Development of Ornithine-Based Nanomedicine for the Management of Acute Liver Injury

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Abstract

Acute liver injury (ALI) is a rapidly deteriorating condition accompanied by elevated systemic ammonia, which causes direct damage to hepatocytes and nervous system. Administration of L-ornithine (Orn) has been considered an effective way to enhance ammonia detoxification via the hepatic urea cycle, which is responsible for nitrogen waste disposal *in vivo*. However, poor pharmacokinetics of oral Orn administration results in unfavorable clinical outcomes of ammonia-lowering effect. Herein, to improve the therapeutic effect of Orn, an Orn-based amphiphilic block copolymer, [poly(ethylene glycol)-*block*-polyOrn] modified with isobutyryl (*i*Bu) groups (PEG-*b*-POrn(*i*Bu)) was prepared. This amphiphilic polymer can self-assemble into uniform nanoparticles ($Nano^{Orn(iBu)}$) suitable for oral administration. In an acetaminophen (APAP)-induced ALI mouse model with hyperammonemia, daily oral administration of $Nano^{Orn(iBu)}$ significantly reduced blood ammonia levels and liver damage compared to Orn treatment. Therefore, $Nano^{Orn(iBu)}$ may become a promising candidate for the oral hepatoprotective agent.

1. Introduction

Liver is a major organ for ammonia (NH_3) detoxification via the hepatic urea cycle. In a condition of acute liver injury (ALI), which is often induced by overdose of antipyretic and analgesic acetaminophen (APAP), dysfunction of the urea cycle leads to excess ammonia accumulation *in vivo*, known as hyperammonemia. L-Ornithine (Orn) is a key molecule controlling the urea cycle, and it has been widely applied in the clinical treatment of hyperammonemia. However, oral administration of low-molecular-weight (LMW) Orn is ineffective due to the rapid elimination and unspecific diffusion; hence, long-time intravenous infusion at a high dose is preferred in clinical applications, which significantly decreases patient's quality of life. To improve the treatment efficacy and patient compliance, an Orn-based amphiphilic block copolymer, [poly(ethylene glycol)-*block*-polyOrn(isobutyryl)] (PEG-*b*-POrn(*i*Bu)), which can spontaneously form nanoparticles ($Nano^{Orn(iBu)}$) in aqueous media due to amphiphilic feature, was designed for oral administration. Our strategy is to accumulate $Nano^{Orn(iBu)}$ in the intestine via oral administration, gradually release Orn from $Nano^{Orn(iBu)}$ catalyzed by intestinal enzymes and deliver Orn to the liver via the hepatic portal vein. The design of $Nano^{Orn(iBu)}$ and its therapeutic effect in an APAP-induced ALI model with

hyperammonemia via oral administration were investigated.

2. Experiment

2.1 Polymer synthesis and nanoparticles preparation

Amphiphilic block copolymer PEG-*b*-POrn(*i*Bu) was synthesized by following steps: (i) ring-opening polymerization of *N*-carboxy anhydride of *N*-δ-carbobenzoxy-Orn (Orn(Z)-NCA) initiated with PEG-NH_2 to obtain PEG-*b*-POrn(Z), (ii) reaction of PEG-*b*-POrn(Z) in a mixture of trifluoroacetic acid and hydrobromic acid to obtain PEG-*b*-POrn, and (iii) modification of exposed amino groups in the PEG-*b*-POrn with isobutyric anhydride to obtain PEG-*b*-POrn(*i*Bu). The synthesis was characterized by GPC and ^{1}H NMR. Nano$^{Orn(iBu)}$ was prepared by dissolving PEG-*b*-POrn(*i*Bu) in DMSO, followed by dialysis against water. The hydrodynamic diameter (D_H) and zeta potential of Nano$^{Orn(iBu)}$ were evaluated by Zetasizer Nano ZS.

2.2 Therapeutic effect of Nano$^{Orn(iBu)}$ in an APAP-induced acute liver injury model

C57BL/6N mice (male, 8 weeks old, body weight (BW): 25–27 g) were divided into 4 groups: Healthy (n = 15), APAP + Water (n = 18), APAP + LMW Orn (n = 15), APAP + Nano$^{Orn(iBu)}$ (n = 15). Test samples were orally administered every day for 4 days at a dose of 400 mg-Orn/kg-BW (3.0 mmol-Orn/kg-BW). On the 3rd day, mice were fasted for 24 h. On the 4th day, APAP was intraperitoneally injected into mice at 300 mg/kg-BW to induce ALI. Mice were euthanized at 6 h post-injection of APAP, and the blood was collected immediately for ammonia measurement. Liver injury marker, alanine transaminase (ALT) was analyzed by FUJI DRI-CHEM.

3. Results and discussion

The successful synthesis of PEG-*b*-POrn(*i*Bu) was confirmed by GPC (M_n(GPC) = 7300; M_w/M_n(GPC) = 1.07) and ^{1}H NMR analyses. Through dialysis, PEG-*b*-POrn(*i*Bu) self-assembled into uniform nanoparticles (Nano$^{Orn(iBu)}$) with the average D_H of 155 nm in water and exhibiting almost neutral charge (−0.47 mV). The symptoms of ALI with hyperammonemia were successfully induced by APAP overdose, which was verified by significantly higher levels of NH_3 and ALT in [APAP + Water] group compared to [Healthy] group (Table I). Oral treatment with Nano$^{Orn(iBu)}$ markedly decreased levels of NH_3 and ALT, whereas LMW Orn treatment failed to produce the same effect. Such a superior protection effect from Nano$^{Orn(iBu)}$ would be resulted from the adequate supply of Orn after oral injection, whereas LMW Orn eliminates very fast *in vivo*.

Table I. Hepatoprotective effect of oral Nano$^{Orn(iBu)}$ treatment in APAP-induced ALI.

	Healthy	APAP + Water	APAP + LMW Orn	APAP + Nano$^{Orn(iBu)}$
NH_3 / μg·dL^{-1}	42 ± 4.3****	127 ± 7.8	126 ± 9.1^{ns}	100 ± 4.7*
ALT / U·l^{-1}	39 ± 2.8****	10723 ± 1227	11466 ± 1031^{ns}	5716 ± 757**

Results are expressed as mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$, “ns” represents non-significance, all groups are compared with [APAP + Water] group.

4. Conclusions

The Orn-based amphiphilic block copolymer was successfully prepared and spontaneously formed nanoparticles (Nano$^{Orn(iBu)}$) in water. Oral Nano$^{Orn(iBu)}$ showed a favorable effect on improving ammonia removal and liver damage in the APAP-induced ALI model compared to LMW Orn, which could offer a novel therapeutic option in the clinical studies of ALI.