redox/pH dual stimuli-responsive biodegradable nanohydrogel using PMAA for a cancer therapy study.

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In this paper, a nanogel based on poly(methacrylic acid) (PMAA) for loading and sustained release of MTZ was prepared through precipitation polymerisation. Its size and morphology were observed using a scanning electron microscope (SEM), and the loading of MTZ and the *in vitro* drug release behaviour in the simulated gastrointestinal medium were evaluated. The long-term antibacterial performance, cytotoxicity, stability and activity of this novel MTZ/PMAA nanogel were investigated.

The novelty of this study includes a synthesis of the nanogel and a three-phase study of the release profile, which might provide a useful reference for other researchers in this field. The use of the PMAA nanogel as an MTZ carrier produces MTZ in a more useful dosage form than MTZ alone for mild-to-moderate Clostridium difficile infections. The specific benefits of this novel hybrid included the following: (i) controlled release of MTZ with higher activity; (ii) dose control to achieve the desired antibacterial effect; (iii) sustained release capability to achieve a long-term antibacterial effect; and (iv) dose limitation to avoid eukaryotic toxicity.

2. Experiment

2.1. Materials

The methacrylic acid (MAA) purchased from Sigma–Aldrich (Shanghai, China) was distilled before use. The ethylene glycol dimethacrylate (EGDMA) from TCI and the metronidazole (MTZ) from Sigma–Aldrich were used as received, and the 2,2′-azobisisbutyronitrile (AIBN) from the Damao Chemical Reagent Factory (Tianjin, China) was recrystallised from ethanol three times before use. The HPLC-grade acetonitrile and methanol were of analytical grade and were used without purification. *B. fragilis* ATCC 25285 was supplied by the China Center of Industrial Culture Collection (Beijing, China). The Luria–Bertani (LB) broth and nutrient agar culture medium were supplied by the Huankai Microorganism Co., Ltd. (Guangzhou, China). The thiazolyl blue tetrazolium bromide (MTT) was purchased from Sigma–Aldrich (Shanghai, China). The human nasopharyngeal carcinoma L929 (L929) cells were supplied by the General Hospital of Guangzhou Military Command. All the other reagents and solvents were obtained from commercial suppliers. All the aqueous solutions were prepared with ultrapure water (>18 MΩ) from a Milli-Q Plus system (Millipore).

2.2. Preparation of the PMAA nanogel

A series of PMAA nanogels with different formula ratios were obtained *via* thermally initiated precipitation polymerisation [20]; *e.g.*, 0.87 mmol MAA was added to 50.0 mL of CH_3CN in a 100 mL round bottom flask. Then, 2.03 mmol of EGDMA and 25.83 mg of AIBN were added under stirring. The pre-solution was purged with nitrogen for 15 min to expel oxygen and sealed rapidly, and the reaction flask was placed in a pre-heated oil bath at 60 °C to polymerise for 24 h with moderate stirring. The product was collected by centrifugation at 8000 rpm, and purified with methanol three times to remove the unreacted MAA or EGDMA. The purified product was dried in a blast drying oven at 40 °C for 2 days to obtain the PMAA-1 nanogel (W_{MAA}:W_{EGDMA} = 67:33), PMAA-2 nanogel (W_{MAA}:W_{EGDMA} = 50:50) and PMAA-3 nanogel (W_{MAA}:W_{EGDMA} = 33:67).

2.3. Preparation of the MTZ/PMAA nanogel

In the drug loading experiment, MTZ was loaded into the PMAA nanogel *via* an immersion method. Briefly, the 10.0 mg PMAA nanogel was soaked in 4.0 mL of 0.9 wt% NaCl solution (pH = 7.0) that contained saturated MTZ. The mixture was ultrasonicated for 10 min to ensure the nanogel dispersion and kept in a digital shaking air bath at 37 °C for 24 h to obtain the MTZ/PMAA-1 nanogel, MTZ/PMAA-2 nanogel and MTZ/PMAA-3 nanogel. The MTZ-loaded nanogel was collected by centrifugation at 8000 rpm, and the supernatant was withdrawn.

The morphology of the obtained nanogel was observed using a SEM (PHILIPS, ESEMXL 30, Holland). Each sample was coated with gold by sputter coating (BAL-TEC, SCD 005, Liechtenstein) at 30 mÅ for 90 s. The number-average diameter (D_n) was calculated with an iTEM Soft Imaging System 5.0 (Olympus Soft Imaging Solution GmbH, build 1186). The calculation formula was as follows [18]:

$$D_n = \sum_{i=1}^{N} \left(\frac{n_i D_i}{n} \right) \quad (1)$$

D_i denoted the diameter of the i nanogel, n_i was the number of the particle with the diameter of D_i, and n was no <100. The results were expressed as the mean ± SD.

The MTZ concentration in the supernatant was dialysed (immersed in PBS (pH = 7.4) with a dialysis bag, molecular weight cut off of 3000) and detected with an analysis of the active ingredient by an HPLC (Agilent 1260) with the external standard method. The chromatograph conditions of the HPLC were as follows: the separation was performed in a AQ-C18 column (size: 4.6 mm × 250 mm, Ultimate™), the detection wavelength was set at 320 nm (the reference wavelength was set at 360 nm), the detection temperature was 25 °C, the flow rate was 1.0 mL/min, the injection volume was 20 μL, the mobile phase was 15% (v:v) acetonitrile (the solvent was a 0.1% (v:v) acetic acid solution in phosphate buffer) and was degassed using helium (purity 5.0), and the detection limit was 0.4 μg/mL. The retention time of MTZ was 5.16 min (Fig. S2).

The drug loading content (*LC*) and drug loading efficiency (*LE*) were calculated using the following Eqs. (2) and (3), respectively. Each sample was performed three times, and the results were expressed as the mean ± SD.

$$LC \ (\mu g/mg) = \frac{m_1 - m_2}{m} \quad (2)$$

$$\text{LE} \ (\%) = \left(\frac{m_1 - m_2}{m} \right) \times 100\% \quad (3)$$

Here m, m_1 and m_2 denoted the weight of the nanogel, the total feed of MTZ and the MTZ in supernatant, respectively.

2.4. In vitro release of MTZ from the nanogel

The simulated gastrointestinal medium and the amount of MTZ/PMAA nanogel that was added were determined from previous reports [21,22]. Briefly, the simulated gastric fluid was a solution of 0.1-mol/L hydrochloric acid (pH = 1.2), and 140 mg of the MTZ/PMAA nanogel was added to 10 mL of the simulated stomach fluid. The simulated small intestinal fluid was prepared with pH = 6.8 PBS containing 1% of pancreatin, and 95 mg of MTZ/PMAA nanogel was added to 10 mL of the simulated small intestine medium. The simulated colon fluid was prepared as a pH = 7.2 buffer medium that was composed of 0.15 wt% potassium dihydrogen orthophosphate, 0.15 wt% dipotassium hydrogen orthophosphate, 0.45 wt% sodium chloride, 0.05 wt% magnesium chloride hexahydrate, 0.005 wt% ferrous sulphate heptahydrate, 0.015 wt% calcium chloride dihydrate and 0.015 wt% sufficient sodium hydroxide, and 1 g of MTZ/PMAA nanogel was added to 10 mL of the simulated colon medium.

The release studies were conducted at 37 °C under slow stirring (50 rpm). The supernatant (2.0 mL) was withdrawn periodically at predetermined time intervals, and another 2.0 mL of simulated gastrointestinal medium was added to continue the experiment. The